Cluster 2 – Non-confidential Summary

1. CLUSTER PROJECT DETAILS

Project number: CL03 J.000556
Name of Project: Pulse Science Cluster 2
Activity #: T1.G6.V1
Name of Activity: Pea Root Rot: Distribution, Genetic Variability, Resistance, and Management
Name of Sub-activity: Sub-Activity 1: Develop and assess molecular diagnostic procedures for the rapid, specific and sensitive detection of new and emerging root rot pathogens in symptomatic field pea roots.
Project research period: April 1, 2013 to March 31, 2018
Principal investigator and research collaborators: Lead: Debra McLaren

NON-CONFIDENTIAL ABSTRACT/SUMMARY (For use in publications and pulse grower websites)

- Overall project objectives, methodology, research design & findings from project start to March 31, 2017.
- **500 words** in lay language.
- To be used "as is" with no additional permissions sought prior to use.

The objective of Sub-Activity 1 was to develop and assess molecular diagnostic procedures for the rapid, specific, and sensitive detection of root rot pathogens in symptomatic pea roots. As part of this research, pea root disease surveys were conducted annually in Manitoba and root rot was more severe for the five-year period of 2013-2017 than in the previous five years (2008-2012). Fusarium root rot was detected in all fields assessed in each year except in 2015 (98%). To monitor changes in pathogen populations over time and to create pathogen profiles, in-depth studies on root rot pathogen identification were conducted each year for two years (2011 and 2015), in three different commercial pea fields from the major production areas in Manitoba. The in-depth studies revealed seven (2011) and nine (2015) different *Fusarium* spp.

A set of *Fusarium* spp. were screened for pathogenicity on the cultivar Admiral and *F. avenaceum* was the most aggressive. Although many Fusarium isolates of other species were not as aggressive as those of *F. avenaceum*, the abundance of some of these less aggressive, but pathogenic isolates would have had an impact on pea productivity as part of the root rot complex. Generally, an average of two (2011) and three (2015) different *Fusarium* spp. were isolated per symptomatic pea root.

Combined molecular and pathology research input was provided by Drs. M.A. Henriquez (2013-2014) and Y.M. Kim (2015-2017) and resulted in the development of new molecular detection techniques for root pathogens. In order to accomplish advanced qualitative and quantitative molecular detection of *Fusarium* spp., new sets of primers for quantitative real-time PCR methods were developed and modified for *F. avenaceum*, and *F. acuminatum.* The quantitative PCR data showed significant variation in the inoculum concentrations of these *Fusarium* spp. among fields, as well as variation among years and geographical areas.

Furthermore, for simultaneous qualitative and quantitative multiplex detection of multiple *Fusarium* spp. and other root pathogen(s) using the Bio-Plex 3D system, species-specific probes for *F. avenaceum*, *F. acuminatum*, and *R. solani* were newly designed and modified. The new assay has been validated for the assessment of genomic DNA concentrations of these *Fusarium* spp. and *R. solani* from pure culture and is

ready to be tested on pea root samples using the Bio-Plex 3D system.

A set of primers specific for *R. solani* has been developed and validated for detection and quantification of this pathogen. In addition, a set of primers for *Aphanomyces euteiches* has been optimized and standardized with successful detection of *A. euteiches* from roots and stems in conventional PCR testing. The primer sets now can be used for the molecular detection of these pathogens by quantitative real-time PCR and digital droplet PCR in pea root samples.

Development of the new primers and probes for pea root pathogens has the potential to support future opportunities for the most advanced and accurate high-throughput digital droplet PCR applications to detect and quantify root pathogens in complex samples in order to monitor disease pressure and make fast and precise disease management decisions.

Completed report to be sent to stoms@saskpulse.com by March 30, 2018