Soybean Root Rot
Identification of the pathogens associated with root rot of soybean

Final Report to MPGA
March 26, 2015

Debra McLaren, (204) 578-6561, debra.mclaren@agr.gc.ca
Robert Conner, (204) 822-7521, robert.conner@agr.gc.ca
Maria Antonia Henriquez, (204) 578-6599, mariaantonia.henriquez@agr.gc.ca
Kan-Fa Chang, (780) 644-8352, kan.fa.chang@gov.ab.ca

Project Duration: April 1, 2012 – March 31, 2015
Budget: $8,000/year

Executive Summary:
Root rot is a serious disease of soybean in Canada for which successful control has been elusive. In a preliminary examination of soybean roots collected from one Manitoba field and four Alberta fields in 2011, *Fusarium* species were predominantly isolated from infected roots. The long-term and most economical approach for managing Fusarium root rot is the use of resistant cultivars, but soybean cultivars with high levels of resistance are not yet available. Furthermore, little information is available on the root rot pathogens associated with this crop in western Canada.

To acquire new information on root rot pathogens in Manitoba and Alberta, commercial soybean fields were surveyed (2012-2014) for the incidence and severity of root rot. Root rot was observed in all fields surveyed and the most frequently isolated pathogens from symptomatic roots were *Fusarium* spp. They were identified using visual assessment, microscopic examination and morphological assessment and included *F. acuminatum, F. avenaceum, F. oxysporum, F. solani, F. redolens, F. tabacinum* as well as additional, but less prevalent, *Fusarium* spp. A new species, *F. proliferatum,* has been reported in the USA, but was first reported in Alberta as a result of this survey. This research provides new information on Fusarium root rot pathogens in order to screen for host resistance and design effective control measures.

Background Information:
Fusarium root rot is a major disease of soybean in Canada and the United States (Wrather et al. 2001) and can cause significant yield reductions due to reduced plant stands, stunted seedlings and weakened root systems. Control of Fusarium root rot is difficult and cultivars with high levels of resistance are not yet available. According to previous studies, *Fusarium solani* and *F. oxysporum* have been reported as the major pathogens causing soybean root rot in North America (Nelson, 1999). Four *Fusarium* species (*F. oxysporum, F. graminearum, F. avenaceum* and *F. tricinctum*) have been frequently isolated from soybean roots in eastern Ontario (Zhang et al. 2010). Of these four species, *F. avenaceum* was the most pathogenic, followed by *F. graminearum.* In a preliminary examination of soybean roots collected from five soybean fields in 2011, *Fusarium* species were predominately isolated from infected roots. This disease is more common and severe when soybeans are seeded into cold soils. For this reason, Fusarium root rot

may be more prevalent on the prairies than it is in Ontario. The long-term and most economical approach for managing Fusarium root rot is the use of resistant cultivars, but soybean cultivars with high levels of resistance are not yet available. In addition, little information is available on the root rot pathogens associated with this crop in western Canada. More information on Fusarium root rot pathogens is needed in order to develop best management practices for this root rot complex.

Soybean production continues to increase with 354,000 ha (875,000 acres) and over 400,000 ha (>one million acres) seeded in Manitoba in 2012, 2013 and 2014. This represents the seventh consecutive annual increase in soybean area in Manitoba. Limited information has been available from Manitoba and Alberta on possible disease risks to this crop, but root rot is a constraint in other areas of Canada where soybean production is established (OMAFRA, 2011).

To acquire new information on root rot pathogens in western Canada, this project was funded to survey commercial soybean fields for the incidence and severity of root rot, identify root rot pathogens and conduct pathogenicity tests using a susceptible soybean cultivar. This research will provide new information on Fusarium root rot pathogens in order to screen for host resistance and design effective control measures.

Project Objectives:
(1) evaluate commercial crops of soybeans in Manitoba and Alberta for root rot;
(2) isolate and identify fungal colonies from root rot lesions;
(3) conduct pathogenicity tests for *Fusarium* isolates from infected soybean root tissue;
(4) provide annual and final reports.

Results:

Survey of commercial crops of soybean

In 2012, 2013 and 2014, approximately 40 soybean crops were surveyed in Manitoba for root diseases, with crops randomly chosen from regions in south-central and southwest Manitoba, where soybean is commonly grown. The surveys for root diseases were generally conducted during late July when most plants were at the early flowering stage. At least ten plants were sampled at each of three random sites in each crop surveyed. Root diseases were rated on a scale of 0 (no disease) to 9 (death of plant). To confirm the visual disease identification, 15 symptomatic roots were collected per field (2012) or from a sub-sample of 10 fields (2013 and 2014) for fungal isolation and identification. Fifteen roots from each soybean crop surveyed in each year were frozen for future PCR analysis of root rot pathogens.

In 2012, the majority of crops were either at or ahead of their expected stages of development due to an earlier than normal start to seeding because of minimal snow cover and higher than average late winter temperatures. A period of cold, wet weather followed in May, and excess moisture prevailed into early July in the regions where most soybean is grown. Root rot was evident in many crops, both early on, and later in the season when soils dried out. In 2013 the cropping season in Manitoba started with excessive spring moisture in some areas and cool conditions. Spring seeding was later than average for most crops. Crop growth continued to be suppressed by lower temperatures and frequent rainfall in areas of the province, which favoured

the prevalence and severity of some diseases. Later in the summer, warmer weather with frequent rainfall prevailed. However in some soybean crops, maturity was a concern and harvest did not start until October. In 2014, the majority of crops were either close to or behind their expected stages of development due to a later than normal start to seeding in many areas because of cool, wet weather. As in 2013, warmer weather developed over the summer but maturity and late harvest were a concern with some soybean crops.

Root rot was observed in all soybean crops surveyed in Manitoba during 2012, 2013 and 2014. The microorganisms most frequently isolated from roots of infected plants were *Fusarium* spp. In 2012, crops in which *Fusarium* spp. were isolated had root rot severity ratings that ranged from 0.6 to 5.7 with a mean of 2.1. Similarly in 2013, root rot ratings for crops in which *Fusarium* spp. were isolated ranged from 0.3 to 4.9 with a mean severity of 2.2. In 2014, root rot severity increased with ratings ranging from 1.9 to 6.8 with a mean of 4.6. Rhizoctonia root rot (*Rhizoctonia solani*) was detected in one crop surveyed in 2012, with a severity rating of 1.7, and in two crops surveyed in 2013, with a severity range of 1.2 to 2.6 and a mean of 1.9. Rhizoctonia root rot was not detected in any of the crops surveyed in 2014. The lower recovery rate of *R. solani* in 2013 and lack of recovery in 2014, suggest that in Manitoba this fungus may not be as important a root rot pathogen of soybean as are *Fusarium* spp., in contrast with other regions in western Canada.

In Alberta, Chang et al. (2013) reported *Fusarium* spp. to be the most frequently isolated microorganisms (100%) from roots of all infected plants followed by *Rhizoctonia solani* (23%). Twenty-eight fields were surveyed across nine locations with 100 root samples collected per field in 2014. Plants were also collected outside the sampling points (primarily in low lying areas of the field), where they were observed to be severely stunted or dead. Diseased plants in most cases could be easily pulled out of the ground, especially when the soil was at field capacity. This was a result of severe pruning of the root system. Root rot occurred in all locations with severity averaging 0.9 and ranging from 0.3 to 1.2 on a scale of 0-4 where 0 = no symptoms and 4 = plant dead. Similar results were evident in 2012 and 2013 with severity ratings ranging from 0.2 to 4.0 and 0.5 to 3.4, respectively. Overall, root rot disease incidence and severity in the surveyed locations were lower in 2014 than in 2013, but nodulation was superior. This suggests that low disease pressure enables soybean plants to form more nodules through their symbiotic associations with *Bradyrhizobium japonicum*.

Identification of *Fusarium* spp. from root rot lesions

To confirm the visual disease identification, 15 symptomatic roots were selected from all Manitoba fields (2012) and a sub-sample of 10 fields (2013 and 2014) for immediate fungal isolation and identification. An additional fifteen roots from each field were frozen for future PCR analysis of root rot pathogens. From the fresh samples, the identification of *Fusarium* species involved visual assessment, microscopic examination and morphological characterization using the criteria of Leslie and Summerell (2006). *Fusarium* spp. identified through this process included *F. avenaceum, F. acuminatum, F. oxysporum, F. solani, F. redolens, F. tabacinum* as well as additional, but less prevalent, *Fusarium* spp.

Approximately 100 isolates cultured from the Alberta surveys of soybean fields were identified as *Fusarium* spp. with *F. acuminatum* being the most predominant pathogen, followed by *F.*

avenaceum, F. oxysporum and *F. culmorum*. A new species, *F. proliferatum*, has been reported in the USA, but was first reported in Alberta as a result of this survey (Chang et al. 2015).

Pathogenicity testing of *Fusarium* isolates from root rot lesions

Pathogenicity screenings of thirty-one isolates were performed using sterilized seed of a susceptible soybean variety. Seeds were germinated on moist filter paper for 3 days at 25°C and then inoculated by immersion in a prepared conidial suspension (2.5 × 100,000 conidia/mL) for 5 minutes. Seeds of the controls were immersed in sterile water. After inoculation, the germinated seeds were planted in 10-cm diameter pots, filled with sterile soilless mix (Sunshine #3). In the greenhouse, the experiment was arranged as a completely randomized design with three replicates and disease assessment was performed 35 days after inoculation. Infected plants displayed dark brown lesions on the hypocotyl and primary root with disease severity ratings that ranged from 0 to 3.5 using the 0-9 rating scale described previously. Based on disease severity, *Fusarium oxysporum* and *Fusarium avenaceum* were the most pathogenic isolates, showing a disease severity that ranged from 1.3 to 3.0 and 0.4 to 3.5 respectively. From thirty-one isolates evaluated, twenty-nine were reisolated from roots of symptomatic plants. The study was repeated and isolates of *F. oxysporum* were again found to be the most pathogenic with a mean disease severity of 2.0. In Alberta, pathogenicity testing of *Fusarium* isolates indicated that *F. proliferatum* was the most aggressive isolate against soybean, causing the greatest reduction in seedling emergence and the greatest root rot severity. *Fusarium avenaceum* was the second most damaging followed by *F. oxysporum*.

Discussion:

The need to identify the major *Fusarium* species causing soybean root rot in Manitoba and Alberta is important in order to provide more effective disease management strategies and systems to producers. This research will assist in the development of soybean germplasm and resistance to Fusarium root rot. Improved disease control will ultimately result in increased competitiveness and profits by increasing yields, reducing risk and enhancing opportunities for using crops such as soybean.

References:

Chang, K.F., Hwang, S.F., Conner, R.L., Ahmed, H.U., Zhou, Q-X., Turnbull, G.D., Strelkov, S.E., McLaren, D.L. and Gossen, B.D. 2015. First report of *Fusarium proliferatum* causing root rot in soybean (*Glycine max* L.) in Canada. Crop Protection 67: 52-58.

Chang, K.F., Nyandoro, R., Howard, R.J., Hwang, S.F., Turnbull, G.D., Laflamme, P., Strelkov, S.E. and McLaren, D.L. 2013. Occurrence of soybean root rot in southern Alberta, Canada in 2011 and 2012. Can. Plant Dis. Surv. 93: 170-173.

Leslie J.F. and Summerell B.A. (2006). The Fusarium Laboratory Manual. Blackwell Publishing Ltd, Iowa. 388 p.

Nelson, B.D. 1999. Fusarium blight or wilt, root rot, and pod and collar rot. Pages 35-36. *in* G.L. Hartman, J.B. Sinclair, and J.C. Rupe, eds. Compendium of soybean diseases. 4th ed. APS, St. Paul, MN. 100 pp.

Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA). 2011. Agronomy Guide for Field Crops: Soybean Diseases. Publication #811. 28 pp. (http://www.omafra.gov.on.ca/english/crops/pub811/14soybean.htm)

Wrather, J.A., Stienstra, W.C. and Koenning, S.R. 2001. Soybean disease loss estimates for the United States from 1996 to 1998. Can. J. Plant Pathol. 23: 122-131.

Zhang, J.X., Xue, A.G., Zhang, H.J., Nagasawa, A.E. and Tambong, J.T. 2010. Response of soybean cultivars to root rot caused by *Fusarium* species. Can. J. Plant Sci. 90: 767-776.

Publications:

Chang, K.F., Hwang, S.F., Conner, R.L., Ahmed, H.U., Zhou, Q-X., Turnbull, G.D., Strelkov, S.E., McLaren, D.L. and Gossen, B.D. 2015. First report of *Fusarium proliferatum* causing root rot in soybean (*Glycine max* L.) in Canada. Crop Protection 67: 52-58.

McLaren, D.L., Henriquez, M.A., Conner, R.L., Chang, K.F., Henderson, T.L. and Kerley, T.J. 2014. Soybean root rot in Manitoba in 2012 and 2013. Can. Plant Dis. Surv. 94: 201-202.

Chang, K.F., Nyandoro, R., Howard, R.J., Hwang, S.F., Turnbull, G.D., Laflamme, P., Strelkov, S.E. and McLaren, D.L. 2013. Occurrence of soybean root rot in southern Alberta, Canada in 2011 and 2012. Can. Plant Dis. Surv. 93: 170-173.

McLaren, D.L., Henriquez, M.A., Conner, R.L. and Chang, K.F. 2013. 2012 field pea, dry bean and soybean research. The Pulse Beat Spring (March 2013) 68: 35-36.

McLaren, D.L., Henriquez, M.A., Conner, R.L., Chang, K.F., Henderson, T.L., Penner, W.C. and Kerley, T.J. 2015. Soybean root rot and phytophthora rot in Manitoba in 2014. Can. Plant Dis. Surv. 95: (in press).

Nyandoro, R., Chang, K.F., Hwang, S.F., Strelkov, S.E., Turnbull, G.D., Howard, R.J., Harding, M.W., McLaren, D.L. and Conner, R.L. 2014. The occurrence of soybean root rot in southern Alberta, Canada in 2013. Can. Plant Dis. Surv. 94: 198-200.

Acknowledgements:

The technical assistance for this research was provided by T.L. Henderson, T.J. Kerley, D.J. Hausermann, W.C. Penner, R. Nyandoro and D.B. Stoesz. We greatly appreciate the financial support provided by the Manitoba Pulse Growers Association.