Canadian Agri-Science Cluster for Horticulture 3

Update to Industry

Semi-Annual – Spring 2021

Activity title: CanPEDNet – The Canadian Potato Early Dying Network

Name of Lead Researcher: Mario Tenuta

Names of Collaborators and Institutions:

Non-AAFC: Khalil Al-Mughrabi, Department of Agriculture, Aquaculture and Fisheries; Ryan Barrett, Prince Edward Island Potato Board; Sebastian Ibarra, PEI Department of Agriculture and Fisheries; Katrina Jordan, University of Guelph; Mathuresh Singh, Agricultural Certification Services; Dmytro Yevtushenko, University of Lethbridge; Herve van der Heyden, Phytodata.

AAFC: Dahu Chen (lead for AAFC team), Fredericton Research and Development Centre; Tanya Arseneault, Saint-Jean-sur Richelieu Research and Development Centre; Louis-Pierre Comeau, Fredericton Research and Development Centre; Claudia Goyer, Fredericton Research and Development Centre; Benjamin Mimee, Saint-Jean-sur Richelieu Research and Development Centre; Oscar Molina, Morden Research and Development Centre; Judith Nyiraneza, Charlottetown Research and Development Centre; Cameron Wagg, Fredericton Research and Development Centre.

Activity Objectives (as per approved workplan):

Sub-activity 1 (SA1): Verticillium and root lesion nematode survey and relation to PED disease and yield
Sub-activity 2 (SA2): characterization of isolates of V. dahliae and V. albo-atrum
Sub-activity 3 (SA3): Root lesion nematode species and interaction with V. dahliae isolates
Sub-activity 4 (SA4): Other soil-borne pathogens associated with the PED complex
Sub-activity 5 (SA5): Validation of real time PCR protocol for Verticillium quantification in soils across Canada
Sub-activity 6 (SA6): Reducing analysis variability through development of high-throughput system for large soil samples
Sub-activity 7 (SA7): PED control through disease control products
Sub-activity 8 (SA8): PED control through improved cropping systems
Sub-activity 9 (SA9): Soil building and health: Preventing reliance on sustained chemical fumigation
Sub-activity 10 (SA10): Field scale evaluation and demonstration of practices to manage PED
Sub-activity 11 (SA11): Soil health property analysis
Sub-activity 12 (SA12): Soil microbial and micro-arthropod communities

Research Progress to Date (use plain language, not to exceed 500 words):

Sub-activity 1

Composite soil samples each from at least 20 commercial fields in Alberta, Quebec, New Brunswick and PEI were collected and analyzed for *Verticillium* and Nematodes species and densities in fall 2019. Samples were taken in spring 2020 from commercial fields in Manitoba and Ontario. Eight fields per province were revisited for disease ratings, yield and soil sampling.

From the fields surveyed, eight fields in each province representing different pathogen population density levels were selected to evaluate the effect of pathogen density on potato PED severity and yield in 2020. Soil samples from five plots were taken in each field to quantify the pathogen density prior to potato planting. Data on pathogen density,

%wilt, soil property and yield were collected. Due to the COVID-19 restriction, only one disease rating was done before harvesting.
In fall 2020, soil samples from 30 commercial fields in Alberta, Quebec, New Brunswick and PEI were collected for Verticillium and Nematode analyses. Field were sampled in early summer in Manitoba. The samples were also provided to Tanya and Benjamin (Saint-jean-sur-Richelieu) for Verticillium and nematode characterization and to Loui-Pierre Comeau for soil property characterization.

Sub-activity 2
A Master's student hired for this project has begun working in Fall 2020. Restrictions to laboratory and greenhouse access for over 9 months in total have severly limited our ability to progress. We are working very hard to catch up. If no other majors delays occur, we are on track to complete our milestone by 2023 as stated. Soil samples were obtained from NB (30), PEI (31), QC (36), ON (32), AB (31) and MB (8) in Fall 2020 and are currently being conserved at 4 degrees until we are able to process them. Soil samples obtained in Fall 2019 and Spring 2020 from NB, PEI, QC, AB ON and MB were partially processed. QC and ON were done and PEI and NB are currently being used for baiting experiments to isolate strains of *Verticillium* (aiming for *dahliae* and *albo-atrum*). Currently, we have isolated 18 strains of *Verticillium* from ON and 7 strains from QC with morphological characteristics compatible with *V. dahliae*. Single spore cultures are currently being done to obtain a pure isolate, and we are ready for the subsequent DNA extractions. We will wait to obtain all Canadian strains from 2019 before sending to sequencing and proceed with 2020 soil samples after that.

Sub-activity 3
Soil samples were obtained from NB, PEI, QC, ON and AB in Fall 2020 and are currently being processed.
Soil samples obtained in Fall 2019 and Spring 2020 from NB, PEI, QC, AB ON and MB were all processed. Currently, we have isolated 306, 574, 203, 447, 5 and 1 larvae (NB, PEI, QC, AB, ON and MB respectively) with morphological characteristics compatible with the *Pratylenchus* genus. They have been handpicked and transferred into individual wells on ELISA plate for DNA extraction. In vitro cultures have also been initiated for 4 provinces. A workflow was established to amplify different genes from thousands of isolates (starting with the COI mt gene) and to sequence them by Sanger seq in collaboration with Genome Quebec. The first steps to sequence and assemble a reference genome for *P. penetrans* using single-nematode sequencing were also carried out (extraction from a single female and genome amplification using Repli-g). This material will be sequenced using short- (Miseq) and long-reads (Oxford nanopore) in the coming weeks.

Sub-activity 4
A subsample of soils from Sub-activity 1 were transferred to Phytodata and analyzed by real-time PCR for various plant pathogens other than *Verticillium* and root lesion nematode. They included *Collectotrichum coccodes* (black dot of potato), *Spongospora subterranean* (powdery scab), *Helminthosporium solani* (silver scurf), *Rhizoctonia solani*, *Phytophthora erythroseptica* (pink rot), *Fusarium solani* (Fusarium dry rot), and *P. ultimum* (Pythium leak of potato). For the Quebec samples, all the pathogens except *H. solani* and *P. erythroseptica* were present in at least one of the soil samples.
Four commercial DNA extraction kits were examined for quantity and quality of DNA extracted from sand, organic and mineral soils from Quebec. The kits were also compared for quantification of *V. dahliae*, *S. subteranea* and *P. ultimum* diluted and undiluted and including an internal standard.
Soils sampled in in sub-activity 1 were analyzed for *V. dahliae, S. subteranea, F. solani, P. ultimum, R. solani AG3, and C. coccodes*.

Sub-activity 5
Several commercial DNA extraction kits were compared using potato soils from NB, PEI and Manitoba by ACS. The E.Z.N.A Soil DNA extraction kit from Omega Bio-Tek provided the best recovery of quality DNA. Soil samples from Sub-activity 1 were then extracted and quantified for Verticillium species (*V. dahliae, V. albo-atrum, V. tricorpus, and V. nonalfalfae*) based on real-time PCR. *Verticillium* DNA concentrations were low and varied with sample. Using the standard dilution plating method for colony counts for microsclerotial producing *Verticillium*, 44% of samples produced colony counts. However, the PCR method found that 86% of the samples had quantifiable *Verticillium* levels. The relationship between colonies and DNA levels in soil samples was not strong. The PCR matched well with colonies for only 1 of 4 provinces, this is likely because of the presence of microsclerotia forming *Verticillium* species other than *V.*

dahliae in those provinces. There was a better relation between DNA levels and soil amended with microsclerotia of *V. dahliae*. DNA levels of soils from Sub-activity 1 matched well with quantification by Phytodata.

Sub-activity 6

This activity was not planned to begin in 2020-2021.

Sub-activity 7

NB. Late funding allocation and wet soil conditions in 2019 preventing conducting a field trial in NB by AAFC. A replicated greenhouse trial was conducted in NB. The treatments included a control, the fungicide Aprovia (Syngenta), the nematicide and fungicide Velum Prime (Bayer), and the fungicide Senator PSPT (Nippon Soda Co.). Aprovia and Velum Prime were applied in-furrow at the recommended rate while Senator PSPT was applied as a dust on the surface of the potato seed. The cultivar Russet Burbank was grown. Soil samples (one soil sample/replicate) were collected at the beginning and at the end of the growth cycle. Verticillium wilt severity was also scored. Analysis of results continues.
ON. Late funding allocation meant a field study in ON by the University of Guelph was not possible. Studies for May 2020 were done but there was bad emergence, and the trial didn't yield good data. It will be repeated in 2021.
MB. Exceptionally late harvest and wet soil conditions prevented conducting a field study in MB. Two trials were carried out in MB in 2020 successfully by the University of Manitoba. The trials were to be done by AAFC according to the workplan but approval for field work wasn't available, so the University of Manitoba stepped in to ensure this activity progressed. Treatments were Velum Prime, Velum/Serenade, Velum/Aprovia/Qadris, Velum/Aprovia/Quadris/Serenade and Aprovia/Quadris. Verticillium and yield determinations were conducted.
Two field trials were set up in fall 2020 in MB by AAFC. The experiments having 12 different treatments including soil fumigant, fungicides and nematicides were established in heavily *Verticillium* spp infested and poorly producing potato fields, in Manitoba. Soil fumigation treatments (6) were applied in September 2020. Fungicide and nematicide treatments were applied at planting in 2020. Analysis of soil samples initially collected from experiments is a work in-progress.

Sub-activity 8

NB. Two-year rotation study: First cycle of crop rotation– potato was completed in 2018 and 2019 at the FRDC research Farm. A second two-year rotation study was initiated in 2020 to confirm the results obtained in the first cycle. Twelve rotation crop treatments were planted in June with a standard field management. Soil samples were taken prior to planting and in the fall after cropping for quantification of root lesion nematodes and *Verticillium dahliae.*
The second year of the rotation of 24 different 5-year cropping systems were planted and weed and crop biomass were collected. Potato yield data was also collected. One plot was not sown properly and this has been noted for future data analyses.
Green Manure Study- a field study was initiated at the FRDC research farm in 2019. Fields were planted Pearl Millet, Brown Mustard and a mix of the two to compare to current land management and soil building practices (typical red clover-barley rotations and grain rotation crops). Biomass and yield data have been collected in plots where appropriate in 2019. The same green manures or crops were grown on plots in 2020. Potato will be planted in 2021. The biomass samples of the weeds and sown crops were taken during the growth. In the fall, soil samples were taken for pathogen density quantification and the rotation crops were remained in the plots to prevent from soil erosion.
PEI. Rotation Study- an experiment was initiated in 2017 at the Harrington Research Farm. Eight rotation systems and a control (bare soil) with and without manure were treatments in 2017 and potato in 2018 the test crop. In 2019 a second phase of a 3yr rotation was initiated and a potato test crop planned in 2021. Cover crop biomass and their total nitrogen and total carbon accumulations, soil nitrate dynamics during the potato phase were monitored monthly, selected physical-chemical soil properties and potato yield and quality were assessed. In 2020, the plots were in cover crop phase. Soil samples were also taken for *V. dahliae* and root lesion nematode assessments.

Sub-activity 9

ON. Late funding allocation did not allow establishment of a study. Establishment of the trial was done by the University of Guelph in 2020 but emergence was poor. The trial will be re-initiated in 2021.
MB. An exceptionally late harvest and wet soil conditions did not allow establishment of this study in 2019. The study was initiated in fall 2020 by the University of Manitoba with fumigation of treatment plots. The research site is on an irrigated commercial processing potato field near Shilo, MB. The experiment is slated to operate fall 2020 to fall 2023. There is intention to find resources to continue the study as soil building and health effects take many years to see.

There are 6 treatments to the study. A Potato-Corn-Spring Wheat rotation is the control and then varying combinations of those crops, biofumigation crops, compost, cover crops and fumigation investigated. The next common potato year will be 2024.

Sub-activity 10

Cavendish and McCain have conducted demonstration trials in PEI, NB, MB and AB since 2018 on various control products of Potato Early Dying.

Sub-activity 11

The soil samples from several sites/provinces had been delivered to the soil carbon on yearly basis as planned for soil property analysis. However, soil samples from the other provinces/sites in the project were incomplete or have yet arrived due to COVID restrictions.

The soil samples delivered to the soil carbon for the 2019 growing season were almost completely analysed. However, since March 2020, the processing of the soil samples from this project at the FRDC was not approved by local management due to COVID restrictions. Some samples were shipped and analysed in private laboratores for standard analyses and other specific analyses (e.g. active carbon POX) are on hold. The completion of the planned analyses will depend on when these samples are approved to be processed at the FRDC. The FRDC soil carbon lab as currently a backlog analyses to more than a 1000 samples representing more than a year of full time work.

Sub-activity 12

Samples from Sub-activity 8 have been frozen at -80 C. Analyses are scheduled to occur in 2021-2022 after potato test crops are completed in those studies.

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

Dahu Chen, August 11, 2020. Progress on Management of Potato Early Dying Complex in NB and PEI. Virtual Presentation to PEI Potato Board.

Dahu Chen, Kamrun Nahar, Cameron Wagg, Louis-Pierre Comeau, Claudia Goyer, Judith Nyiraneza, Mathuresh Singh, Helen Tai, Bernie Zebarth (2020). Effect of rotation crops on potato early dying complex and potato yield. Poster presentation at the American Plant Heath 2020 (Virtual), August 8-12, 2020.

Mario Tenuta, 2020. CanPEDNet Update. Manitoba Potato Research Meeting. Portage La Prairie, MB and by Zoom. Nov 17, 2020.

Dahu Chen, Bernie Zebarth, Louis-Pierre Comeau, Tom Dixon, Claudia Goyer, Kamrun Nahar, Ryan Barrett, and Sebastian Ibarra (2020). Population Density of Potato Early Dying Pathogens in PEI and New Brunswick and Effect of Biofumigation and Fumigation on PED Management. Oral presentation to the 2020 Atlantic CPS Annual Meeting (Virtual), December 10, 2020.

Dahu Chen, Bernie Zebarth, Louis-Pierre Comeau, Kamrun Nahar, Ryan Barrett, and Sebastian Ibarra (2021). Population Density of Potato Early Dying Pathogens and Their Potential Effect on Potato Yield in Prince Edward Island and New Brunswick. Oral presentation at the Northeast Potato Technology Forum (Virtual), March 23-24, 2021.

Dahu Chen, Louis-Pierre Comeau, Tom Dixon, Claudia Goyer, Kamrun Nahar, Mohammad Islam, Bernie Zebarth (2021). Effect of Biofumigation and Fumigation on Population Density of Root-Lesion Nematodes, *Verticillium dahliae* and Potato Yield in New Brunswick. Oral presentation at the North East Potato Technology forum (Virtual), March 23-24, 2021.

COVID-19 Related Challenges:

Activities have been affected by COVID restrictions. In laboratory activities within AAFC were limited to only essential activities. This meant experiments and analysis of samples could not be done. Our private partners (ACS and Phytodata) were not affected by restrictions and maintained on schedule. Our University researchers were challenged with restrictions on the number of personnel working and closure of laboratories over the summer. Field activities were maintained. However, approval for field work under COVID protocols came late for the University of Guelph resulting in poor trial establishments. Restrictions are still in effect for AAFC and university laboratories. It is hoped that these will be lifted come fall 2021 to allow for processing of samples and laboratory experiments.

Key Message(s):

We have successfully continued the CanPEDNet project despite severe challenges presented by COVID restrictions. We have had several group meetings and many email and phone discussions among researchers. The project is moving along. Trials except for in Ontario have been established. COVID-19 restrictions have stopped particularly laboratory analyses and experiments. The soil survey and development of molecular diagnostic methods is nearing completion. Several control products examined show good results in improving yield and reducing PED. Good progress has been made in molecular diagnostic and quantification methodology with commercial options soon to be available to growers and industry.

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