Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

2019-2020

Activity title: Investigating the occurrence and distribution of potato tuber necrosis-inducing viruses in Canada and studies on varietal responses to the viruses for minimizing economic losses cause by the pathogens

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Activity Objectives (as per approved workplan):
FY 2019-2020
1. Unveiling the incidences/occurrences of necrotic viruses (mainly PMTV and PVYNTN) in potatoes in the participating provinces (mainly Manitoba and New Brunswick) in 2019;
2. Understanding the sensitivity of up to 6 potato cultivars to Alfalfa mosaic virus-induced internal necrosis and the sensitivity of up to 5 newly released potato clones/cultivars to PVYNTN-induced potato tuber necrotic diseases;
3. Initiation of the studies on the sensitivity to PMTV-induced necrosis in up to 12 potato cultivars – first year field trial of group one cultivars.

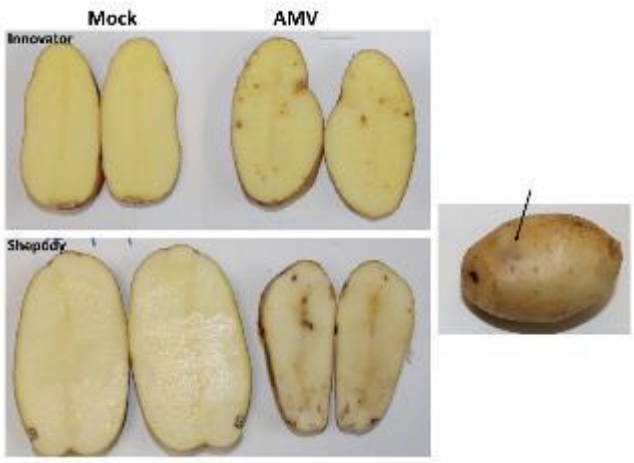
Research Progress to Date (use plain language):
1. Investigating the incidences/occurrences of necrotic viruses (mainly PMTV and PVYNTN) in potatoes in the participating provinces (mainly Manitoba and New Brunswick) in 2018;
We completed the analysis of 248 tubers received from Manitoba (248, via Vikram Bisht of Manitoba Agriculture) in March 2019 (2018's crop) for all four targeted viruses, namely potato mop-top virus (PMTV), tobacco rattle virus (TRV), alfalfa mosaic virus (AMV) and potato virus Y strain NTN (PVY^{NTN}) by PCR and ELISA. Among the samples, 6 tested positive for PMTV by both PCR and ELISA. Interesting, none of the positive tubers exhibited visible spraing symptoms (internal necrosis).
We yet to test the balk potato samples for the 2019 crop from Manitoba (379 random tubers, received in early March 2020) and New Brunswick (10 symptomatic tubers, also received in early March 2020) due to COVID-19 pandemic caused facility closure. Nevertheless, we tested 88 tubers that exhibited a certain degree of internal or external necrosis upon screening ~2,400 tubers (0 month storage) harvested from the field PMTV trial in NB (see below) by ELISA for PMTV. Out of the 88 tubers, 7 was confirmed to be PMTV-positive.

2. Understanding the sensitivity of up to 6 potato cultivars to Alfalfa mosaic virus (AMV)-induced internal necrosis and the sensitivity of up to 5 newly released potato clones/cultivars to PVYNTN-induced potato tuber necrotic diseases

2a. Greenhouse trial on the sensitivity of potato cultivars to alfalfa mosaic virus (AMV).

In the previous progress report (2018-2019), we reported the characterization of an isolate of AMV (designated as isolate CaM) that was initially deemed an "necrotic" strain (Nie et al. 2015. Plant Disease 99:1658-1658) similar to that reported in California back to 1950 (Oswald 1950. Phytopathology 40:973-991). Six potato cultivars (Innovator, Yukon Gold, Rochdale Gold-Dorée, Chieftain, Shepody and Jemseg) and one breeding clone (F87084) were analyzed for their responses to the virus infection. All tubers of CaM-infected Innovator and Shepody plants developed sporadic internal necrotic spots (see Figure below), as did approximately 23% and 8% tubers of CaM-infected Yukon Gold and Rochdale Gold-Dorée, respectively. Tubers from the remaining cultivars were necrosis-free. Interestingly, an AMV isolate (Ca175-1) that was deemed "non-necrotic" (Xu and Nie 2006. 96:1237-1242) also caused necrosis in tubers of Innovator and Shepody, suggesting that the internal necrosis in AMV-infected tubers depends on potato cultivar rather than on AMV strain/haplotype, and CaM is just a "regular" isolate of AMV. This portion of the study is now published in Plant Disease (Nie et al. 2019. Potato tuber necrosis induced by alfalfa mosaic virus depends on potato cultivar rather than on virus strain. Plant Disease 104:340-347 https://doi.org/10.1094/PDIS-04-19-0827-RE).

Figure 1. Example necrotic symptoms on tubers of plants infected with alfalfa mosaic virus (AMV) isolate CaM. Arrow indicates the external bruise-like symptom on a tuber (cv. Shepody) that exhibited internal necrosis. Mock, tubers of mock-inoculated plants; AMV, tubers of AMV isolate CaM-infected plants.



In addition, a set of 8 cultivars (Lamoka, Russet Burbank, Goldrush, Russet Norkotah, Atlantic, Kennebec, Snowden, Dark Red Norland), along with the AMV necrosis-sensitive cultivars Innovator and Shepody, are being tested for their sensitivities to AMV-induced internal necrosis at FRDC. The results yet to be analyzed.

2b. Greenhouse trial on the sensitivity of the new releases to PVYntn-induced tuber necrotic ringspot disease (PTNRD).

Eleven (11) advanced clones/new releases (F14002, F14021, VF14016, VF14017, VF14018, CV011010-2, F15062, 1-4, 12-4, 9-7, 12-7) from the AAFC breeding program, along with the PTNRD-susceptible cv. Yukon Gold, are being tested for their sensitivity to PVYntn-induced tuber necrosis. Virus-free tubers were planted in the greenhouse at the AAFC's Fredericton Research and Development Centre (FRDC) in July 2019. Five (5) plants each of the clones were mechanically inoculated with PVYntn whist 5 plants each of the un-inoculated were set as healthy controls. The plants were lab-tested by ELISA for PVY at 2 weeks-after inoculation; and the uninfected plants were re-inoculated with the virus by graft-inoculation. All but the clone 12-7 were successfully infected with the virus. The tubers were harvested in mid-October, and a visual observation of the tubers for PTNRD was performed immediately after the harvesting. At harvest, only tubers from Yukon Gold developed PTNRD. However, after 3-month-storage at 4°C, tubers of F15062 developed PTNRD as well (Fig. 2). Analysis of the pedigree of the clones indicates that one of the parents of F15062 is "AC Chaleur", a popular cultivar that is susceptible to PTNRD (Nie and Singh 2003). Secondary infection is on hold due to the COVID caused facility closure.

Fig. 2. Assessment of AAFC new releases/advanced clones to PVYntn induced potato tuber necrotic ringspot disease (PTNRD). OF the 11 clones tested, only F15062 exhibited susceptibility to PNTRD along with the Yukon Gold after 3 months of storage.

3. Initiation of the studies on the sensitivity to PMTV-induced necrosis in up to 12 potato cultivars – first year field trial of group one cultivars.

In the previous report (2018-2019 annual report), we reported the progress in the development of a PCR based assay termed "high resolution DNA melting (HRM) analysis" for simultaneous detection of potato mop-top virus (PMTV) and its fungal vector *Spongospora subterranea* (Sss, the potato powdery scab disease causal pathogen) from soil directly. For this fiscal year (2019-2020), we further fine-tuned the method, and moreover, we used this method to verify the infestation of PMVT and Sss in a potato filed that was suspected to be infested with the pathogens (Fig. 3). The HRM assay, which can be achieved in 2-3 days after a soil sample is collected, produced similar results to the conventional baiting-plant assay, a method that takes at least 2 weeks after the baiting plants being transplanted to the soil (Davey 2009. Methods in Molecular Biology volume 508 - Plant Pathology: 259-265. Also Fig. 4). The method has excellent potential to be used for large scale survey of fields for PMTV and Sss infestation.

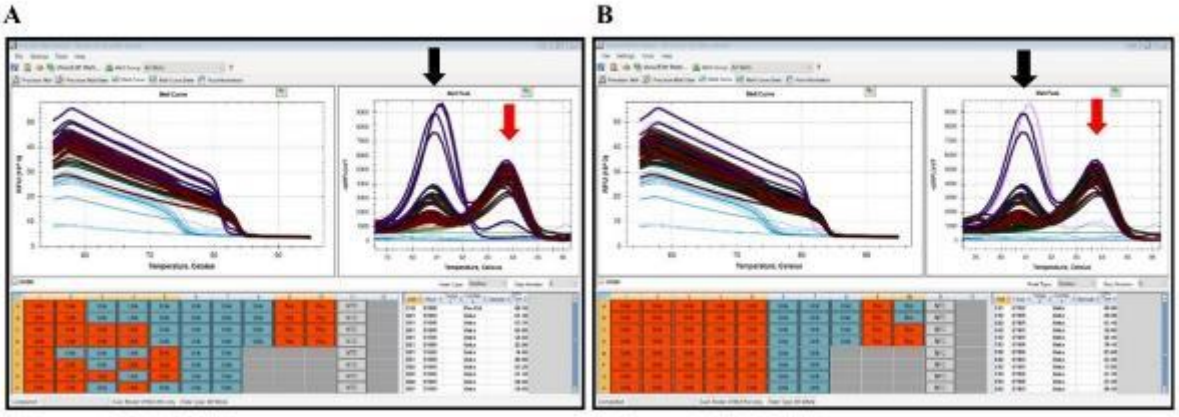


Figure 3. Application of duplex high resolution DNA melting (HRM) assay for detection of potato mop-top virus (PMTV) and *Spongospora Subterranea* (Sss) in soil. **A**. PMTV. Samples detected positive for PMTV is highlighted in red in the 96-well plate layout. **B**. Sss. Samples detected positive for Sss are highlighted in red in the 96-well plate layout. Columns 1-3, soil samples from Field A; columns 4-5: samples from Field B; columns 6-8, samples from Field C; column 9, Sss and PMTV positive soil controls; Column 10, PMTV-positive plant material controls (10A, PMTV-infected tobacco leaf; 10D, PMTV-tobacco infected root; 10B and C, PMTV-positive potato tuber).

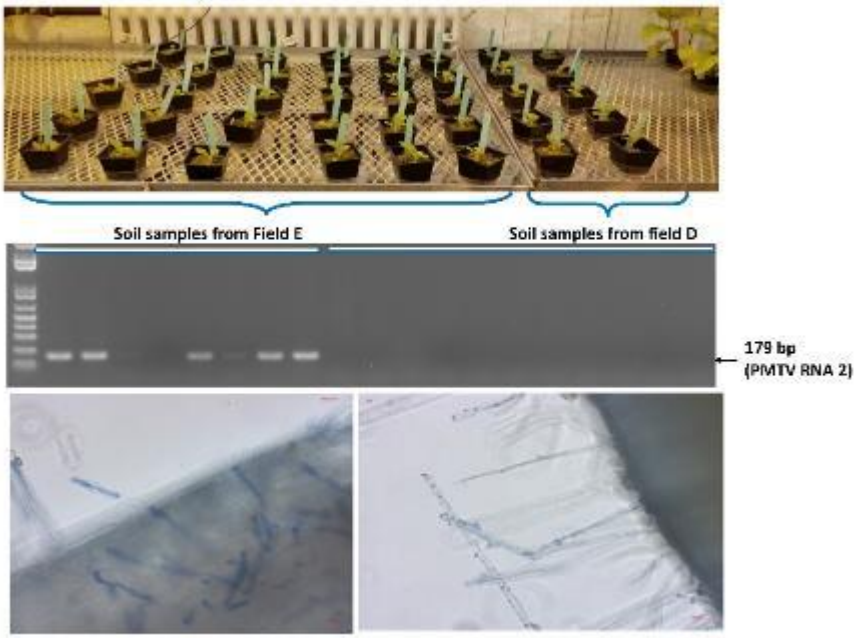


Figure 4. Bait tobacco bioassay for confirmation of potato mop-top virus (PMTV) and *Spongospora subterranea* (Sss) infestation in soil detected by high resolution DNA melting (HRM) assay. Top panel. PMTV baiting. Seedlings of common tobacco cv. Samsun at ~2 leaf stage were transplanted into flat tray cells containing the soil samples to be assayed. The plants were tested at three weeks post-transplanting for PMTV by conventional RT-PCR (**Middle panel**) and ELISA and for Sss by root hair staining (**Bottom panel**). Left side of all panels, tobacco plants were grown in soil samples from Field E; right of all panels, tobacco plants were grown in soil samples from Field D.

A field trial on the sensitivity of potato cultivars to PMTV-induced necrosis is on-going. The trial was performed in a potato field in New Brunswick. The field was chosen partially because in which a high incidence of PMTV-induced spraing disease was observed in potatoes produced there. When soil samples collected from the field were subjected to the newly developed HRM assay, PMTV- and Sss-positive results was obtained. Eleven (11) common potato cultivars (Russet Burbank, Shepody, Goldrush, Russet Norkotah, Atlantic, Lamoka, Snowden, Chieftain, Yukon Gold, Kennebec, Dark Red Norland) and four (4) AAFC new releases (F13049, F13015, F13007, and F13014) were included in the trial. The trial was performed in a randomized complete block design with four replications. Each replication consisted of 15 seed tubers each planted 0.3 m apart with potato spacers. The tubers were planted in the beginning of June 2019; and the resulting plants were managed as other plants in the adjacent fields. Potatoes were harvested in early October 2019. Potatoes from each treatment were divided into 4 groups evenly (ca. 2400 tubers in total each group), two (0 and 3 month-storage) were examined for visible external and internal necrosis whereas three are being stored at 4-7^{o}C for different time periods (i.e., 6 and 9 months). At 0 month, 91 exhibited a varying degree of internal necrosis. Of the 91 tubers, 7 and 20 tested positive for PMTV by ELISA and RT-PCR, respectively. At 3 month-month-postharvest, 148 tubers exhibited internal necrotic symptoms, and among them, 27 and 50 tubers tested PMTV-positive by ELISA and RT-PCR, respectively. This preliminary result indicates that as storage time increases, the number of ELISA positive and RT-PCR positive tubers increase, consistent with general observation by the industry.

We also randomly selected 5 tubers each of the 4 replicates of all cultivars, and tested them for PMTV by ELISA and RT-PCR at 0 and 3 months after harvesting. At 0 month, 9% of the tubers tested positive by ELISA and 13% positive by RT-PCR. Unlike that in the symptomatic tubers, the number of ELISA and RT-PCR positive tubers remained largely at the same level (11% by ELISA and 12% by RT-PCR) after 3 months' storage. Nevertheless, the number of tubers exhibiting spraing-like disease (internal necrosis) increased from 1.7% at 0-month to 4.3% at 3-month. The sensitivity of different cultivars appeared to be varied, but more solid conclusion can only be reached after all the time course study is completed.

Due to the COVID-19 pandemic caused facility closure, the 6 month storage was unable to be properly completed as scheduled. The 9-month storage is also pending.

Extension Activities (presentations to growers, articles, poster presentations, etc.):

One (1) peer reviewed article: Nie X, Dickison V, Singh M, De Koeyer D, Xu H, Bai Y, and Hawkins G. 2019. Potato tuber necrosis induced by alfalfa mosaic virus depends on potato cultivar rather than on virus strain. ***Plant Disease*** DOI: 10.1094/PDIS-04-19-0827-RE.

One (1) poster presentation in a scientific conference: Nie X, Dickison V, Singh M, De Koeyer D, Xu H, Bai Y, and Hawkins G. 2019. Potato tuber necrosis induced by alfalfa mosaic virus depends on potato cultivars rather than on virus strains. Plant Health 2019 – American Phytopathological Society Annual Meeting, Cleveland, OH, 3-7 August 2019. Presentation No. 401-P2.

Six (6) viral genome sequences in GenBank/National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/)

- **Nie X**, and Dickison V. 2019. Alfalfa mosaic virus isolate Ca175-1 RNA 3, complete sequence. Accession number: MK607978
- **Nie X**, and Dickison V. 2019. Alfalfa mosaic virus isolate CaM RNA 3, complete sequence. Accession number: MK607977
- **Nie X**, and Dickison V. 2019. Alfalfa mosaic virus isolate Ca175-1 RNA 2, complete sequence. Accession number: MK607976
- **Nie X**, and Dickison V. 2019. Alfalfa mosaic virus isolate CaM RNA 2, complete sequence. Accession number: MK607975
- **Nie X**, and Dickison V. 2019. Alfalfa mosaic virus isolate Ca175-1 RNA 1, complete sequence. Accession number: MK607974
- **Nie X**, and Dickison V. 2019. Alfalfa mosaic virus isolate CaM RNA 1, complete sequence. Accession number: MK607973

Early Outcomes (if any) or Challenges:

We developed a PCR-based method termed high-resolution DNA melting (HRM) analysis for simultaneous detection of potato mop-top virus (PMTV) and its fugal vector *Spongospora subterranea* (Sss, the powdery scab-causing pathogen) from soil samples directly. The method is reliable and efficient, and has the potential to be used for large scale survey of fields for PMTV and Sss infestation.

Key Message(s):

All progresses well.

This project is generously funded through the Canadian Agri-Science Cluster for Horticulture 3, in cooperation with Agriculture and Agri-Food Canada's AgriScience Program, a Canadian Agricultural Partnership initiative, the Canadian Horticultural Council, and industry contributors.

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