University of Lethbridge
Faculty of Arts & Science

Potato Research at the University of Lethbridge

Period covered: January - December 2020

January 28, 2021

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I. Current Research Activities

Full-time lab members:

1. Dmytro Yevtushenko, PhD, Principal Investigator;
2. Maria Munawar, Ph.D., Postdoctoral Fellow (May 2020 –);
3. Mariana Vetrici, Ph.D., Research Associate (January 2020 –);
4. Colby Robertson, M.Sc. candidate (defended thesis in March 2020);
5. Christie Stephen, M.Sc. candidate (May 2020 –);
6. Nick Schimpf, M.Sc. candidate (September 2020 –);
7. Atta Ur Rahman, M.Sc. candidate (September 2020 –);

Part-time lab members (U of L undergraduate students):

1. Andrea Gelene Abenoja, 4th-year undergraduate student (Jan.–Sept. 2020);
2. Brendon DeGroot, 3rd-year undergraduate student (May–September 2020);
3. Dylan Jones, 4th-year undergraduate student (May–September 2020);
4. Becky Davidson, 3rd-year undergraduate student (May–September 2020);
5. Gobind Sundhu, 3rd-year undergraduate student (January–April 2020);
6. Karan Sundhu, 3rd-year undergraduate student (January–April 2020);
7. Madison Slomp, 3rd-year undergraduate student (May–September 2020);
8. Melissa Telfer, 4th-year undergraduate student (September – December 2020).

Co-supervised graduate students (the Lethbridge Research and Developmental Centre):

1. Salina Kaphle, M.Sc. candidate (defended thesis in August 2020);
2. Max Erickson, M.Sc. candidate (September 2019 –).

The impact of COVID-19 on my research activities. Although I tried to minimize the impact of nationwide lockdown on my research projects, I must admit that the COVID-19 pandemic affected my capacity to conduct my regular research and training activities. For example, my postdoctoral

fellow Dr. Maria Munawar had to join my lab in January 2020. Because she was in China when the pandemic started, she was able to arrive in Canada only in the end of March, on the day when Canada closed its borders. Furthermore, she had to be on self-isolation for 14 days upon arrival in Lethbridge, and even small actions, like applying for social insurance number or opening a bank account were impossible for several weeks. The start of M.Sc. study by Atta Ur Rahman was deterred from May to September 2020, and he was able to arrive in Canada only in December 2020. Another M.Sc. student, Christie Stephen, started in May 2020, but had to work from home. In May and June 2020, the U of L granted permission to several lab members, including me, to continue critical research in our lab on campus. Yet, even simple task of delivering a vital piece of research equipment (e.g., Zeiss Axicam 208 camera) took 2 months instead of 10 days. Also, travel restrictions, which started in March 2020, cancelled all my professional meetings and visits.

Increased teaching load. Starting from 2021, my teaching load will increase from 2 courses per year to full load of 4 courses per year, including new large-size classes. This change will greatly affect my time dedicated to research.

Project 1

Title: Canadian network for control of potato early dying complex (CanPEDNet).
Date: April 2019 – March 2024
Team: Dr. Dmytro Yevtushenko, Co-Investigator.
Brief details: The U of L potato lab represents the Province of Alberta in this network, which involves over 30 scientists across Canada. The overall objective is to reduce the severity of potato early dying (PED) and increase potato productivity and profitability in Canada. On a nationwide scale, the project aims to: (i) improve our understanding of the causal agents of PED; (ii) develop commercial soil tests for *Verticillium* species; (iii) improve management for control of PED; (iv) improve soil health to enhance resilience to PED, and (v) disseminate the results to potato growers and the industry. At the provincial scale, our lab works on understanding the causal agents of PED: distribution and levels of *Verticillium* species and root lesion nematodes in commercial potato fields of Alberta, relation of PED disease severity to yield loss, interaction of the root lesion nematode species with *V. dahliae*, and the role of other soil-borne pathogens in the PED disease complex.
Results. Eight fields (four with the highest and four with the lowest pathogen/pest populations, based on the results from the fall 2019 soil samplings in 32 fields) were sampled in spring 2020 prior to a potato crop to quantify pathogen/pest levels at the beginning of the growing season. Prior to harvest, each location was assessed for disease severity based on visual symptom development. At harvest, each area was hand-sampled for tuber yield, and tubers are graded according to processing contract specifications. An important outcome of this research is to compare fall and spring pest and pathogen populations to disease and yield so as to provide recommendation to potato growers on sample timing.
Thirty-one fields, planned for planting Russet Burbank in spring 2021, were sampled in the fall 2020. The soil samples were sent to the project-designated labs for *Verticillium* and nematodes analyses: the Agricultural Certification Services Inc. in Fredericton (NB), the PEI Potato Quality Institute in Charlottetown (PEI), and the Saint-Jean-sur-Richelieu Research and Development Centre, Agriculture and Agri-Food Canada (QC).
Next steps: The continuation of soil samplings for the *Verticillium* and root nematode surveys in spring 2021, visual assessments of potato plants for disease severity in summer 2021, hand-harvesting of the selected areas for tuber yield and grading, soil

sampling from the next round of a minimum of 30 fields in fall 2021, and other project-associated activities according to the CanPEDNet workplan.

Project 2

Title: Management strategies of potato early dying (PED) complex in Southern Alberta.
Date: May 2018 – April 2020.
Team: Colby Robertson, M.Sc. student;
Dr. Dmytro Yevtushenko, Principal Investigator;
Dr. Michael Harding, Co-supervisor (Alberta Agriculture and Forestry).
Brief details: This project was designed as a part of a larger CRD project on pathogens, population levels and control strategies for Verticillium wilt disease development in potato (the CRD in Potato Pathology). It focused on: (i) identification of the causal agents of Verticillium wilt (PED) in potato fields of Alberta; (ii) evaluation of new commercial products – Elatus, Velum Prime and Pic Plus – to control PED under local environmental conditions; and (iii) the impact of the fumigant on the soil microbiome and potato yields. The study was conducted in commercial potato fields (two fields per season) selected with help from local agronomists and participating field owners.
Results and dissemination of knowledge: All experiments were completed by the end of 2019. Since then, time was entirely devoted to writing and revising the M.Sc. thesis, and dissemination of the results.

- Colby successfully defended his M.Sc. thesis at the University of Lethbridge on March 31, 2020.
- Published research article titled “Impact of fumigation on soil microbial communities under potato cultivation in Southern Alberta” in *American Journal of Potato Research* (published online on January 22, 2020).
- Presented his research results at the Biology Graduate Symposium at the University of Lethbridge (January 31, 2020).
- Submitted research article titled “A survey of *Helicotylenchus*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus* in potato fields in Alberta, 2018 and 2019” to the Vegetable Section of the 2021 Plant Disease Survey of *The Canadian Journal of Plant Pathology*.

After the thesis defense, Colby has been employed with Cavendish Farms in Lethbridge, AB. Colby stays in touch with our lab: we conduct in-lab tests for Cavendish Farms on after-cooking darkening of potato and help the company with molecular diagnostics of potato diseases.
Practical applications: The knowledge of the relationship between pathogen population levels (*Verticillium* and *Pratylenchus* species) and crop yield will improve disease management decisions. Evaluation of the efficacy of new commercial products to control PED under local environment, and the impact of fumigation on soil microbiome and potato yields will help the industry to mitigate PED severity and improve potato production in Alberta.
Next steps: The PED research will be continued by new M.Sc. student (see Project 3).

Project 3

Title: Population biology and control strategies for potato early dying disease in Alberta.

Date: September 2020 – August 2022.
Team: Atta Ur Rahman, M.Sc. student;
Dr. Dmytro Yevtushenko, Principal Investigator;
Dr. Michael Harding, Co-supervisor (Alberta Agriculture and Forestry);
Dr. Maria Munawar, postdoctoral fellow.
Brief details: This project is a continuation of our lab research on PED, started in Projects 1 and 2. This project was submitted as the Results Driven Agriculture Research (RDAR) grant application in October 2020 (a copy of our RDAR grant application is attached to this document). *We hope for the positive outcome on our RDAR grant submission.* The major goal is to accelerate the research on PED in Alberta to determine region-specific thresholds for the disease development under local environmental conditions. The major difference of this study from Project 1 (CanPEDNet) is that we will focus on identification of PED causative agents at the molecular, rather than on morphological level. This will allow us to identify all pathogens, involved in PED, at the species level rather than on the genus level (in contrast to Project 1). Other objectives include: (i) Characterization of *Verticillium* isolates for aggressiveness on the cultivar Russet Burbank, which dominates processing potato production in Alberta; and (ii) Analysis of relationships between the pathogen population levels in soils, severity of disease symptoms, and potato yields to determine Alberta's potato-specific thresholds for disease development.
Anticipated results: The study will establish region-specific thresholds for PED disease development in Alberta. Other novelties include the development of simple and reliable PCR-based molecular diagnostic protocol(s) for rapid screening, identification, and quantification of Verticillium species and plant-parasitic nematodes, and establishment of correlation between initial inoculum of the pathogens in soils and PED symptoms.
Next steps: Because of pandemic-related travel restrictions, the M.Sc. student (Atta Ur Rahman) arrived in Canada in December 2020, although his official start date was September 2020. Currently, he is working on the PED literature and experimental designs. We are in process of assembling a M.Sc. committee for this study, and consulting with other plant pathologists (e.g., Dr. Mario Tenuta) on the most efficient ways to achieve the objectives.

Project 4

Title: The Potato Pest Monitoring Program
Date: May – September 2020
Team: Dr. Dmytro Yevtushenko, the University's Technical Representative;
Andrea Gelene Abenoja, 4th-year undergraduate student;
Brendon DeGroot, 3rd-year undergraduate student;
Dylan Jones, 4th-year undergraduate student;
Becky Davidson, 3rd-year undergraduate student;
Brief details: The U of L potato lab performs diagnostics of potato pathogens and pests for Promax Agronomy Services Ltd. and the Potato Growers of Alberta to provide early warning of major epidemics. The program focuses on improvement of disease management decisions through monitoring the occurrence of major potato pathogens – the late blight pathogen *Phytophthora infestans*, the early blight pathogen *Alternaria solani*, *Fusarium* species, and the zebra chip pathogen *Candidatus* Liberbacter

solanacearum – in potato fields of Alberta. The program runs each year from May to the first week of September, and employs four U of L undergraduate students. Pathogen diagnostics includes identification and counting fungal spores collected from active spore traps in commercial potato fields, using both traditional and molecular methods. Pest diagnostics involves identification and quantification of potato psyllids *(Bactericera cockerelli*), as well as other pests (green peach aphids) and pest predators, caught on sticky traps at field edges. The potato psyllids were further analyzed for the presence of the zebra chip pathogen *Candidatus Liberbacter solanacearum,* using polymerase chain reaction (PCR) and pathogen-specific DNA primers. The results were reported to Promax company to assess the risks for disease developments.

Results: The numbers of *Phytophthora, Fusarium* and *Alternaria* spores were reported daily (Monday through Friday) to Promax company. The number of potato psyllids, as well as the presence and number of non-potato psyllids and green peach aphids, were reported twice a week (Thursday and Friday). These data served as a risk assessment tool to improve disease management decisions. The program resulted in a spin-off project on isolation of *P. infestans*-like spores (every time when they are found in spore trap samples from potato fields), their germination *in vitro,* and morphological and molecular identification. The spin-off project was used to develop a technology transfer protocols for molecular diagnostics of *P. infestans*-like spores to confirm their species identity:

- **Morphological and molecular identification of unknown potential fungal pathogens of potato.**
  The protocol describes step-by-step procedure for germination of spores *in vitro*, fungal culture and identification to the species level using morphological criteria (light microscopy) and molecular diagnostics (PCR analyses with universal DNA primers). The protocol is simple, fast, reproducible and can be completed within 96 hours from DNA extraction to receipt of sequencing results, allowing for critical decisions to be made in a short amount of time.

Next steps: The U of L Potato Pest Monitoring Program has been successful and will be continued in its 3rd season in summer 2021. In addition, Dr. Maria Munawar will spend at least 50% of her time on molecular diagnostics in this program to ensure timely analyses that are critical to growers.

Project 5

Title: Development of culture media for high density micropropagation of potato plants *in vitro.*

Date: January – April 2020, September – December 2020.

Team: Dr. Dmytro Yevtushenko, Principal Investigator;
Andrea Abenoja, 4th-year undergraduate student

Brief details: Production of high quality, disease-free seed potatoes is the first and most expensive of inputs, as it takes several growing seasons to multiply seed tubers to quantities sufficient for the end users. All nuclear stock seed potatoes originate from certified virus-free potato plants that are grown *in vitro* in the lab. The U of L potato lab continues the development and improvement of media compositions for high density micropropagation of potato plants *in vitro.* The research is conducted by U of L undergraduate student Andrea Abenoja in a series of Research Internship and Independent Study projects. The overall objective is to develop new, low-cost culture

media to improve potato growth and multiplication under commercial tissue culture lab conditions. It extends our previous findings on Russet Burbank to other cultivars, such as Ranger Russet and Shepody. It also incorporated more detailed morphological and biochemical analyses, including measuring chlorophyll content, plant biomass, root development, etc.

Results and dissemination of knowledge: In cultivars Russet Burbank and Shepody, an increase of mesos ($CaCl_2$, KH_2PO_4 and $MgSO_4$) and micronutrients to concentrations four times higher than in the original MS medium was confirmed to be the most significant factor associated with plant quality, multiplication and shoot length in all plants. In cultivar Ranger Russet, the most vigorous and healthy plants were obtained on the medium containing four times higher concentrations of both mesos and macronutrients (NH_4NO_3, KNO_3). The latter indicated genotype-specific response of potato cultivars to culture conditions.

- Poster and oral presentation on improvement of potato clonal micropropagation, presented at *the 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020, online (2nd Place Poster Award, Session P1).

Practical applications: The developed media compositions can greatly improve the efficiency of large-scale propagation of tested cultivars (Russet Burbank, Shepody, and Ranger Russet) under commercial lab conditions.

Next steps: We now have sufficient data and replications to publish a manuscript. I will start working on it shortly. Meanwhile, this project will be continued on other cultivars. I am planning to prepare these media in Magenta culture vessels and distribute them to our commercial labs, so they can confirm the benefits of using these media for potato micropropagation under their lab conditions.

Project 6

Title: Nematode diversity and population levels in Southern Alberta.

Date: May 2020 –

Team: Dr. Maria Munawar, postdoctoral fellow;
Dr. Dmytro Yevtushenko, Principal Investigator.

Brief details: The U of L potato lab has started a comprehensive survey for plant parasitic and other nematodes in agricultural fields of Alberta. The study originates from our PED research and the need for better understanding of soil nematodes contributing to PED and, possibly, to other potato diseases. The research is conducted by plant pathologist/nematologist Dr. Maria Munawar and focused on morphological and molecular characterization of nematode species in potato fields, found both in soil and in plants. All soil samples collected in the CanPEDNet project and analysed for nematodes (to a genus level) in a designated lab, are also processed in our lab to identify the presence of not only the root-lesion nematode *Pratylenchus penetrans,* which is the known contributor to PED, but also other plant parasitic nematodes, such as *P. neglectus,* pin nematodes, stunt nematodes, etc. Special attention is paid to those nematode groups that are found in potato fields in relatively high numbers. The findings have resulted in discovery of new pin nematode species and two published manuscripts on stunt and pin nematodes in Alberta:

- Munawar M, **Yevtushenko DP**, Castillo P. 2020. Integrative taxonomy, distribution and host associations of *Geocenamus brevidens* and *Quinisulcius*

capitatus from southern Alberta, Canada. *The Journal of Nematology*. Accepted for publication.

- Munawar M, **Yevtushenko DP**, Palomares-Rius JE, Castillo P. 2020. Species diversity of pin nematodes (*Paratylenchus* spp.) from potato growing regions of southern Alberta, Canada. *Plants*. Accepted for publication.

Next steps: We are the only lab in Alberta with expertise on nematodes. Combined with largely unknown nematode populations in the province, we have a unique opportunity to conduct comprehensive study of plant-parasitic and other, including beneficial, nematodes in the region. Because root-lesion nematodes are involved in PED, this nematode project contributes to our PED projects (particularly, Project 3). Also, we received a request from Perry's farm if we could process their soil samples for nematodes (~10-15 samples per season) in our lab, rather than sending them to the PEI Potato Quality Institute. We agreed.

Project 7

Title: Plant host defense peptides.
Date: May, September 2020 –
Team: Christie Stephen, M.Sc. candidate (May 2020 –);
Nick Schimpf, M.Sc. candidate (September 2020 –);
Dr. Dmytro Yevtushenko, Principal Investigator.
Brief details: This is NSERC Discovery grant project that represents fundamental academic research on plant host defense peptides (HDPs) and their role in the plant innate immunity. The overarching goal of this research includes the elucidation of the molecular mechanism(s) of HDP-mediated disease resistance in plants. Namely, exploration of the mode of action of HDPs and their structural motifs essential for antimicrobial activity, their intracellular localization and transport within the host plant, and the role of HDPs in modulating the plant innate defense. The research is conducted by two M.Sc. students, Christie Stephen and Nick Schimpf. The studies will fill a critical gap in our understanding of the mechanisms involved in plant innate immune system, and the findings may be used in the conventional potato breeding programs to improve disease resistance in the established cultivars. This is purely fundamental research, required by academia.

Short, small-scale projects

These projects were designed as Independent study projects for the U of L undergraduate students.

Title: Potato protoplast culture and plant regeneration *in vitro.*
Date: January – April 2020
Team: Christie Stephen, 4th-year undergraduate student, now M.Sc. candidate
Dr. Dmytro Yevtushenko, Principal Investigator.
Brief details: Plant protoplasts present a convenient cell-based experimental model to study many aspects of plant biology, including the rapid analysis of gene expression, protein subcellular localization, protein activity and protein–protein interactions *in vivo*. The experimental work will include isolation of protoplasts from leaves of potato plants grown *in vitro* and culturing them on nutrient medium to induce cell divisions, colony

formations, and plant regeneration. Christie learnt a variety of plant cell and tissue culture techniques, including working with plant material under aseptic conditions in laminar flow cabinet, design and preparation of plant regeneration media, isolation and culture of protoplasts, plant regeneration from callus, clonal micropropagation of plants, etc. Christie applied for the M.Sc. program in my lab to work on an HDP project of the Discovery grant and started her M.Sc. study (Project 7) in May 2020.

- Poster and oral presentation on the isolation and cultivation of Russet Burbank potato leaf protoplasts at *the 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020, online.

Title: Pathogenicity of *Fusarium* spp. to potato tubers.
Date: January – April 2020
Team: Karan Sundhu, 4th-year undergraduate student;
Dr. Dmytro Yevtushenko, Principal Investigator.
Brief details: The project was based on using fungal culture *in vitro,* and included identification and characterization of *Fusarium* species responsible for causing potato dry rot, and assessment of pathogenicity of *Fusarium* isolates to major potato cultivars. During the course of this project, the student learnt a variety of plant pathology techniques, including working with fungi under aseptic conditions in biosafety cabinet, culturing fungal species *in vitro*, preparation of culture media, isolation of fungal conidia and inoculation of plants, assessment of disease development and pathogenicity of fungal isolates.

- Abstract presentation on pathogenicity of *Fusarium* spp. to potatoes at *the 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020, online.

Title: Molecular cloning of expression vectors containing an HDP gene.
Date: January – April 2020
Team: Karan Sundhu, 4th-year undergraduate student;
Mariana Vetrici, Ph.D., Research Associate;
Dr. Dmytro Yevtushenko, Principal Investigator.
Brief details: The project is a part of the Discovery grant, and focused on learning molecular cloning techniques, including bacterial cultures, isolation of plasmid DNA, DNA restriction analyses, design of PCR primers, DNA amplification, sequence analysis, etc.

- Abstract presentation on molecular characterization and verification of two plant expression vectors at *the 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020, online.

Title: Molecular identification of *Phytophthora infestans*-like fungi/oomycetes isolated from the potato pest monitoring spore traps.
Date: September – December 2020
Team: Melissa Telfer, 4th-year undergraduate student;
Dr. Dmytro Yevtushenko, Principal Investigator.
Brief details: In our Potato Pest Monitoring program (May-September 2020), we isolated 15 *Phytophthora infestans*-like spores from spore traps set up across the potato

fields in Alberta. These spores were germinated *in vitro* and identified to a species level using morphological criteria (light microscopy) and molecular diagnostics (PCR analyses with ITS-specific DNA primers). The study included preparation of culture media, growing fungi/oomycetes *in vitro*, isolation of fungal DNA, PCR primer selection or design, PCR amplification, horizontal gel-electrophoresis, gene alignment and interpretation of sequence analysis data. This project resulted in the development of a technology transfer protocol for molecular diagnostics of *P. infestans*-like spores to confirm their species identity (a copy of his protocol is attached)
Practical applications: The results of this study will help to improve spore diagnostics in our Potato Pest Monitoring program.

II. Anticipated Research Activity

Title: Population biology and control strategies for powdery scab in potato.
Putative start date: September 2021
Team: Dr. Dmytro Yevtushenko, Principal Investigator;
Dr. Michael Harding, Co-supervisor (Alberta Agriculture and Forestry).
Brief details: In December 2020, Syngenta representatives Sterling Mitchell (the Agronomic Service Representative, Syngenta, in Taber) initiated talks with Dr. Michael Harding and me on Syngenta-sponsored project on powdery scab in potato. During zoom meeting, which also included Dr. Jen Foster (the Agronomic Services Manager, Syngenta, Guelph, Ontario), we discussed the scientific and financial parts of the project and scheduled next meeting for March 1, 2021. The project will be designed as a M.Sc. Study, with room for extension into a larger project, and will focus on management strategies to control powdery scab in Alberta. It will involve field testing of some new control products manufactured by Syngenta. I have already prepared an advertisement for a grad student.

Title: Physiological age of the seed potato (general running title)
Putative start date: September 2021
Team: Dr. Jonathan Neilson, Research Scientist (Lethbridge Research and Development Centre, Agriculture and Agri-Food Canada);
Dr. Dmytro Yevtushenko, Principal Investigator.
Brief details: The project focuses on transcriptome analyses and identification of genes that are activated during dormancy break and sprouting. During two-year period, we collected tubers of Elite 2 generation of seed potato cultivars Russet Burbank, Ranger Russet, Umatilla Russet, Atlantic, NO2000, Ivory Russet, Perline, and Shepody. These tubers were stored at different temperatures (4°C and 10°C), and sampling of tuber eye tissues was performed on a monthly basis, freeze-dried (lyophilized) and stored at -80°C. The ultimate goal of this project(s) is to help growers to make science-based decision on physiological readiness of seed tubers to grow, which affects all aspects of crop production.
I have a promising M.Sc. candidate for this project, Brett Phillips. He was in my Plant Breeding and Genetics class (A+ student). He is still deciding which potato project to choose in my lab.

Title: Molecular diagnostics of major potato pathogens
Putative start date: March 2021

Team: Dr. Maria Munawar, postdoctoral fellow;
Dr. Mariana Vetrici, Research Associate;
Dr. Dmytro Yevtushenko, Principal Investigator.
Brief details: I have noted that there is a demand for local diagnostics of potato diseases. Currently, we perform molecular diagnostics that is related to our Potato Pest Monitoring program: *Phytophthora infestans* and potato psyllids (both *Bactericera cockerelli* and the zebra chip pathogen *Candidatus Liberbacter solanacearum*). We are planning to widen the list of major potato pathogens that we can identify by molecular methods. The plan is to start with identification of several major fungal, oomycete, and bacterial pathogens. This will be done in collaboration with Dr. Jie Feng from the Plant Health Lab at the Crop diversification Centre, North, in Edmonton, AB. Depending on the lab workload, we will conduct molecular diagnostics of plant material at the University of Lethbridge or send it overnight to the lab in Edmonton.

III. Research Funding Applications

The NSERC Collaborative Research and Development (CRD) grant application
Title: CRD in Potato Pathology.
Date of submission: May 30, 2019.
Decision: Not funded. In March 2020, I received the NSERC decision letter, stating that "Although your application was recommended for funding, it is decided not to be funded by NSERC due to budgetary constraints". According to NSERC, they received unusually high number of CRD applications and had limited funds available. I want to emphasize that my CRD grant application was well-prepared, evaluated by several key experts in the field, and edited multiple times before the final submission.

Results Driven Agriculture Research (RDAR) grant application
Title: Population biology and control strategies for potato early dying disease in Alberta.
Date of submission: November 19, 2020.
Decision: Not available yet.
Brief details: The project is built on my NSERC CRD in Potato Pathology grant application, which was not funded "due to budgetary constraints" (see details above). It relates to Project 3 above. We already have M.Sc. student, Atta Ur Rahman, who has started working on this project. The major goal is to accelerate the research on PED in Alberta to determine region-specific thresholds for the disease development under local environmental conditions.
We hope for the positive outcome on our RDAR grant submission.

IV. Publications

a. Published manuscripts:

Neilson JAD, Robertson CJ, Snowdon EW, **Yevtushenko DP**. 2020. Impact of fumigation on soil microbial communities under potato cultivation in Southern Alberta. *The American Journal of Potato Research* **97**, 115–126. This manuscript is based on data obtained from Colby's M.Sc. research.

Liyanage DWK, **Yevtushenko PD**, Konschuh M, Bizimungu B, Lu Z-X. 2020. Processing strategies to decrease acrylamide formation, reducing sugars and free asparagine content in potato chips from three commercial cultivars. *Food Control*. Accepted for publication on June 25, 2020. This manuscript is based on data obtained from Dilumi's M.Sc. research.

Munawar M, **Yevtushenko DP**, Castillo P. 2020. Integrative taxonomy, distribution and host associations of *Geocenamus brevidens* and *Quinisulcius capitatus* from southern Alberta, Canada. *The Journal of Nematology*. Accepted for publication. This manuscript is based on data obtained from our lab research on nematodes.

Munawar M, **Yevtushenko DP**, Palomares-Rius JE, Castillo P. 2020. Species diversity of pin nematodes (*Paratylenchus* spp.) from potato growing regions of southern Alberta, Canada. *Plants*. Accepted for publication. This manuscript is based on data obtained from our lab research on nematodes.

b. Submitted manuscripts:

Vetrici M, **Yevtushenko DP**, Misra S. 2020. Characterization of *LEAFY COTYLEDON1* (*PmLEC1*) during zygotic and somatic embryogenesis. *Plant Physiology*.

Robertson CJ, **Yevtushenko DP**, Snowdon EW, Harding MW. 2020. A survey of *Helicotylenchus*, *Paratylenchus*, *Pratylenchus* and *Tylenchorhynchus* in potato fields in Alberta, 2018 and 2019. *The Canadian Journal of Plant Pathology.*

Erickson M, Shi J, **Yevtushenko DP**, Lu Z-X. 2020. Oxidation and thermal degradation of oil in frying conditions: a review of natural antioxidant use. *Food Reviews International*.

c. Manuscripts in preparation:

Liyanage DWK, **Yevtushenko DY**, Konschuh M, Bizimungu B, Lu Z-X. 2021. Effect of nitrogen fertilization strategies and surface-area-to-volume ratio on acrylamide formation in French fries. *Journal of Food Science and Technology*.

Munawar M, **Yevtushenko DP**, Harding M et al. 2021. Running title: Fungicide-sensitivity of *Fusarium* isolates.

d. Poster and oral presentations at scientific conferences, symposia and meetings:

Vetrici M, **Yevtushenko DP**, Misra S. 2020. *LEAFY COTYLEDON1* as a central processing unit during seed development. In: *The Plant Biology 2020 Worldwide Summit*, Washington, DC, USA, July 27-31, 2020. Online poster presentation.

Vetrici M, **Yevtushenko DP,** Misra S. 2020. Characterization of *LEAFY COTELIDON1 (PMLEC1)* gene expression during zygotic and somatic embryogenesis. In: *The 2020*

World Congress on In Vitro Biology, San Diego, California, USA, June 6-10, 2020. P-2036. Online poster presentation.

Stephen C, **Yevtushenko DP**. 2020. Isolation and cultivation of Russet Burbank potato leaf protoplasts. In: *The 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020. Online poster presentation.

Abenoja AG, **Yevtushenko DP.** 2020. Improvement of potato clonal micropropagation. In: *The 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020. Online poster presentation (2nd Place Poster Award, Session P1).

Sundhu G, Vetrici M, **Yevtushenko DP.** 2020. Molecular characterization and verification of two plant expression vectors. In: *The 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020.

Sundhu K, Vetrici M, **Yevtushenko DP.** 2020. Pathogenicity of *Fusarium* spp. to potato tubers. In: *The 6th Annual Undergraduate Research in Science Conference of Alberta* at the University of Lethbridge, Alberta, Canada, May 25-26, 2020.

Roberson C, Snowdon E, Harding M, **Yevtushenko DP**. 2020. Improving Alberta potato production with soil treatments. In: *The 9th Annual U of L Biology Graduate Research Symposium* at the University of Lethbridge, Alberta, Canada, January 31, 2020.

V. Technology Transfer Protocols:

1. **Yevtushenko DP.** 2020. Identification of unknown plant material in processed potato products using DNA barcodes.
   This protocol was developed in my lab in response to the request of Cavendish Farms company to identify foreign plant material that was accidentally incorporated into potato food products during deep-fry processing. The diagnostic protocol is based on PCR amplification of four gene regions, namely *matK*, ITS, *trnH-psbQ* and *rbcL*. It combines the strengths of molecular genetics, sequencing technologies and bioinformatics to quickly identify an unknown plant contaminant in thermally processed foods in a rapid, reproducible, and reliable manner.
   A copy of this technology transfer protocol is included with this report.

2. **Yevtushenko DP.** 2020. Acclimatization of *in vitro* grown potato plants to the greenhouse.
   This protocol was developed to maximize the survival rate of potato plantlets transferred from tissue culture to the greenhouse. The *in vitro* environment is characterized by controlled temperature, high humidity, low light intensity, minimal stress, and aseptic conditions. Because *in vitro* plants lack protective waxy cuticles, stomatal regulation and stomatal hairs, transfer to soil often lead to rapid desiccation, wilting and death. Our developed protocol describes a simple and reliable step-by-

step procedure for acclimatization of *in vitro* grown potato shoots to the greenhouse. The protocol results in 100% survival rate of transferred *in vitro* plants.
A copy of this technology transfer protocol is included with this report.

3. **Yevtushenko DP.** 2020. Rapid isolation and examination of root lesion nematode from soil and roots of infected potato plants.
This protocol was developed as a part of the Canadian network for control of potato early dying complex (CanPEDNet) program to detect plant-parasitic nematodes. The protocol describes step-by-step extraction procedure that allows us to isolate all worm-form stages of nematodes from the soil samples in less than 20 minutes. It also describes a procedure for staining of nematodes in plant tissues using acid fuchsin, and a guideline to morphological diagnostics. This protocol is a prerequisite for the quantification of nematodes and establishing pest economic thresholds.
A copy of this technology transfer protocol is included with this report.

4. **Yevtushenko DP.** 2020. Permanent mounts of plant-parasitic nematodes for long term preservation.
This protocol was developed to preserve the specimens so they can be transported safely and remain in good condition for species identification and taxonomic purposes. The developed protocol is based on fixing the nematodes with hot formalin. As a result of the heat shock, the nematodes assume a characteristic shape, depending on the species, and the subsequent ethanol-glycerol treatments clear the nematode internal structures for easy identification to the species level.
A copy of this technology transfer protocol is included with this report.

5. **Yevtushenko DP.** 2020. Morphological and molecular identification of unknown potential fungal pathogens of potato.
This protocol was developed in my lab as a part of the Potato Pest Monitoring Program to help the industry to improve potato disease management. It is designed to identify *Phytophthora infestans*-like spores collected from spore traps set up in potato fields across Alberta. The protocol describes step-by-step procedure for spore germination of spores *in vitro*, fungal culture and identification to the species level using morphological criteria (light microscopy) and molecular diagnostics (PCR analyses with universal DNA primers). The protocol is simple, fast, reproducible and can be completed within 96 hours from DNA extraction to receipt of sequencing results, allowing for critical decisions to be made in a short amount of time.
A copy of this technology transfer protocol is included with this report.

VI. Teaching Activity

As the Research Chair, I was required to teach two courses per academic year (2016-2020).

In Fall 2020 semester (September – December 2020), I taught two courses:
1. BIOL 4500 B: Agricultural Biotechnology;
2. BIOL 5500/7500: Current Topics in Biological Sciences.

The BIOL 5500/7500 is a graduate level course (M.S. and Ph.D. students), which requires lots of time to grade and write comments on students' research proposals (their first and final drafts), evaluate their research presentations, rebuttals, critiques, journal clubs, etc. As a result, practically all my time in September-December 2020 was dedicated to teaching, particularly the BIOL 5500/7500 class.

I use teaching as an opportunity to identify and recruit students for research in my lab. For example, the four U of L Independent Study students, who conducted small-scale project in my lab in Spring and Fall 2020, came to my lab from my BIOL 3850 Plant Breeding and Genetics class. One of them, Christie Stephen, started her M.Sc. study in my lab (Project 7). Another promising student, Brett Phillips, will join our lab as a M.Sc. student in September 2021.

VII. Professional Meetings, Trainings and Visits

Because of the COVID-19 pandemic, all professional meetings in 2020 were either cancelled or moved to online format. As a result, I did not travel during 2020. The participation in online conferences included:

- Online poster and oral presentations at the 6th Annual Undergraduate Research in Science Conference of Alberta at the University of Lethbridge, Alberta, Canada, May 25-26, 2020.
- Online poster and oral presentation at the 2020 World Congress on In Vitro Biology, San Diego, California, USA, June 6-10, 2020. P-2036.
- Online poster and oral presentation at the Plant Biology 2020 Worldwide Summit, Washington, DC, USA, July 27-31, 2020.
- Provided written research update for the 2020 Alberta Potato Conference and Tradeshow that was held online in November 2020.
- Recruiting M.Sc. students for future research projects.

VIII. Service Activities

As a faculty member I am required to dedicate up to 20% of my time in service activities, which includes graduate student supervision, participation on internal committees, and general public outreach. The following is an example of such services I have provided to the University of Lethbridge and society in addition to research and teaching:

- Member of the Supervisory Committee for one Ph.D. and two M.Sc. students (non-potato projects).
- Member of the AgBiotech Committee program at the University of Lethbridge.
- Member of the Examination Committee for PhD Comprehensive Examination of Aleksei Sorokin, Ph.D. candidate with Dr. Igor Kovalchuk (Department of Biological Sciences, University of Lethbridge).
- Contributed to online issues of the "Potato Minute – Potato Growers of Alberta" on a monthly basis, or when required. These articles update potato growers and the industry on the current status of potato research and projects at the University of Lethbridge. They are written in a simple language aimed at the

broad audience. This communication is an important link between the university and the community of farmers. These articles usually consist of 1-2 pages of text with photographs of lab members at work.

Signed:

Dmytro Yevtushenko, PhD