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# MPSG FINAL EXTENSION REPORT

**PROJECT TITLE:** **Manitoba survey and molecular quantification of soybean cyst nematode**

**PROJECT START DATE: 1 April 2017** **PROJECT END DATE: 30 September 2019**

**DATE SUBMITTED: 16 October 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.*

This project continues the proactive role of the Univerity of Manitoba and MPSG in knowing when SCN has arrived in the Province, outreach activities to inform growers about SCN and develop capacity within the Province for future surveys and research to mitigate damage due to SCN after its arrival. The project has three objectives;
1) Survey soybean fields for SCN
2) Publish results of this and the past two SCN surveys
3) Develop accurate and quick molecular method for quantification of SCN in soil

## *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.*

1) In October 2017, 30 commercial soybean fields in Manitoba near the U.S. border with history of soybean and edible bean cultivation were sampled after harvest. Three soil samples were collected per field from several risk areas yielding a total of 90 composite samples. A modified Fenwick elutriator (soil washing unit) (Fig 1, a) based on the USDA soil cyst extractor was first tested in terms of recovery efficiency (65.5%±5.1 standard deviation) and then 2.2 kg of each soil sample was used to recover nematode cysts. Overall, 17 of the composite samples from 12 fields were reported positive for nematode cysts. In total, 42 cysts were recovered and almost half the number of cysts from seven fields was brown and lemon-shaped, as expected of Soybean Cyst Nematode (SCN). Cysts that were intact were used for morphological (Fig 1, b-c) and molecular identification. DNA sequences were compared with those in the NCBI Gene bank database using BLAST to find the best matches with the nematode species. Based on the morphological characters, PCR with species-specific primer sets for SCN; CoxIII and SCAR, and DNA sequencing of multiple regions, we found four fields (fields #3, field #8, field #18 and field #28 with having 2,1,14 and 4 cysts/ 2.2 kg soil, respectively) were positive for SCN (Table 1). The sequences obtained in our samples had best matches (100%) with *Heterodera glycines* in the sequence database. The fields in which the nematode was found occur in the RMs of Grey, Rhineland, and Montcalm. In order to verify the presence of the pest, the four positive fields were reanalysed and resampled. Soil samples of the four fields held in storage were extracted for cysts. Based on the morphological characters and molecular analyses, two of the fields again yielded soybean cyst nematode cysts (5 lemon-shaped cysts). Using PCR with species-specific primer sets, these cysts produced an expected band size of about 252 bp (CoxIIIF1- CoxIIIR1) when visualized on agarose gel. Further, in May 2019, the four fields were re-visited, and soil sampled again. A total of 12 composite soil samples were obtained. Overall, 10 of the 12 composite samples from four fields had 36 nematode cysts. Twenty two of the 36 recovered cysts from the same two fields were brown and lemon shaped as a possible indicator of SCN, but none of them were positive for soybean cyst nematode based on molecular analysis. Recently in August 2019, the field with the highest number of cysts was investigated twice for presence of SCN on soybean roots as soybean is planted this year. On the second examination of roots, few white to yellow females of cyst nematodes were observed (Fig 1, d). Using a bioassay method, the soil samples from above mentioned positive fields were used for planting a soybean variety susceptible to SCN in growth chamber (Fig 1, e). Roots were checked for presence of SCN cysts at 60 and 90 days from planting. No cyst was recovered in this cycle, study will be continued for a second cycle using the same soil. This study confirms the first occurrence of SCN in commercial soybean fields in Manitoba.

2) Results of this research have been made available to growers and industry as a map of RMs in Manitoba found positive for SCN in the current and past studies (see https://www.gov.mb.ca/agriculture/crops/seasonal-reports/pubs/soybean-cyst-nematode-notice.pdf). In addition, a short manuscript describing results of this study has been submitted to Plant Disease (attached to this report).

3) Conventional methods of SCN quantification in soil rely upon the manual sorting and counting of eggs. In these methods, diagnosis of SCN infestation in soil requires crushing extracted cyst to reveal the eggs inside and counting the number of the eggs under a microscope (Ingham, 1994). In this study we propose to move past the most laborious steps (crushing cysts and counting eggs) by developing molecular protocols for quantification of SCN using the CoxIII and SCAR genes correlated to traditional microscopic counting of eggs for a variety of soil types. In July 2019, twenty fields in Ontario with a range of SCN levels and covering a range of soil types and regions in Southern Ontario were sampled. Three to four composite soil samples were collected per field yielding a total of 71 composite samples. From each composite sample, a 100 $cm^3$ subsample was measured by water volumetric displacement to determine the number of SCN cysts/100 $cm^3$ of soil. Cyst extraction was performed by using manual cyst extraction method. The eggs

## *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 has resulted in very important benefits to soybean growers. It has shown for the first time the presence of the SCN in Manitoba. At this early stage of infestation for the province growers can start to manage the pest before it results in yield reductions. The results of the project have been presented to growers and industry and we have more presentations planned for the new year. Dr. Tenuta also help John Wilson of the University of Nebraska in his talk on SCN at the Manitoba Agronomist Conference providing his results and helping to tailor Mr. Wilson's talk to Manitoba growers. The emphasis now to growers is to slow the spread of the pest to other fields using biosecurity protocols. Also reducing the buildup of the pest by using resistant varieties of soybean. It also puts emphasis on growers to have their fields tested.

A recommendation for research from this project is to continue surveys to determine the spread and levels of SCN in Manitoba. Also to determine the HG type of the pest to determine what soybean resistance genetics will be effective against the pest.

## PART 5: COMMUNICATION

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

**Presentations & Meetings**

M. Tenuta. (2019). ???????? Webinar hosted by the Saskatchewan Pulse Growers.

M Tenuta, N Ghavami*. (2019). Soybean Cyst Nematode. Manitoba Pulse and Soybean Growers – Getting It Right - Crop Production Meeting, Winkler, Canada.

M Tenuta, N Ghavami*. (2019). Soybean Cyst Nematode. NorthStar Genetics Grower Meeting, Morris, Canada.

N Ghavami*, M Tenuta, D Lange. (2018). Preliminary Results of the 2017 Manitoba Soybean Cyst Nematode Survey. Canadian Phytopathological Society Annual Meeting, Quebec City, Canada.

M Tenuta. (2018). Soybean Cyst Nematode: Coming to a Field Near You? Manitoba Agriculture Soybean Production Meeting, Portage la Prairie, MB, Canada.

M Tenuta. (2018). SCN Coming to a Place Near You? Agvise Laboratories - 29th Annual Soil Fertility Seminar, Portage la Prairie, MB, Canada.

M Madani*, N Ghavami*, M Tenuta. (2017). Survey and Development of Molecular Soil Diagnostics for Soybean Cyst Nematode in Manitoba. Canadian Phytopathological Society Annual Meeting, Winnipeg, Canada.

M Tenuta, M Madani*, N Ghavami*. (2017). Soybean Cyst Nematode and What Manitoba Growers Need to Know. Canterra Seeds Agronomy Workshop, Carberry, Canada.

**Media / Press Interviews**

Caution Urged After Soy Disease Found in Man., Robert Arnason, The Western Producer, October 3, 2019

Soybean Cyst Nematode Confirmed in Manitoba, Top Crop Manager, September 20, 2019

Soybean Cyst Nematode Confirmed in Manitoba - Pest Confirmed at Low Levels in Four Southern RMs, Glacier FarmMedia Network Staff, AGCanada, September 19, 2019

Soybean Cyst Nematode, Manitoba Pulse & Soybean Growers, September 16, 2019

Recent Field Findings of Soybean Cyst Nematode in Manitoba, Manitoba Agriculture Seasonal Reports Press Release, September 16, 2019

Four Cases of Soybean Cyst Nematode Confirmed in Southern Manitoba, RealAgriculture, September 16, 2019

Soybean Cyst Nematode Confirmed in Southern Manitoba, Cory Knutt, PortageOnline, September 16, 2019

In Search of the Soybean Cyst Nematode, Mario Tenuta, Pulse Beat, January 1, 2019

Soybean School: Preparing to battle SCN when (not if) it is found in Western Canada, Real Agriculture, Broadcast interview with Kelvin Heppner, March 20, 2018

Manitoba poised to meet advancing soybean cyst nematode, Richard Kamchen, Manitoba Co-operator, March 12, 2018

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

Table 1. Nematode cyst samples from grower fields in Manitoba positive for being soybean cyst nematode.

| Field Number | #Lemon-shaped cyst found | Cone top analysis | Cyst content | PCR Positive for CoxIII & SCAR Primers | ITS Sequence Positive Match in % |
|---|---|---|---|---|---|
| 3 | 3 | | | | |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| 8 | 1 | Bifenestrate | Eggs & Juveniles | + | 100 |
| 18 | 14 | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 78 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | | | | |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | | | | |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | | | | |
| 28 | 4 | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |
| | | | | | |
| | | Bifenestrate | Eggs & Juveniles | + | 100 |

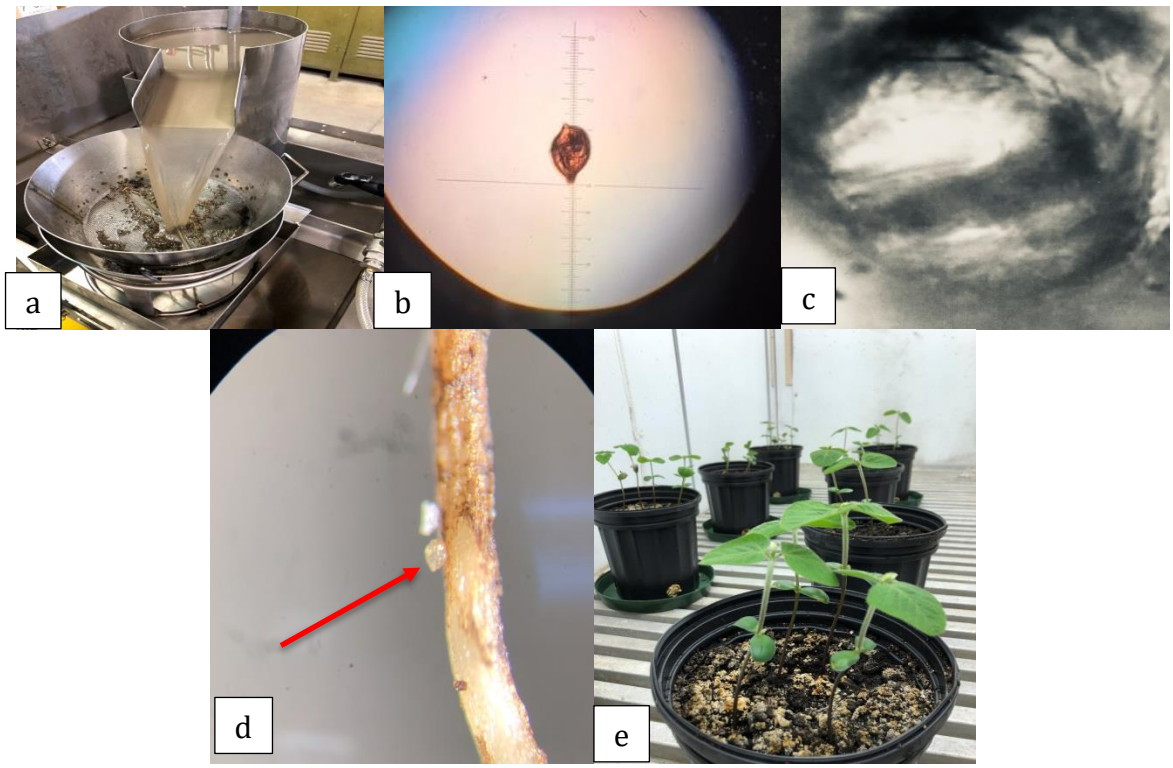


Figure 1. a) Cyst extraction using the USDA Cyst Extractor that MPSGA, b) a SCN lemon shaped cyst under a dissecting microscope, c) Bifenestrate vulval cone pattern of a SCN cyst, d) an immature SCN cyst on a soybean root from a field, e) Bioassay of soybean plants in soil from fields having SCN

Figure 2. Map of Rural Municipalities that Soybean Cyst Nematode was found and surveyed in this project.