CANADIAN PHYTOPATHOLOGICAL SOCIETY

ATLANTIC REGION MEETING 2019

CHARLOTTETOWN RESEARCH AND DEVELOPMENT CENTRE
AGRICULTURE AND AGRI-FOOD CANADA
440 UNIVERSITY AVE., CHARLOTTETOWN, PE

Atlantic Region Meeting
November 21, 2019
Charlottetown Research and Development Centre, Charlottetown, PE

INTRODUCTION

Welcome to Charlottetown and to the 2019 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 7 oral and 4 poster presentations on various topics in phytopathology scheduled for the afternoon. A dinner will take place at 6 pm at the Old Triangle Irish Alehouse, 189 Great George St., Charlottetown, PE following the meeting.

I am pleased to welcome **Dr. Hai Nguyen**, Research Scientist, Ottawa Research and Development Centre, Ottawa, ON, as our keynote speaker. Dr. Nguyen is known for his work in the areas of biosystematics, comparative genomics, and bioinformatics. His presentation is entitled: **A Canadian history of potato wart disease caused by *Synchytrium endobioticum***.

Many thanks to all who are attending the meeting. I would also like to thank the **Canadian Phytopathological Society** for sponsoring this event. I trust we will be able to get together on a regular basis in future, and on that note, plans are developing to host the 2020 meetings in Fredericton, New Brunswick.

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 Atlantic Canada.

Rick Peters
Atlantic Region Rep, CPS

The Canadian Phytopathological Society, Atlantic Region Meeting 2019

Thursday, 21 November 2019
Boardrooms A-B, CRDC, Agriculture and Agri-Food Canada, Charlottetown, PE

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

13:15-14:00 **Keynote Speaker: Dr. Hai Nguyen,** Ottawa Research and Development Centre, Ottawa, ON
A Canadian history of potato wart disease caused by *Synchytrium endobioticum*.

14:00-14:20 Expression of *Synchytrium endobioticum* genes involved in cell signaling, communication, and DNA integration is enhanced in zoospores compared to resting spores.
Donna Smith*, Mark Annett, Hai Nguyen, and Kasia Dadej

14:20-14:40 Can potato zebra chip disease related pathogens be transmitted through true seeds?
Xiang (Sean) Li*, Jingbai Nie, Eric Jiacheng Chuan, Desmond Hammill, Huimin Xu, and Christian Lacroix

14:40-15:00 Root rot of field peas and its potential impact on potatoes in New Brunswick and Prince Edward Island.
Kamrun Nahar, Louis-Pierre Comeau, Claudia Goyer, Tandra Fraser, Aaron Mills, and Dahu Chen*

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

Posters

Carry-over effects of cover cropping on disease and the soil microbiome. (GS)
Harini Balasundaram*, Lindsey Clairmont, Bourlaye Fofana, Claude Caldwell, and Adam Foster

A survey of Fusarium head blight of barley in the Maritime provinces in 2018.
Emma Halliday, Petra Larsen, Ron Matters, Justin Renaud, Matk Sumarah, and Adam Foster*

Characterization of Septoria leaf spot and stem canker of lowbush blueberry.
Shawkat Ali*, Pervaiz Abbasi, Paul Hildebrand, and Willy Renderos

A bacterial classification toolkit and its application in *Clavibacter* spp. and *Liberibacter* spp. (GS)
Eric Jiacheng Chuan*, Wang Li, Qian Tian, Jingbai Nie, Xianchao Sun, Wenjun Zhao, and Xiang (Sean) Li

15:30-15:50 A next-generation sequencing approach for the simultaneous detection of plant viruses for plant quarantine testing. (GS)
Desmond Hammill*, Huimin Xu, and Fred Kibenge

15:50-16:10 First report of Tomato brown rugose fruit virus on tomato in Canada.
Huimin Xu*, Desmond Hammill, Xuechan (Shannon) Shan, and Tongyang Tian

16:10 -16:30 Development of multiplex RT-PCR based diagnostic procedures for detecting eight viruses and one viroid in the potato nuclear stocks certification program. (GS)
Shuchen Elena Yan*, Huimin Xu, Mathuresh Singh, and Pat Quilty

16:30 -16:45 **Closing remarks:** Rick Peters

18:00-21:00 **Dinner** (Old Triangle Irish Alehouse, 189 Great George St., Charlottetown, PE)

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

ABSTRACTS

A Canadian history of potato wart disease caused by *Synchytrium endobioticum*. H.D.T. NGUYEN. *Ottawa Research and Development Centre* (*ORDC*), *Agriculture and Agri-Food Canada* (*AAFC*), *960 Carling Ave., Ottawa, ON K1A 0C6, Canada*
Synchytrium endobioticum (Schilberszky) Percival is the fungus that causes potato wart disease. Although recognized and described over 100 years ago in Hungary, in modern day, it is considered a quarantine plant pathogen. It has the ability to cause great social and economic impact for countries that have it, including Canada and particularly in Prince Edward Island, where potatoes are produced and exported. This fungus causes horror for growers because the disease it causes is impossible to eradicate and has impeded their ability to export potatoes. This keynote gives a short summary of the biology of the fungus and the historical background of the disease in a Canadian context.

Expression of *Synchytrium endobioticum* genes involved in cell signaling, communication, and DNA integration is enhanced in zoospores compared to resting spores. D. SMITH, M. ANNETT, H.D.T. NGUYEN, AND K. DADEJ. *Canadian Food Inspection Agency, Charlottetown Laboratory, Charlottetown, PE C1A 5T1, Canada; and (H.D.T.N., K.D.) Agriculture and Agri-Food Canada, K.W. Neatby Building, Ottawa, ON K1A 0C6, Canada*
The fungal pathogen, *Synchytrium endobioticum*, (Schilbersky) Percival is the causal agent of potato wart disease, and is regulated in Canada as a quarantine pest. The disease is typically characterized by a proliferation of wart tissue on the surface of infected tubers. These warts contain sori of *S. endobioticum*, which lives as an obligate biotroph within the host tissue. During dry periods, or over winter, the sori develop thick walls and can remain viable in decaying plant tissue and soil for several decades, even in the absence of a host. Under cool, damp conditions, however, warts continue to grow and the sori release zoospores that infect growing potato sprouts and stolons in the surrounding soil. This study was undertaken to determine if transcriptomics could be used to reveal some insights into the early process of infection, and to characterize the genes differentially expressed in the infectious zoospores compared with those expressed in the resting, thick-walled, overwintering stage. Whole mRNA transcriptome libraries were prepared and sequenced from four replicates of both resting spores and free swimming zoospores of the LEV6574 isolate from St. Eleanors, Prince Edward Island. The sequence data was mapped to an annotated reference whole-genome assembly of the LEV6574 isolate of *S. endobioticum* and differentially expressed genes were identified. Fisher's exact test, a functional analysis, revealed that expression of genes involved in cell signaling, communication, and DNA integration was significantly enhanced in zoospores.

Can potato zebra chip disease related pathogens be transmitted through true seeds?

X. LI, J. NIE, E.J. CHUAN, D.L. HAMMILL, H. XU, AND C. LACROIX. *Canadian Food Inspection Agency, Charlottetown Laboratory, Charlottetown, PE, Canada; and (E.J.C., C.L.) Biology Department, University of Prince Edward Island, Charlottetown, PE, Canada*

Potato Zebra Chip Disease (ZCD) and related pathogens, '*Candidatus* Liberibacter solanacearum' (CLso) haplotypes, continue to spread globally in recent years. The latest cases of discovery include potato and Parsley in Australia (Jan 2018), potato in Spain (Aug 2017) and carrot in Portugal (Jan 2018), potato in Alberta, Canada (July 2018), carrot in Greece (July, 2016) and Israel (Jan 2017). The botanical seeds of carrot and parsley are suspected to be a pathway for continental transmission, while psyllids are considered the vectors for local spread of ZCD in North America. To date, only a single isolated case of seed-transmission was reported by Spanish scientists. Data presented here demonstrate that CLso can be transmitted through carrot seeds, at least in the earlier stage after harvesting. The carrot seeds harbouring CLso produced from Europe were germinated and planted under containment facility in Canada. The diseased materials were successfully graft-transmitted to tomato plants (cv. Moneymaker). Both CLso and *Potato Virus S* were detected simultaneously in carrot and tomato plants using NGS and bioinformatics approaches followed by confirmation using PCR and RT-PCR. As for potato zebra chip pathogen, the phloem limited fastidious bacterium can be visualized under electron microscope. The ZCD pathogen was detected in the freshly harvested tomato fruit at peduncle end, pericarp, style end and columella placenta with seeds using PCR. It remains to be tested whether the pathogen can be transmitted through the botanical seeds of tomato and potato.

Root rot of field peas and its potential impact on potatoes in New Brunswick and Prince Edward Island. K. NAHAR, L.-P. COMEAU, C. GOYER, T. FRASER, A. MILLS, AND D. CHEN. *Fredericton Research and Development Centre (FRDC), Agriculture and Agri-Food Canada (AAFC), 850 Lincoln Road, Fredericton, NB E3B 4Z7, Canada; and (T.F., A.M.) Charlottetown Research and Development Centre (CRDC), Agriculture and Agri-Food Canada (AAFC), 850 Lincoln Road, Fredericton, NB E3B 4Z7, Canada*

Legume crops including field peas are common rotation crops to improve soil fertility and to reduce pathogen levels in crop productions in Western Canada. Peas as rotation crops in potato production system have recently increased in New Brunswick (NB) and Prince Edward Island (PEI) because new markets are now available. Little is known about the causal organisms of the root rot complex of peas and their potential impact on potato production in the Maritimes. This study aims to identify the causal organisms of the pea root rot and to determine its potential impact on the subsequent crop potato in Maritimes. Symptomatic plants were taken for pathogen isolation from a total of 18 commercial pea fields from NB and PEI in 2018. Pure isolates were obtained either using single spore isolation or hyphal tip technique. Species identification was based on morphological characteristics and DNA sequences of ITS and translation elongation factor 1-alpha. Root rot was present in every pea field surveyed in the two provinces with 255 and 46 fungal isolates recovered from NB and PEI, respectively. *Fusarium* spp. accounted for ~ 80 % of the isolates and were grouped into 14 different groups based on the morphology. Species of *Pythium,* and *Rhizoctonia* were identified using PCR primers targeting and sequencing of the amplicons. Among the *Fusarium* spp. at least five isolates per field were randomly selected to test pathogenicity in Russet Burbank. About 10% of the tested isolates were pathogenic, causing Fusarium dry rot in Russet Burbank. This study indicated that the root rot complex of field peas are mainly caused by *Fusarium* spp. in the Canadian Maritimes and have a potential impact on potato Fusarium dry rot.

Carry-over effects of cover cropping on disease and the soil microbiome.

H. BALASUNDARAM, L. CLAIRMONT, B. FOFANA, C. CALDWELL, AND A. FOSTER.
Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, 440 University Avenue, Charlottetown, PE C1A 4N6, Canada; and (H.B., C.C.) Dalhousie University - Agricultural Campus, PO Box 550, Truro, NS B2N 5E3, Canada

Cover crops within rotational cropping systems have been used in both conventional and organic agricultural practices for many years. They play a vital role in preventing soil erosion, restoring nutrients, managing weeds, and reducing damage by insects and pathogens. The purpose of this project is to study the cover crops' ability to change soil microbiome diversity and how this affects plant disease in subsequent years. Soil samples were collected from field trials in Prince Edward Island containing replicated plots in year 1 with cover crops as well as a non-cover crop control and in year 2 with barley or soybean. Metagenomics sequencing of soil DNA extracted from these samples were conducted in order to assess treatment carry-over effects on the soil microbiome. As well, various beneficial and pathogenic organisms present in the soil were identified. Preliminary analysis of the metagenomics data revealed that cover crops influenced bacterial and fungal communities differently. Based on visual assessment, the main root disease observed in barley and soybean was Fusarium crown rot. Next steps include quantification of the different pathogens and beneficial endophytes present in the root tissue through quantitative PCR, to validate the metagenomics sequencing results.

A survey of Fusarium head blight of barley in the Maritime provinces in 2018. E. HALLIDAY, P. LARSEN, R. MATTERS, J. RENAUD, M. SUMARAH, AND A. FOSTER. *Charlottetown Research and Development Centre, Agriculture and Agri-Food Canada, 440 University Avenue, Charlottetown, PE C1A 4N6;* (E.H.) *Department of Forensic Science Saint Mary's University 923 Robie St, Halifax, NS B3H 3C3; and* (J.R., M.S.) *London Research and Development Centre, Agriculture and Agri-Food Canada, 1391 Sandford St, London, ON N5V 4T3*

Fusarium head blight (FHB) is an economically important disease affecting barley worldwide causing both yield loss and contamination of grain with mycotoxins such as deoxynivalenol (DON). To better understand the populations of *Fusarium* spp. causing FHB in barley in the Maritime provinces of Canada, seeds were collected from 36 fields from 10 regions by our partners at the Atlantic Grains Council. Seed samples were split with 100 seeds per field being surface sterilized and plated to isolate *Fusarium spp.* and a second sample of seeds were ground for both DNA extraction for species specific QPCR analysis and DON analysis. In total, 336 isolates were collected with 70% being identified as *F. graminearum*, 18% *F. sporotrichoides*, 7% *F. poae* and 2% *F. avenaceum.* DON contamination ranged from 0 to 15.6 ppm and the number of isolates collected ranged from 0 to 54 from each field sample. Overall, Nova Scotia sites had the highest FHB levels based on DNA, isolate collection and DON while less FHB was observed in PEI and New Brunswick, but the ratios of different *Fusarium* spp. was similar in all provinces. Isolates showed differences in virulence on barley, but no association was observed between collection site. *F. graminearum* QPCR analysis correlated significantly with both DON (R^2=0.92) and the number of isolates collected (R^2=0.62), but QPCR targeting other species did not correlate with mycotoxin or culture data. QPCR was a rapid and accurate method to quantify *F. graminearum* in barley seeds and will be utilized in future surveys.

Characterization of Septoria leaf spot and stem canker of lowbush blueberry. S. ALI, P.A. ABBASI, P. HILDEBRAND, AND W. RENDEROS. *Kentville Research and Development Centre, Agriculture and Agri-Food Canada, 32 Main Street, Kentville, NS B4N 1J5, Canada*
In Canada, wild lowbush blueberry is a valuable horticultural crop that is managed on a 2-year production cycle with the harvesting of fruits, which occurs every other year, follows pruning of the stems. Several fungal diseases cause premature defoliation in fruiting fields and negatively affect fruit quality and yields. A Septoria-like leaf spot and stem canker have been described from both sprout and fruiting fields as one of the major causes of leaf and fruit drop. The pathogen was isolated from leaf and stem tissues of plants naturally infected in the field and based on morphology, the causal agent was described as Septoria-like pathogen. The pathogen was further characterized based on molecular analysis of several gene sequences. Analysis of the ITS, TEF-1α, β-tubulin, Calmodulin and LSU genes of the fungal isolates from lowbush blueberry revealed its identity as *Sphaerulina amelanchier*. The pathogenicity of the representative leaf and stem isolates was confirmed on lowbush blueberry clones, *Vaccinium angustifolium* and *V. myrtilloides*, in a growth chamber. Symptoms such as minute water-soaked specks to irregular reddish purple spots like those observed in the field appeared on leaves and stem followed by defoliation. In highbush blueberry similar symptoms are produced by *Septoria albopunctata* that is very different from *S. amelanchier* based on both morphology and sequences of several genes.

A bacterial classification toolkit and its application in *Clavibacter* spp. and *Liberbacter* spp. J. CHUAN, W. LI, Q. TIAN, J. NIE, X. SUN, W. ZHAO, AND X. LI. *Canadian Food Inspection Agency, Charlottetown Lab, 93 Mount Edward Road, Charlottetown, PE C1A 5T1, Canada; (J.C.) Biology Department, University of Prince Edward Road, 550 University Avenue, Charlottetown, PE C1A 4P3, Canada; (W.L., X.S.) Southwest University, Beibei District, Chongqing 400715, China; and (W.L., Q.T., W.Z.) Chinese Academy of Inspection and Quarantine, Chaoyang District, Beijing 100123, China*

Bacterial classification methods heavily rely on the rRNA/rDNA sequences, but the variations are sometimes not sufficient for the identification of closely-related species. Here, a bioinformatics toolkit PolyChrome Classifier (PCC) is proposed to integrate whole-genome sequence comparison (ANI) and phylogenetic analysis to provide accurate identification of bacteria. In total, 53 *Clavibacter* and 27 *Liberibacter* assemblies obtained from GenBank were analyzed to create the PCC database and ANI matrixes for interspecies and intraspecies classification and identification. The PCC was applied to identify five cultivated isolates (DM1, DM2, DM4, TJ7 and NDM1) with NGS genome sequences and one non-culturable sample with metagenomic sequence dataset. PCC trimmed the adaptors and low-quality bases of the NGS sequence data. After host genome removal, PCC assembles the reads to create contigs and computes ANI and phylogenetic trees for interspecies and intraspecies comparison. In conclusion, DM4 and TJ7 share more than 99% ANI values with *Clavibacter tessellarius* type strain ATCC 33566, and were classified as such, whereas DM1 can be classified as a new *Clavibacter* species sharing less than 93.8% ANI values with other authentic *Clavibacter* spp. DM2 is clustered within a group with three other strains in NCBI (AY1A6, CASJ009 and AY1B3) within another unknown species. The two *C. tessellarius* strains, ATCC 33566 and DOAB 609 in GenBank may belong to two separate species since the two share ANI value less than 93.7%. As for the non-culturable pathogen of potato zebra chip disease, PCC classified the causal agent in infected tomato as *Candidatus* Liberibacter solanacearum haplotype A (ANI > 99%). Additionally, *Clavibacter* species AY2B8 and BS5 and '*Can* Liberibacter asiaticus' SGCA1 and SGpsy may be misidentified respectively due to low ANI values with authentic strains, respectively.

A next-generation sequencing approach for the simultaneous detection of plant viruses for plant quarantine testing. D. HAMMILL, H. XU, AND F. KIBENGE. *Canadian Food Inspection Agency (CFIA), 93 Mt. Edward Rd., Charlottetown, PE C1A 5T1, Canada; and (F.K.) Department of Pathology and Microbiology, Atlantic Veterinary College, University of Prince Edward Island 550 University Ave., Charlottetown, PE C1A 4P3, Canada*

In recent years, next-generation sequencing (NGS) technology has been widely accepted as a high-throughput, unbiased diagnostic tool that has attractive features in the field of plant pathogen diagnostics. NGS has opened the door to very sensitive and specific testing with the opportunity to detect multiple pathogens in a single sample. In this study, various RNA extraction methods were evaluated to acquire high quality non-fragmented RNA. The application of NGS was then explored to determine if plant pathogenic viral genomes could be generated *de novo* via a bioinformatic pathway when mapped to the whole host genome without *a priori* knowledge of their presence. Potato mini tubers imported from Bangladesh were screened by NGS and viral contigs from mixed infections of *Potato aucuba mosaic virus*, *Potato virus Y*, *Potato virus X*, *Potato virus S*, *Potato virus M*, *Potato leafroll virus*, and *Tomato chlorosis virus* could be detected simultaneously with high specificity. Similarly, NGS was successfully applied to determine the causal agents of unknown etiology in tomato (*Solanum lycopersicum*) without *a priori* knowledge of their existence in the sample and confirmed the first report of *Southern tomato virus* in Canada. All viruses revealed by NGS were confirmed by at least one other test. NGS followed by confirmation will aid in the diagnosis of plant pathogens that were otherwise unable to be tested for within the current Potato Post-Entry Quarantine program as well as identify unknown agents in other regulatory testing allowing for timely management strategies and regulatory actions to be executed.

First report of *Tomato brown rugose fruit virus* on tomato in Canada. H. XU, D.L. HAMMILL, X. SHAN, AND T. TIAN. *Canadian Food Inspection Agency, 93 Mount Edward Road, Charlottetown, PE C1A 5T1, Canada; (X.S.) Plant Disease Clinic, University of Guelph, 95 Stone Road W., Guelph, ON N1G 2Z4, Canada; and (T.T.) California Department of Food and Agriculture, Sacramento, CA95832, USA*

In early 2019, *Tomato brown rugose fruit virus* (ToBRFV) was detected in greenhouse tomato crops in southern Ontario by the Plant Disease Clinic, University of Guelph. The infected tomato showed brown rugose patches, yellow blotches, and chlorotic spots on fruits and mosaic, mottling, yellow patches or spots on leaves. Deformed and narrow leaves were also noticed. Three bags of samples, each containing 3-5 leaves and 2 fruits, were collected from the infested greenhouse and shipped to Canadian Food Inspection Agency, Charlottetown Laboratory for further testing. These leaf samples were screened for ToBRFV by RT-PCR using several sets of primers either designed in this research or based on published data. The leaf saps of these samples were also inoculated onto healthy greenhouse grown tomato plants (cv. Yellow Pear). The inoculated plants kept were visually inspected for up to 35 days post inoculation (dpi) and symptoms similar to those described above were observed. RT-PCR again confirmed ToBRFV infection of the inoculated tomato plants (up to 35 dpi). RNA extracted from the inoculated plants (28 dpi) using the optimized column based extraction procedures was used for cDNA library construction followed by next generation sequencing using an Illumina MiSeq platform. The assembly of *de novo* contigs was achieved using CLC Genomics Workbench (v11). Complete sequences of ToBRFV were obtained from all three samples and the virus identity of these sequences was confirmed by Blast search (NCBI GenBank) and Clustal (v1.2.4) analysis. This is the first report of ToBRFV on tomato in Canada.

Development of multiplex RT-PCR based diagnostic procedures for detecting eight viruses and one viroid in the potato nuclear stocks certification program. S. YAN, H. XU, M. SINGH, AND P. QUILTY. *Canadian Food Inspection Agency (CFIA), Charlottetown Laboratory, 93 Mount Edward Road, Charlottetown, PE C1A 5T1, Canada; (M.S.) Agricultural Certification Services, 1030 Lincoln Road, Fredericton, NB E3B 8B7, Canada; and (P.Q.) PEI Potato Quality Institute Inc., 98 Hillstrom Ave., Charlottetown, PE C1E 2C6, Canada*

The Canadian Food Inspection Agency implements the Potato nuclear stock certification program to ensure the production of high quality and disease-free seed potatoes. Currently, this program employs double and triple antibody sandwich enzyme-linked immunosorbent assay (DAS- or TAS-ELISA) and return-polyacrylamide gel electrophoresis (R-PAGE) for detecting targeted viruses and *Potato spindle tuber viroid* (PSTVd), respectively. Pathogen-specific antibodies for ELISA are commercially available for detecting potato virus A, M, S, X, Y (all strain types), potato leafroll, latent, and mop-top viruses. However, the aforementioned methods are highly labor-intensive and time-consuming procedures with low sensitivity. In this project, conventional and real-time quantitative RT-PCR procedures were developed and validated for indexing potato nuclear stocks, whereas viral RNAs in microplants were readily detectable. As little as 1 µl of tissue sap from a microplant or 1 pg of total RNA extract was sufficient for reliable RT-PCR detection. Virus-specific RNA could be detected in RNA extracts with almost no non-specific amplification in the presence of other viral RNAs. The use of potato genome-specific primer sets provided a reference for assessing the quality of RNA extracts and the amplification of the targeted RNAs. The genomes of virus and viroid isolates or strains used in this research were verified using next-generation sequencing (NGS) technology to ensure the accuracy of the primers and probes employed. All tests conducted using the standard RT-PCR and RT-qPCR protocols (multiplex format) were validated using another diagnostic assay and known/unknown microplants, and the standard protocols developed can be implemented for indexing potato nuclear stocks.

NOTES

NOTES