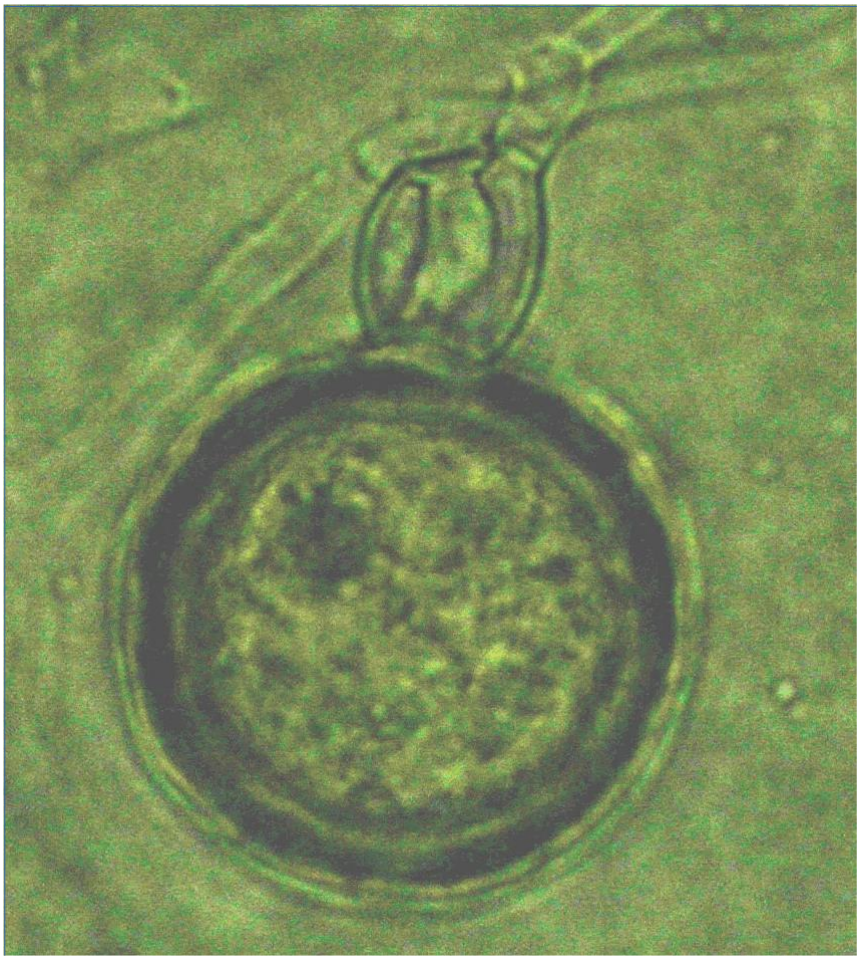
CANADIAN PHYTOPATHOLOGICAL SOCIETY



MARITIME REGION MEETING - 2004

The Delta Prince Edward Hotel, Charlottetown, PEI

DECEMBER 2, 2004

Canadian Phytopathological Society
Maritime Region Meeting
December 2, 2004
Delta Prince Edward Hotel, Charlottetown, PE

INTRODUCTION

Welcome to Charlottetown and to the 2004 Canadian Phytopathological Society Maritime Region Meeting. It has been some time since we got together as a group, and I look forward to a stimulating afternoon of scientific discussion and fellowship. There are 10 oral presentations on various topics in phytopathology scheduled for the afternoon. A dinner and presentations will take place at the Merchantman Pub following the meeting.

I am pleased to welcome Dr. David Patriquin, Biology Professor at Dalhousie University, as our keynote speaker. His Ph.D. work on nutrient cycling in tropical seagrass beds in the late 1960s led to postdoctoral and collaborative work with soil microbiologists in Canada and Brazil and that in turn to an interest in the challenges of farming organically. Prof. Patriquin and his students have worked with individual farmers, groups of farmers and landscapers on strategies for transition to organic agriculture and horticulture and for reducing limitations to production in fully organic regimes. The related field observations and experiments frequently pointed to a close linkage between fertilization practices and pests. Dr. Patriquin has been involved in development and implementation of certification codes for organic farming and has served on the editorial board of Biological Agriculture and Horticulture and on the federal Pest Management Advisory Council. His presentation is entitled: "**Managing nitrogen for control of pests and diseases**". A copy of a related article "**Managing soils for effective pest control**" is included at the end of this booklet.

Many thanks to all who attended the meeting. I hope we will be able to get together on a regular basis in future. I would particularly like to thank the Canadian Phytopathological Society, represented by president Richard Hamelin, and the Prince Edward Island Dept. of Agriculture, Fisheries and Aquaculture, represented by Dr. Tony Sturz, for sponsoring this event. I would also like to thank Agriculture and Agri-Food Canada and its staff for helping to prepare for the meeting, including this book of abstracts. Special thanks go to Tommy Gallant for acting as the audio-visual technician for this event.

This booklet contains abstracts of the 10 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 ongoing development of phytopathological research in the Maritimes.

Rick D. Peters
Maritime Region Rep, Canadian Phytopathological Society

2004 - Scientific Program

Thursday, December 2
Valient Room, Delta Prince Edward

1:00-1:10 **Welcome:** Rick D. Peters, CPS Rep - Maritime Region

1:10-1:45 **Keynote Speaker: Dr. David Patriquin**, Professor of Biology, Dalhousie University, NS
Managing nitrogen for control of pests and diseases

Session A: Viruses

1:45-2:00 PVA-CP transgene suppresses PVA-induced hypersensitive response in potato cultivar Shepody
Xianzhou Nie, Rudra P. Singh, Jan Zeng and Teresa Molen

2:00-2:15 Identification of a Canadian alfalfa mosaic virus potato isolate and its detection in potato by RT-PCR and RFLP procedures
Huimin Xu

2:15-2:30 Investigations on the potential use of reflectance spectrophotometry for detection of virus infected potato plants
Matt Crane, **Robert Coffin**, William Hardy, Mathuresh Singh and H. Anderson MacEwen

Session B: Bacteria

2:30-2:45 Epidemiology and management of fire blight in red raspberry
Gordon Braun and Paul Hildebrand

2:45-3:00 Detection and differentiation of pectolytic erwinias in potato by multiplex PCR
Solke De Boer and Jeanette D'Aubin

3:00-3:15 Comparison of PCR primers for the detection of bacterial ring rot
Mohsen Taghavi, Solke De Boer and Len Ward

3:15-4:00 **Nutrition Break**
Valient Room, Delta Prince Edward

Session C: Fungi and Oomycetes

4:00-4:15 Assessing the *in vitro* sensitivity of *Alternaria solani* and *A. alternata* isolates from Prince Edward Island to azoxystrobin
William MacDonald, Rick Peters, Robert Coffin and Chris Lacroix
(Graduate Student Presentation)

4:15-4:30 Recent investigations regarding the soil-borne pathogen *Phytophthora erythroseptica* and the management of potato pink rot
Rick Peters, Tony Sturz, Bud Platt and Walter Arsenault

4:30-4:45 Ultra-sensitive methods for PCR detection of potato wart in soil
Len Ward, Solke De Boer and André Lévesque

4:45-5:00 Wrap-up

6:00-10:00 **Dinner and Presentations**
Merchantman Pub, across the street from the Delta Prince Edward

Note to Presenters: Please ensure that your presentation is given to the Audio/Visual Co-ordinator at least 1 hour prior to the start of the session

2004 - Meeting Abstracts

Keynote Speaker
1:10-1:45

Managing nitrogen for control of pests and diseases. D.G. Patriquin. *Dalhousie University, Halifax, NS B3H 4J1, Canada.*
Excess N in soil and plants stimulates pests through a variety of mechanisms, while some pests are stimulated by plant N deficiency. Maintenance of soil inorganic N at low levels while avoiding N deficiency is a key to controlling pests without use of pesticides and/or the effective use of soft pesticides such as soap. Examples of pests and diseases stimulated by an excess or deficiency of N will be given and strategies for managing N discussed.

Session A: Viruses
1:45-2:00

PVA-CP transgene suppresses PVA-induced hypersensitive response in potato cultivar Shepody. X. Nie, R.P. Singh, J. Zeng, and T. Molen. *Potato Research Centre, Agriculture and Agri-Food Canada, P.O. Box 20280, 850 Lincoln Road, Fredericton, NB E3B 4Z7, Canada.* Previous studies have demonstrated that the potato cv. Shepody is resistant to *Potato virus A* (PVA). The resistance is featured with field and mechanical inoculation immunity against the virus. Graft-inoculation of Shepody plants with PVA-containing scions results in hypersensitive response (HR)-like reactions in stems and tubers with characteristic necrosis. The number of tubers with necrosis is correlated with the number of PVA-containing scions and the duration of scions on the stock plants: increasing the number of scions or increasing the duration of scions on the stock plants resulted in an increased number of tubers showing necrosis. Transgenic Shepody plants with a sense or antisense copy of PVA coat protein (CP) gene under the direction of the *Cauliflower mosaic virus* promoter were developed. The plants were subjected to graft-inoculation with PVA-containing scions in the greenhouse, and were monitored by detecting PVA using ELISA and RT-PCR during the growing season. No PVA was detected in either the wild or the transgenic lines, indicating that the transgenes do not compromise the resistance against the virus. Potato tubers from graft-inoculated plants were harvested three months after inoculation, and were examined for necrosis. Approximately half of the tubers (8/17) from the wild type plants (i.e., untransformed Shepody) showed necrosis, so did 1/3 to1/2 tubers from the plants transformed with the antisense PVA-CP. However, no necrosis was found in the tubers from the plants that had been transformed with the sense PVA-CP gene, indicating that the transgene blocks the PVA-mediated hypersensitive response. The results also suggest that the coat protein of PVA is unlikely to be the elicitor of the HR in Shepody plants during the course of Shepody-PVA interaction.

Session A: Viruses
2:00-2:15

Identification of a Canadian alfalfa mosaic virus potato isolate and its detection in potato by RT-PCR and RFLP procedures. H. Xu. *Canadian Food Inspection Agency, Centre for Animal and Plant Health, 93 Mount Edward Road, Charlottetown, PE C1A 5T1, Canada.*
A Canadian alfalfa mosaic virus (AMV) isolate, AMV03175, was obtained from a commercial potato field and characterized by bioassay and ELISA. Its identity as AMV was confirmed by sequence analysis of the coat protein (CP) gene. Nucleotide and amino acid sequences of the AMV03175 CP gene were compared to that of several known AMV strains. Phylogenetic analysis was performed and the dendrograms obtained indicated that AMV03175 was closely related to AMV strains 425, KR1, KR2 and Danza. All sequenced AMV strains and isolates were divided into four groups. A pair of primers, AMV-F and AMV-R, specific to AMV CP gene was designed based on nucleotide sequence alignment of 20 AMV strains/isolates. Subsequent evaluations showed that RT-PCR using this primer set was specific and sensitive for detecting AMV in potato leaf tissue. To screen potato leaf samples on a large scale, composite leaf samples were used for total RNA extraction followed by RT-PCR amplification. AMV RNAs were easily detected in composite samples of 400 to 800 potato leaves. RFLP based on *Sac*I digestion of PCR amplicons, showed that the RFLP procedure was an efficient method for specific confirmation of PCR amplification products and for rapid identification of AMV RNA. RT-PCR followed by RFLP analysis may be a useful approach for screening potato samples on a large scale for the detection and identification of AMV.

Session A: Viruses
2:15-2:30

Investigations on the potential use of reflectance spectrophotometry for detection of virus infected potato plants. M.B. Crane, R.H Coffin, W. Hardy, M. Singh, and H.A. MacEwen.
Gentec Ltd., Kensington, PE C0B 1M0, Canada; (R.H.C., W.H., H.A.M.) Cavendish Farms, Kensington, PE, Canada; and (M.S.) Agr. Certification Services, Fredericton, NB, Canada.
The potato crop is susceptible to colonization by a wide range of insect pests and diseases (fungi, bacteria, and virus). The two major virus diseases are Potato Virus Y (PVY) and Potato Leaf Roll virus (PLRV).Virus colonized plants generally have lower yields of tubers. There is growing interest in post harvest testing to assure that certified seed stocks have less than specified tolerances of virus infection. The production of virus free or low virus seed requires isolation from infected stocks (inoculum) and low amounts of aphids (virus vectors). Seed potato growers inspect and remove (rogue) infected plants from their seed fields to reduce/prevent current season spread of viruses. Some varieties develop "easy to see" symptoms when colonized by PVY whereas other varieties (Shepody and Russet Norkotah) develop only faint mottling (mosaic) symptoms in leaves. Human roguers differ in their ability to visually detect virus infected plants.
The physiological status of the potato plant is manifested by the spectral characteristics - reflectance, transmittance and absorption - of incoming radiation (light). Virus infected plants can be observed by the human eye to differ slightly from healthy plants in their color, specular reflection, and leaf geometry. In 2004, field trials were conducted with PVY infected and healthy Shepody, Russet Burbank and Russet Norkotah. Readings were taken at canopy level using a hand held spectrometer sensitive to reflected light in the visible and near-infrared bands. This "detector" offers an opportunity to investigate differences in the specific spectral features observed in virus infected plants and healthy plants of the same variety. The collected reflectance data is then normalized and analyzed using regression analysis so that characteristic spectral features of virus infected plants may be identified. These features may aid in the future development of an automated detection system.

Session B: Bacteria
2:30-2:45

Epidemiology and management of fire blight in red raspberry. P.G. Braun and P.D. Hildebrand. *Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, Kentville, NS B4N 1J5,Canada.*
Fire blight of red raspberry, caused by *Erwinia amylovora*, is not a new disease but has received less attention than fire blight of apple and pear because it is sporadic and affects a minor crop. However, the effects of fire blight in raspberry can be just as destructive, killing flowers, developing fruit and new canes. The bacteria over-winter in infected canes and the initial inoculum in spring arises from systemically infected fruiting laterals on floricanes. Healthy flowers are infected primarily through nectaries while leaves, canes and green fruit are infected through wounds. Insects are the primary vectors of the pathogen in raspberry fire blight. Infected canes can produce copious amounts of bacterial exudates days before other symptoms of fire blight become evident. Insect vectors can spread the bacteria before the source is detected. For this reason a program to predict fire blight in raspberry may facilitate the application of management strategies. MaryBlyt™ 4.3, a predictive program for apple and pear fire blight, was evaluated for use in raspberry fire blight in 2000 - 2004. In three of five years MaryBlyt 4.3 accurately predicted primary inoculum production, flower infection periods and the appearance of fire blight symptoms on flowers. The results of control strategies tested will be discussed.

Session B: Bacteria
2:45-3:00

Detection and differentiation of pectolytic erwinias in potato by multiplex PCR. S.H. De Boer and J.A. D'Aubin. *Centre for Animal and Plant Health, Canadian Food Inspection Agency, Charlottetown, PE, Canada.*

The pectolytic erwinias cause several diseases in potato including blackleg, aerial stem rot, stem wet rot, and bacterial soft rot. Several species and subspecies of pectolytic erwinia are involved. *Erwinia carotovora* subsp. *atroseptica* (Eca) (syn. *Pectobacterium atrosepticum*) is the usual causal agent of blackleg although recently *E. carotovora* subsp. *brasiliensis* (Ecbr) was described as the causal agent of blackleg in Brazil. *Erwinia chrysanthemi* (Ech) causes a stem wet rot disease in several European countries and has been described as a pathogen of potato in Japan, Australia, Peru, and South Africa. *E. carotovora* subsp. *carotovora* (Ecc) is the main contributor to bacterial soft rot of potato tubers although the other pectolytic erwinias may also cause soft rot of tubers in storage. Since the erwinias are tuber-borne, use of clean seed is an important control strategy. Various methods to index seed lots for erwinia have been published. We developed a multiplex PCR for the simultaneous detection of four pectolytic erwinias. Published primer pairs for each of Eca, Ecbr, Ecc, and Ech were combined into a single multiplex PCR test. Amplicons of 690, 550, 420, and 322 base pairs representing Eca, Ecc, Ech, and Ecbr indicated the presence of the respective erwinia taxons. To test seed potato lots, tissue extracts from composite tuber samples were enriched in a minimal growth medium for 3-4 days and subjected to the multiplex PCR. Multiplex PCR performed on samples from Canadian seed potato lots revealed the presence or absence of Eca and Ecc in the lots. Ecbr and Ech were not detected in field samples, although they were readily detected in comparable samples spiked with cells of these bacteria.

Session B: Bacteria
3:00-3:15

Comparison of PCR primers for the detection of bacterial ring rot. M. Taghavi, S. H. De Boer, and L.J. Ward. *College of Agriculture, Shiraz University, Shiraz, Iran; and (S.H.D.B., L.J.W) Centre for Animal and Plant Health, Canadian Food Inspection Agency, Charlottetown, PE C1A 5T1, Canada.*

Bacterial ring rot, caused by *Clavibacter michiganensis* subsp. *sepedonicus* (Cms), is one of the most devastating diseases of potato. The pathogen is spread by infected tubers and, therefore, it is critical that only certified seed be used. Eradication and control of the spread of ring rot is dependent on reliable methods of detection. Since infected seed potatoes are the major source of initial inoculum, detection of low level of Cms in seed potatoes plays an important role in disease control and eradication. Several PCR primer sets were selected from the literature and evaluated for the sensitive and specific detection of the bacterial ring rot pathogen. Two PCR primer sets directed against plasmid sequences and three primer pairs against genomic sequences were used to amplify Cms DNA in separate PCR reactions. Cms isolates CmsR14, CmsR13, CS2, P45 and CS2889^T were used for the evaluations. *Clavibacter michiganensis* subsp. *michiganensis* (Cmm) LGM7333^T was used as a negative control for specificity. DNA was extracted using Magnesil KF Genomic System (Promega) or an in-house extraction method involving proteinase K treatment followed by ammonium acetate precipitation of proteinaceous matter and subsequent DNA precipitation and washing with isopropanol and ethanol, respectively. Strain P45 which purportedly lacks the plasmid was, as expected, not amplified by PCR using the plasmid directed primer sets. All other Cms strains were amplified by PCR with all but one of the primer sets and none amplified the Cmm negative control strain. PCR with one primer set had a sensitivity of detection of 10^3 cells/ml, while PCR sensitivity of detection was 10^4 cells/ml with the other primer sets.

Session C: Fungi and Oomycetes
4:00-4:15

Assessing the *in vitro* sensitivity of *Alternaria solani* and *A. alternata* isolates from Prince Edward Island to azoxystrobin. W. MacDonald, R.D. Peters, R.H. Coffin, and C. Lacroix. *Department of Biology, University of Prince Edward Island, 550 University Ave., Charlottetown PE C1A 4P3, Canada; (R.D.P.) Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, 440 University Avenue, Charlottetown, PE C1A 4N6, Canada; and (R.H.C.) Cavendish Farms, Kensington, PE, Canada.*
Isolates of *Alternaria solani*, causal agent of potato early blight, and *A. alternata*, another potato leaf spot pathogen, were collected from several potato fields in Prince Edward Island in 2003. An *in vitro* spore germination assay was used to measure the sensitivity of these isolates to azoxystrobin, the active ingredient in a novel strobilurin fungicide. The effective concentration that inhibited spore germination by 50% (EC_{50}) was determined for each isolate. EC_{50} values ranged from 0.003 to 0.014 parts per million (ppm) for *A. solani*, while the values for *A. alternata* ranged from 0.001 to 0.023 ppm. This result suggests that the isolates tested are sensitive to azoxystrobin; no indication of resistance was observed. This sensitivity is likely due to the limited exposure of these two pathogens (*A. solani* and *A. alternata*) to strobilurin chemistry in Prince Edward Island potato fields.

Session C: Fungi and Oomycetes
4:15-4:30

Recent investigations regarding the soil-borne pathogen *Phytophthora erythroseptica* and the management of potato pink rot. R.D. Peters, A.V. Sturz, H.W. (Bud) Platt, and W.J. Arsenault. *Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, 440 University Avenue, Charlottetown, PE C1A 4N6, Canada; and (A.V.S.) PEI Department of Agriculture and Forestry, Plant Health Research and Diagnostics, P.O. Box 1600, Charlottetown, PE C1A 7N3, Canada.*

Pink rot, caused by *Phytophthora erythroseptica*, has reemerged as an important disease of potatoes in North America. All underground potato tissues may be affected, but diseased tubers are most commonly observed. Most soils in potato-growing regions of North America contain propagules of *P. erythroseptica*. However, infected seed tubers can also spread new strains of the pathogen between regions. There is some evidence that crop rotation can reduce levels of inoculum in the soil. Other cultural practices such as providing good soil drainage, minimizing tuber injury at harvest and grading out diseased tubers prior to storage are also important. Although no commonly-grown potato cultivars are immune to pink rot, differences in cultivar response to disease exist. In general, potato cultivars with late-season field maturity are more resistant to disease than those with early or mid-season maturity. The two pathogens *P.infestans* (late blight) and *P. erythroseptica* can commonly be found co-infecting potato tubers and tuber rot severity can be significantly increased in the presence of both pathogens. Therefore, successful management of late blight may also aid in the management of pink rot. Chemical control of pink rot relies on the use of the systemic fungicide mefenoxam (Ridomil ® Gold). In-furrow applications of Ridomil Gold have generally provided better suppression of pink rot than foliar applications, although efficacy varies among regions. In recent years, the development of mefenoxam-resistant strains of *P. erythroseptica* in the U.S.A. has caused concern. Surveys conducted from 1999 to 2001 in Prince Edward Island (PEI) indicate that populations of the pathogen in PEI are still sensitive to mefenoxam. Continued monitoring of the mefenoxam resistance of populations of *P. erythroseptica* will ensure that mefenoxam is only applied where mefenoxam-sensitive populations of the pathogen occur.

Session C: Fungi and Oomycetes
4:30-4:45

Ultra-sensitive methods for PCR detection of potato wart in soil. L.J. Ward, S.H. De Boer, and C.A. Lévesque. *Canadian Food Inspection Agency, Charlottetown, PE, Canada; and (C.A.L.) Agriculture and Agri-Food Canada, ECORC, Ottawa, ON, Canada.*
Potato wart is caused by the obligate pathogen *Synchytrium endobioticum*. Sporangia from this organism can survive in soil for decades providing a readily available source of inoculum. In the fall of 2000 potato wart was identified on Prince Edward Island (PEI) resulting in the closure of all US markets to PEI potatoes. An extensive soil surveillance program was initiated to identify possible sources of over-wintering sporangia using traditional methodologies of soil sampling followed by sieving and microscopic identification. To develop a molecular test for the potato wart pathogen, the internally transcribed (ITS) region of ribosomal DNA of *S. endobioticum* was identified and sequenced . Primers suitable for sensitive detection of this obligate parasite using the polymerase chain reaction (PCR) were selected and tested using DNA extracted from herbarium and culture collections containing *S. endobioticum* and closely related chytrids as well as sieved and raw soil fractions and potato material obtained from PEI and Newfoundland. Initial tests involved DNA extraction using a commercial kit followed by amplification of the ITS1, ITS2 and 5.8S contiguous regions of the ribosomal DNA target. The amplicon was blotted to charged modified nylon membrane and probed with an ITS2- digoxigenin-PCR labelled probe. PCR was also used to amplify DNA extracted from hand-picked single spores and spores added to 0.5g of negative soil. These results along with sequence data confirmed the specificity of the selected primers for *S. endobioticum.* PCR amplification had a sensitivity of at least one spore per 0.5g of soil. In order to increase this sensitivity, template enrichment and serial PCR was tested using dilutions of DNA template and both identical and nested primer sequences. A two-step nested PCR produced the highest end-point dilution of template DNA.