CANADIAN PHYTOPATHOLOGICAL SOCIETY

MARITIME REGION MEETING 2015

Potato Research Centre, AAFC, 850 Lincoln Road, Fredericton, New Brunswick

The Canadian Phytopathological Society Maritime Region Meeting 2015

Thursday, 16 April 2015
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
J. Edward Hurley, Associate Director, Research, Development and Technology, Potato Research Centre

13:10-13:30 **Deena Errampalli**, President, Canadian Phytopathological Society
Opening remarks and update from the CPS President (Video conference)

13:30-14:00 **Keynote Speaker: Mathuresh Singh,** Agricultural Certification Services, 1030 Lincoln Road, Fredericton, NB, Canada E3B 8B7
Agricultural Certification Services Inc. – a private not-for-profit laboratory playing a major role in disease diagnostics and research

14:00-14:15 **Updates on Potato Late Blight Research in New Brunswick during 2013-2014.** K.I. Al-Mughrabi, K. Jayasuriya*, R. Poirier, L.M. Kawchuk, R.D. Peters, F. Daayf, and B. Prithiviraj

14:15-14:30 **What's in a wart? Insights from the genome of the potato wart pathogen *Synchytrium endobioticum*.** D. L. Joly*, J. Cullis, C. Lewis, D. S. Smith, M.-C. Gagnon, M. P. E. Van Gent-Pelzer, H. Van De Geest, G. J. Bilodeau, T. A. J. Van Der Lee, X. Li, P. J. M. Bonants, and C. A. Lévesque

14:30-14:45 **Detection and quantification of *Verticillium dahliae* in soil of potato and strawberry fields and its distribution in PEI and Nova Scotia.** G. Wang-Pruski*, T. Borza, A. Govindarajan, X. Gao, B. Beaton, K. Best, Z. Ganga, and K. Pruski

14:45-15:00 **Detection and quantification of *Verticillium dahliae* and *Verticillium albo-atrum* in potato and strawberry plants.** T. Borza*, A. Govindarajan, X. Gao, R. Peters, Z. Ganga, J. Rand, B. Beaton, K. Best, K. Pruski, and G. Wang-Pruski

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

PEI Analytic Laboratory – Plant disease diagnostic submissions. M.M. Clark and A. M. Driscoll*

Survey of Metalaxyl-m Resistance in Populations of the Potato Pink Rot Pathogen (*Phytophthora erythroseptica*) in Canada and Alternative Disease

Management Strategies. B. Crane*, R.D. Peters, L.M. Kawchuk, A. MacPhail, K.A. Drake, D. Gregory, and K. MacDonald

Identification of Pathogenic Fusarium Species in Carrots and Their Qualification in Soil. H. Lu*, G. Wang-Pruski, R.D. Peters, J. Driscoll, and S. Asiedu

15:30-15:45 **Re-classification of potato isolates of *Pectobacterium wasabiae* as *Pectobacterium kelmani.*** X. Li*, K. Yuan, and S.H. De Boer

15:45-16:00 **Detection and identification of *Xylella fastidiosa* from maple trees.** K. Yuan* and X. Li

16:00 -16:15 **Characterization of the molecular mechanisms involved in the *Pseudomonas fluorescens* LBUM223 / *Streptomyces scabies* interaction leading to the biocontrol of potato common scab.** M. Filion*, T. Arsenault, and C. Goyer

16:15 -16:30 **Viruses detected in Canadian potato lots – a brief summary of potato regulatory testing for the past 10 years**. H. Xu*

16:30-16:45 **Potato virus Y strains transmit mechanically from one plant to another.** M. Fageria*, X. Nie, A. Gallagher, and M. Singh

16:45-17:00 **Development of a PCR-based detection system for five prominent viruses of strawberry.** T.D.B. Mackenzie*, A.G.E. Gallagher, and M. Singh

17:00-17:15 **The changing epidemiology of tomato and potato late blight in Canada.** R.D. Peters*, L.M. Kawchuk, F. Daayf, K.I. Al-Mughrabi, A. MacPhail, D. Gregory, K.A. Drake, M. Trenholm, and B. Crane.

17:15-17:30 **Occurrence of potato tuber damaging viruses in Canada and evidence of genome reassortment and RNA recombination in *Potato mop-top virus*.** X. Nie*, V. Dickison, X. Hu, X. Xiong, and M. Singh

17:30–17:35 **Closing remarks** Rick Peters

18:30 **Dinner** (Isaac's Way: 649 Queen St, Fredericton. Tel (506) 474-7222)

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

Keynote 13:30-14:00

Agricultural Certification Services Inc. - a private not-for-profit laboratory playing a major role in disease diagnostics and research

M. SINGH

Agricultural Certification Services Inc., 1030 Lincoln Road, Fredericton, NB, E3B 8B7, Canada

Agricultural Certification Services Inc. (ACS) is a not-for-profit laboratory owned by New Brunswick potato growers and located in Fredericton, NB. ACS has been in operation since 1996 providing Canadian Food Inspection Agency (CFIA) approved testing services to potato industries in NB, PEI, NS, ON, QC and AB. ACS is equipped for microbiological, serological and molecular research work and uses novel technologies for testing, such as PCR and real-time PCR along with ELISA. Services offered by ACS include: Bacterial Ring Rot testing; testing for potato viruses A, M, S, X, Y, potato leafroll virus, potato latent virus, potato mop-top virus, Potato Spindle Tuber Viroid, Late Blight, Pink rot testing and virus eradication from accessions of potatoes. In addition to potato testing, ACS offers Vomitoxin test in grains, such as Wheat, Barley, Oat and Corn. Recently, ACS started new diagnostic services using RT-PCR testing for strawberry viruses, such as *Strawberry mild yellow edge virus*, *Strawberry mottle virus*, *Strawberry vein banding virus*, *Strawberry pallidosis virus* and *Strawberry crinkle virus*. ACS has been involved in conducting contract research in collaboration with different organizations, such as the New Brunswick Department of Agriculture, Aquaculture and Fisheries, Agriculture & Agri-Food Canada, McCain's, Cavendish Farms, National Research Council, Canadian Horticultural Council, DuPont and the CFIA. Currently, ACS is either leading or collaborating on seven different research projects related with PVY strain characterization to management using insecticides and mineral oil.

Updates on Potato Late Blight Research in New Brunswick during 2013-2014

K.I. AL-MUGHRABI[1], K. JAYASURIYA[1], R. POIRIER[1], L.M. KAWCHUK[2], R.D. PETERS[3], F. DAAYF[4] and B. PRITHIVIRAJ[5].

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During 2013-2014, we evaluated the susceptibility of alternative hosts (tomato, pepper, eggplant, petunia, and nightshade) to late blight caused by *Phytophthora infestans* US-23 strain. We also assessed the efficacy of fungicides registered in Canada against late blight, both *in vitro* and *in situ*. When detached leaves of alternate hosts were inoculated with *P. infestans* US-23 isolates, nightshade was the most susceptible (Disease Severity "DS" = 9.5 mm) and the severity was comparable to that on potato (DS=8.9 mm). Late blight severity on petunia (4.7 mm) and tomatoes (4.3-4.7 mm) was significantly ($p<0.05$) less than that on nightshade. Eggplant had a DS of 2.1 mm while pepper was the least susceptible (DS=0.03 mm). Field trials conducted in New Brunswick over 2 seasons examined the following treatments: 1) Control; 2) Confine™; 3) Phostrol™; 4) Zampro™; 5) Bravo®ZN/Dithane™DG; 6) Allegro500F; 7) Curzate®DF; 8) Acrobat®50WP; 9) Ridomil®GoldMZ68WP; 10) Tattoo®C; 11) Gavel®75DF; 12) Ranman400SC; 13) Headline®EC; 14) Reason®500SC; 15) Revus®; and 16) Cabrio®Plus, alternated with BravoZN/DithaneDG. The effect of seaweed extract alone or alternated with other fungicides was assessed against both early and late blight diseases. Treatments included: 1) Control; 2) seaweed extract (@3.71 L/ha) alternated with Dithane™DG/Bravo®ZN; 3) seaweed extract (@3.71 L/ha, and 4) QuadrisTop™ (@1 L/ha) alternated with Dithane™DG/Bravo®ZN. All fungicides tested in the field suppressed late blight which prevailed during the latter part of the two growing seasons. Seaweed extract alone was ineffective in controlling late blight. Seaweed extract alternated with Dithane™DG and Bravo®ZN or the treatment with QuadrisTop™ alternated with Dithane™DG and Bravo®ZN significantly reduced late blight severity and incidence, as well as early blight severity. In all treatments, marketable yield did not vary significantly.

What's in a wart? Insights from the genome of the potato wart pathogen *Synchytrium endobioticum*.

D. L. JOLY, J. CULLIS, C. LEWIS, D. S. SMITH, M.-C. GAGNON, M. P. E. VAN GENT-PELZER, H. VAN DE GEEST, G. J. BILODEAU, T. A. J. VAN DER LEE, X. LI, P. J. M. BONANTS AND C. A. LÉVESQUE.

Département de biologie, Université de Moncton, 18 avenue Antonine-Maillet, Moncton, NB E1A 3E9, Canada; (J.C., C.L., C.A.L.) Eastern Cereal and Oilseed Research Centre (ECORC), Agriculture and Agri-Food Canada (AAFC), 960 Carling Avenue, Ottawa, ON K1A 0C6, Canada; (D.S.S., X.L.) Canadian Food Inspection Agency (CFIA), 93 Mount Edward Road, Charlottetown, PE C1A 5T1, Canada; (M.-C.G., G.J.B.) Canadian Food Inspection Agency (CFIA), 3851 Fallowfield Road, Ottawa, ON K2H 8P9, Canada; and (M.P.E.v.G.-P., H.v.d.G, T.A.J.v.d.L, P.J.M.B.) Plant Research International, P.O. Box 16, NL-6700 AA, Wageningen, The Netherlands.

Potato wart is a serious disease of cultivated potato (*Solanum tuberosum* L.) caused by the obligate biotrophic soil-borne chytrid fungus *Synchytrium endobioticum* (Schilb.) Perc. Even though the disease has been repeatedly reported in numerous locations around the world, its global distribution has been limited by strict quarantine and regulatory measures. In 2000, the US temporarily closed its border to imports of PEI potatoes following the discovery of the fungus, which has then been found subsequently in 2002, 2003, 2007, 2012 and 2014. Genome sequencing of plant pathogens is being increasingly used to improve our understanding of virulence mechanisms, as well as assisting regulatory agencies to develop molecular markers towards more sensitive and accurate detection methods. Isolates of *S. endobioticum* have been independently sequenced using Next-Generation Sequencing technologies, unravelling a reasonably small genome of ~20 megabases, for which educated annotation revealed under 6,000 genes. Being an intracellular holocarpic pathogen, *S. endobioticum* secreted proteins are prime candidates to look for virulence factors. Preliminary analyses revealed a relatively small secretome of ~250 putative secreted proteins. Interestingly, one family of secreted proteins represented almost a fifth of the entire secretome. Within this family, high rates of amino acid changes have been identified between two isolates, which suggests diversifying selection is acting on these genes. Functional assays will be developed to screen the function of these putative secreted proteins within plant tissues.

Detection and quantification of *Verticillium dahliae* in soil of potato and strawberry fields and its distribution in PEI and Nova Scotia

G. WANG-PRUSKI, T. BORZA, A. GOVINDARAJAN, X. GAO, B. BEATON, K BEST, Z. GANGA, K. PRUSKI.

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Verticillium dahliae Kleb., in combination with other fungi, is responsible for the early dying syndrome of potatoes which causes important reduction of tuber yields. When present in strawberry fields, *V. dahliae* can also cause severe economic losses in production. As this fungus has a wide host range and microsclerotia has a long viability, assessing the amount of the inoculum in soil is needed for disease managementThe aim of this study was to detect and quantify the levels of *V. dalhiae* propagules in soil samples cultivated with potatoes, strawberries and rotating crops. Molecular methods, such as real-time quantitative PCR (qPCR), provide a faster quantification of *V. dalhiae* in soil samples compared to plating methods. Using a qPCR method, the distribution of *V. dalhiae* in soil was analyzed in four strawberry fields and two potato fields in Nova Scotia, and seven potato fields or fields with rotation crops in Prince Edward Island. The results showed that the qPCR allows the detection of as little as one cell per gram of soil. Higher levels of *V. dalhiae* propagules were found in potato fields and in fields previously cultivated with potatoes. The pathogen was also found in strawberries fields, but lower levels of the pathogen were determined, very likely due to the fumigation practices. Since fumigation is not permitted in Prince Edward Island, the pathogen will continue to represent a significant threat to the potato production.

Detection and quantification of *Verticillium dahliae* and *Verticillium albo-atrum* in potato and strawberry plants

T. BORZA, A. GOVINDARAJAN, X. GAO, R. PETERS, Z. GANGA, J. RAND, B. BEATON, K. BEST, K. PRUSKI AND G. WANG-PRUSKI

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Verticillium spp. infect a broad range of plants. In potatoes and strawberries, *Verticillium* wilt causes decreased tuber and berries yields, respectively. In Atlantic Canada, the disease is caused mainly by two species, *V. dahliae* Kleb. and *V. albo-atrum* Reinke & Berthold. Previous work carried out in this geographical area, more than a decade ago, indicated high levels of infestation of potatoes with both *V. dahliae* and *V. albo-atrum*, while no such data is available for strawberries. A real-time quantitative PCR (qPCR) method was used for the detection and quantification of *V. dahliae* and *V. albo-atrum* in potato stems collected from fields in Prince Edward Island (PEI) and Nova Scotia (NS) and in strawberry plants harvested in fields from NS. For both pathogens, the detection limit using qPCR was 1 to 2 cells/gram of fresh tissue. All potato plants tested from PEI and NS were found positive for *V. dahliae* (generally > 1000 cells/grams of plant tissue), but not for *V. albo-atrum*. *V. albo-atrum* was identified only in an experimental field in which plants were artificially inoculated with *V. albo-atrum* strain 1856. However, *V. dahlia*e was also identified in these plants, providing additional evidence that presently this pathogen has a ubiquitous distribution in potato plants grown in PEI and NS. The screening of strawberry plants allowed the detection of a few plants infested with *V. dahliae*, but not with *V. albo-atrum.* The level of infestation of strawberry plants was found to be very low compared to that observed in potato plants.

Survey of Metalaxyl-m Resistance in Populations of the Potato Pink Rot Pathogen (*Phytophthora erythroseptica*) in Canada and Alternative Disease Management Strategies

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Pink rot, caused by the pathogen *Phytophthora erythroseptica,* is a common disease of potatoes in Canada. Pink rot is most prevalent in wet growing conditions in autumn which contribute to pathogen spore release and tuber infections. Traditionally, pink rot has been managed with metalaxyl-m (Ridomil Gold ®) applications either in-furrow at planting or as a foliar spray during the growing season. In recent years, isolates of *P. erythroseptica* resistant to metalaxyl-m have appeared in potato growing regions of Canada, including the Maritime provinces. In 2013, a two-year national survey was initiated to assess the distribution of resistant strains of *P. erythroseptica* in Canada. Samples of infected tubers from across Canada were used to obtain isolates of the pathogen for testing for metalaxyl-m resistance using an *in vitro* agar assay. Resistant strains were recovered from Prince Edward Island, New Brunswick, Nova Scotia, Ontario, and Manitoba in 2013. This indicates an expansion in range and distribution of metalaxyl-m resistant isolates of *P. erythroseptica* is occurring in Canada. Therefore, alternative management strategies need to be assessed to provide protection against tuber infection by *P. erythroseptica*. In 2014, a field study was conducted to test the efficacy of in-furrow applications of Serenade SOIL ®, Ridomil Gold ®, Presidio ®, Phostrol ®, an experimental treatment, and foliar applications of Phostrol ® to suppress tuber infections by metalaxyl-m sensitive or metalaxyl-m resistant isolates of *P. erythroseptica* during planting and in wet growing conditions in autumn. Ridomil Gold ® provided the greatest suppression of seed piece decay by the metalaxyl-m sensitive isolate in the study, but did not suppress infection by the resistant isolate. Plots with other chemical treatments yielded seed piece decay similar to that of the inoculated control for both the metalaxyl-m sensitive and resistant *P. erythroseptica* isolates. All treatments suppressed tuber infection by the metalaxyl-m sensitive isolate during wet autumn growing conditions compared to the inoculated control. Foliar Phostrol ®, Presidio ®, and an experimental treatment provided the greatest suppression of tuber infection by the metalaxyl-m resistant isolate during wet autumn growing conditions relative to the inoculated control. In-furrow treatment of Phostrol ®, Serenade ® or Ridomil Gold ® did not provide control of tuber infection by the metalaxyl-m resistant strain. These results suggest that there are some potential new management strategies to control pink rot infections caused by metalaxyl-m resistant strains of *P. erythroseptica* that continue to spread across Canada.

Identification of Pathogenic *Fusarium* Species in Carrots and Their Qualification in Soil

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In recent years, the carrot producers in Prince Edward Island (PEI) suffered a great loss due to the development of dry rot caused by *Fusarium* pathogens. *Fusarium* spp. is the major pathogen that threatens the PEI carrot industry. Two major pathogenic species, *F. avenaceum* and *F. oxysporum*, have been detected in carrot fields in PEI. To distinguish pathogenic *Fusarium* spp. from other non-pathogenic fungi and monitor the infection level of those pathogens in soil, a highly sensitive detection method is required. To achieve this goal, a fast and sensitive real-time quantitative polymerase chain reaction (qPCR) method has been developed. Species-specific primers targeting a short fragment (less than 200 bp) of DNA were designed for distinguish the pathogenic *Fusarium* spp. from other soil fungi. This method would be able to accurately detect and quantify *F. avenaceum* and *F. oxysporum* and the number of fungal propagules presented in soil of carrot fields, which will be valuable for developing and testing management strategies to control the pathogenic *Fusarium* spp.

Key words: *Fusarium* dry rot, Detection and quantification of soil pathogens, qPCR

Re-classification of potato isolates of *Pectobacterium wasabiae* as *Pectobacterium kelmani*

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With a narrow host range, *Pectobacterium wasabiae* (formerly *Erwinia carotovora* subsp. *wasabiae*) was originally described as the causal agent of soft rot of horseradish (*Eutrema wasabi* Maxim.) back in 1987 in Japan. Recently, a group of pectolytic bacteria causing potato tuber decay, aerial stem rot, and/or blackleg-like symptoms in Europe, NA and some other potato production regions were classified as *P. wasabiae* based mainly on phylogenetic and molecular methodologies. Further investigation on phenotypical characterization indicated that these potato-associated strains differed from the four typical horseradish *P. wasabia* strains. The potato strains were positive in utilizing or acidification of lactose, melibiose and raffinose, while the four horseradish *P. wasabiae* strains from Japan were all negative. Revisiting the phylogenetic trees in published studies indicated the presence of phylogenetical differences between these two groups of bacteria. In order to define the classification of the potato strains of the pathogen, we selected a typical isolate CFIA 1002 for decoding its genome sequences using paired-end Illumina HiSeq sequencing technology with TrueSeq V3 chemistry. Sequencing resulted in 8,682,640 reads (insert size of 300 bp) totaled 876,946,640 bp, with approximately 175X genome coverage. Comparative genomic analysis of the draft genome sequences of CFIA 1002 with WPP163 from USA, SCC3193 from Europe, and the original *P. wasabiae* strain CFBP3394 suggested a need for reclassification of potato isolates, including potato isolates from the US and European, as a new species, we suggest naming *Pectobacterium kelmani*. Species-specific real-time PCR assays were developed for diagnostic purpose.

Detection and identification of *Xylella fastidiosa* from maple trees

KAT YUAN, XIANG LI

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Xylella fastidiosa causes leaf scorch in more than 100 plant species of agricultural importance. For instance, *X. fastidiosa* subsp. *fastidiosa*, the causal agent of Pierce's Disease (PD) of grapevine, has been considered a regulated pathogen due to the economic losses caused to the grape industry. As a North American indigenous pathogen, *X. fastidiosa* subsp. *multiplex* infects many shade trees and other plant hosts, some of which are also the host plants of the PD pathogen, such as almond. Therefore, rapid diagnostics and differentiation of these two closely related pathogens are important for effective management and prevention of PD caused by *X. f. fastidiosa.* In this study, four samples (M1, M2, U1 and U2) were collected from maple trees showing typical symptoms of bacterial leaf scorch. Isolation of *X. fastidiosa* was attempted using selective media. *X. fastidiosa*-like colonies were obtained on isolation plates from one of the samples after more than 15 days incubation. The isolate was identified as *X. fastidiosa* in a species-specific PCR assay using a common primer set RST31/RST33, resulting in amplification of a 737bp DNA amplicon which was cloned for detailed sequencing analysis. Further analysis of the isolate using a PD-specific TaqMan real-time PCR assay indicated that this bacterium was not the PD pathogen *X. fastidiosa* subsp. *fastidiosa.* Based on comparison with published literature, it is likely that the causal agent of the diseased maple trees belongs to *X. fastidiosa* subsp. *multiplex.*

Characterization of the molecular mechanisms involved in the *Pseudomonas fluorescens* LBUM223 / *Streptomyces scabies* interaction leading to the biocontrol of potato common scab

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Common scab of potato, caused by *Streptomyces scabies*, is an economically important disease. There are currently no efficient measures to control common scab. We have previously determined that *Pseudomonas fluorescens* LBUM223 showed antagonistic properties against *S. scabies* under *in vitro* conditions, mainly due to the production of the antimicrobial compound phenazine-1-carboxilic acid (PCA). In this study, we investigated if LBUM223 was able to control common scab under growth chamber and field conditions, and also evaluated the contribution of three mechanisms in controlling common scab development: 1) antibiosis; 2) induced systemic resistance; 3) and transcriptional alteration of the pathogen's virulence gene expression. The results obtained in growth chamber experiments and field trials were congruent and suggested that PCA production by LBUM223 is required to significantly reduce scab development on potato. Although no reduction in *S. scabies* population was observed in the rhizosphere or geocaulosphere in the presence of LBUM223, a significant reduction in *txtA* expression in *S. scabies*, encoding the production of thaxtomin A, a phytotoxic virulence factor, was observed in the geocaulosphere starting at 10 weeks following planting. Furthermore, LBUM223 did induce long-term overexpression of several defense-related genes in potato, but this systemic response did not seem to significantly contribute to disease control. Taken together, these results suggest that *Pseudomonas fluorescens* LBUM223 controls common scab development not by antibiosis or by stimulating plant defense responses, but instead reduces *S. scabies* thaxtomin A production in the geocaulosphere, leading to reduced virulence and symptoms development.

Viruses detected in Canadian potato lots – a brief summary of potato regulatory testing for the past 10 years

H. XU

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Over fifty viruses have been reported world-wide to infect potato naturally. Most of them have plus-sense ssRNA as their genome that is composed of one or more RNA molecules. Five species in the family of *Geminiviridae* have ssDNA as their genome and 3 species in the families of *Rhabdovirudae* and *Bunyaviridae* have negative-sense ssRNA as their genome. In the seed potato certification program in Canada, seed potato lots are indexed for all viruses and a viroid – *Potato spindle tuber viroid*. Eleven viruses have been confirmed to infect potato naturally. Four of the 11 viruses, *Potato virus S* (PVS), *Potato virus X*, *Potato virus Y* (PVY, the common strain) and *Potato leafroll virus*, have a wide distribution across the country and their incidence in seed potato lots is quite low except that of PVS. The diversity of PVY strains appears to be increasing. *Potato latent virus, Tobacco rattle virus* and *Alfalfa mosaic virus* have been detected in some potato fields in isolated areas. *Potato mop-top v*irus (PMTV) was confirmed to have a nation-wide distribution 10 years ago. However, the incidence of PMTV is quite low and tuber necrotic symptoms induced by PMTV infection was only detected from tubers of a few susceptible potato cultivars. *Potato virus M* and *Potato virus A* have become minor potato pathogens and are hardly detected in seed potato lots for the past 10 years. All regulatory potato testing indicated that *Potato aucuba mosaic virus* was not present in commercial potato plot. Some viruses stated in published literatures to be present in North America may not present in Canadian potato lot at all.

Potato virus Y strains transmit mechanically from one plant to another

MANPHOOL FAGERIA, X. NIE, A. GALLAGHER, AND MATHURESH SINGH

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Emergence of different PVY strains including tobacco veinal necrosis (PVY^{N}), recombinant N:O ($PVY^{N:O}$), and potato tuber necrosis (PVY^{NTN}) are of increasing concern for potato crops across North America. There are several reports confirming aphid-mediated current season spread of PVY but information on mechanical transmission of PVY from plant to plant is inadequate. The goal of this study was to determine whether PVY strains transmit mechanically through tuber cutting and plant wounding in Shepody and Russet Norkotah. In the case of mechanical transmission of PVY through tuber cutting, after one infected tuber was cut with a knife, four uninfected tubers were cut consecutively with the same knife. The knife was not disinfected between these cuts. Wounding was induced mechanically in the plants maintained in the greenhouse using six methods described in brief below: Bouncing healthy plants against PVY infected ones; metal brushing of the leaflet of an infected plant followed by brushing on the leaflet of the healthy plant; hammering of the leaflet from an infected and a healthy plant together; carborundum rubbing between the leaflet of the healthy and the leaflet of the infected plant; squeezing of the twigs of the healthy and the infected plant together, and untreated control. These methods facilitated sap exchange between the healthy and infected plants. In particular, we intended to design these methods such that they mimic field operations to the greatest extent possible. Results demonstrated that there was no PVY transmission due to seed cutting but there was a high level of PVY transmission due to all the plant wounding methods except in the control. The second part of the investigation is in progress where we aim to investigate the pattern of PVY movement in the potato plant following mechanical inoculation.

Development of a PCR based detection system for five prominent viruses of strawberry.

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The cultivated strawberry (*Fragaria x ananassa*) is a valuable perennial crop, whose productive lifespan and berry yield is often limited by a number of viruses. We have developed a sensitive PCR detection procedure for five of these viruses: Strawberry Mild Yellow Edge (SMYEV), Mottle (SMoV), Vein Banding (SVBV), Crinkle (SCV), and Pallidosis Associated (SPaV) viruses. The five viruses can be transmitted by vegetative propagation of the plants, with varying persistence in different insect vectors, and are usually asymptomatic alone, only causing disease with combined infections, thus virus detection and epidemiology in strawberry is complex and difficult. Our procedure allows for screening large numbers of strawberry leaves harvested at any time in the season, growth stage or level of symptomology of the plant, and can also be used to detect viruses in wild strawberry plants, which can act as a natural virus reservoir. Processing typically takes two days, separated into viral nucleic acid extraction first, followed by a two-step cDNA synthesis/PCR protocol with proprietary primers targeting each virus. The full five-virus screen can be processed simultaneously as two multiplex PCRs for SMYEV, SMoV, SPaV and SVBV, SCV respectively, although simplex or other custom multiplex PCR protocols can also be performed. We routinely process composite samples of several plants mixed together to more cost-effectively screen for uncommon viruses across a large number of plants. This procedure has been commercialized by Agricultural Certification Services, Inc. to service private industry, governmental and research needs beginning in the 2014 growing season.

The changing epidemiology of tomato and potato late blight in Canada

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Late blight of tomato and potato, caused by *Phytophthora infestans*, has caused significant economic losses in Canada in recent years. National surveys conducted since 2010 have documented the introduction and spread of novel genotypes of the pathogen, including US-22, US-23 and US-24, which have been responsible for most documented cases of disease. Long-distance transmission of genotypes on infected tomato transplants or potato seed has occurred, in addition to the common local transport of sporangia via wind and rain. In isolated instances, evidence for the generation of novel strains via sexual recombination has also been obtained. These novel pathogen strains have shown differences in host preference as well as sensitivity to fungicides used to control late blight. For example, US-23, which now dominates most pathogen populations in Canada, is highly aggressive on tomato foliage and fruit and potato tubers, but less aggressive on potato foliage. The spectrum of sensitivity to metalaxyl-m found in populations of US-23 is shifting from sensitive to increased resistance to this chemical. Tomatoes, either as infected transplants in garden centres or in home gardens, have become significant sources of infective propagules that can initiate disease in adjacent potato crops. The changing dynamic of late blight in Canada has forced both tomato and potato industries to reconsider their disease management strategies.

Occurrence of potato tuber damaging viruses in Canada and evidence of genome reassortment and RNA recombination in *Potato mop-top virus*

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In recent years, incidences of potato tubers exhibiting external and/or internal necrosis have been observed in potatoes in Canada as well as many other countries. Several viruses including *Potato virus Y* tuber necrosis strain (PVY^{NTN}), *Potato mop-top virus* (PMTV) and *Tobacco rattle virus* (TRV) have been linked to the tuber necrosis. Various biological, serological and molecular approaches have been used to unveil the tuber necrosis-causing viral pathogens. The complete genome comprising three genomic RNAs of three Canadian and two Chinese isolates of potato mop-top virus were sequenced and analyzed. Two ORFs were found in RNA1 of 6.1 kb, encoding a readthrough RdRp. A CP-readthrough protein was encoded by RNA2 of 3.1 kb. Four ORFs that encoded the triple-gene-block protein (TGBp) and a cysteine-rich protein were found in RNA3 (2.9 kb) of the two Chinese isolates; whereas in the Canadian isolates, only three ORFs encoding TGBps were observed in RNA3. A single nucleotide mutation of A_{2462} to G_{2462} abolished the start codon "AUG" for the fourth putative ORF in RNA3 of the Canadian isolates. Based on phylogenetic and sequence similarity analysis at the complete RNA sequence level, each of RNA1, RNA2 and RNA3 could be divided into at least two groups; and interestingly, not all the RNAs in each of the analyzed isolates exhibited the same phylogenetic relationship with that of other isolates. In Canadian isolates Ch9/Ch10/Ch20, all genomic RNAs belonged to group A; and in Chinese isolate Yunnan, all of its RNA belonged to group B; and in Swedish isolate Sw, RNA1 and RNA2 belonged to group A while RNA3 belonged to group B. In Chinese isolate Guangdong, RNA1 and RNA2 belonged to group A, and RNA3 was a hybrid of A and B, possessing a recombinant event at ~nt 1782. Taken together, this research, for the first time, demonstrates that genome reassortment and RNA recombination had taken place during PMTV evolution and diversification process.

Appendix

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