

## MPSG FINAL EXTENSION REPORT

**PROJECT TITLE:** Characterizing the Fusarium Species that Affect Major Crops in Manitoba

**PROJECT START DATE:** 1 June 2016

**PROJECT END DATE:** 31 May 2018

**DATE SUBMITTED:** 12 June 2018

### PART 1: PRINCIPAL RESEARCHER

#### PRINCIPAL

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### PART 2: EXECUTIVE SUMMARY

*Outline the project objectives, a summary of the activities and results, and their relevancy to pulse and soybean farmers.*

Soybean is one of the most important food crops in Canada, but it faces wilting and root rot diseases caused by different Fusarium species. The isolation of a toxin-producing Fusarium species from soybean in MB has been concerning. Therefore, the aim of this project was (i) to study the cross-pathogenicity of some Fusarium species between pulse and cereal crops, (ii) to test the in vitro and in planta competitiveness between isolates collected from these two types of crops, as well as (iii) to investigate the ability of selected Fusarium species to produce mycotoxins within infected soybean tissues. Further objectives were (iv) to study the genetic diversity of the Fusarium community associated with soybean root rots in Manitoba and (v) to design molecular markers to detect and differentiate these pathogens.

The results in this project can be summarized as follows:

1. The tested Fusarium strains that infect wheat/barley/oat and cause FHB can also infect soybean and cause root rot symptoms.
2. There is an increasing trend of the 3-ADON genotype within the Fusarium isolates infecting cereals and soybean in Manitoba. Selected *F. graminearum* isolates (from soybean and wheat) can infect soybean and express the Tri5 gene within the infected soybean plants, strongly indicating the potential of these isolates to accumulate trichothecene mycotoxins in infected soybeans;
3. Diversity was shown among the tested Fusarium species/isolates, and
4. A PCR-RFLP protocol has been developed to differentiate between three important soybean Fusarium pathogens: *F. graminearum*, *F. poae*, and *F. avenaceum*.

### **PART 3: EXPERIMENT DESCRIPTION & RESULTS**

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*Concisely describe the experimental methods and results to date. You may include up to 3 graphs/tables/pictures in the Appendix.*

#### 1- Cross-pathogenicity of Fusarium isolates collected from cereals and pulses:

Several Fusarium isolates belonging to nine species collected from cereal crops (wheat, barley & oat), soybean, and pea crops were used in the current project to assess their capability to infect both types of crops (i.e. wheat and soybean) and cause FHB to wheat and root rot to soybean. The results showed that all Fusarium isolates from cereal and pulse crops were able to infect soybean plants and cause root rot symptoms at the V3 growth stage with different severity degrees. The statistical analysis indicated that disease severity ranged between 0.5 and 3.5 on a 1-9 scale. *F. graminearum* and *F. poae* isolates from wheat were the most aggressive (in a concurrent soybean project, our lab published the first report of *F. cerealis* causing root rot disease on soybean anywhere in the globe). Other cross-pathogenicity results involving peas, barley, and wheat from our collaborators are still under analysis.

#### 2- Competitiveness of Fusarium species isolated from pulse/soybean or cereal crops:

In this part, the competitiveness of different Fusarium *graminearum* isolates collected from different crops (cereals, soybean, peas) to infect soybean and cause root rot symptoms has been investigated. At the V3 growth stage, these pathogens were detected in the soybean tissues using standard microbiological methods as well as molecular methods with strain-specific primers based on the trichothecene genotype. The Fg isolated from soybean was more aggressive and generated severe root rot symptoms on soybean when compared to the other Fg strains isolated from wheat. Fungal pathogens have been re-isolated from the infected tissues and confirmed by morphological, microscopic and molecular tools.

#### 3- Investigate toxin producing potential of the *F. graminearum* isolates from soybean versus those from cereals:

Sixty-five Fusarium isolates were examined for the trichothecene mycotoxin genotype based on the amplification of portions of Tri3 and Tri12 genes. A multiplex PCR protocol based on sequence differences in major genes involved in trichothecene biosynthesis (Tri3 and Tri12) has been used and overall, 42% of the strains were NIV, 23% were 3-ADON and 15.4% were 15-ADON, while the trichothecene genotype for the remaining isolates (19.2%) has not been resolved. The results also revealed an increasing trend of 3-ADON genotype within the Fusarium isolates infecting cereals and soybean, but this result is based mainly on the limited number of isolates used in this study.

Another experiment has been conducted to investigate the ability of different Fusarium species isolated from cereal and soybeans to express the Tri5 gene within the infected soybean plants. Our results showed that certain *F. graminearum* isolates (from soybean and wheat) can infect soybean and express the Tri5 gene within the infected soybean plants, which provides a strong indication about the ability of these isolates to accumulate trichothecene mycotoxins in infected plants.

#### 4- Investigate genetic diversity of selected Fusarium species infecting these crops:

- Phylogenetic studies showed diversity of the Fusarium species that can infect soybean and cause root rot.
- A PCR-RFLP protocol has been developed to differentiate between three important soybean Fusarium pathogens: *F. graminearum*, *F. poae* and *F. avenaceum*.
- A unique restriction site found in *F. graminearum* will be used to develop a novel marker to differentiate *F. graminearum* from other Fusarium pathogens in the *F. graminearum* species complex (FGSC) and other Fusarium species associated with FHB in cereals and root rots in soybean.



## **PART 4: RELEVANCE TO FARMERS AND FUTURE RESEARCH**

*Describe how the project results can be captured to benefit pulse and soybean farmers (production recommendations, innovation items, marketing plans, commercialization of technology etc). Identify any future research opportunities.*

Fusarium is a very common soil fungus, and more than 10 different species are known to infect soybean roots and cause root rots in Manitoba. The number of Fusarium species involved in soybean is rapidly increasing with more Fusarium pathogens added to the list every year. Our lab has reported soybean root rots caused by *F. cerealis* for the first time in Canada and anywhere in the globe. Accurate identification of fungal phytopathogens is essential for virtually all aspects of plant disease, including both basic and applied research leading to the development of disease management methods. Developing tools for rapid and accurate detection and differentiation between possible pathogens will help in prevent disease spread and follow the appropriate control method. During the current project, we have developed several molecular tools (i.e. PCR-RFLP and Multiplex PCR) as well as novel species-specific primers for rapid detection and differentiation between different Fusarium pathogens that cause soybean root rots. These findings are instrumental for our future research, which will include:

- 1- Comparative genomic analysis for Fusarium pathogens belonging to different species using several genes or informative genome areas to find signature markers for each species.
- 2- Designing molecular PCR-based tools like PCR-RFLP and multiplex-PCR for rapid and accurate detection of the different Fusarium pathogens.
- 3- Optimizing a molecular tool to detect specific Fusarium pathogen directly from the infected plant tissues.
- 4- Identify toxigenic Fusarium species associated with soybean during crop vegetative and reproductive stages.
- 5- Determine the genotype and chemotype of selected Fusarium strains using molecular and chemical analysis.
- 6- Investigate the occurrence of trichothecene mycotoxins in soybean tissues (leaves and seeds).

Continuous assessment of the Fusarium activity in cereals and pulses is crucial in monitoring the threats they represent to these crops, the growers and the industry in general.

## **PART 5: COMMUNICATION**

*List extension meetings, papers produced, conference presentations made, project materials developed.*

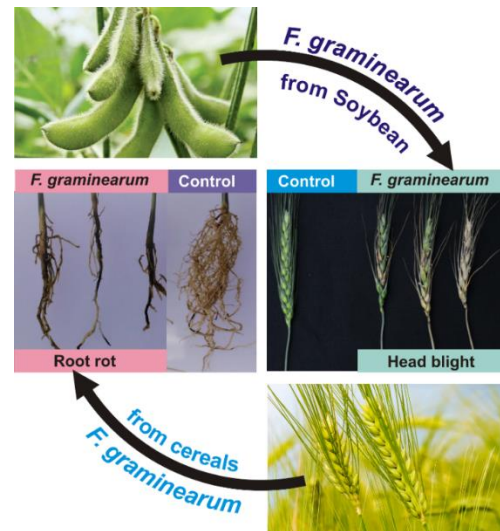
- Hafez et al. (2017): Cross-pathogenicity of Fusarium isolates infecting pulse and cereal crops in Manitoba. Canadian Phytopathological Society regional meeting, Winnipeg MB (Oral presentation)
- Hafez et al. (2018): Studies on cross-pathogenicity of Fusarium sp. between cereal and soybean in MB. CropConnect Conference, Winnipeg MB (poster)
- Hafez et al. (in prep) A unique PCR-RFLP marker for specific detection of Fusarium graminearum
- Hafez et al. (in prep) Species-specific primers and multiplex PCR to detect and differentiate Fusarium species causing soybean root rots
- Hafez et al. (in prep) Competitiveness of *F. graminearum* from different crops on soybean and wheat
- Hafez et al. (in prep) Cross-pathogenicity of selected Fusarium species between soybean and wheat
- Hafez et al. (in prep) Production of trichothecene mycotoxins in soybean by toxigenic Fusarium species from soybean and cereal crops.



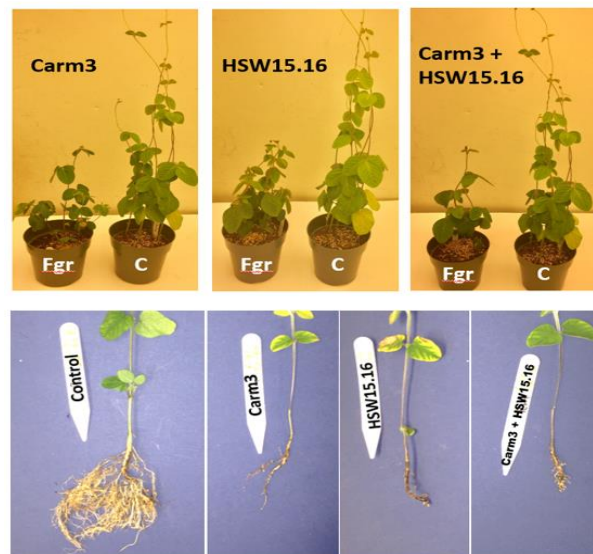
APPENDIX

Include up to 1 page of tables, graphs, pictures.

**Figure 1:** Cross pathogenicity of some Fusarium species between wheat and soybean. A *F. graminearum* isolated from soybean caused head blight to wheat, and a *F. graminearum* from barley caused soybean root rot.



**Figure 2:** In planta competitiveness of *F. graminearum* Carm3 (isolated from soybean) and HSW15.16 (isolated from wheat). Both isolates showed capability to infect soybean and cause root rot symptoms in separate and co-infection settings.



**Figure 3:** PCR-RFLP for specific detection of *F. graminearum*. The gene amplified with newly designed primers that give a 450 bp PCR fragment (un-cut). The PCR fragments digested with a restriction enzyme, showing only *F. graminearum* contains the restriction site for this enzyme. *F. graminearum* show a unique restriction pattern (two fragments: 200 and 250 bp) and can be easily recognized from the other Fusarium species.

- 1: *F. graminearum*
- 2: *F. avenaceum*
- 3: *F. oxysporum*
- 4: *F. cerealis*
- 5: *F. poae*
- 6: *F. sporotrichioides*
- 7: *F. equiseti*
- 8: *F. incarnatum*

