Funding

Survey and Development of Molecular Soil Test for Soybean Cyst Nematode in Manitoba

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Time zone in Winnipeg: CDT North America

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INTRODUCTION

This study presents the first occurrence of SCN in commercial soybean fields in the province of Manitoba, Canada, and the development of real-time PCR assays for the quantification of densities in soil. Soybean Cyst Nematode (SCN), *Heterodera glycines* Ichinohe, is one of the most devastating disease/pest organisms of soybean worldwide. In Canada, the SCN is present in the province of Ontario and Quebec. The nematode is expected to soon be found in Manitoba, as it is spreading rapidly from the neighboring counties of North Dakota and Minnesota (United States).Precise identification of SCN and the infested fields is crucial to reducing the spread of the pathogen and limiting yield losses.In Manitoba. Prior to this study, our laboratory surveyed 76 soybean fields from 2012 to 2015, and did not find the nematode.

OBJECTIVES

survey soybean fields in Manitoba for the presence of SCN, and develop molecular protocols for quantification of SCN calibrated to traditional microscopic counting of eggs.

MATERIALS AND METHODS

Study One

- In October 2017, 30 commercial soybean fields in Manitoba near the U.S. border with a history of soybean and edible bean cultivation were sampled. (Fig 1),
- Each field was segregated into three risk areas for SCN introduction such as entranceways, depressions, and headlands,
- A soil washing unit, a modified Fenwick elutriator based on the USDA soil cyst extractor was used to recover nematode cysts (Fig 2),
- In July of 2021, an additional soybean field showed signs of SCN. A sampling grid was conducted in an infested patch and cysts were extracted by the wet-sieving and decanting method.
- Cysts were identified based on morphological characters, PCR with species-specific primers for *H. glycines* (CoxIII primer set which was validated in our laboratory for their ability to identify SCN populations and the published SCAR), and DNA sequencing of several genes (Ou et al. 1993, Subbotin et al. 2001, Madani et al. 2013).

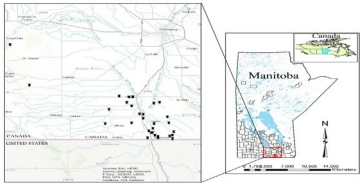

Fig 1. Locations of soybean fields sampled

Fig 2. Modified Fenwick elutriator

Fig 3. The adult SCN females on an infected soybean root

Study Two

- In July 2019, 20 fields with a range of SCN levels from Southern Ontario were sampled and used to optimize cysts, eggs, and DNA extractions as well as PCR reaction procedures,
- Cyst and egg extraction was performed in a 100 cm ^{3} subsample.
- Total genomic DNA was extracted from soil debris using the modified PowerSoil DNA Isolation Kit,
- A SYBR Green-based real-time PCR assay was optimized using the CoxIII, as well as SCAR-based primer set,
- A standard curve was obtained by preparing six serial dilutions (ten-fold each) of 1:10 diluted standard DNA.
- Calibration curves were obtained by adding a different number of SCN eggs(10,100,500,1000) in three replicates to both suspension and soil debris in which the absence of *H.glycines* confirmed,
- The melting profile further supported the specific detection of the SCN.

RESULTS

Study one

- Seven fields out of 30, sampled in 2017, had lemon-shaped cysts in good enough condition for morphological and molecular identifications.
- PCR products of DNA extracted from lemon-shaped cysts (fig 4) having ambifenestrate vulval cone structure (Fig 5), with measurements within the range for SCN, produced an expected band size of about 252 bp (CoxIII), 477 bp (SCAR), and 800 bp (D2-D3) when visualized on an agarose gel. (Fig 6).
- All sequenced samples had identity matches of 99-100% for the ITS, and 18S regions, and of those, few cysts also yielded >97% identity match for the CoxIII region of *H. glycines* in the GenBank nucleotide database.
- Overall, based on the morphological characters of cysts, PCR with species-specific primer sets for *H. glycines*, and DNA sequencing of several genes, four fields out of 30, were found positive for the SCN and had 2, 1, 14, and 4 cysts/ 2.2 kg of soil.
- The SCN population density of samples collected in 2021 from the infected patch ranged from 0 to 7797 eggs/100 cm ^{3} of soil. indicating population build-up.
- The five positive fields were from one of the Rural Municipalities of Thompson, Norfolk-Treherne, Rhineland, Emerson-Franklin, and Montcalm.

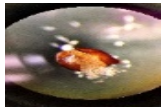

Fig 4. Cyst broken open to expose eggs and juveniles

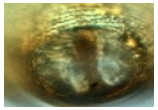

Fig 5. Ambifenestrate vulval cone structure.

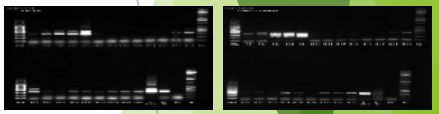

Fig 6. Duplex PCR amplification products obtained using universal and *SCN* specific primers

Study Two

- The relationship between Cq values and log of DNA concentration (dilution series) on the standard curve for SCAR and COXIII primer sets were linear with amplification efficiency of % 102.82 and % 100.3, respectively. Cq values increased proportionally with the dilution rate (Fig 7).
- The melt curves revealed species-specificity of the assay by generating melt temperatures of 80±0.5 °C and 75±0.5 °C for DNA extracts positive for *H. glycines* when amplified with SCAR (Fig 8), and COXIII, respectively.
- There were highly significant negative correlations between the number of eggs added and Cq values in nematode suspension (Fig 9), as well as in soil debris (Fig 10) at all the inoculation levels for both Primer sets, indicating the sensitivity, and quantification of the method.

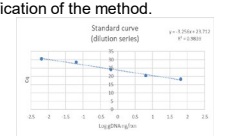

Fig 7. Real-time PCR standard curve of 10-fold serially diluted genomic DNA of H. glycines using SCAR primer

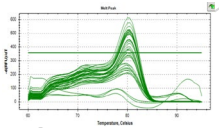

Fig 8. Melt curve generated in qPCR using SCAR primerset

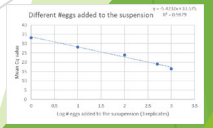

Fig 9. Relationship between the number of SCN eggs added to suspension and Cq values

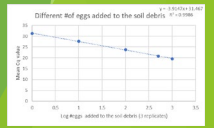

Fig 10. Relationship between the number of SCN eggs added to soil debris and Cq values