Final Report to the Grain Farmers of Ontario

Project Title: Identifying sources of partial resistance and tolerance to Phytophthora and Pythium root rots in short-season soybean in Ontario

Project Number: S2009ID06

Researchers: Dr. Allen Xue, ECORC-AAFC, 960 Carling Avenue, Ottawa, Ontario, K1A 0C6. Tel: 613-759-1513. Email: allen.xue@agr.gc.c

Dr. Elroy Cober, AAFC-ECORC. Tel: 613-759-1610. E-mail: elroy.cober@agr.gc.ca
Dr. Vaino Poysa, AAFC-GPCRC. Tel: 519-738-2251. E-mail: vaino.poysa@agr.gc.ca.
Dr. Isvan Rajcan. University of Guelph. Tel: 519-824-4120 ext. 53564. E-mail: irajcan@uoguelph.ca
Dr. Raymond Cerkauskas, AAFC-GPCRC. Tel: 519-738-1227. E-mail: raymond.cerkauskas@agr.gc.ca.

Executive Summary: (Can use same summary as provided for the research guide)

Should be maximum one page and include a brief summary of activities to date, objectives or goals accomplished and highlights of achievements and reach of project to date and/or issues that have affected success of the project to date.

Phytophthora and Pythium root rots are destructive disease of soybean in short-season regions of Ontario. In the present research, we screened 87 new and old short-season soybean varieties for resistance to three common races of *Phytophthora sojae* (Races 1, 3, and 5) and for partial resistance (multi-gene resistance) and tolerance to Phytophthora and Pythium root rots in Ontario. Of the 87 varieties evaluated to *P. sojae*, 29 were resistant to at least one of the three races. Cultivars AC Brant, AC Orford, Beechwood, Maple Arrow, Maple Donovan, and OAC Gretna were resistant to all three races. We developed a primary root inoculation method to screen soybean varieties for partial resistance to Phytophthora root rot in the controlled environment. Using this new method, 27 of the 87 varieties were identified with high level of partial resistance. Twelve of the 27 partially resistant cultivars were further selected for field trials in disease nurseries in both Harrow and Ottawa each year during 2009, 2010, and 2011. All of the 12 cultivars showed consistent high levels of partial resistance with plant mortality ranging from 9.5 to 16.7%, which were significantly lower than the susceptible cultivar OX20-8 that was used as control and had a mean of 56.1% plant mortality over the three years. Yield reductions of the 12 cultivars ranged from 29.4 to 58.4% on average of three years. AC Bravor, Maple Donovan, Renfrew and AC Hercule had yield reduction of 29.4, 32.1, 37.0, and 37.4%, respectively, and were more tolerant than the remaining cultivars (39.2-58.4%). These cultivars can be used as sources of partial resistance and tolerance to Phytophthora root rot for future cultivar development for Canada.

Twenty-four isolates from eight *Pythium* spp. were evaluated for their pathogenicity in causing soybean seed rot (SR) and damping-off (DO) in a greenhouse environment, and the effect of temperature on the ability of these isolates to cause SR was also studied. There were significant differences among the eight *Pythium* spp. in both SR and DO. When tested at 25°C, *Pythium ultimum* were the most pathogenic species, causing 97.0% SR, and 46.4% DO, on verage over

two soybean cultivars used. *Pythium aphanidermatum* was the second most pathogenic species, resulting in 88.5% SR and 41.8% DO. The two species resulted in significantly greater SR and DO than the other six species tested and were considered highly pathogenic. Temperature had a significant influence on SR by the *Pythium* spp. At all four temperatures (4°C, 12°C, 20°C, and 28°C) used, *P. ultimum* was highly pathogenic, while *P. arrenomanes, P. coloratum, and P. dissotocum* were the least pathogenic. The temperature by *Pythium* spp. interactions were more pronounced for *P. aphanidermatum* which showed an increased percentage of SR with an increase of temperature and for *P. irregulare, P. macrosporum, and P. sylvaticum* which showed a decreased percentage of SR with an increase of temperature.

A total of 94 soybean cultivars were screened in both laboratory and greenhouse trials under artificial inoculations with a highly pathogenic strain of *Pythium ultimum* var. *ultimum* isolated from a diseased soybean plant in Ontario in 2010. None of the 94 cultivars were resistant to either Pythium seed rot or Pythium seedling blight. Cultivars Maple Arrow Brown and Maple Ridge Brown showed moderate levels of resistance to these diseases. The two cultivars showed no significant reduction on seed germination and plant emergence compared to the non-inoculated controls. Both cultivars had less than 37% mortality after 7 days on the inoculated growth media, in comparison with the non-inoculated controls. The remaining cultivars had greater mortality rates, ranging from 60 to 100%. These cultivars have not been reported previously to possess resistance or tolerance to Pythium seed rot or Pythium seedling blight and may be used in resistance breeding for development of new Pythium resistant soybean cultivars in Canada.

Detailed Description of the Project: Provide an update on project objectives and those reached to date.
Include an update of all activities undertaken to reach the project objectives.
Provide a summary of the results to date.
Identify the public good/benefit and/or benefit to Ontario farmers of the project to date.

Phytophthora root rot.

Phytophthora root rot, caused by *Phytophthora sojae*, is a destructive disease which attacks soybean at any stage of plant development and commonly reduces yield by 10-40% in Canada. Although one of the major priorities of soybean breeding in Canada has been the improvement of disease resistance, particularly Phytophthora resistance, the genetic basis of resistance to Phytophthora root rot in most Canadian soybean cultivars has not been determined. The objective of this study was to evaluate 87 short-season soybean cultivars released in Canada for their reactions to three *P. sojae* races (races 1, 3, 5) commonly observed in Ontario. Resistant cultivars identified from this study could be used for future cultivar development for Canada.

The percentage of mortality for the 87 soybean cultivars to each of the three *P. sojae* races was determined in two experiments and 29 cultivars were resistant ($\leq$25% mortality) to at least one of the three races. Of the resistant cultivars, AC Brant, AC Orford, Beechwood, Maple Arrow, Maple Donovan, and OAC Gretna were resistant to all three races.

A primary root inoculation method was developed and used to screen the 87 soybean varieties for partial resistance to Phytophthora root rot in the controlled environment. Using this new method, 27 of the 87 varieties were identified with high level of partial resistance. Twelve partially resistant cultivars including AC Brant, AC Bravor, AC Hercule, AC Orford, Alta, Beechwood, Crest, Maple Arrow, Maple Donovan, Micron, Nattawa, and Renfrew were further selected for field trials in both Harrow and Ottawa in Phytophthora disease nurseries during 2009, 2010, and 2011. A Phytophthora susceptible cultivar OX20-8 was included control in the nurseries. All of the 12 cultivars showed consistent high levels of partial resistance with plant mortality ranging from 9.5 to 16.7%, which were significantly lower than the susceptible check cultivar OX20-8 that had a mean of 56.1% plant mortality over the three years.

Yield reductions of the 12 selected partially resistant cultivars were examined by comparing the yield of the respective cultivars grown under a nature field adjacent to the disease nursery at each location during the three years of field trials. Yield of the 12 cultivars was reduced by an average of 42.2%, ranging from 29.4 to 58.4% over three years. AC Bravor, Maple Donovan, Renfrew and AC Hercule had yield reduction of 29.4, 32.1, 37.0, and 37.4%, respectively, and were more tolerant than the remaining cultivars (39.2-58.4%). Partial resistance is characterized as incomplete resistance and it is believed to be controlled polygenically and may be more durable for Phytophthora resistance in soybean. These cultivars with partial resistance and tolerance identified in the present research have not been reported previously to possess resistance and tolerance to Phytophthora root rot and may be used in resistance breeding for development of new soybean cultivars in Canada.

Pythium seed rot and seedling blight

Pythium seed rot and seedling blight caused by *Pythium* species are serious problems of soybean seedling establishment in Ontario and Manitoba where the majority of Canadian soybean is grown. Typical symptoms of infection by *Pythium* species include soft and decayed seed before germination, pre- or post-emergence damping-off in the seedling stage, and hypocotyl discoloration and root rot in advanced growth stages. The disease severity increases with cool and moist conditions, minimum tillage, and earlier planting. There is little information in aggressiveness among isolates within a *Pythium* species and difference in pathogenicity among *Pythium* species to soybean. The objectives of this research were to compare the pathogenicity of 24 isolates from eight *Pythium* species in causing seed rot and damping-off of soybean and to determine the influence of temperature on seed rot caused by the eight *Pythium* species.

There were significant differences among the eight *Pythium* species in both seed rot and damping-off . *P. aphanidermatum* and *P. ultimum* were the most pathogenic species, causing seed rot by 84.6% and 94.9%, and damping-off by 28.8% and 21.7%, respectively; *P. irregulare* and *P. sylvaticum* were intermediate, having seed rot of 35.7% and 37.2%, and damping-off of 5.2% and 3.6%, respectively; *P. arrenomanes*, *P. coloratum*, *P. dissotocum*, and *P. macrosporum* were least pathogenic, causing seed rot ranging from 7.8 to 16.6% and damping-off of 0.4 to 3.1%.

Temperature had significant influences on seed rot by the *Pythium* species At all four temperatures (4°C, 12°C, 20°C, and 28°C) used, *P. ultimum* was highly pathogenic while *P. arrenomanes, P. coloratum, and P. dissotocum* were least pathogenic. The temperature by *Pythium* species interactions were more obvious for *P. aphanidermatum* which showed an increased seed rot values with the increase of temperature and for *P. irregulare, P. macrosporum, and P. sylvaticum* which showed a decreased seed rot values with the increase of temperature (Fig. 2). These results suggest that *P. aphanidermatum* is not likely responsible for soybean root rot and damping-off in short-season soybean growing regions of Eastern Ontario and Manitoba where the soil temperature is often below 20°C during the crop emergence and the early seedling development stage. While *P. macrosporum*, *P. irregulare* and *P. sylvaticum* are likely responsible for the seed decay, seedling blight and root rot of short-season soybeans. The high level of pathogenicity of *P. macrosporum* to soybean at the low temperatures has not been previously reported. Further studies with a large number of *P. macrosporum* isolates from soybean and various host plants are needed to better understand the *P. macrosporum* isolates × soybean cultivar interaction as affected by temperature.

A total of 94 soybean cultivars were screened in both laboratory and greenhouse trials under artificial inoculations with a highly pathogenic strain of *P. ultimum* var. *ultimum*, isolated from a diseased soybean plant in Ontario in 2010. A total of 80 seeds in four replicate water agar plates per cultivar were used in laboratory trials and 80 seeds in four replicate plant trays per cultivars used in greenhouse trials. The same amount of seeds that were not inoculated with the pathogen but plated or planted in the same growth media were used as controls for each cultivar in each trial. The experiments were repeated once for both the laboratory and greenhouse trials.

In the laboratory trials, none of the 94 cultivars were resistant to Pythium seed rot. Cultivars Maple Arrow Brown and Maple Ridge Brown showed moderate levels of resistance. The two cultivars showed no significant reduction on seed germination and less than 37% mortality after 7 days on the inoculated water agar plates, in comparison with the non-inoculated controls. The remaining cultivars had greater mortality rates, ranging from 60 to 100%.

In the greenhouse trials, 13 cultivars showed moderately resistant or moderately susceptible to Pythium seedling blight (Table 1). Maple Arrow Brown and Maple Ridge Brown were the most tolerant cultivars, with emergence reduction by less than 3% compared to the non-inoculated controls, and were significantly better than other cultivars (18-100%) (Fig. 1). In addition, Maple Arrow Brown was better than Maple Ridge Brown in resistance to root rot and reductions to plant height and dry weight. The remaining cultivars including AC Glengary, Ceryx RR, Electron, Flambeau, Maple Glen, Male Isle, OAC Gretna, OT06-13, OT06-22, PS44, and PS46 were considered moderately susceptible, having root rot severity of 2.4-3.9 on a 0-5 scale, and emergence reduced by 18-39%, plant height by 57-72%, and plant dry weight by 12-60%. These cultivars have not been reported previously to possess resistance or tolerance to PRR&SR and may be used in resistance breeding for development of new Pythium resistant soybean cultivars in Canada.

Knowledge Transfer: Indicate how the information has been communicated with industry to date, including the development of communication materials, presentations made, scientific and popular press articles developed, and any other communication activities.

Results have also been conveyed to Ontario soybean growers, industry, and the general public in the following publications:

Scientific papers:

Zhang, H.J., **Xue, A.G.,** Zhang, J.X., and Xu, Y.L. 2009. Effect of biocontrol *Trichoderma* on soybean seedling and the growth of MM7 in soybean rhizosphere. Soybean Sci. 28: 511-515.

Zhang, J.X., **Xue, A.G.,** and Tambong, J. 2009. Evaluation of *Bacillus subtilis* strains for biocontrol of soybean root rots caused by *Fusarium oxysporum* and *F. graminearum.* Plant Dis. 93: 1317-1323.

Zhang, S.Z., and **Xue, A.G.** 2009. Population biology and management strategies of *Phytophthora sojae* causing Phytophthora root and stem rots of soybean. *In* Arya A. and Perello A. Eds. Management of Fungal Pathogens: Current Trends and Progress. CAB International, UK. pp. 318-328.

Zhang, S.Z., **Xue, A.G.**, Zhang, J.X., Cober, E., Anderson, T.R.. Poysa, V., and Rajcan, I. 2010. Reactions of Canadian short-season soybean cultivars to three races of *Phytophthora sojae*. Can. J. Plant Sci. 90:207-210.

Zhang, J.X., and **Xue, A.G.** 2010. Biocontrol of Sclerotinia stem rot (*Sclerotinia sclerotiorum*) of soybean suing novel *Bacillus subtilis* strain SB24 under controlled conditions. Plant Pathol. 59:382-391.

Zhang, S.Z., Xu, P.F., Wu, J.J., **Xue, A.G.**, Li, W.B., Chen, W.Y., Zhang, J.X., Lv, H.Y., and Chen, C. 2010. Races of *Phytophthora sojae* and their virulences on soybean cultivars in Heilongjiang, China. Plant Dis. 94: 87-91.

Pan, F.J., McLaughlin, Yu, Q., **Xue, A.G.**, Xu, Y.L., Han, X.Z., Li, C.J., and Zhao, D. 2010. Responses of soil nematode community structure to different long-term fertilizer stragegies in the soybean phase of a soybean-wheat-corn rotation. European J. Soil Biology 46: 105-111.

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.

Xue, A.G., Rioux, S., Morrison, M.J., Chen, Y., Zhang, J.X., and Yan, W. 200x. Resistance and tolerance to Sclerotinia stem rot in selected short season soybean cultivars in Canada. Americas J. Plant Sci. Biotech. 4: 48-54.

Zhang, J.X., **Xue, A.G.,** and Morrison, M. 2010. Impact of time between the field application of Bacillus subtilis strains SB01 and SB24 and the inoculation with Sclerotinia sclerotiorum on the suppression of Sclerotinia stem rot in soybean. Euro. J. Plant Pathol. In press.

Hwang, S.F., Feng, J., Chang, K.F., Conner, R., Strelkow, S.E., Gossen, B.D., McLaren, D.L., and Xue, A.G. 2011. Identification of microsetellite molecularLink to quantitative trait loci controlling resistance to Fusarium root rot in field pea. Can. J. Plant Sci. 91: 199-204.

Zhang, H.J., **Xue, A.G.**, Zhang, J.X., Xu, Y.L., and Yu, D.C. 2011. Biocontrol of soybean root rot caused by *F. oxysporum* and *F. graminearum*. Soybean Sci. 30: 113-118.

Zhang, J.X., **Xue, A.G.**, Morrison, M., and Meng, Y. 2011. Impact of time between the field application of *Bacillus subtilis* strains SB01 and SB24 and the inoculation with *Sclerotinia sclerotiorum* on the suppression of Sclerotinia stem rot in soybean. Euro. J. Plant Pathol. 131: 95-102.

Wei, L., **Xue, A.G.**, Cober, E., Babcock, C., Zhang, J., Zhang, S.Z., Li, W., Wu, J.J., and Liu, L.J. 2011. Pathogenicity of *Pythium* species causing seed rot and damping-off on soybean under controlled conditions. Phytoprotection 91: 3-10.

Wang, L.F., Xu, Y.L., Li, S.X., Li, C.J., and **Xue, A.G.** 2011. Effects of metabolite of *Gliocladium roseum* on egg hatching and juvenile activity of *Meloidogyne incognita.* Soybean Sci. 30: 818-822.

Conference proceedings

Wang, L.F., Xu, Y.L., Li, C.J., and **Xue, A.G.** 2009. Effects of metabolin of *Gliocladium roseum* on egg hatch of *Heterodera glycines*. Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 32.

Zhang, J.X., and **Xue, A.G.** 2009. Biocontrol of soybean Sclerotinia stem rot using *Bacillus subtilis* strain sb24. Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 93.

Zhang, J.X., **Xue, A.G.**, and Tambong, J. 2009. Evaluation of *Bacillus subtilis* strains for biocontrol of soybean root rots caused by *Fusarium oxysporum* and *F. graminearum.* Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 162.

Zhang, S.Z., Xu, P.F., Wu, J.J., **Xue, A.G.**, Li, W.B., Chen, W.Y., Zhang, J.X., Lv, H.Y., and Chen, C. 2009. Races of *Phytophthora sojae* in Heilongjiang province and resistance of soybean cultivars. Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 198.

Pan, F.J., Xu, Y.L., Yu, Q., **Xue, A.G.**, Liu, W. and Han, X.Z. 2009. Community structure and seasonal fluctuation of soil nematodes in soybean rhizosphere under different manure nodes. Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 249.

Xue, A.G., Rioux, S., Morrison, M.J., Chen, Y., Zhang, J.X., and Yan, W. 2009. Resistance and tolerance to Sclerotinia stem rot in selected short season soybean cultivars in Canada. Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 265.

Zhang, H.J., **Xue, A.G.,** Zhang, J.X., Xu, Y.L., and Li, C.J. 2009. Biological control of root rots caused by *Fusarium graminearum* and *Fusarium oxysporum* in soybean in Canada Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 29.

Zhang, S.Z., **Xue, A.G.**, Zhang, J.X., Cober, E., Anderson, T.R. and Rajcan, I. 2009. Reactions of Canadian short-season soybean cultivars to three races of *Phytophthora sojae*. Proc. World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China. p. 106.

Xu, P.F., Wu, J.J., **Xue, A.G.**, Zhang, S.Z., Li, W.B.,Chen, W.Y., Lv, H.Y., Lin, S.F., Li, N.H., Fan, S.J., and Wang, X. 2010. Differentially expressed cDNAs of soybean during infection by *Phytophthora sojae*. Proc. 3rd International Conference of Plant Molecular Breeding, September 5-9, 2010, Beijing, China. pp. 184-185.

Other publications and reports:

Xue, A.G. 2010. Evaluation of the biocontrol agent *Clonostachys rosea* (strain ACM941) for Fusarium root rot and seedling blight management in soybean. Final report to PMC-AAFC. 14 pp.

Xue, A.G. 2010. Identifying sources of partial resistance and tolerance to Phytophthora and Pythium root rots in short-season soybean (SSS) in Ontario. 2009 Annual Report to the Grain Farmers of Ontario. 7 pp.

Xue, A.G., Wei, L., Cober, E., and Babcock, C. 2010. Pathogenicity of *Pythium* species in causing seed rot and damping-off of soybean. Pulse Beat 61:29.

Xue, A.G., Zhang, S.Z., Cober, E., Poysa, V., and Rajcan, I. 2010. Sources of resistance to Phytophthora root rot in Canadian short-season soybean cultivars. Pulse Beat 61:30-31.

Xue, A.G. 2010. Evaluation of biological and chemical seed treatments for control of Fusarium and Rhizoctonia root rots of soybean, seed borne phase of FHB in wheat, and loose smut of barley. Confidential report to Chemtura Corporation. 17 pp.

Xue, A.G. 2011. Evaluation of biological and chemical seed treatments for control of Fusarium and Rhizoctonia root rots of soybean, seed borne phase of FHB in wheat. Confidential report to Chemtura Corporation. 12 pp.

Invited speaker in scientific conferences, growers meetings, and seminars and media interviews:

Xue, **A.G**. 2009. Identifying sources of partial resistance and tolerance to Phytophthora and Pythium root rots in short-season soybean in Ontario. Invited presentation at the joint meeting of the Ontario Oil and Protein Seed Crops Committee and Ontario Soybean Growers in Guelph, ON, Jun. 25, 2009.

Xue, **A.G**. 2009. Resistance and tolerance to Sclerotinia stem rot in selected short season soybean cultivars in Canada. Invited speaker at the World Soybean Research Conference VIII, Aug. 10-15, 2009, Beijing China.

Xue, **A.G**. 2009. Genetic resistance to Phytophthora and Pythium root rots in Canadian soybean. Invited speaker at the 2009 Soybean Breeding Whorkshop, Aug. 16-18, Harbin, China.

Xue, **A.G**. 2010. Soybean production and disease control in Canada. Invited seminar at Department of Plant Pathology, Northeast Agricultural University, Harbin, China, September 2, 2010.

Xue, **A.G**. 2010. Soybean production and disease control in Canada. Invited seminar at Department of Plant Pathology, Nanjing Agricultural University, Nanjing, China, August 25, 2010.

Xue, A.G. 2011. Soybean root rots and control methods. Invited speaker at the 5th Manitoba Special Crops Symposiuim, February 9-10, 2011, Winnipeg, MB, Canada. Expenses fully paid by the conference/MPGA.

Xue, A.G. 2011. Pythium and Phytophthora root rots of soybean in Ontario. Invited speaker at the Annual OOPSCC and Ontario Soybean Growers's Research Meeting, June 28, 2011, London, Ontario.

Xue, A.G. 2011. Phytophthora and Pythium root rots and control strategies. Invited speaker at the 22nd Annual Conference of Soybean Science, August 21-24, 2011, Yan An, China. Expenses partially paid by the conference for invited speakers.

Xue, A.G. 2011. Soybean root rots and control strategies in Canada. Invited seminar at College of Agronomy, Heilongjiang Bayi Agricultural University, Daqing, China, August 29, 2011. (in conjunction with the 22nd Annual Conference of Soybean Science in Yan An, China, August 21-24, 2011).

Xue, A.G. 2011. Soybean diseases and their control strategies in Canada. Invited seminar at Plant Protection Institute, Heilongjiang Academy of Agricultural Sciences, Harbin, China, August 31, 2011. (in conjunction with the 22nd Annual Conference of Soybean Science in Yan An, China, August 21-24, 2011).

Interviewed by Gord Gimour, FBC Staff on January 6, 2011. Title: World's worst soybean disease has not arrived here yet. The article published on Page 20 of the Manitoba Cooperator, February 17, 2011.

Interviewed by John Dietz, Freelance writer for Top Crop Manager on August 3, 2011. Title: Soybean pest expected soon - Soybean cyst namatode could be your worst nightmare. The article published in Top Crop Manager November 2011. Pages 52-54. Available online at: www.topcropmanager.com.