Canadian Phytopathological Society
Maritime Region Meeting
November 27, 2008
Potato Research Centre, Fredericton, NB

INTRODUCTION

Welcome to Fredericton and to the 2008 Canadian Phytopathological Society Maritime Region Meeting. It has been a full year since we got together as a group, and I look forward to a stimulating afternoon of scientific discussion and fellowship.

There are 7 oral presentations on various topics in phytopathology scheduled for the afternoon. A dinner and presentations will take place in downtown Fredericton following the meeting.

I am pleased to welcome Mary Leggett, current president of CPS, as our keynote speaker. Her presentation is entitled: "**What it takes to develop commercial microbial products**".

Many thanks to all who attended the meeting. I would particularly like to thank Dr. Xianzhou Nie, AAFC, Fredericton and his assistants for hosting this year's meeting and taking on the responsibilities of organizing the meeting, scheduling the presentations and preparing the book of abstracts. 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 underway to have the 2009 meeting in Nova Scotia.

This booklet contains abstracts of the 7 oral 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.

Rick D. Peters
Maritime Region Rep
Canadian Phytopathological Society

The Canadian Phytopathological Society Maritime Regional Meeting 2008

Thursday, 27 November 2008
Conference Room, Potato Research Centre-Agriculture and Agri-Food Canada
Fredericton, NB

13:00-13:10 **Introduction/welcome: Rick D. Peters**, CPS representative – Maritime region

13:10-14:00 **Keynote Speaker: Mary Leggett, President of CPS,** Novozymes Bio-Ag Saskatoon, Saskatchewan.
What it Takes to Develop Commercial Microbial Products

14:00-14:20 **Analysis of gene expression of two potato cultivars during common scab infection using microarrays.** C. Goyer*, D. De Koeyer, H. Tai, V. Gustafson, R. Griffith, C. Rothwell.

14:20-14:40 **Does ethylene play a role in tomato in response to viroid invasion?** (Student paper) X. Hu*, H. Tai and X. Nie

14:40-15:00 **Current research at the Potato Research Centre: Resistance to Golden Nematod**e. A.M. Murphy*, D. De Koeyer, S. Wood, K. Douglass, T. Dalton, and D.H. Wilson.

15:00-15:30 Nutrition Break

15:30-15:50 **A single procedure for the isolation of viruses, PSTVd and BRR from potato plants for RT-PCR detection** R. P. Singh, M. Singh* and A. Sullivan

15:50-16:10 **Dynamics of Phage and *Streptomyces Scabiei* Populations in Soil and their Efficacy in Controlling Common Scab.** M. Ramirez* and C. Goyer.

16:10-16:30 **Management of potato seed-piece decay caused by fungicide-resistant strains of *Fusarium* species.** R.D. Peters*, K.A. Drake and I.K. Macdonald

16:30-16:40 Wrap-up

18:00 – Dinner and award presentation (to be determined)

Note to presenters: please ensure that your presentation is given to the audio/visual coordinator 30 mins prior to the start of the meeting.

Analysis of gene expression of two potato cultivars during common scab infection using microarrays. C. Goyer[1], D. De Koeyer[1], H. Tai[1], V. Gustafson[2], R. Griffith[1], C. Rothwell[2]. *[1]Agriculture and Agri-Food Canada, Potato Research Centre, P.O. Box 20280, Fredericton, NB, Canada, E3B 4Z7; [2]BioAtlantech, P.O. Box 636, Station "A", 921 College Hill Road, Fredericton, NB, Canada*

Streptomyces scabiei, a filamentous bacterium causes common scab, a disease characterized by brownish lesions on potato tubers. Potato defense responses to *S. scabies* infection are not well understood. The objective of this study was to investigate changes in gene expression profiles between healthy or *S. scabiei* infected potato tubers of cultivar Atlantic and Shepody using the 44 K oligomer POCI (Potato Oligo Chip Initiative) microarray chip. Atlantic and Shepody tubers were planted and inoculated with a mix of vermiculite- *S. scabiei* or sterile vermiculite (control) than tubers were harvested at 10 and 14 weeks after planting. A three-way ANOVA was used to filter genes showing significant differences in gene expression between cultivar, status and time showed that 5846 ($p \leq 0.01$). K-means clustering was used to group genes into ten clusters based on patterns of expression in infected and healthy tubers of Atlantic and Shepody at 10 and 14 weeks of incubation. Genes included in clusters 2 and 3 were up-regulated in infected tubers of Atlantic while clusters 4 and 6 grouped genes that were up-regulated in infected tubers of Shepody. As examples, several genes coding for ubiquitin conjugating proteins and protease inhibitors were up-regulated in infected tubers of Atlantic while defense-related genes encoding ascorbate peroxidase, polyphenol oxidase and wound inducible protease inhibitors were up-regulated in tubers of Shepody. The results have demonstrated that potato cultivars differed in their response to infection by *S. scabiei* and that their responses changed over time.

Does ethylene play a role in tomato in response to viroid invasion?

Xinxi Hu, Helen Tai and Xianzhou Nie
Potato Research Centre, Agriculture and Agri-Food Canada, P.O. Box 20280, 850 Lincoln Road, Fredericton, New Brunswick, Canada E3B 4Z7 (X.H., H.T., X.N.); College of Horticulture and Landscape, Hunan Agricultural University, Changsha, Hunan, China 410128 (X.H.)

The phytohormone ethylene has been demonstrated to play a role in plant resistance against various pests including fungal and bacterial pathogens as well as insects. To investigate whether the hormone is involved in tomato plants in response to the invasion of viroid, the smallest plant pathogen whose genome is a single stranded circular RNA of 200-460 nucleotides, tomato cv. 'Pearson' and its ethylene-insensitive mutant Never ripe (Nr) were challenged with *Tomato chlorotic dwarf viroid* (TCDVd), a close relative of the well know viroid *Potato spindle tuber viroid* (PSTVd). Both 'Pearson' and Nr plants developed various symptoms including dwarf, bunch, leaf chlorosis and necrosis at two-weeks-post-inoculation and thereafter. The sizes of leaves and fruits were also significantly reduced in the viroid-infected plants. In addition, the viroid invasion induced the basic and acidic PR genes. Further investigation revealed that Nr developed leaf necrosis earlier and more severely than 'Pearson' did, which appears to be coupled with smaller fruits and lower yield in the TCDVd-infected mutants over the viroid-infected wild type plants. Interestingly, the viroid concentration in Nr was lower than that in 'Pearson'. Treatments of 'Pearson' and Nr with ethephon, an ethylene-releasing compound, and/or the anionic silver thiosulfate complex (STS), an ethylene action inhibitor, were carried out to further assess the possible involvement of ethylene in the viroid-tomato interactions. Results indicated that STS-treated 'Pearson' mimicked Nr in symptom expression and viroid titre, supporting that ethylene plays a role in tomato reactions to TCDVd.

Current research at the Potato Research Centre: Resistance to Golden Nematode

Murphy, AM., De Koeyer, D.,Wood, S., Douglass, K., Dalton, T., and Wilson, DH.

Golden nematode, (GN, *G. rostochiensis* has been identified from four areas in Canada (Vancouver Island, Newfoundland, Québec and Alberta). For some of the regulated areas, a management plan has been devised that includes the use of GN resistant varieties. The number of Canadian varieties with commercial merit is limited and the majority of them have resistance to just one race of GN (Ro1). The reliance on Ro1 resistant varieties could lead to the emergence of new pathotypes, so there is a need to develop varieties presenting resistance to more than one race.

The objectives are to develop and select parents with resistance to GN and to develop and use DNA markers linked to GN resistance genes to strengthen potato breeding efforts. If successful, this research will lead to the release of commercially acceptable GN resistant varieties allowing potato producers from regulated areas to follow the management plan. These varieties will also be available to other potato producers wishing to use them preventively.

Marker-assisted selection (MAS) is particularly desirable for selecting GN resistance in Canada due to the expense of greenhouse evaluations and time and risk associated with field evaluations. Because of the quarantine significance of the pest, in locations such as NB where pest has not been reported, a method that can be applied in the absence of the pest is the only option. The results from the MAS assays will be validated by assessing resistance in field and pot tests in infested locations.

Improved molecular marker systems are required to enhance the adoption of MAS by potato breeders and to improve cost effectiveness. In medical diagnostic applications, high-resolution DNA melting (HRM) analysis has been shown to have several advantages over other genotyping methods. Recently, Dr. De Koeyer's Lab has established protocols for detecting allele dosage in tetraploid potatoes using HRM. Selecting potato lines with multiple resistance alleles is important for the development of superior parents within a potato breeding program because their use would increase the frequency of resistant progeny.

Recent research findings will be presented.

A single procedure for the isolation of viruses, PSTVd and BRR from potato plants for RT-PCR detection

Rudra P. Singh, Mathuresh Singh and Andrew Sullivan

A variety of tests are performed to assess the presence of disease in newly received potato samples to an *in-vitro* potato tissue culture lab. Examples of these tests include Enzyme-linked immunosorbent assay (ELISA) for viruses, immunofluoresence for Bacterial ring rot, return-polyacrylamide gel electrophoresis (R-PAGE) for Potato spindle tuber viroid, nucleic acid hybridization (NAH) and reverse transcription polymerase chain reaction (RT-PCR) for viruses and viroids. As the use of RT-PCR or PCR becomes more prevalent in current testing programs, it was prudent to develop a single procedure for the extraction of RNA and DNA for use in the latter assays. As a result, a procedure was developed which is applicable for the preparation of both RNA and DNA with nucleic acids quality suitable for PCR and RT-PCR. The procedure utilizes an extracting solution consisting of a NaOH-EDTA-LiCl solution containing 0.5% triton 405R. The plant tissues (leaves, tubers) are homogenized in the extraction solution and the homogenate is briefly centrifuged and supernatant collected. The nucleic acids are precipitated from the supernatant with isopropanol/sodium acetate at -20^{o}C overnight and precipitate is collected by centrifugation. The precipitate is washed with 70% ethanol, vacuum dried and dissolved in sterile distilled water for use in RT-PCR or PCR reactions. The nucleic acids have been used to detect potato viruses and viroid (PVA, PVM, PLRV, PVY, PVX, PLCV, PVS, PMTV and PSTVd) in simplex, duplex, triplex, tetraplex and pentaplex combinations in conventional RT-PCR and up to triplex in real time PCR. BRR is detected in simplex PCR. The procedure is simple, rapid and inexpensive.

Dynamics of Phage and *Streptomyces Scabiei* Populations in Soil and their Efficacy in Controlling Common Scab. M. Ramirez and C. Goyer. *Potato Research Center, Agriculture and Agri-Food Canada, 850 Lincoln Road, Fredericton, NB E3B 4Z7, Canada.*

Common scab is a bacterial disease responsible for significant economic losses estimated at 15-17M$ each year in Canada. The causal agent *S. scabiei* is commonly found in soil and once established in a field, there are few control means to reduce common scab. Use of biological control such as phages is an alternative to decrease plant disease. The long-term aim of this project is to develop biopesticides using phages to infect *S. scabiei*. Four genetically different phages infecting *S. scabiei* named Stsc1, Stsc3, LN1 and LN4 were isolated from potato field soil. Phages were shown to infect most strains of *S. scabiei* and other pathogenic *Streptomyces*. The specific objectives of this study are to quantify changes in populations of *S. scabiei* and phages over time and assess the efficiency of phage Stsc1 to control common scab. The phage and pathogen populations were quantified in sterile soil microcosms over time. Stsc1 and Stsc3 decreased the *S. scabiei* population by approximately a 10 fold factor after 14 days of incubation although this reduction was not observed with LN1. To determine the ability of phages to decrease common scab, seed pieces of the susceptible potato cultivar Shepody were planted in 15cm pots inoculated with 1) *S. scabiei*, 2) phage Stsc1 and 3) both *S. scabiei* and Stsc1. Common scab severity on potato tubers was recorded at harvest time. Phage Stsc1 reduced the common scab lesion coverage by 52% and decreased the severity of scab lesions. Further experiments are underway to quantify S. *scabiei* and phage populations using molecular tools. These results represent a first step in developing a strategy to control common scab using phages.

Management of potato seed-piece decay caused by fungicide-resistant strains of *Fusarium* species. R.D. Peters*, K.A. Drake and I.K. Macdonald. *Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, 440 University Ave., Charlottetown, PE Canada.*

Strains of *Fusarium* spp. with resistance to commonly-used fungicides have become commonplace in Canada. Populations of *F. sambucinum* with resistance to thiabendazole (post-harvest treatment) and thiophanate-methyl (seed-piece treatment) were reported in western Canada in the 1990s and now occur in all potato production regions in the country. More recently, populations of *F. sambucinum* and *F. coeruleum* with resistance to fludioxonil (seed-piece treatment) have been recovered from various provinces. In addition, multi-class (benzimidazole and pyrrole) resistance has also been documented. These pathogen populations have caused an increased incidence of seed-piece decay resulting in poor crop stands and reduced yields. Field and storage studies were conducted in Prince Edward Island, Canada to ascertain the impact of fungicide-resistant strains on crop loss and to define potential management strategies. In summary, inoculation of potato seed pieces with pathogen isolates resistant to a specific product followed by application of that product, resulted in loss of efficacy of that product with concomitant yield loss. In all cases, treatment of potato seed pieces with mancozeb at various rates completely controlled seed-piece decay caused by strains of *F. sambucinum* possessing multi-class fungicide resistance. Strategies to manage fungicide resistance in pathogen populations, including product rotations and mixtures, will be discussed.