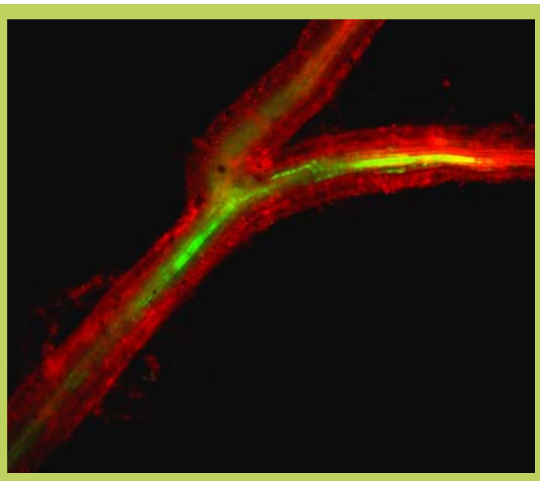
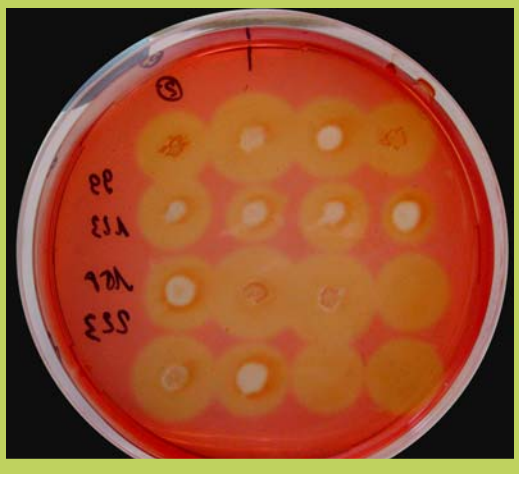
Euphresco

Euphresco III

“Dickeya & Pectobacterium meeting”








22 - 24 November 2015, Gdansk, Poland

Local organizer:
Prof. PhD E. Lojkowska (IFB UG & MUG, Gdansk, PL)
PhD Robert Czajkowski, PhD Marta Potrykus,
PhD Wojciech Sledz, PhD Małgorzata Waleron
Agata Motyka, Sabina Zołedowska

Project coordinators: **PhD Maria Bergsma (NVWA, NL) and PhD Jan van der Wolf (WUR, NL)**

IFB

The MOBI4Health project has received funding from the European Union's Seventh Framework Programme for reserarch, technological development and demonstration under grant agreement no 316094 and from the Ministry of Science and Higher Education

MOBI4Health

Sunday, 22th November 2015

10:00 13:00 **Guided tour city of Gdańsk** - We meet you at Dom Muzyka reception 9.45.
We meet with the guide at Novotel Hotel 10.00

16:00 21:00 **Visit to castle of Malbork** - Bus leaving from Novotel Hotel at 16:00

Monday, 23th November 2015

08:30 08:45 **Wellcome from University of Gdansk**

Opening Ceremony

08:45 **Introduction Euphresco III** - Jan van der Wolf

Scientific contributions

09:00 09:20 **Mass spectrometry in microbial research**
Katarzyna Macur, Mobi4Health

Session 1: Plant Pathogen Interactions

09:20 09:40 **The metabolic phases of the soft-rot disease**
Nicole Hugouvieux-Cotte-Pattat, Mobi4Health

09:40 10:00 **Plant phenolic acids affect the virulence of *Pectobacterium* spp. via quorum-sensing regulation.**
Janak Raj Joshi, Lipsky A., Shaked Yariv and Yedidia I.

10:00 10:20 **Comparison of *Dickeyas* expression profiles in macerated tubers and *Dickeya solani* diversity.**
Pédron J., Raoudes Essart Y., Khayi S., Hélias V., Faure D. and Van Gijsegem F.

10:20 10:50 Coffee & Tea break

Session 2: Epidemiology and control Part 1

10:50 11:10 **Spreading of soft rot bacteria with nematodes and winter flies.**
Minna Pirhonen, Nykyri J. and Pasanen M.

11:10 11:30 **Characterization of Finnish *Pectobacterium carotovorum* isolates from potato stems showing blackleg symptoms.**
Miia Pasanen and Pirhonen M.

11:30 11:50 **The effect of soil temperature and moisture on survival of *Pectobacterium carotovorum* subsp. *brasiliense* in fallow soil: Preliminary results.**
J.E. van der Waals, Laughton N. and Nhukarume L.

11:50 12:10 **Understanding the epidemiology of *Pectobacterium* in-field spread and blackleg disease.**
Emma Campbell, Humphris S., Cahill G., Nova Y., Saddler G., Elphinstone J., Wale S., and Toth I.

12:10 12:30 **Effect of lenticels and vascular infection with Dickeya spp. on blackleg symptom expression in potato fields**
Brice Dupuis, Riot G., Kellenberger I. and Schaerer S.

12:30 12:50 **Lytic bacteriophages against soft rot *Enterobacteriaceae* – isolation, characterization and future perspective.**
Robert Czajkowski, Ozymko Z., De Jager V. and Lojkowska E.

12:50 13:40 Lunch

Session 2: Epidemiology and control Part 2

13:40 14:00 **Steam versus DET sterilization in liquid media**
Enbio Technology

14:00 14:20 **Characterizing genetic resources to control the potato pathogen complex species belonging to *Pectobacterium* and *Dickeya*.**
Hélias Valérie, Quêtu-Laurent A., Pellé R., Le Hingrat Y., Marhadour S., Andrivon D., and Kerlan M.C.

14:20 14:40 **Implementation of research results on *Erwinia* into the seed production in The Netherlands.**
Kees Kristelijn

14:40 15:00 **Mechanism of antimicrobial activity of *Pseudomonas* sp. P482 against soft rot pathogens: a study aided by genome mining.**
Dorota M. Krzyzanowska, Ossowicki A., Rajewska M., Maciag T. and Jafra S.

15:00 15:20 **Virulence of Pectobacterium and Dickeya species in potato under climate conditions in Western Europe.**
Jan van der Wolf, de Haan E.G., Kastelein P., Krijger M., de Haas B.H., Velvis H., Mendes O. and Van der Zouwen P.S.

15:20 15:50 Coffee &Tea break

The MOBI4Health project has received funding from the European Union's Seventh Framework Programme for reserarch, technological development and demonstration under grant agreement no 316094 and from the Ministry of Science and Higher Education

MOBI4Health

Session 3: Diagnostics

15:50	16:10	**Whole-genome comparison-based classification of the soft rot *Enterobacteriaceae*.** Leighton Pritchard, Humphris S., Glover R., Elphinstone J. and Toth I.
16:10	16:30	**Monitoring of imported and national seed lots in the control of pectinolytic bacteria in the Swiss potato Industry (2013-2015).** Patrice de Werra and Keiser A.
16:30	16:50	***Dickeya solani* and *Pectobacterium* spp. in Israel.** Leah Tsror
16:50	17:15	**Bacterial diseases of potatoes in Chile: A re-emergent sanitary problem.** Ivette Acuña, Sandoval C. and Mancilla S.
19:30		**Workshop Dinner 19:30** PANORAMA RESTAURANT, Wały Piastowskie 1, 80-854 Gdańsk

Tuesday, 24th November 2015

Session 4: Surveys

08:30	08:50	**Test methods and field correlations for *Dickeya* and *Pectobacterium* spp.** Eisse de Haan and Kooman M.
08:50	09:10	**Survey of grower practice and identification of contributory factors which may be causing an increase in blackleg occurrence in Scotland.** Gerry Saddler, Davey T. and Hodge C.
09:10	09:30	***Dickeya solani*, the game changer in the 2015 cropping season potato blackleg outbreaks in north Finland.** Yeshitila Degefu and Holappa, O.
09:30	09:50	**Characterization of pectinolytic bacteria isolated in potato in Poland and Norway in 2013.** Renata Lebecka, Grupa A., Wiken Dees M., Perminow J., Næstad R., Brurberg M. B., Motyka A., Zoledowska S., Sledz W., Potrykus M., Golanowska M., Czajkowski R. and Lojkowska E.
09:50	10:10	**Biodiversity of *Dickeya* spp. isolated from potato plants and water sources in temperate climate.** Marta Potrykus, Golanowska M., Sledz W., Zoledowska S., Motyka A., Kolodziejska A., Butrymowicz J. and Lojkowska E.
10:10	10:30	Coffee & Tea break

Plenary discussion - Report Euphresco II

10:30	• **Euphresco III** • **Proficiency test** • **Panel of strains** • **Planning next meeting** • **Others** **Closure of the meeting**
12:30	Lunch
14:00	Departure

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MOBI4Health

Mass spectrometry in microbiological research.

Katarzyna Macur, Paulina Czaplewska

Laboratory of Mass Spectrometry, Core Facility Laboratories, Intercollegiate Faculty of Biotechnology UG-MUG, ul. Kładki 24, 80-822 Gdańsk, Poland

Mass spectrometry (MS) is a powerful tool to investigate microbial world. New developments in MS instrumentation and analytical workflows have been contributing to wider application of this technique in different areas of microbiology, such as taxonomic identification or microbial interactions [1]. The presentation will focus on different applications of mass spectrometry equipment of the Laboratory of Mass Spectrometry, supported by the FP7 MOBI4Health project, in microbial protein research performed at the at the Intercollegiate Faculty of Biotechnology UG-MUG. The examples will include application top-down and bottom-up proteomic approaches for identification of intact proteins and protein digests, respectively. The SILAC-based protein identification and quantification of differently treated *Candida albicans* cells will also be described. Apart from presently used at the IFB UG-MUG MS workflows, new applications available on our instrumentation of particular interest for microbiological studies will be shown.

[1] T. Luzzatto-Knaan, A.V. Melnik, P.C. Dorrestein: Mass spectrometry tools and workflows for revealing microbial chemistry. *Analyst* 2015, 140:4949.

The metabolic phases of the soft-rot disease.

Nicole Hugouvieux-Cotte-Pattat

CNRS UMR5240 Microbiologie Adaptation et Pathogénie, Université de Lyon, Université Claude Bernard Lyon 1, INSA de Lyon, F-69621 Villeurbanne.

The soft-rot disease caused by *Dickeya* dadantii is a dynamic process usually divided in two main phases. Bacteria penetrate their host using wounds or natural openings. During the first hours, bacteria reside latently in the plant intercellular space without provoking any symptoms. This "asymptomatic phase" is followed by a short transition to the "symptomatic phase" corresponding to appearance of the soft-rot symptoms.

Metabolomics (^{13}C-NMR spectroscopy) was used to analyze the metabolic activity of the pathogen. After infection of chicory leaves by *D. dadantii* 3937, the asymptomatic and the symptomatic phases corresponded to 1-8 hours and 12-24 hours, respectively. A kinetic analysis, with 4 h intervals, was performed to follow the infectious phases. The modifications observed during infection gave a direct image of the bacterial metabolism. During the asymptomatic phase, bacteria began to assimilate some easily metabolizable plant soluble sugars. A controlled repression of aggressive factors allows the bacteria to multiply without being recognized by their host. The transition step corresponds to a strong metabolic change including induction of pectate lyase production. The symptomatic phase corresponds to the expansion of soft-rot due to the pectate lyase burst. Bacteria multiply to large population sizes by obtaining more nutrients through the degradation of plant cell wall polysaccharides.

Plant phenolic acids affect the virulence of *Pectobacterium* spp. via quorum-sensing regulation.

Janak Raj Joshi[1, 2], Alexander Lipsky[2], Shaked Yariv[2] and Iris Yedidia[2]*

[1] *Department of Plant Pathology and Microbiology and the Otto Warburg Minerva Center for Agricultural Biotechnology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot, Israel*
[2] *Department of Plant Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel*
[*] *Correspondence. E-mail: irisy@volcani.agri.gov.il*

The virulence strategy of *Pectobacteria* is based on the secretion of exoenzymes that degrade the cell wall of their hosts, providing nutrients to the bacteria on the one hand, but conversely exposing the bacteria to plant defense compounds on the other. To better understand the effects of these plant-derived antimicrobial molecules, we have screened phenolic acids and polyphenols, for their ability to affect virulence in several *Pectobacteria*. The results revealed a significant effect of some phenolic compounds on virulence determinants such as motility, biofilm formation and extracellular enzyme activities. Moreover, short exposure to some phenolics prior to infection reduced disease severity by 20 to 100%, as shown in infection assays in three different hosts. The effect was not species specific and was proven effective in *P. carotovorum*, *P. carotovorum* subsp. *basiliense*, *P. atrosepticum* and *P. aroidearum*. To explore the mechanism underlying these processes, the effect of cinnamic acid (CA) and salicylic acid (SA) on virulence was further investigated in *P. aroidearum* and *P. carotovorum* subsp *brasiliense*. The results clearly indicated that both compounds interfered with the quorum-sensing (QS) machinery of the two species, consequently altering the expression of bacterial virulence factors. While in control treatments, expression of QS-related genes increased over time, exposure of bacteria to nonlethal concentrations of CA or SA inhibited the expression of QS genes, including *expI*, *expR*, PC1_1442 (*luxR* transcriptional regulator) and luxS (a component of the AI-2 system). Other virulence genes known to be regulated by the QS system, such as *pecS*, *pel*, *peh* and *yheO*, were also down-regulated relative to the control. In agreement with the low levels of expression of *expI* and *expR*, CA and SA also reduced the level of N-acyl-homoserine lactone (AHL) signal. The effects of CA and SA on AHL signaling was confirmed in compensation assays, in which exogenous application of N-(β-ketocaproyl)-L-homoserine lactone (eAHL) led to the recovery of the reduction in virulence caused by the two phenolic acids. The results support a mechanism by which plant phenolics interfere with *Pectobacterium virulence* via the QS machinery.

Comparison of *Dickeyas* expression profiles in macerated tubers and *Dickeya solani* diversity.

Jacques Pédron[1], Yannick Raoul des Essart[2,3], Slimane Khayi[2,4], Valérie Hélias[3], Denis Faure[2] and Frédérique Van Gijsegem[1]

[1] *iEES Paris (Institute of Ecology and Environmental Sciences), INRA-UPMC Université Paris 06, UMR 1392, 7 quai Saint-Bernard F-75005, Paris, France*
[2] *Institut for Integrative Biology of the Cell (I2BC), CNRS-CEA-Université Paris-Sud, Saclay Plant Sciences, UMR9198, Avenue de la Terrasse, 91198 Gif-sur-Yvette cedex, France*
[3] *Research, Development, Promotion of Seed Potatoes - French Federation of Seed Potato Growers (RD3PT-FN3PT), 43-45 Rue de Naples, 75008, Paris, France*
[4] *Université Moulay Ismaïl, Faculté des Sciences, Département de Biologie, Meknès, Maroc*

Comparison of the complete genome sequence of one *D. solani* and one *D. dianthicola* strains, both isolated from diseased potato in France, revealed a high synteny between these genomes. Both genomes only harbour a few hundred genes present in only one of the two species. Interestingly, these species-specific genes are often clustered in genomic regions, frequently associated with mobile elements in *D. dianthicola*. Most of these genomic regions regroup genes that are predicted to be involved in metabolism/transport as well as regulatory genes. Comparison of gene expression profiles after tuber infection reveals that the expression of several of these species-specific genes is modulated in planta as compared to in vitro bacterial growth pointing to an involvement in plant-bacteria interactions. A different modulation of several genes that are common to both species was also observed in planta. This indicates specificities in gene regulation between both species and highlights the importance of expression analysis to unravel the complete diversity of related strains/species in their interaction with their hosts.
The *D. solani* diversity was analysed by comparing the complete genome sequence of *D. solani* strain 3337 with 19 other *D. solani* genomes of strains isolated in different years, from various geographical locations and from different hosts. This population genomic analysis highlighted an unexpected variability among *D. solani* isolates. Indeed, in addition to scattered SNP/InDel variations, replacing and additive horizontal gene transfers (HGT) were observed by inter-species introgression of *D. dianthicola* genomic regions and plasmid acquisition. Furthermore, this analysis led to the characterization of two distinct sub-groups within the *D. solani* species differing to each other by variations (mainly SNP/InDels) in about one half of the genes. Replacing HGTs were also observed between the two *D. solani* sub-groups.

Spreading of soft rot bacteria with nematodes and winter flies.

Minna Pirhonen, Nykyri J. & Pasanen M.

Department of Agricultural sciences, University of Helsinki, Finland.

We have investigated the role of free-living nematodes and winter crane flies multiplying in storage rooms in the spreading of soft rot bacteria. Soft-rot enterobacteria were able to withstand nematode grazing, colonize the gut of *Caenorhabditis elegans* and subsequently disperse to plant material while remaining virulent. Two nematode species were also isolated from a rotten potato sample, and one of these isolates (*Pristionchus* sp.) was shown to be able to disperse soft-rot enterobacteria to plant material (Nykyri et al. 2013). The winter crane flies were collected from storage rooms and identified as *Trichocera maculipennis*. This species is known of antropogenic associations - in localities of cold climate it chooses relatively warm shelters, as cellars and caves, and often migrates with humans. No soft rot bacteria were identified in the adult winter crane flies collected from potato storage rooms. However, when the flies were exposed to potato tuber tissue containing soft rot bacteria, they were able to transfer the bacteria onto surface sterilized potato tubers. Furthermore, when the exposed potato tubers were incubated in rot-promoting conditions, they rotted faster than the control tubers. In conclusion, nematodes may have a role in the short-distance spreading of soft rot bacteria in soil, whereas the winter crane flies may spread the bacteria in potato storage rooms to clean harvest if rotten tubers are present in the same storage.

Nykyri et al. 2013 Plant Pathology 63:747-757

Characterization of Finnish *Pectobacterium carotovorum* isolates from potato stems showing blackleg symptoms.

Miia Pasanen & Minna Pirhonen

Department of Agricultural sciences, University of Helsinki, Finland.

Pectinolytic enterobacteria isolated from potato stems and tubers in Finland were identified as *Pectobacterium atrosepticum*, *Pectobacterium wasabiae* and *Pectobacterium carotovorum* subsp *carotovorum* using phylogenetic analyses and biochemical tests (Pasanen et al. 2013). *P. c*. subsp. *carotovorum* strains clustered into two distinct groups in phylogenetic analyses. One of these groups mainly contained strains isolated from potato tubers and the other one mainly included isolates from potato stems. However, none of the *P. c.* subsp. *carotovorum* isolates differed statistically from the water-treated control in the amount of blackleg symptoms in a field assays with vacuum--inoculated seed tubers. Stem isolates were not able to elicit a hypersensitive response in tobacco leaves and they also produced barely a measurable levels of autoinducers in the stationary phase in vitro. Biolog phenotypic tests showed no differences between stem and tuber isolates. In DNA--DNA hybridization analysis stem isolate strain showed around 70% DNA-DNA relatedness with *P. c.* subsp. *carotovorum* type strain and in average nucleotide analysis they had less than 95% pairwise similarity value with any *P. carotovorum* species or subspecies, which is less than the cut off value (>95%) for species. Stem isolates clearly differ from *P. c.* subsp. *carotovorum* type strain and do not clearly resemble any other the *P. c. subspecies*, including *P. carotovorum* subsp. *brasiliense*. However, the stem isolates produced the expected amplicon with *P. c.* subsp. *brasiliensis* subspecies-specific primers, suggesting that these kind of strains can be mistakenly identified as *P. carotovorum* subsp. *brasiliense*.

Pasanen et al. (2013) Annals of Applied Biology 163(3):403-419.

The effect of soil temperature and moisture on survival of *Pectobacterium carotovorum* subsp. *brasiliense* in fallow soil: Preliminary results.

J.E. van der Waals, N. Laughton & L. Nhukarume

Department of Plant Science, University of Pretoria, Private Bag X20, Hatfield, Pretoria, 0028, South Africa

Monitoring of pathogen levels in soil is an important tool for disease management and understanding pathogen ecology. One of the most important pathogens in the South African potato industry is *Pectobacterium carotovorum* subsp. *brasiliense* (Pcb), the causal agent of soft rot and blackleg of potatoes. Although many articles have stated that pectinolytic bacteria are not able to survive for extended periods of time in soil in the absence of a host, none of these studies were conducted on Pcb. The aim of this experiment is thus to determine how long and under which environmental conditions Pcb is able to survive in fallow soil. Pilot pot trials using a clay-loam soil were set up at three different temperatures, 10, 20 and 30°C. The soil in each of the temperature treatments was maintained at two different moisture conditions i) dry (no watering at all) and ii) wet (saturation point). A 108 cfu/ml suspension of a GFP-tagged Pcb isolate was used to inoculate the soil. Soil samples were taken regularly after inoculation from each temperature / moisture treatment to determine survival of the bacterium in the soil. A dilution series was made of each soil samples and plated out onto selective medium. Resultant colonies were counted and viewed under fluorescent light to confirm the presence of the GFP-tagged isolate. The concentration of bacteria in samples was determined also determined by qPCR. Preliminary results showed that four to six weeks after inoculation Pcb was no longer detectable in soil by conventional plating out. However, bacteria were still detected by qPCR up for five months after inoculation of the soil. This is probably due to amplification of non-living bacterial DNA in the soil. Pcb survived longest in the wet soil and at warmer temperatures than in dry soil or at 10°C. This trial confirms results of various other studies on survival of pectinolytic bacteria, which indicate that these bacteria are unable to survive in soil for extended periods of time in the absence of a host. A second trial is currently underway to verify the results of the first.

Understanding the epidemiology of *Pectobacterium* in-field spread and blackleg disease.

Emma Campbell[1], Sonia Humphris[1], Greig Cahill[2], Yvonne Nova[2], Gerry Saddler[2], John Elphinstone[3], Stuart Wale[4] and Ian Toth[1]

[1]James Hutton Institute, Invergowrie, Dundee DD2 5DA
[2]Science and Advice for Scottish Agriculture (SASA), Edinburgh EH12 9FJ
[3]Fera Science Ltd., York YO41 1LZ
[4]Scotland's Rural College, (SRUC), Aberdeen AB21 9YA

Experimental plots were used to track the movement of a streptomycin resistant strain of *Pectobacterium atrosepticum* (Pba) from an infected central zone to healthy neighbouring plants. During the growing season, leaf, stem, root and tuber samples were taken from plants at radiating points from the central infector zone and tested for the presence of the marked strain and naturally occurring isolates. At the end of the growing season samples were taken from the stem of all plants showing blackleg symptoms and the pathogen present was identified. After haulm destruction the tubers designated to be sampled for assessment of infection were hand dug from the plot and tested to determine whether *Pectobacterium* infection was systemic (found in the vascular tissue of stolon end), or as lenticel infection in tuber peel. Our results confirmed movement of the streptomycin resistant Pba strain from infected to healthy plants. However, the data also revealed that natural isolates of *Pectobacterium*, including but not only Pba, were more prevalent than Pba 1039 strep^{R} in terms of both contamination and in causing blackleg disease. Pba 1039 strep^{R} and natural isolates of *Pectobacterium* were detected in both the peel and stolon end of the harvested tubers.

Effect of lenticels and vascular infection with *Dickeya* spp. on blackleg symptom expression in potato fields

Brice Dupuis, Gaétan Riot, Isabelle Kellenberger and Santiago Schaerer

Agroscope, Institute for Plant Production Sciences IPS, 1260 Nyon 1, Switzerland

Potato blackleg is known to be a seed borne disease caused by pectinolytic bacteria belonging to the genera *Pectobacterium* and *Dickeya*. The seed tuber can be infected through the lenticels and/ or through the vascular system of the plant. Inoculation through the lenticels is broadly used to assess the aggressiveness of *Pectobacterium* and *Dickeya* strains and is also commonly used to assess the susceptibility of the cultivars to blackleg. Nevertheless, little is known about the relevance of those results to assess the risk of transmission of the inoculum to the progeny tubers, through the vascular system of the plant. Field experiments were set up in Switzerland to answer this question: 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, planted in the field (year 1) and then, 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 results revealed that the correlation is better for aggressiveness trials (R-squared of 0.51 and 0.38) than for cultivar susceptibility studies (R-squared of 0.07, 0.06 and 0.21). This work indicates that 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 reach to wrong conclusions if the objective of the trial is to distinguish the susceptibility of different cultivars to those bacteria.

Lytic bacteriophages against soft rot Enterobacteriaceae – isolation, characterization and future perspective.

Robert Czajkowski[1], Z. Ozymko[1], V. de Jager[2], E. Lojkowska[1],

[1] *Department of Biotechnology, Intercollegiate Faculty of Biotechnology UG and MUG, Kladki 24, 80-822 Gdansk, Poland*
[2] *Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, the Netherlands*

Pectobacterium spp. and *Dickeya* spp. are necrotrophic bacterial pathogens of many important crops worldwide. The effective strategies to control pectinolytic bacteria have not yet been developed. Consequently, the management of *Pectobacterium* spp. and *Dickeya* spp. is based mainly on the exclusion of infected plant material and the use of hygienic practices during potato cultivation and in storage. This study reports on the isolation and characterization of broad host lytic bacteriophages able to infect the dominant Pectobacterium spp. and Dickeya spp. affecting potato in Europe viz. *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), *P. wasabiae* (Pwa) and *Dickeya solani* (Dso) with the objective to assess their potential as biological disease control agents. The lytic bacteriophages were isolated from potato samples collected in different potato fields in Poland. The phages were characterized for features that are potentially important for successful biological control applications and bacteriophages' stability in the environment. Transmission electron microscopy was used to study bacteriophage morphology. In the interaction studies, the phages were characterized for optimal multiplicity of infection, the rate of adsorption to the bacterial cells, the latent period and the burst size. The selected bacteriophages were also genotypically characterized with RAPD-PCR and RFLP techniques. The structural proteomes of selected phages were obtained by fractionation of phage proteins by SDS-PAGE. Phage protein identification was performed by liquid chromatography-mass spectrometry (LC-MS) analysis. Pulsed-field gel electrophoresis (PFGE), genome sequencing and comparative genome analysis were used to gain knowledge of the length, organization and function of the genomes.

Characterizing genetic resources to control the potato pathogen complex species belonging to *Pectobacterium* and *Dickeya*.

Hélias Valérie [1, 2, 4], Quêtu-Laurent Angélique [1, 2, 4], Pellé Roland [3, 4], Le Hingrat Yves [1, 4], Marhadour Sylvie [1, 3, 4], Andrivon Didier [2, 4], Kerlan Marie-Claire [3, 4]

[1] *FN3PT/RD3PT, French Federation of Seed Potato Growers- Research, Development, Promotion of Seed Potatoes, 43-45 rue de Naples, F-75008 Paris, France*
[2] *INRA, UMR1349 IGEPP, F-35653 Le Rheu Cedex, France*
[3] *INRA, UMR1349 IGEPP, Keraiber, F-29260 Ploudaniel, France*
[4] *UMT Innoplant, UMR1349 IGEPP, F-35653 Le Rheu Cedex*

Pectobacterium and *Dickeya* are the two bacterial genera involved in blackleg and soft rot symptoms in potato. They cause major damage and costly losses in Europe and beyond. The use of genetic resistance constitutes an interesting prospect to control these diseases, which currently relies exclusively on prophylactic measures.

Progenitors with resistance to *P. atrosepticum* have been identified by INRA in the late 1990's, made available to the French potato breeders and introduced in useful genetic material (cultivars or hybrids) [1] [2]. However, annual surveys and diversity studies conducted by FN3PT-RD3PTin France over the last decade have shown evolutions in the prevalence and distribution of the species/subspecies complex responsible for soft-rot and blackleg [3]. One part of the National Research Program on *Pectobacterium* and *Dickeya* (FN3PT-RD3PT-INRA) aim at evaluating the performance of the resistance sources identified some years ago against *P. atrosepticum* to control the other members of this pathogenic bacterial complex. A collection of 24 hybrids from different genetic backgrounds was tested against representative strains of the main taxa responsible for soft rot and blackleg. Ten of these clones showed very low soft rot severity in front of two to five species/subspecies, and 9 additional clones showed specific resistance to one of these species. Experiments to test the resistance level of clones against blackleg (stem infections) will be conducted. These promising results open new hopes for the sustainable control of the soft rot/backleg complex in potato.

[1] Andrivon et al., 2003. Amer J Potato Res 80: 125-134

[2] Pasco et al., 2006. Potato Res 49 : 91-98.

[3] Hélias et al., 2014, 19th Triennial Conference of the EAPR, Brussels : p.110

Implementation of research results on *Erwinia* into the seed production in The Netherlands.

Kees Kristelijn

HZPC, Edisonweg 5, 8501 XG Joure

In the Netherlands, after the *Erwinia* problems in the early 2000's, a survey was held under 300 seed potato growers. After a scan of the problems, and the answers of the farmers in the survey, a project with all major stakeholders was started in 2005. During the periods 2005 – 2009 ("Bacterievrije pootgoedteelt") and 2009 – 2013 ("Deltaplan Erwinia") research was done on almost all aspects of growing seed potatoes. All this research lead to a list of do's and don'ts which had to be implemented within the process of growing seed potatoes. A group of 8 companies , with more than 90% of the total acreage of seed potato production in the Netherlands, started a new project called Deltaplan Erwinia 2.0. HZPC started to work with the results of the first project in 2009. Adjustments in advice's to growers, and testing of basic seed material, have lead to less problems in degrading and rejection of seed lots in the last years in the Netherlands. This, despite the growing concern about the development of P.c. subsp. *Brasiliense*. In the years after 2009 the development of Erwinia in the different HZPC seed lots was monitored. In my presentation I will highlight some results of the first year of Deltaplan 2.0 and go in to more detail in the way HZPC acts on the implementation of research results on Erwinia into the seed potato production in the different seed production area's in Europe.

Mechanism of antimicrobial activity of *Pseudomonas* sp. P482 against soft rot pathogens: a study aided by genome mining.

Dorota M. Krzyzanowska, Adam Ossowicki, Magdalena Rajewska, Tomasz Maciąg & Sylwia Jafra

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Pectobacterium and *Dickeya* are plant-pathogenic bacteria causing soft rot disease of many vegetables and ornamental plants. Despite serious economic losses caused by these bacteria, the means to fight these pathogens are limited. *Pseudomonas* sp. P482, a Gram-negative bacterium originating from the rhizosphere of tomato, is able to inhibit the growth of various species of *Pectobacterium* and *Dickeya* in a plate assay and to attenuate plant tissue maceration caused by the selected strains of these pathogens.

To reveal the mechanisms involved in the antagonism between *Pseudomonas* sp. P482 and the soft rot pathogens, a transposon mutagenesis and a genome mining approach in combination with the site-specific mutagenesis were applied. The genome sequence of *Pseudomonas* sp. P482 was obtained, automatically annotated and subjected to data mining in search for secondary metabolite biosynthetic clusters. The search was performed both manually, with the blastp tool, and automatically, with the antiSMASH 2.0 software. Five genes were selected based on the *in silico* study, each representing one biosynthetic cluster predicted by the antiSMASH 2.0 as a result of a default search against its internal database. Knock out of these genes showed that they are not responsible for the antimicrobial activity of P482 against the soft rot bacteria. A more unrestricted antiSMASH 2.0 search based on Pfam domain probabilities revealed 18 more clusters designated as "hypothetical". A transposon mutant of P482 not inhibiting the growth of *Dickeya* spp. in vitro, carries an insertion in one of these "hypothetical" clusters. Thus, genome mining for secondary metabolite clusters is a powerful approach although when novel mechanism are at stake, it should be used in combination with other methods

Virulence of *Pectobacterium* and *Dickeya* species in potato under climate conditions in Western Europe.

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Dickeya and *Pectobacterium* strains, isolated from potato or from surface water in the Netherlands were tested in field experiments for their virulence in potato, Seed potatoes were vacuum-infiltrated and planted in 2013 and 2014 in randomized blocks in a clay soil in the Netherlands. Blackleg and slow wilt symptoms were recorded during the growing season. In both seasons, inoculation with strains of *Pectobacterium atrosepticum* and *P. carotovorum* subsp. *brasiliense* resulted in the highest disease incidences of between 75 and 95 percent of the plants that emerged. Inoculation with strains of *D. solani* and *P. wasabiae* resulted in disease incidences between 5 and 25 percent. Hardly any or no disease was observed in the treatments with four strains of *P. c* subsp. *carotovorum* strains, a strain of *D. dianthicola* and the water control. Co-inoculations of seed potatoes with a strain of *P. c.* subsp. *brasiliense* and *D. solani* gave a similar disease incidence as inoculation with *P. c.* subsp. *brasiliense* only. However, co-inoculation of *P. carotovorum* subsp. *brasiliense* with *P. wasabiae* decreased the disease incidence compared to inoculation with *P. c.* subsp. *brasiliense.* The presence of pathogens in progeny tubers of plants was confirmed with enrichment-TaqMan assays and for some treatments with dilution plating followed by a colony-TaqMan assay. For this, specific TaqMan assays were developed and evaluated for detection of *P. c.* subsp. *brasiliense* and *P. wasabiae* whereas a TaqMan for detection of *P. atrosepticum* previously developed previously at FERA (UK) was evaluated.

Whole-genome comparison-based classification of the soft rot *Enterobacteriaceae*.

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Effective detection and diagnosis of the soft rot *Enterobacteriaceae* (SRE) is dependent on the ability to classify pathogenic isolates accurately. Diagnostics and classification are made more difficult by the influence of horizontal gene transfer on phenotype, and historically complex and sometimes inaccurate nomenclatural and taxonomic assignments that persist in strain collections and online sequence databases. We present novel whole-genome classifications of the SRE using average nucleotide identity (ANI), illustrating inconsistencies between the established taxonomies and evidence from completely sequenced isolates

Monitoring of imported and national seed lots in the control of pectinolytic bacteria in the Swiss potato Industry (2013-2015).

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During recent years, possibilities of minimizing the impact of blackleg in Swiss seed potato production have been discussed. Complementary to the official seed potato certification scheme based on visual inspection in the field, a reliable routine method to assess the health status of seed tuber lots is being sought.

In an on-farm project conducted on behalf of the Swiss Federation of Seed Potato Growers (swisssem) from 2013 to 2015, the relationship between seed infection and blackleg incidence in the field was investigated. PCR/qPCR tests were performed to detect latent infections in seed lots by the causal agent of blackleg. The focus was on Dickeya sp. (Dsp), P*ectobacterium atrosepticum* (Patr), *Pectobacterium wasabiae* (Pwas) and *Pectobacterium carotovorum* subsp. *brasiliense* (Pcbr), which are the most problematic pectinolytic bacteria encountered recently in Switzerland. Having been in steep decline in Switzerland since 2012, Dsp and Patr were almost absent in 2015, in both seed latent infection and in the field. Since 2013, Pcbr has gained in importance and was still the most problematic agent of blackleg this year.
Although the reliability and usefulness of PCR/qPCR tests as a diagnostic tool seem to be acceptable for Dsp and Patr, this is not the case for Pcbr. This bacterium was found in latent infections in a very large part of the seed lots analyzed, but was not consistently seen in the field. This led to an increased number of false positive analyses, which weakened the reliability of the test for this bacterium.

Dickeya solani and *Pectobacterium* spp. in Israel.

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Potato is being cultivated in two major seasons in Israel; seed lots are imported from Europe for the spring each year whereas for the fall-winter season the growers use their own seeds (produced in spring under inspection of PPIS). Spring begins with low temperatures followed by increase, while the fall starts with high temp which decrease later. Pectinolytic bacteria cause economic losses with symptoms including pre-emergence tuber rot, blackleg and wilt. Although the pectinolytic bacteria are transmitted mostly by latent infection of seed tubers, disease symptoms vary with climate. *Dickeya solani* (Ds), *Pectobacterium carotovorum* (Pc), *P. c. brasiliense* (Pcb) and P. wassabiae (Pw) can grow at low and high temperatures with higher maximal growth temperature compared with *D. dianthicola* or *P. atrosepticum* (Pa). Development and expression of the disease in field may be particularly favored under warm-climate conditions.

The protocol for detecting *Dickeya*-latent infection in seed lots by enrichment RT-PCR was used to detect also other *Pectobacterium* spp. In 2015, 77 commercial imported seed lots were checked for latent *D. solani* and *Pectobacterium* spp. infections. Field surveys indicated that 69% of seed lots that were Ds-negative had no disease symptoms in fields (-L/-F), 19% were Ds-positive lots with field symptoms (+L/+F), 9% of seed lots were Ds-negative but had symptoms (false negative; -L/+F) and 3% were false positive (+L/-F). In a field trial conducted in 2015, seed tubers of randomly selected imported seed lots (10 lots of cv. Sifra, 6 Mozart, one of each cvs. Winston, Marabel, Rodeo, Canberra) were planted at Gilat (4 reps X 50 tubers). Wilt and blackleg symptoms were weekly monitored and the presence of pectinolytic bacteria was checked in seed tubers, plants and progeny tubers. In the selected seed lots, Ds and Pcb were detected in 75% and 55%, respectively. At the end of the experiment all sampled plants were contaminated with Ds, and 45% had Pcb. In progeny tubers, 75% were infected with Ds, and 70% with Pcb. Ware potatoes were recently exported to Jordan but only after lab test for Ds. Out of 39 tested lots 11 were Ds-positive (28%) and therefore rejected (among them: Sifra - 20 lots with 7 rejected; Winston 8 lots, 2 rejected; Panamera 2 lots, one rejected). The presence of Ds and Pcb in imported seed material directly affect yields in the spring crop in Israel, as well as on export of ware potato produced in this season in addition to the fall crop which might be also affected. Pathogenicity of Pcb isolates using the potato tuber maceration test was evaluated and further characterization will be carried out.

Bacterial diseases of potatoes in Chile: A re-emergent sanitary problem.

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Potato crop in Chile is positioned in fourth place with an average area in the last 10 years of 51.929 ha, reaching a production and yield average of 1.130.405 tons and 21.9 t / ha (ODEPA, 2014). Considering fresh consumption, potato represents about 50 kg/inhabitant per year. In addition, this crop has a great economic and social importance being produced by nearly 60.000 farmers, 50% of them in southern Chile. Today, the main sanitary problems in potato are late blight, PVY and bacterial diseases. During the 90´s, *Pectobacterium atrosepticum* (*Erwinia carotovora* sp *atroseptica*) was described as the main causal agent of soft rot and black leg, with a disease incidence of 40% to 100% as tuber latent infection and producing 20% of yield losses. At that time, the problem was managed using in vitro multiplication in the potato seed production system. However, black leg and soft rot is increasing again in the last 5 years, producing 15% of yield losses mainly due to the use of new irrigation systems, favorable weather conditions and new varieties, among others. During the last potato seasons a new survey was performed to do a collection of plant and tuber isolates. Strains were characterized using specific PCR primer ECA1f + ECA2r (de Boer et al, 1995) and EXPCCF+EXPCCR (Kang et al, 2003) for *P. atrosepticum* and *P. carorotovorum* subsp *carotovorum*. PCR shows that 13.5% and 64.8% of the strains amplified with ECA1f + ECA2r or EXPCF+EXPCR primer, respectively. However, 10% of strains did not shows reaction with these primers. Then, these results indicate that new strains are associated with potato causing black leg and soft rot, however, new studies need to be performed to have conclusive results.

Test methods and field correlations for *Dickeya* and *Pectobacterium* spp.

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Results of the yearly *Pectobacterium* and *Dickeya* survey showed a the relative increase of *Pectobacterium carotovorum* subsp. *brasiliense* as causal agent of blackleg. Therefore, we performed field experiments to determine the aggressiveness of Pcb as compared to *Pectobacterium atrosepticum* and *Dickeya solani*. In addition, different test methods were compared and field correlations of these methods were determined. Results of these experiments will be presented.

Survey of grower practice and identification of contributory factors which may be causing an increase in blackleg occurrence in Scotland.

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Blackleg remains the biggest cause of down-grading and rejection in the Scottish Seed Potato Classification Scheme. Currently, control is reliant on initiating production from disease free planting material, industry good practice and the implementation of the Classification Scheme itself. In Scotland, healthy planting material arises from disease-tested micro-plants produced by SASA, which are multiplied at 8 mini-tuber production facilities, under controlled conditions, across the country. Field grown potatoes are initially produced as pre-basic (PB) seed by 28 registered growers, where a zero-tolerance for blackleg (and other faults) is applied. Crop inspections during every year of field multiplication serve to limit/remove heavily diseased crops from the production chain.

Up until 2007 this approach was judged to be effective however more recently blackleg incidence has risen steadily, peaking in 2011 to levels not seen for more than 20 years. In addition, post--harvest tuber testing of PB stocks has shown that some crops can become infected with *Pectobacterium atrospeticum* (in the absence of symptoms) as early as FG1. It is also clear that by looking at the national picture from field inspections returns of basic crops, the incidence of blackleg generally increases with each subsequent field generation.

The purpose of this study was therefore to investigate what influence, if any, social changes/industry practices may have on blackleg incidence with a view to identifying contributing factors, should they exist. The survey initially focused on Scotland's 28 PB growers (alongside 1 PB grower each from England and Northern Ireland) and was based on one-to-one interviews in which a series of questions around social, financial and economic change were addressed. Results from this part of the study highlight possible contributory factors which may in part be contributing to the rise in blackleg incidence. The next stage in the process will be described from which, it is hoped, a refined approach to blackleg control will be derived.

Dickeya solani, the game changer in the 2015 cropping season potato blackleg outbreaks in north Finland.

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During the last three decades, the etiology of blackleg and soft rot in Finland has shifted from a serotype of one species - *Pectobacterium atrosepticum* to multiple species. To date *Pectobacterium atrosepticum*, *Pectobacterium carotovorum* subsp. *brasiliense*, *Pectobacterium carotovorum*, *Pectobacterium wasabiae* and *Dickeya solani* are frequently detected from potato with typical blackleg symptoms. Furthermore, it is intriguing to observe a significant number of samples with typical blackleg where none of these currently known potato blackleg and soft rot bacteria are detected. A circumstance tempting to suggest "unknowns" to the list and a revisit to the etiology of the disease complex. The *Dickeya* and *Pectobacterium* species listed above are known to be adapted to different temperature optimums and nowadays disease has become a likely phenomenon in Finland irrespective of the prevailing summer weather- warm or cool.

According to the 2015 weather statistics report by Finnish Meteorological Institute (http://en.ilmatieteenlaitos.fi/press-release/98978129) June was unusually cold 1-2 degrees colder than usual with exceptionally high precipitation especially in North Finland. July was exceptionally cold. The monthly average temperature in July remained below the long-term average in the whole country, varying from just over 15°C in southern parts of the country to less than 11°C in northern Lapland. A July as cool as this year occurs in the region on average once in 30 years. After two months of colder-than-average weather, August turned out to be warmer than average across the whole country. The average temperature for the whole country was 14.9°C, which was 1.4°C above normal levels. The month also saw a total of 15 hot days (where temperature rose over 25°C). The last time that August was the hottest month of the summer was in 2006.

Until the end of July, no blackleg symptoms were reported by farmers in the region. Just in the first and second week of August, severe and rapidly spreading blackleg outbreaks were reported from two separate fields of the same variety and seed background. The game changer was *D.solani*. Further characterization of the outbreaks and analysis of weather data will be discussed.

Characterization of pectinolytic bacteria isolated in potato in Poland and Norway in 2013.

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Bacteria belonging to *Pectobacterium* and *Dickeya* spp. cause soft rot diseases of a great variety of crops and ornamentals worldwide. In the last 5 years potato losses caused by soft rot have increased significantly and it appears that warmer climate escalate the disease development. We investigated the population structure of pectinolytic bacteria (*Dickeya/Pectobacterium* species) in Poland and Norway. The survey was conducted in 2013, and a representative selection of species and isolates from both countries, were examined in detail. From Poland, 17 Pectobacterium wasabiae (Pw), 14 *P. atrosepticum* (Pba), 9 *P. carotovorum* subsp. *carotovorum* (Pcc), and 2 - *Dickeya solani* (Ds) were selected. From Norway, 21 Pcc and 18 Pa isolates were selected. Single isolates of Pw and Ds were found. The isolates were characterized for several phenotypic traits: the aggressiveness on potato tubers, production of cellulases, pectinases, proteases, siderophores, motility, and growth in the presence of high salt concentration (5% NaCl). A maceration test on tubers of the susceptible potato cultivar Irys, at 26oC, showed high variability in aggressiveness within each bacterial species. The isolates from Norway were in average more aggressive than the Polish ones. The Pcc isolates were significantly more aggressive than the Pba isolates, and these were more aggressive than the Pw isolates. Polish strains of Pba, Pcc, Pwa and Ds did not differ significantly from Norwegian strains in other analyzed phenotypic traits. Individual isolates exhibited differences especially in siderophore, protease production and motility. Only three of the analyzed Pba strains produced proteases. The prediction of bacterial species based on discriminant analysis of preliminary data of all phenotypic traits ranged from 0.667 (for Pwa) to 1.0 (for Ds). The work is in progress within Polish-Norwegian POTPAT project.

Biodiversity of *Dickeya* spp. isolated from potato plants and water sources in temperate climate.

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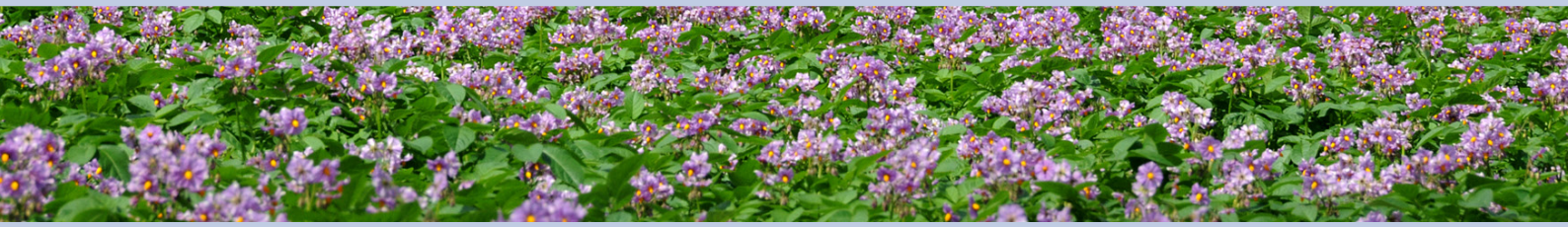
Bacteria from the genera *Dickeya* (formerly *Erwinia chrysanthemi*) and *Pectobacterium* (formerly *Erwinia carotovora*) are the agents of blackleg and soft rot disease on many important crops. In 2005, *D. solani* was isolated for the first time in Poland from a symptomatic potato plant. This motivated us to study the presence and diversity of *Dickeya* spp. in potato fields and water sources (including surface waters near potato fields, and water from potato processing facilities and sewage plants) in temperate regions of Poland (Central Europe). Only *D. dianthicola* and *D. solani* were isolated from symptomatic potatoes, and only *D. zeae* and *D. chrysanthemi* were isolated from water sources. The *Dickeya* spp. isolated from potato formed a relatively homogenous group, while those from water sources were quite diverse. To our knowledge, this is the first comprehensive characterization of *Dickeya* spp. isolated from regions with a temperate climate in Central Europe.

Pathology Section Meeting

Sunday 7th – Thursday 11th August 2016
Dundee, Scotland



It gives us great pleasure to announce that the next meeting of the European Association for Potato Research (EAPR) Pathology Section will be held in Dundee, Scotland.

This meeting covers research into potato pests and diseases with a focus on applied outcomes and attracts world-class speakers.

The program allows ample time for scientific presentations and an opportunity for participants to interact socially. The meeting will also include the chance to attend 'Potatoes in Practice', the largest field-based potato event in the UK.

To register your interest in the meeting or for any other enquiries please email:
eaprpathology2016@hutton.ac.uk

For further information please visit:
http://www.eaprpathology2016.eu

We look forward to welcoming you to Dundee in 2016.

Organising Committee Chair: Ian Toth
Organising Committee: Alison Lees and Sonia Humphris
Pathology Section Chairperson: Leah Tsror