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MPSG FINAL EXTENSION REPORT

PROJECT TITLE: The Most Comprehensive Survey of Foliar Diseases in Manitoba soybean

PROJECT START DATE: 1 March 2016 **PROJECT END DATE: 28 February 2019**

DATE SUBMITTED: 30 January 2019

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.

Each year soybean farmers across Manitoba face economic hardships from disease-causing microorganisms (called pathogens), and in many cases yield losses are underestimated due to misdiagnosis or underreporting of disease. Our project objective was to carry out a two-year (2016 and 2017) survey of foliar diseases infecting Manitoba soybean at two growth stages (V2/3 and R6). To do this, we used a fairly new and powerful diagnostic tool called next-generation sequencing. In collaboration with MPSG and MAFRD, we surveyed soybean fields (81 in 2016; 67 in 2017) throughout the province growing region. Our major findings were:

1) We uncovered a long list of foliar pathogens, including bacteria, fungi, and viruses. Some pathogens were not previously reported in Manitoba (e.g., Cercospora sojina) or even known to infect soybean (e.g., Brome mosaic virus). It is important that these new pathogens are closely monitored in future disease surveys.
2) We identified a long list of residual pathogens (microorganisms that infect crops other than soybean) and insects pests. This data will be useful for the development of integrated pest management and crop rotation strategies.
3) We developed rapid, sensitive, and cost-efficient molecular diagnostics for some of the most common and important pathogens. These new diagnostics will improve the accuracy of pathogen identification in future surveys at a minimal cost.
4) We produced an Extension Handbook, which includes a photo of the symptomatic leaf and a description of the infecting pathogen(s) and disease. The handbook contains 96 leaf samples (44 V2/3; 52 R6) and will be freely available to commodity groups, farmers, and researchers.

PART 3: EXPERIMENT DESCRIPTION & RESULTS

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

Fields Surveyed: Soybean fields were scouted throughout the southern Manitoba growing regions in late June (V2/3) and in late August (R6). At least 5 plants showing foliar disease-like symptoms (e.g. chlorosis, spots, necrotic areas, deformation) were taken from each field. In total, we collected samples from 81 fields in 2016 and 67 fields in 2017 (see Figure 1).

Processing of Samples: A single leaf was plucked from each diseased plant, photo-indexed, preserved in a special solvent, and brought back to lab for next generation sequencing (NGS) using multiple approaches (e.g., RNA sequencing; 16S and ITS amplicon sequencing). This teases out DNA (genetic code) from the plant and any other organism that may be in or on it. After multiple stages "DNA work" we generate the data – billions of pieces of DNA. Using powerful computers, these are pieced together just like a massive jigsaw puzzle. The result is a comprehensive and precise catalogue of the disease-causing microorganisms (called pathogens) present in each leaf sample.

Identification of Foliar Pathogens: Our survey uncovered an variety of pathogens known to cause foliar disease in soybean, and also identified their range distribution throughout the province (see Figure 2). This includes pathogens not previously reported in Manitoba (e.g., Cercospora sojina) and even a virus not known to infect soybean (Brome mosaic virus). We were even able to unravel the different pathovars present for some of the pathogens.

Identification of Residual Pathogens: In many cases, foliar pathogens overwinter in the soil and can re-infect next year's crop. Crop rotation helps protect against this, as many pathogens are host-specific and cannot successfully invade the foliar tissues of the newly planted crop. We uncovered pathogens important to other major crops in the province but not known to infect soybean. These are mostly parasitic to cereals and brassicas. Awareness that these microorganisms are active in soybean fields will be valuable for the development of crop rotation strategies.

Identification of Arthropod Vectors: Many arthropods cause damage to host plants through their feeding behaviour, or serve as vectors that spread pathogens plant-to-plant. We identified a variety of pest and vector species, including aphids, thrips, mites, and armyworms.

Development of Molecular Diagnostics: While NGS has proven to be an exceptional tool, it is impractical for use as a routine diagnostic. A major benefit of our approach is it generated DNA sequence data for strains of pathogens specific to Manitoba. We used this information to develop PCR-based diagnostics for seven of the most prevalent and important soybean foliar pathogens present in the province. Each diagnostic was tested and validated on 70 fields from our 2016 survey.

The Extension Handbook of Foliar Soybean Diseases: We produced an extension handbook from the samples collected during the 2016 and 2017 surveys. The handbook includes a description of the pathogen(s) infecting the sample, the resultant disease, and photos of the symptomatic leaves taken directly from Manitoba fields. The handbook contains 96 individual leaf samples (44 of V2/3 and 52 of V6).

Next Steps: Our final plans for the project are to publish the 2017 soybean survey in a peer-reviewed journal. We will also disseminate the Extension Handbook to commodity groups, farmers, researchers, and extension specialists who may be interested, and also make it freely available online.

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.

The project results are a benefit to soybean farmers for a variety of reasons:
1) The newly identified pathogens should be closely monitored in future disease surveys. Since they were presumably misdiagnosed or unreported in previous surveys, their potential impact on yield losses have not yet been taken into account.
2) The residual pathogens detected will be important for the development of crop rotation strategies. The fact that several corn, barley, and canola pathogens are active in fields one year after harvest should be taken into consideration by farmers on a two crop rotation.
3) The rapid, sensitive, and cost-efficient PCR diagnostics we developed are superior to culturing methods. We recommend they be used in future disease surveys to validate visual assessments of symptom development.
4) Our Extension Handbook will be made freely available and is an informative resource for use by commodity groups, farmers, researchers, and extension specialists.

In terms of future research, this project set forth with a list of specific objectives, which were all achieved. It is impractical to use next generation sequencing as a routine diagnostic tool; however, the information generated from this project will prove to be extremely beneficial for subsequent surveys, which are carried out on an annual basis by MAFRD.

PART 5: COMMUNICATION

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

Conference Meetings:
2016 Canadian Phytopathological Society in Winnipeg.
2018 Canadian Phytopathological Society- Atlantic meeting. St. John's, NL.

Published Papers (thus far):
Díaz Cruz G, Smith CM, Wiebe KF, Villanueva SM, Klonowski AR, Cassone BJ. Applications of next generation sequencing for large scale pathogen diagnoses in soybean. Plant Disease. In press.
Díaz Cruz G, Smith CM, Wiebe KF, Charette JM, Cassone BJ. First Report of Brome mosaic virus infecting soybean, isolated in Manitoba, Canada. Plant Disease 102(2):460.
Díaz Cruz G, Smith CM, Wiebe KF, Cassone E BJ. First complete genome sequence of Tobacco necrosis virus D isolated from North America. Genome Announcements 5(32):e00781-17.

Project Materials Developed:
Foliar Disease Extension Handbook

APPENDIX

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

Figure 1. Regional distribution of soybean fields surveyed in Manitoba in 2016 (left, red dots) and 2017 (right, green dots). The first survey denotes the V2/3 stage; the second survey denotes the R6 stage.

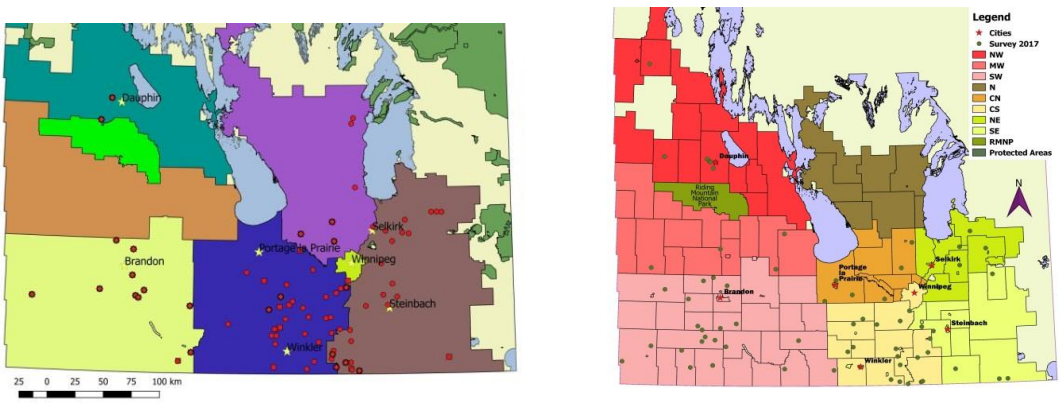

Figure 2. Foliar pathogens identified during the 2016 (1: V2/3 ; 2: R6)and 2017 (3: V2/3; 4: R6) surveys. Regions positive for a given pathogen are shown in green; regions where the pathogen is absent are in red. Note: we were unable to sample from eastern Manitoba in 2016 (V2/3) due to poor weather conditions.

			WESTERN				CENTRAL				EASTERN			
Type	**Pathogen**	**Disease**	**1**	**2**	**3**	4	**1**	**2**	**3**	4	**1**	**2**	**3**	4
Fungal/ Oomycete	*Alternaria* spp.	Leaf spot												
	Cercospora kikuchii	Leaf blight												
	Cercospora sojina	Frogeye												
	Colletotrichum spp.	Anthracnose												
	Didymella pinodes	Ascochyta blight												
	Drechslera spp.	Leaf spot												
	Peronospora manshurica	Downy mildew												
	Pleospora herbarum	Leaf blight												
	Septoria glycines	Brown spot												
Bacteria	*Pseudomonas syringae*	Bacterial pustule												
	Xanthomonas spp.	Bacterial pustule												
Virus	*Tobacco necrosis virus* D	Necrotic spots												
	Alfalfa mosaic virus	Alfalfa mosaic												
	Bean yellow mosaic virus	Viral infection												