Canadian Phytopathological Society
Maritime Region Meeting
November 25, 2010
Canadian Food Inspection Agency, Charlottetown Laboratory, Charlottetown, PEI

CONTENT : PROGRAM AND ABSTRACTS

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Charlottetown, PE

INTRODUCTION

Welcome to Charlottetown and to the 2010 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 11 oral presentations and 2 poster presentations on various topics in phytopathology scheduled for the afternoon. A dinner and presentations will take place at **The Old Triangle Irish Alehouse** following the meeting.

I am pleased and honoured to welcome Dr. Robert Coffin, retired scientist with Cavendish Farms in Prince Edward Island, as our keynote speaker. Dr. Coffin is well-known for his research on many aspects of potato production and for his enthusiastic and informative presentations. His presentation is entitled: "Politics, Perception and Science."

Many thanks to all who attended the meeting. I would particularly like to thank Drs. Sean Li and Huimin Xu, Canadian Food Inspection Agency-Charlottetown Laboratory, Charlottetown, PE and their colleagues 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, represented by president Jeannie Gilbert, for sponsoring this event. I trust we will be able to get together on a regular basis in future, and on that note, we will host the 2011 meetings in Fredericton, NB.

This booklet contains abstracts of the 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.

Rick D. Peters
Maritime Region Rep
Canadian Phytopathological Society

Canadian Phytopathological Society
Annual Maritime Region Meeting – 2010
Canadian Food Inspection Agency-Charlottetown Laboratory

Scientific Program
Thursday, November 25
Conference Room – Canadian Food Inspection Agency, Charlottetown Laboratory, Charlottetown, PEI

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

1:10-1:45 **Keynote Speaker: Dr. Robert Coffin**
Politics, Perception and Science.

Session A: **Fungal Plant Pathogens (Chair: Dr. Rick Peters)**

1:45-2:00 **Nova Scotia Agriculture College's Late Blight Research Program Update. G. Wang-Pruski*, S. Lim, R. Coffin, R. Peters, D. Pinto, K. Al-Mughrabi, H. Platt, Zenaida Ganga, S. Veenhuis-MacNeill, W. Hardy, I. Macdonald, K. Drake and T. Hamill**

2:00-2:15 **Susceptibility of Lowbush Blueberry in the Sprout and Crop Phase to Valdensinia Leaf Spot. P.D. Hildebrand*, W.E. Renderos, S.A.E. Fillmore and B. Walker**

2:15-2:30 **Survey of *Fusarium* Species Causing Potato Seed-Piece Decay in Canada and Their Resistance to Fungicides. K. Lugosch*, R.D. Peters, T. Barasubiye and K. Drake**

2:30-2:45 **The Validation of Phosphorous Acid-Responsive Proteins by MRM. Lim, S.*, G. Wang-Pruski, D. Pinto, R.H. Coffin, K.I. Al-Mughrabi and R.D Peters**

2:45-3:00 **Infection of Potato Tubers by *Phytophthora infestans* via Stolons. D.H. Lambert, R.D. Peters* and H.W. Platt**

3:00-3:45 **Nutrition Break and Poster Session (Lab Visitation Lead by Dr. Umadatt Singh and other CL staff)**

Session B: **Posters**

PEI Plant Disease Diagnostic Service.
M. Clark*

Characterization and Molecular Analysis of the Ribosomal DNA Intergenic Spacer (IGS) in the Genomes of the Potato and Tobacco Cyst Nematodes, *Globodera pallida, G. rostochiensisis* and *G. tabacum*. M. Madani*, A. Vierstraete and S. H. De Boer

Session C: **Plant Virology and Bacteriology (Chair: Dr. Harvinder Bennypaul)**

3:45-4:00 **Molecular Detection and Characterization of *Potato Virus S* Isolates Collected from Potato Lots in the Province of Prince Edward Island. H. Xu*, S. Cody, J. Nie and J. D'Aubin**

4:00-4:15 **Multiple Pectobacteria Associated with Potato Stem Rot. S.H. De Boer*, X. Li, A. MacDonald and L.J. Ward**

4:15-4:30 **Molecular Detection and Characterization of *Tobacco Rattle Virus* Isolates Collected from Greenhouse *Epimedium* Plants in Ontario and British Columbia. H. Xu*, L.J. Ward, J. Nickerson and U. Singh**

4:30-4:45 **Detection and Identification of Fastidious Phloem-Limited Bacteria in Potato. X. Li*, J. Nie, P. Ross, D.L. Hammill, J. Nickerson, L. Ward and S. H. De Boer**

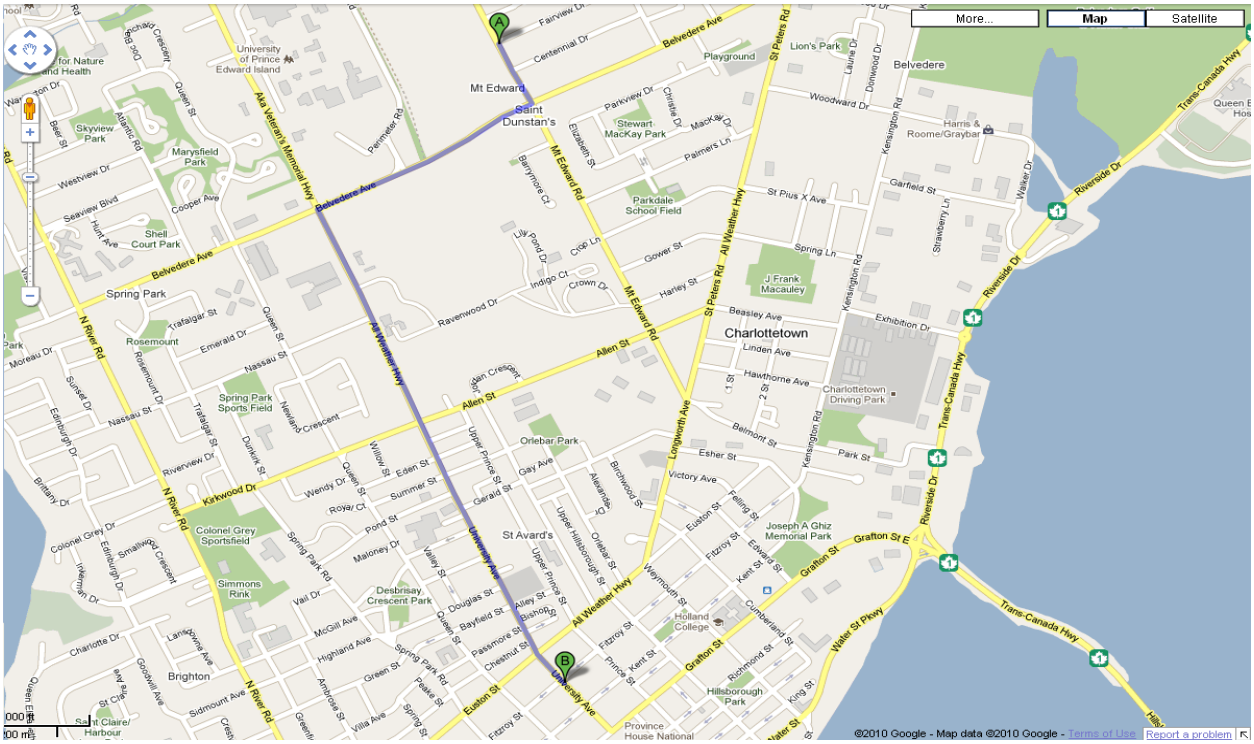
4:45-5:00 **Detection and Characterization of *Potato Virus V* in Potato Tubers Intercepted in a Traveler's Luggage. H. Xu*, B. Jenkins, S. Cody and J. D'aubin**

5:00-5:30 **Wrap-up.**

7:00-10:00 **Dinner and Awards**

The Old Triangle Irish Alehouse (89 University Avenue, See Map)

***** **Presenter**



A : CFIA-Charlottetown laboratory; B : The Old Triangle Irish Alehouse

ABSTRACTS

Susceptibility of Lowbush Blueberry in the Sprout and Crop Phase to Valdensinia Leaf Spot.

P.D. Hildebrand*, W.E. Renderos, S.A.E. Fillmore and B. Walker.

Atlantic Food and Horticulture Research Centre, Agriculture and Agri-Food Canada, 32 Main Street, Kentville, NS B4N 1J5.

Valdensinia leaf spot is becoming a serious disease in lowbush blueberry that causes premature leaf drop in the crop and sprout phases of production. The purpose of this study was to compare the relative susceptibility of foliage on sprout and crop stems over time. Stems were collected randomly from small sites ($2m^2$) in 2 sprout and 2 crop fields periodically beginning in early June to August in 2009 and 2010. The stems were inoculated with *Valdensinia heterodoxa* (800 spores/mL) and incubated in a moist chamber at 20 C for 24 h after which the incidence of infected leaves and lesion diameter were assessed. Incidence of leaf drop was assessed after 6 days, spores per leaf after 14 days and incidence and length of sclerotia after 30 days. The incidence of infected sprout leaves was much higher than crop leaves and remained high through the growing season, compared with crop leaves which became resistant to infection. Lesion size was considerably greater on sprout leaves but decreased rapidly by late July. The incidence of defoliation was not significantly different between leaf types, but tended to decrease over time. Spore production was high in both leaf types early in the season and decreased linearly with time. Incidence of sclerotia in leaves was high for both leaf types in June, but decreased rapidly for crop leaves compared with sprout leaves. Sclerotia length decreased with time and tended to be greater in sprout leaves. These results indicate that the pathogen is most aggressive early in the season and that sprout leaves are more susceptible which has implications for understanding disease development, spread and management.

Survey of *Fusarium* Species Causing Potato Seed-Piece Decay in Canada and Their Resistance to Fungicides.

K. Lugosch*, R.D. Peters, T. Barasubiye and K. Drake.

Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, Charlottetown, PE Canada; (T.B.) Agriculture and Agri-Food Canada, Eastern Cereal and Oilseed Research Centre, Ottawa, ON Canada.

Strains of *Fusarium* spp. with resistance to commonly used potato seed-piece treatment fungicides have been observed in Canada. In particular, populations of *F. sambucinum* and *F. coeruleum* with resistance to thiabendazole, thiophanate-methyl and/or fludioxonil have been frequently recovered. These pathogen populations have caused an increased incidence of seed-piece decay resulting in poor crop stands, reduced yields, and losses as high as 60% in some cases. Potato seed tubers with symptoms of decay were sampled from across Canada in spring 2010. In total, 85 samples were obtained, yielding 171 individual *Fusarium* isolates. Isolates were identified to species level by microscopic examination of morphological features and molecular methods and then tested for sensitivity to thiabendazole and fludioxonil using a fungicide-amended agar assay. The seed decay pathogens *F. sambucinum, F. avenaceum* and *F. coeruleum* were the most common species recovered. Isolates of *F. oxysporum, F. cerealis, F. acuminatum* and *F. sporotrichioides* were also identified. In summary, 84% and 76% of *F. sambucinum* isolates showed resistance to thiabendazole and fludioxonil, respectively, although isolates from Alberta and British Columbia showed more sensitivity to these fungicides than those found in other provinces. Most isolates of *F. avenaceum* and *F. coeruleum* were sensitive to both fungicides, although some fungicide-resistant isolates of these species were recovered in eastern Canada. In summary, the survey confirms the presence of strains of *Fusarium* in Canada resistant to thiabendazole and/or fludioxonil, particularly with respect to *F. sambucinum.* Fungicide resistance has limited the effectiveness of the most common potato seed-piece treatments used to control seed decay caused by *Fusarium* spp. in Canada. A re-evaluation of management practices for control of *Fusarium*-induced potato seed-piece decay is required.

The Validation of Phosphorous Acid-Responsive Proteins by MRM.

Lim, S.*, G. Wang-Pruski, D. Pinto, R.H. Coffin, K.I. Al-Mughrabi and R.D Peters.

Nova Scotia Agricultural College, PO Box 550, Truro, NS B2N 5E3; (D.P.) National Research Council-IMB, Halifax, NS B3H 3Z1; (R.H.C.) Cavendish Farms, Summerside, PE C1N 5J5; (R.D.P.) Agriculture and Agri-Food Canada, Charlottetown, PE C1A 4N6, Canada; (K.I.A.) New Brunswick Department of Agriculture and Aquaculture, Wicklow, NB E7L 3S4.

Phosphonate chemicals contain phosphorous acid (PA) as an active ingredient to suppress late blight. These fungicides generally have a very favorable environmental profile. However, the defense mechanism(s) in the plant induced by PA is still unknown. In our three year field trials, the application of Confine™, a registered potassium phosphate-based fungicide, on potato leaves delayed the spread of late blight disease up to two weeks. Proteins from Confine-treated leaves and water-treated leaves as a control were identified using quantitative comparative proteomics. In total, about 8.7% (103 of 1184 proteins) reproducible proteins were modulated by Confine. Interestingly, the molecular function of more than 60% of up-regulated proteins by Confine was involved in defense related function. These Confine-responsive proteins were validated by multiple reaction monitoring (MRM), a highly specific mass spectrometry technique. Totally, 20 proteins (18 up-regulated proteins and two down-regulated proteins) were quantified by MRM. One or two peptides per protein were used for the analysis. Data by MRM confirmed that the abundance of the peptides for the 18 selected proteins was increased in response to Confine and the abundance of the peptides for the two proteins was decreased after the Confine treatment. Therefore, all proteins investigated by MRM were validated successfully. Previous works have shown that some proteins with defense functions reacted with DAB (3,3'-Diaminobenzidine). Therefore, Confine-treated leaves were tested by DAB assay and results showed that the leaves acquired the defense response. These results demonstrated that the proteins validated by MRM and the biological assay could help us to understand defense mechanism(s) triggered by PA.

Infection of Potato Tubers by *Phytophthora infestans* Via Stolons.

D.H. Lambert, R.D. Peters* and H.W. Platt.

Department of Plant, Soil and Environmental Sciences, Deering Hall, University of Maine, Orono, ME 04469, USA; (R.D.P and H.W.P) Agriculture and Agri-Food Canada, Crops and Livestock Research Centre, 440 University Ave., Charlottetown, PE C1A 4N6, Canada.

The standard model for the infection of potato tubers by *Phytophthora infestans* involves the movement of infective propagules from the foliage through the soil profile during rain events resulting in direct infection of tubers, generally through natural openings such as eyes. However, under some circumstances, a high incidence of tubers infected at the stolon end can occur. To further explore the infection pathway, two greenhouse experiments were established wherein soils of potted plants were drenched with a sporangial suspension of *P. infestans* prior to tuber initiation. Seventy days after planting, plants were carefully removed from pots and underground plant parts including tubers, stolons and roots were assessed for visible symptoms of disease and the presence of the pathogen using microscopy and tissue plating methods. In total, 38% and 53% of formed tubers in experiments 1 and 2, respectively, showed symptoms of late blight tuber rot and were infected with *P. infestans* as determined by tissue platings. In all cases, tubers were infected at the stolon-end via stolons which also showed evidence of necrosis. Pathogen hyphae were observed within infected stolons and isolates of *P. infestans* could be recovered from both diseased tuber and stolon tissues. Growth of *P. infestans* through stolons into tubers provides a novel pathway for the infection of tubers. Thus, growers should place even more importance on early season control of disease.

PEI Plant Disease Diagnostic Service.

M. Clark

PEI Department of Agriculture, Kensington Potato Services, 7 Gerald A. McCarville Dr. PE, C0B 1M0

The Plant Disease Diagnostic Service provides farmers, agri-business and agricultural extension staff with a disease identification and control advisory service. Diagnoses are based on visual examination of symptoms, microscopic observation and explicit laboratory techniques. The objective of this program is to provide clients with a fundamental service, identifying the problem and providing information to control the problem if possible, and to prevent any reoccurrence. All sample results are followed up with a written disease diagnostic report, fact sheet on the disease and a Crop Specialist visit if needed.

The collection of data is recorded using Plant Health Reporting System (PHRS). This computer program can be utilized to query information on specific diseases, and to monitor disease status for new and existing diseases. Through the collection of data (PHRS), all pest levels are recorded for the Crop Specialists, Pest Hotlines and the farmers. Timely information on pest levels help farmers make informed decisions on the utilization of appropriate chemical control measures and management practices.

Characterization and Molecular Analysis of the Ribosomal DNA Intergenic Spacer (IGS) in the Genomes of the Potato and Tobacco Cyst Nematodes, Globodera pallida, G. rostochiensisis and G. tabacum.

M. Madani*, **A. Vierstraete, S. H. De Boer.**

Canadian Food Inspection Agency, Charlottetown Laboratory, 93 Mount Edward Road. C1A 5T1, PE Canada; and (A.V.) Department of Biology, Ghent University, Ledeganckstraat 35, B-9000 Ghent, Belgium

The intergenic spacer (IGS) region between the 18S and 28S rRNA genes in potato cyst nematodes, *G. pallida* and *G. rostochiensis*, and tobacco cyst nematode, *G. tabacum*, was amplified, cloned and sequenced. For sequencing, the IGS segment downstream from the 28S gene was amplified with primer pair 28F2/15R and upstream from the 18S gene using primer pair G18S/9R. On the basis of the preliminary IGS sequence alignment for the three species under study, we designed a set of primers from consensus regions for specific amplification of the entire IGS region. PCR amplification of IGS from *G. tabacum*, *G. pallida*, and *G. rostochiensis* yielded respectively, a single amplicon (3 kb), 3 amplicons (2.5, 2.6 and 2.9 kb), and 2 amplicons (2.8 and 2.9 kb) suggesting that some *Globodera* spp. have more than one variant copy of IGS. Sequence analysis of cloned IGS-PCR products revealed a long repetitive sequence as well as shorter repeats. Short repeats of (T) 6, (A) 6 and (C) 6 were observed in each of the IGS sequences. An insertion/deletion of one to three nucleotide(s) separated each repetitive element and the small sub-repeat units were positioned inside the longer repeats. Regions approximately 400 bp long in *G. rostochiensis* and 350 bp in *G. tabacum*, without any internal repetitive elements, were identified between the two repetitive regions. A short signature sequence specific to each of the three species was also identified. Understanding the molecular structure of the IGS provides additional data that can be helpful for population studies, identification and diagnostic purposes, and perhaps will also reveal differences between subspecies and races of these *Globodera* spp.

Molecular Detection and Characterization of *Potato Virus S* Isolates Collected from Potato Lots in the Province of Prince Edward Island

H. Xu*, S. Cody, J. Nie and J. D'Aubin.

Canadian Food Inspection Agency, Charlottetown Laboratory, 93 Mt. Edward Rd., Charlottetown, PE, C1A 5T1, Canada

Potato virus S (PVS, *Carlavirus*) is considered to be one of the most common potato viruses worldwide. The infected plants of most of the common potato cultivars show little or no symptoms but the yield reduction caused by PVS infection can be up to 20%. There is little sequence variation among PVS isolates and no well defined strain types have ever been proposed. In recent years, however, PVS variants that have unique biological, pathological and molecular features have been detected in some countries, which indicates the presence of possible distinct PVS strain types in some potato growing regions. To understand the strain identity and genetic variability of PVS isolates in Canada, 25 PVS isolates from over 300 PVS positive potato samples were selected for further characterization. The coat protein (CP) gene of these isolates was amplified in RT-PCR and sequences of PCR amplicons were then determined. Phylogenetic analysis indicated that all PVS isolates from Canada and the US clustered in the same group and all known PVS isolates fell into two distinct groups, the common PVS isolates and the Andean PVS isolates. All the Canadian PVS isolates were in the common PVS isolate group. Several sets of primers specific to different segments of PVS genome were designed and evaluated for use in RT-PCR for detecting common PVS isolates in various potato tissues. Evaluation data showed that RT-PCR was highly specific and sensitive for amplifying PVS RNA from potato samples. PVS RNAs were readily detected by RT-PCR in composite samples of 400 to 800 potato leaves or 200 to 400 dormant tubers.

Multiple Pectobacteria Associated with Potato Stem Rot.

S.H. De Boer*, X. Li, A. MacDonald, and L.J. Ward.

Charlottetown Laboratory, Canadian Food Inspection Agency. 93 Mount Edward Road, Charlottetown, PE, C1A 5T1, Canada.

Although *Pectobacterium atrosepticum* (Pa) and *P. carotovorum* (Pc) are known as the causal agents of potato blackleg and aerial stem rot of potato, respectively, we recently also isolated *P. wasabiae* (Pc) and to a lesser extent *P. brasiliensis* (Pb) from stems with blackleg-like symptoms. Isolate identifications by classical microbiological procedures were confirmed by multi-locus sequence typing, and pathogenicity on potato was determined in greenhouse studies. Selected isolates of each *Pectobacterium* spp. were tested for pathogenicity in a field plot experiment. Seed potato tubers (cv. Superior), vacuum infiltrated with one of the four pectobacteria, were grown with and without irrigation and observed for stem decay. Incidence of stem decay was greatest in plots under irrigation, and greater in plots planted with Pa-inoculated seed than plots planted with Pc-, Pb-, or Pw-inoculated seed. Disease incidence was greater with Pw-inoculated than Pb- and Pc-inoculated seed under irrigation, but was greater with Pb-inoculated seed in the unirrigated plot. The inoculated *Pectobacterium* spp. was detected by PCR in every corresponding diseased stem tested during mid-season, but later Pa was only detected in about one-half of the diseased stems grown from Pa-inoculated seed. Pa was also detected mid-season in diseased stems in plots planted with Pc, Pb, and Pw-inoculated seed, and Pc, Pb, and Pw was detected in some of the diseased stems of the Pa-inoculated plot as well as in some stems of all other plots. While inoculated pectobacterial species (except for Pc) were also detected at the stolon end of 10-30% of symptomless progeny tubers, heterologous species were also detected. These preliminary field results suggest the involvement of all four *Pectobacterium* spp. in potato stem rot but the role of each species as a primary pathogen needs further clarification.

Molecular Detection and Characterization of *Tobacco Rattle Virus* Isolates Collected from Greenhouse *Epimedium* Plants in Ontario and British Columbia.

H. Xu*, L.J. Ward, J. Nickerson and U. Singh.

Canadian Food Inspection Agency, Charlottetown Laboratory, 93 Mt. Edward Rd., Charlottetown, PE, C1A 5T1, Canada

Epimediums (*Epimedium* spp., *Berberidaceae*) are annual plants that are widely used in gardens and for medical uses. Epimedium plants of several cultivars showing mosaic, mottling, chlorotic spots and necrotic lesions were obtained from two nursery farms in Ontario and British Columbia in July, 2010. These samples were initially screened by RT-PCR using the primer set targeting the Open Reading Frame 4 (ORF4) of *Tobacco rattle virus* (TRV) RNA 1 and the RT-PCR procedures developed previously for the detection and identification of TRV potato isolates. Amplicons with expected size (463 bp) for TRV were generated from the RNA extracts of the Epimedium plants by RT-PCR. Subsequent digestion of the PCR products with the restriction endonucleases showed that the PCR amplicons of some samples were digested as expected by *Alu*I, but not by *Dde*I, The ORF4 gene of eight TRV Epimedium isolates (named as TRV-E889 to 910) were amplified by RT-PCR followed by sequence analysis that revealed some differences among the these isolates. The analysis indicated that the TRV Epimedium isolates may have two different origins (compared TRV-E889 and 903). Phylogenetic analysis and comparison of the Epimedium TRV isolates with other known TRV isolates showed that some of the isolates (e.g. E888, E889, E906) were closely related to TRV from ornamental species (e.g. tulip) while other Epimedium TRV isolates (e.g. E903, E904, E908-E910) shared a higher identity with European tobacco and potato TRV isolates than with the TRV isolates from Canadian potatoes.

Detection and Identification of Fastidious Phloem-Limited Bacteria in Potato.

X. Li*, J. Nie, P. Ross, D.L. Hammill, J. Nickerson, L.J. Ward and S. H. De Boer.

Canadian Food Inspection Agency, Charlottetown Laboratory, 93 Mt Edward Road, Charlottetown, PE, C1A 5T1, Canada

Fastidious phloem-limited bacteria in potato are phytopathogens that specifically inhabit the phloem tissue, causing leaf stunting, witches' broom and purple top. Among them, phytoplasma have been described since 1967 with worldwide distribution, whereas '*Candidatus* Liberibacter solanacearum' was only recently named as the causal agent of potato zebra chip disease that so far has a limited distribution. Apart from the common symptoms which are similar to potato witches' broom caused by a phytoplasma, the main characteristic of potato zebra chip disease is the severe internal necrosis of potato tuber tissue. However, it was observed that the zebra chip pathogen tended to be eliminated naturally from some tissues such as aerial tubers of greenhouse-grown plants, but remained readily detectable in diseased stem and infected progeny tubers of the same plants. In contrast, age-dependent disappearance of symptoms from ZC-infected tomato plants did not affect infectivity. Potato plants graft-inoculated from latently infected tomato became symptomatic but, interestingly, similarly graft-inoculated tomato plants did not but were PCR-positive. To ensure accurate detection and differentiation of the pathogens, we used a culture-independent systematic approach to clone and sequence the entire 16S rRNA gene, an intergenic spacer region, and partial 23S rRNA gene of the causal agents of both the zebra chip and potato witches' broom diseases. Phylogenetic analyses was used to rapidly differentiate between the potato zebra chip disease and potato witches' broom, which despite very similar symptomologies are caused by very different organisms, the former being classified as an Alphaproteobacteria, while the latter belongs to the Mollicutes. A conventional PCR assay targeting the rrn operon of '*Can.* L. solanacearum' was adapted for routine use in the potato post-entry quarantine program.

Detection and Characterization of *Potato Virus V* in Potato Tubers Intercepted in A Traveler's Luggage.

H. Xu*, B. Jenkins, S. Cody and J. D'Aubin.

Canadian Food Inspection Agency, Charlottetown Laboratory, 93 Mount Edward Road, Charlottetown, PE, C1A 5T1

Over 20 potato tubers were intercepted by the Canada Border Services Agency staff in luggage of a traveler returned from a trip to Peru and submitted to the Charlottetown Laboratory for testing. Some plants grown from these tubers showed systemic mosaic and necrotic lesions on lower leaves. Tubers s harvested from the infected plants did not show any visible necrotic (surface and internal) symptoms. Mechanical inoculation of the leaf sap from two (CL-2, CL-3) diseased plants to a panel of 19 indicator species resulted in the infection of several indicator species. Electron microscopy (EM) examination revealed flexuous filaments with an average length of 760 nm and a diameter of 11 nm. These morphological features resembled virus particles of species in *Potyviridae*. Subsequent ELISA screening showed that these plants were infected with *Potato virus V* (PVV), a species of the genus *Potyvirus* in the family of *Potyviridae*. Several sets of primers specific to PVV coat protein (CP) gene were designed and RT-PCR was evaluated for detecting this virus in various potato tissues. PVV was readily detected by RT-PCR and PCR products were verified easily by RFLP. The entire CP gene of PVV isolates CL-2 and CL-3 were also amplified by RT-PCR followed by sequence analysis. Phylogenetic analysis of PVV isolates based on the amino acid and nucleotide sequences of the CP gene showed that CL-2 and CL-3 were slightly different. PVV-CL3 is closely related to most of the known PVV isolates detected in many European countries, US and Peru. PVV-CL2 has a low identity to most of the known PVV isolates, but almost identical to a PVV isolate detected in Peru. RT-PCR and RFLP approaches were also evaluated for detecting and identifying PVV in potato tissues.