CANADIAN PHYTOPATHOLOGICAL SOCIETY

ATLANTIC REGION MEETING 2017

KENTVILLE RESEARCH AND DEVELOPMENT CENTRE,
AGRICULTURE AND AGRI-FOOD CANADA,
32 MAIN STREET, KENTVILLE, NS

Atlantic Region Meeting
November 23, 2017
Kentville Research and Development Centre, Kentville, NS

INTRODUCTION

Welcome to Kentville and to the 2017 Canadian Phytopathological Society (CPS) Atlantic Region Meeting. It is our pleasure to host this year's CPS regional meeting, and we are looking forward to a stimulating afternoon of scientific discussion and fellowship.

There are 8 oral presentations and one poster presentation on various topics in phytopathology scheduled for the afternoon. A dinner will take place at Old Orchard Inn, 153 Greenwich Rd South, Exit 11, Highway 101, Wolfville NS following the meeting.

I am pleased to welcome **Dr. Shawkat Ali**, Research Scientist, Kentville Research and development Centre, Kentville NS, as our keynote speaker. Dr. Ali is an emerging scientist and known for his work on "effector proteins". His presentation is entitled: '**Role of effectors in inducing and suppressing disease resistance in barley and potato by pathogenic fungi and nematodes**'

Many thanks to all who are attending the meeting. I would particularly like to thank the president of the **Canadian Phytopathological Society**, **Dr. Denis Gaudet**, for sponsoring this event and I'd like to thank Denis for making the time to participate in our event and to bring us up to date on CPS activities. I trust we will be able to get together on a regular basis in future, and on that note, plans are developing for Linda Jewel to host the 2018 meetings in Newfoundland.

This booklet contains abstracts of oral and poster presentations in the order that they were presented. All abstracts will subsequently be published in an upcoming edition of the Canadian Journal of Plant Pathology. The research work represented by these papers forms an important part of the on-going development of phytopathological research in the Maritimes.

Pervaiz A. Abbasi
Chair local arrangements committee

The Canadian Phytopathological Society Atlantic Region Meeting 2017

Thursday, 23 November 2017
Cornwallis Room, KRDC, Agriculture and Agri-Food Canada, Kentville, NS

13:00-13:15 **Introduction/Welcome:**
Dr. Rick D. Peters, CPS representative – Atlantic region
Dr. Mark Hodges, Associate Director, Research, Development and Technology, Kentville Research and Development Centre, Kentville, NS

13:15-13:30 **Dr. Denis Gaudet**, President, Canadian Phytopathological Society
Opening remarks and update from the CPS President

13:30-14:00 **Keynote Speaker: Dr. Shawkat Ali,** Kentville Research and Development Centre, Kentville, NS
Role of effectors in inducing and suppressing disease resistance in barley and potato by pathogenic fungi and nematodes

14:00-14:20 **Characterization of *Fusarium graminearum* putative virulence factors by *CRISPR/Cas9* gene editing.** ADAM J. FOSTER

14:20-14:40 **Bio-protectant development to control potato pathogens.** C.W. KIRBY, M.H. NABUURS, J.N.D. VACON, and S.M. BOYETCHKO**.**

14:40-15:00 **Assessment of the use of biopesticides and phosphite to reduce common scab in potatoes.** G. WANG-PRUSKI, R. COFFIN, J. COFFIN, B. BEATON, C. GOYER, M. Z. ALAM, F. DESAI, T. BORZA, Y. LIU, Y. XI

15:00-15:30 Nutrition Break/Poster Presentations

15:30-15:50 **Review: Marker–assisted selection of disease traits integration into AAFC's Potato Breeding Program.** VIRGINIA DICKISON, BENOIT BIZIMUNGU, DAVID DE KOEYER, KATHERYN DOUGLASS, and XIANZHOU NIE

15:50-16:10 **Estimation of *Verticillium* and *Fusarium* abundance by real-time quantitative PCR using DNA extracted from plant and soil samples – pitfalls and limitations.** T. BORZA, H. LIU, X. GAO AND G. WANG-PRUSKI.

16:10 -16:30 **Foliar selenium application induces defense response in potato plants infected with late blight.** ASHOK SOMALRAJU, JASON MCCALLUM, DAVID MAIN, RICK PETERS, BOURLAYE FOFANA

16:30 -16:50 **Suberization of skin cells from potato tubers infected or not infected with common scab pathogens.** Y. LIN, Z. ZHANG, R. COFFIN, J. COFFIN, T. BORZA, G. WANG-PRUSKI

16:50-17:00 **Closing remarks** Rick Peters

17:00-20:00 **Dinner** (Old Orchard Inn, 153 Greenwich Rd South, Exit 11, Highway 101)

Note to presenters: please ensure that your presentation is given to the audio/visual coordinator prior to the start of the meeting.

Keynote 13:30-14:00

Role of effectors in inducing and suppressing disease resistance in barley and potato by pathogenic fungi and nematodes

S. ALI, P.A. ABBASI, J.D. LAURIE, R. LINNING, J.A. CERVANTES-CHA´VEZ, D. GAUDET, L. JAMSHAID, G. BÉLAIR, P. MOFFETT and G. BAKKEREN

Kentville Research and Development Centre., Agriculture and Agri-Food Canada, 32 Main Street, Kentville, NS, B4N 1J5, Canada; (J.D.L & D.G) Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada Lethbridge, Alberta T1J 4B1, Canada; (R.L, J.A.C & G.Bak) Summerland Research and Development Centre, Agriculture and Agri-Food Canada Summerland, BC, V0H 1Z0, Canada; (G.B) Saint-Jean-sur-Richelieu Research and Development Centre, Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu, J3B 3E6, QC Canada; (P.M) Département de Biologie, Université de Sherbrooke, Sherbrooke J1K 2R1 QC Canada

During infection, plant pathogens secrete effector proteins to reprogram the host plant for its own benefit. In special cases, recognition of certain effectors by resistance genes, essential components of the host surveillance system, induces resistance to infection. No effectors with such avirulence function have been described for basidiomycete fungi infecting cereals. *Ustilago hordei* is a biotrophic basidiomycete fungus that infects barley. *UhAVR1* from *U. hordei* functions as an avirulence protein, rendering it avirulent on barley cultivar Hannchen, having corresponding resistance gene *Ruh1*. We have located *UhAvr1* within the genome using a deletion approach and confirmed its resistance-triggering function. We also provide evidence that transposable element (TE) activity in the *UhAvr1* promoter region and translocation of the coding region are likely responsible for enabling virulence on Hannchen. This region of the genome harbours a cluster of predicted secreted proteins and is syntenic to a cluster in closely-related corn pathogens, *U. maydis* and *Sporisorium reilianum*. In *U. maydis*, deletion of this region results in dramatic reduction in virulence on corn. Potato cyst nematodes, including *Globodera rostochiensis*, are major obstacles to potato production in many areas of the world. Like many other pathogens, *G. rostochiensis* secret effectors, which facilitate colonization of the host plants. These proteins are delivered to the plant intercellular space as well as to the host cell cytoplasm through a specialized structure, known as stylet. We have characterized 30 such effector proteins from *G. rostochiensis* by expressing *in planta*. The results of some of these effectors will be presented.

Characterization of *Fusarium graminearum* putative virulence factors by *CRISPR/Cas9* gene editing

A.J. FOSTER

Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, 440 University Avenue, Charlottetown, PE C1A 4N6, Canada

Pathogenic fungi, such as *Fusarium graminearum* (a causal agent of Fusarium head blight in wheat, barley and oats), utilize small secreted cysteine-rich proteins (SSCRPs) to modulate host immunity. These proteins are commonly referred to as effectors. Currently, the function and role in virulence of only a few SSCRPs is known compared to the vast number that have been identified. One widely used approach to characterize fungal effectors is gene disruption through homologous recombination. This method works by replacing all or part of a target gene with a selectable marker and assessing the phenotype of the new 'knock-out' isolates. This approach is greatly limited, as only one gene can be targeted per transformation whereas many effector proteins are suspected of having functional redundancy and can exist in large gene families. The type II clustered regularly interspaced short palindromic repeat system (CRISPR) together with the CRISPR-associated, protein Cas9 recently emerged as a powerful new tool for gene editing in molecular biology. Among the different uses of the CRISPR/Cas9 system is the ability to target multiple genes for editing through insertion and/or deletion (indels). Methods have been developed and adapted to use CRISPR/Cas9 to assess SSCRP function through the selection of gene targets, the design of sgRNA, the construction of cassettes, vector assembly and fungal transformation. These approaches for CRISPR/Cas9 gene editing of SSCRPs will be discussed.

Bio-protectant development to control potato pathogens

C.W. KIRBY, M.H. NABUURS, J.N.D. VACON, and S.M. BOYETCHKO.

Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, 440 University Avenue, Charlottetown, PE, C1A 4N6, Canada, and (S.M.B) Saskatoon Research and Development Centre, Agriculture and Agri-Food Canada, Saskatoon, SK, S7N 0X2

Bioautography methods are being used to screen for bio-protectant candidates produced from an established soil bacterial collection to control potato pathogens. Current pathogens of interest are *Phytophthora erythroseptica* (Pink Rot), *Colletotrichum coccodes* (Black Dot), *Helminthosporium solani* (Silver Scurf), *Streptomyces scabies* (Common Scab), *Fusarium sambucinum* (Dry Rot), *Alternaria alternata* (Brown Dot), and *Phytophthora infestans* (Late Blight). Direct bioautography methods require large amounts of spores to conduct the assay in a reasonable time-frame; however determining growth conditions to induce sporulation of our target pathogens has proved to be taxing. To overcome this problem, we are using a modified method where the culture can be taken directly from its standard grown conditions on a petri plate to create the microbial suspension for the assay to allow rapid screening for antimicrobial compounds. These ongoing efforts to improve potato viability (both in the field and storage) through the screening and isolation of potential bio-protectant candidates for environmentally-friendly treatment methods will be presented.

Assessment of the use of biopesticides and phosphite to reduce common scab in potatoes

G. WANG-PRUSKI, R. COFFIN, J. COFFIN, B. BEATON, C. GOYER, M. Z. ALAM, F. DESAI, T. BORZA, Y. LIU, Y. XI

Department of Plant and Animal Sciences, Faculty of Agriculture, Dalhousie University, Truro, NS B2N 5E3, Canada; *(RC, GC) Privar Farm Inc., PEI; (BB) Department of Agriculture and Fisheries, PEI; (CG) Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, 850 Lincoln Road, Fredericton, NB, E3B 4Z7, Canada*

Potato common scab (*Streptomyces scabiei* Lambert and Loria) is increasingly becoming a tuber quality issue in PEI. Biopesticides are promising formulations to suppress common scab. Two commercial biopesticides, Microflora Pro™ and Double Nickel™, were examined in 2016 and 2017 on a field infested with common scab at Privar Farm Inc., PEI. Four potato varieties, Goldrush (resistant), Prospect (susceptible), Green Mountain (very susceptible), and Red Pontiac (very susceptible) were used. Four replicated plots were established for each of the four varieties and treatments. Since phosphite products such as Confine, Phostrol and Rampart, are widely used by growers in the region, the effect on common scab development using one of these products was also investigated. The same treatments and field design were used as described above and the plots were sprayed 4 times with Phostrol during the growing season. Common scab rating was recorded for each tuber by determining the percentage of common scab area, number of shallow pits, number of deep pits, and necrotic patches. The number of suberized skin cell layers and the thickness were measured using a protocol developed in our laboratory. Scab rating, shallow pits, deep pits and necrotic area showed significant differences among cultivars, and their response to the treatments also showed significant variations. Histological assessments of suberized tuber skin showed significant variations in the four varieties. Although there were visible reduction of common scab in some of the varieties following the Microflora Pro and Double Nickel treatments, scab severity on tubers of the susceptible varieties was not decreased enough, therefore these tubers are unlikely to pass for premium grade table stock potatoes.

Review: Marker–assisted selection of disease traits integration into AAFC's Potato Breeding Program

V. DICKISON, B. BIZIMUNGU, D. DE KOEYER, K. DOUGLASS, and X. NIE

Fredericton Research and Development Centre, Agriculture and Agri-Food Canada, 850 Lincoln Road, Fredericton, NB, E3B 4Z7, Canada

The development of new potato cultivars using conventional breeding takes on average 10-12 years prior to industry integration. Breeding for disease resistance is complicated by the lack of effective screening tools in order to speed up and improve the selection process. Currently, disease traits are evaluated using phenotypic data collected after exposure to the disease and subsequent rating of the effects of the disease on each line. This process is labour intensive and time-consuming, and can take months or years to ensure accurate assessment of a single trait. Multiple high throughput molecular markers are being developed and validated for use within AAFC's potato breeding program. Currently, high resolution DNA melting (HRM) markers/assays for the resistances against potato cyst nematodes (PCN) (*Globodera rostochiensis* pathotype Ros1), *Potato virus Y* (PVY) and *Potato virus X* (PVX) are being integrated within the program. The HRM assay for PCN resistance has been adopted and part of routine screening for 7 years within AAFC's breeding program; developed, validated and performed by AAFC colleagues, Dr. D. De Koeyer and K. Douglass. The breeding team has recently increased its capacity to do their own evaluation of lines using marker-assisted selection (MAS) and will commence PCN marker evaluation. In addition to the HRM marker for the PCN resistance, respective HRM markers for extreme resistance (ER) against PVY and PVX, developed by X. Nie and his team, are being incorporated into the program's selection process. These gel electrophoresis-free markers/assays were developed either by converting the existing gel electrophoresis-dependent markers such as STS markers or based on ER-linked single nucleotide polymorphisms (SNPs) that were identified by next generation sequencing (NGS). The utilization of the high throughput HRM-based marker-assisted selection within the breeding program will assist AAFCs Breeding Program to incorporate traits of interest sought by industry needs more efficiently and effectively.

Estimation of *Verticillium* and *Fusarium* abundance by real-time quantitative PCR using DNA extracted from plant and soil samples – pitfalls and limitations.

T. BORZA, H. LIU, X. GAO, A. GOVINDARAJAN, and G. WANG-PRUSKI.

Department of Plant, Food and Environmental Sciences, Faculty of Agriculture, Dalhousie University, Truro, NS B2N 5E3, Canada

Verticillium and *Fusarium* spp. infect a wide range of plants. These pathogens affect several crops grown in Atlantic Canada, including potatoes, carrots and strawberries. The main *Verticillium* species pathogenic to potatoes and strawberries are *V. dahliae* Kleb. and *V. albo-atrum* Reinke & Berthold while the main *Fusarium* species pathogenic to carrots are considered *Fusarium avenaceum* (Fr.) Sacc. and *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen. Several assays using real-time quantitative PCR (qPCR) have been designed in our laboratory to assess the incidence and abundance of these pathogens. Subsequently, these approaches have been used to process a large number of plant and soil samples. The identification of pathogens using the qPCR method was much faster and reliable than the traditional methods involving plating on specific media. On the other hand, the estimation of pathogen abundance using the absolute quantification (standard curve) qPCR method, was found to be affected by several pitfalls and limitations. While generating and processing qPCR data from the abovementioned pathogens, it has been found that several factors such as the amount of sample used for DNA isolation, gene copy number, genetic polymorphism, the amount of DNA used as template for amplification, the volume of the qPCR reaction, qPCR amplification efficiency and detection limit, and the statistical processing of pathogen distribution in soil and in plant samples, all greatly affect the outcome of this molecular approach. Therefore, the significance of the results has to be clearly spelled out and the limitations of this molecular approach have to be acknowledged.

Foliar selenium application induces defense response in potato plants infected with late blight

A. SOMALRAJU, J. MCCALLUM, D. MAIN, R. PETERS, and B. FOFANA

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Late blight caused by *Phytophthora infestans* is a devastating disease, leading to complete crop loss in some cases. Although chemical control of potato late blight using fungicides is effective under conventional production systems, organic systems require different and viable disease control practices. Selenium (Se) is a mineral micronutrient, often used in crop and livestock bio-fortification because of its antioxidant and stress tolerance properties. However, the role of selenium in plant growth and defense is not fully understood. This study was designed to evaluate the defense responses induced by foliar selenium application in potato plants infected with late blight under greenhouse setting. Our recent data on disease incidence, secondary metabolites production, and phenylpropanoid pathway genes expression following selenium application will be presented and discussed.

Suberization of skin cells from potato tubers infected or not infected with common scab pathogens

Y. LIN, Z. ZHANG, R. COFFIN, J. COFFIN, T. BORZA, and G. WANG-PRUSKI

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The suberization of potato tuber skin cells is important for water and mineral metabolisms, and disease resistance during growth and development. Observation of structural changes of suberized cell layers during tuber development can be assessed by the number of suberized cells, and the thickness of suberized layers. The outcome can provide a better understanding of the efficacy of this barrier against pathogens such as common scab (*Streptomyces scabiei* Lambert and Loria) in different potato varieties. A quick and effective method to observe the suberized cell layers in potato tuber skin was developed in our laboratory. The simplified method allowed the evaluation of as many as eight tubers a day; as a result, suberization could be compared in real-time throughout tuber development. Two trials were established in the 2017 season, one in the growth chamber and another in the field. Potato tuber suberization was compared in different growth stages in non-infected tubers in the growth chamber trial that comprised of the varieties Goldrush (resistant to common scab) and Prospect (susceptible to common scab). In addition, tubers from these two varieties plus Hindenburg (resistant), Jubel (resistant), and Riverdale Russet (susceptible) were collected periodically from the common scab infested field. Data confirmed that the number of suberized cell layers and the thickness of suberized cell layers were increased during tuber growth and development. Also, common scab resistant varieties were found to have a significantly higher number of suberized cell layers and thicker suberized cell layers as compared to the susceptible varieties.

POSTER PRESENTATIOM

Diseases diagnoses in commercial crops submitted to the PEI plant disease diagnostic laboratory for the 2017 season.

M.M. Clark and A. MacLeod

P.E. Department of Agriculture and Fisheries, Plant Disease Diagnostic Laboratory, P.O. Box 2000, 23 Innovation Way, Charlottetown, Prince Edward Island, C1E 0B7, Canada

A total of 259 disease diagnoses were completed during the period June 1st to November 14th, 2017. Categories of samples received were potatoes (62.55%), cereal and oilseed crops (10.81%), vegetable and fruit crops (25.48%), and other (2.67 %). The 2017 potato growing season started with overall good emergence and vigorous plant stands. However, some uneven emergence and potato seed piece decay became noticeable in potato varieties Gemstar, Russet Burbank, Piccolo, Prospect and Goldrush. The prevalent Fusaria strains involved with the seed piece decay samples were *Fusarium oxysporum* and *Fusarium coeruleum*. Both Fusaria species were found to be resistant to fludioxonil and in most cases sensitive to thiabendazole, difenoconazole and prothioconazole. This Fusarium resistance work was completed by Dr. Rick Peters and his staff at Agriculture and Agri-Food Canada (AAFC/ACC). For the first time in history, there were no confirmed cases of potato foliar late blight. Dr. Sean Lee and Dr. Jingbai Nie from the Canadian Food Inspection Agency (CFIA/ACIA) confirmed an identification of a phytoplasma in a commercial garlic sample and *Pectobacterium atrosepticum* in one potato bacterial blackleg sample. Isolations from potato stem tissue confirmed early dying fungi involved included *Rhizoctonia* sp., *Colletotrichum coccodes*, *Verticillium* spp. and a high level of *Fusarium oxysporum* (Dr. Tharcisse Barasubiye, AAFC/NFIS). Foliar potato leaf spot symptoms started to invade the wilted potato plants of potato varieties FL1879, Atlantic, Innovator, Ranger Russet and Russet Burbank. The causal agents involved included *Alternaria alternata* and some *Alternaria solani.* Isolations from 'highbush' blueberry plants confirmed the presence of the fungal organism, *Phomopsis* sp. The apple acreage on Prince Edward Island is increasing and fire blight symptoms appeared in mid-July in two varieties (confirmation pending).