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Short Communication

First report of *Heterodera glycines* infecting commercial dry beans (*Phaseolus vulgaris*) in Canada

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Abstract

Edible bean is an important human protein source, and Canada is the fifth largest exporter worldwide. Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is a key soybean (*Glycine max* L.) pest, though it also infects dry beans (*Phaseolus* spp.). Cysts were observed on black bean roots in a commercial field in Bruce County, Ontario, in August 2018. Based on morphological characteristics, molecular evidence and pathogenicity experiments, SCN was confirmed. This is the first report of naturally occurring SCN infection associated with visible plant damage in a commercial dry bean field in Canada, which is a potential threat to this important niche industry.

Key words: *Heterodera glycines*, *Phaseolus vulgaris*, soybean cyst nematode

Résumé

Les graines de légumineuses comestibles constituent une importante source de protéines pour l'alimentation humaine et le Canada figure au cinquième rang des plus grands exportateurs de la planète. Le nématode Heterodera glycines est un des principaux ravageurs du soja (Glycine max L.), mais il s'attaque aussi à diverses sortes de haricot sec (Phaseolus spp.). En août 2018, les auteurs ont noté la présence de kystes sur des racines de haricot noir, dans un champ du comté de Bruce, en Ontario. La morphologie du parasite, les données moléculaires ainsi que les résultats des expériences sur la pathogénicité ont confirmé qu'il s'agissait du nématode du soja. Ce cas d'infection naturelle associé à des dommages apparents est le premier à avoir été signalé dans un champ de haricot sec au Canada. Le nématode du soja pourrait devenir une menace pour cette culture secondaire majeure. [Traduit par la Rédaction]

Mots-clés : *Heterodera glycines, Phaseolus vulgaris*, nématode du soja

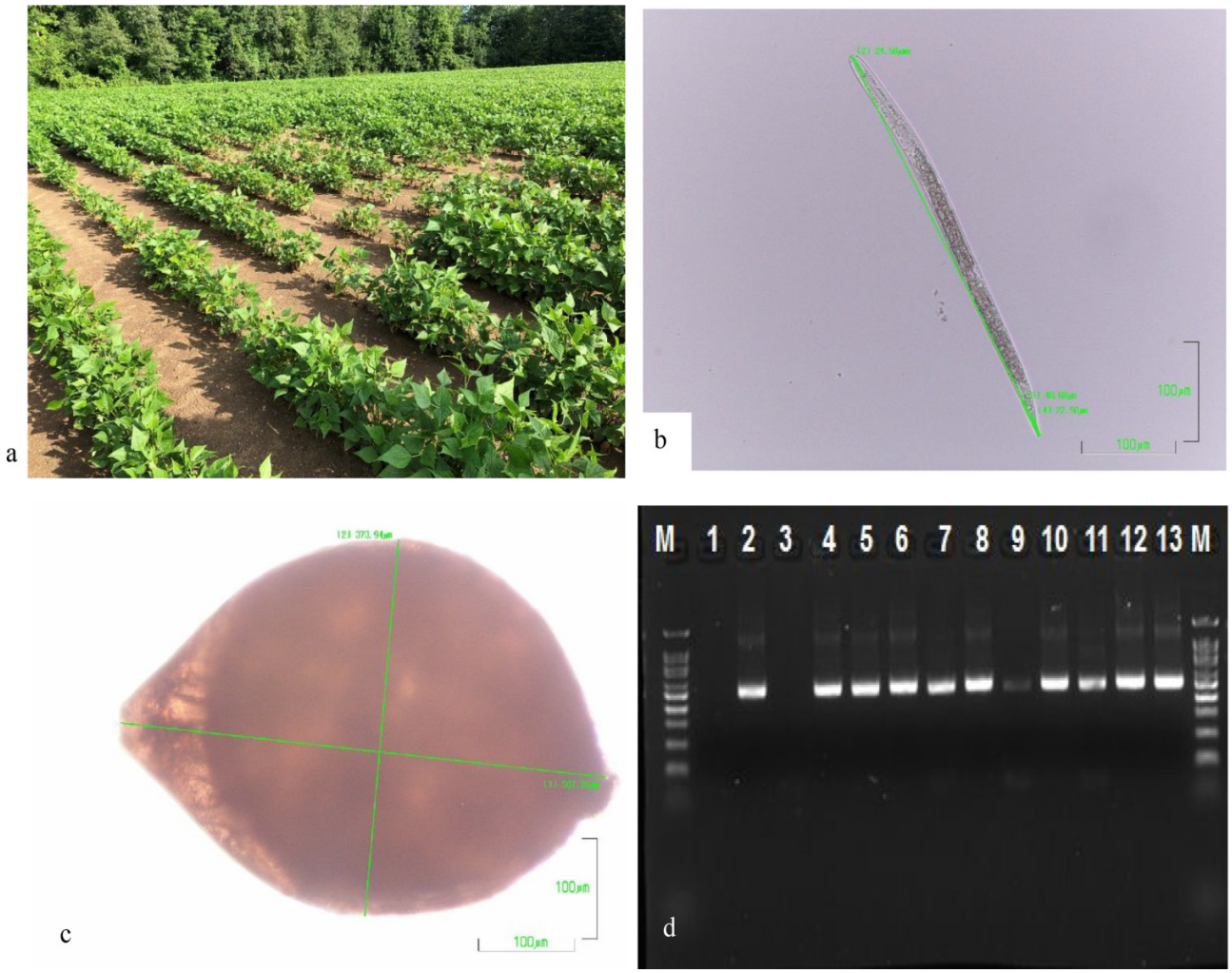
1. Introduction

Soybean cyst nematode (SCN; *Heterodera glycines* Ichinohe) is an economic threat to soybean (*Glycine max* L.), though it also infects dry bean types including *Phaseolus* spp. as well (Poromarto and Nelson 2009). The spread of SCN from the USA to Ontario, Canada, was confirmed in Essex County in 1987 and to Quebec in 2014 (Tylka and Marett 2017). SCN was found in 80% of tested soybean fields in southwestern Ontario (OMAFRA 2021) in a recent survey and it is spreading north and east into the primary dry bean production region, which includes Bruce County. SCN spreads rapidly in soil attached to farm equipment, plants, and seeds and remains viable after passing through a bird's digestive system (Riggs 1977).

Dry bean (*Phaseolus vulgaris* L.) is a major source of dietary protein for various cultures, particularly for subsistence farmers (Hardman et al. 1990). Worldwide dry bean production has increased to more than 20 MMT, worth over 20 billion USD, with 80% of production on farms in developing countries (Goodwin 2003). Canada is the fifth largest dry bean exporter in the world (FAO 2017), valued at more than 300 million CAD. In 2019, Ontario produced 52 025 ha of dry bean across white or navy (24 606 ha), cranberry (5091 ha), black (4226 ha), kidney (6704 ha), otebo (3618 ha), and adzuki (7778 ha) market classes (OMAFRA 2019). Western Ontario which includes Middlesex, Perth, Huron, Bruce, and Simcoe counties, is the primary production region for dry bean in Ontario.

Stunted and chlorotic black beans (cv. "Zorro") were observed on a sandy knoll in a 40 ha commercial field in Bruce County, Ontario in August 2018 (Fig. 1*a*). Upon examination of roots, cysts were observed. Therefore, several experimental methods were used to determine if SCN was present, as this would be the first report of a natural infestation of SCN on commercial dry beans in Canada.

Fig. 1. (*a*) Stunted black bean plants; (*b*) second-stage juvenile; (*c*) female cyst SCNs with morphometric measurements found on the plant roots and in the surrounding soil; and (*d*) agarose gel electrophoresis of species-specific sequence-characterized amplified region (SCAR) primers SCNF1/SCNR1 from *Heterodera schachtii* Schmidt (negative control; lane 1), *Heterodera glycines* (positive control; lane 2), DEPC-water (negative control; lane 4), and *Heterodera glycines* cysts MK817506.1–MK817515.1 (lanes 4–13) from a commercial dry bean field in Bruce County, Ontario, Canada in 2018.

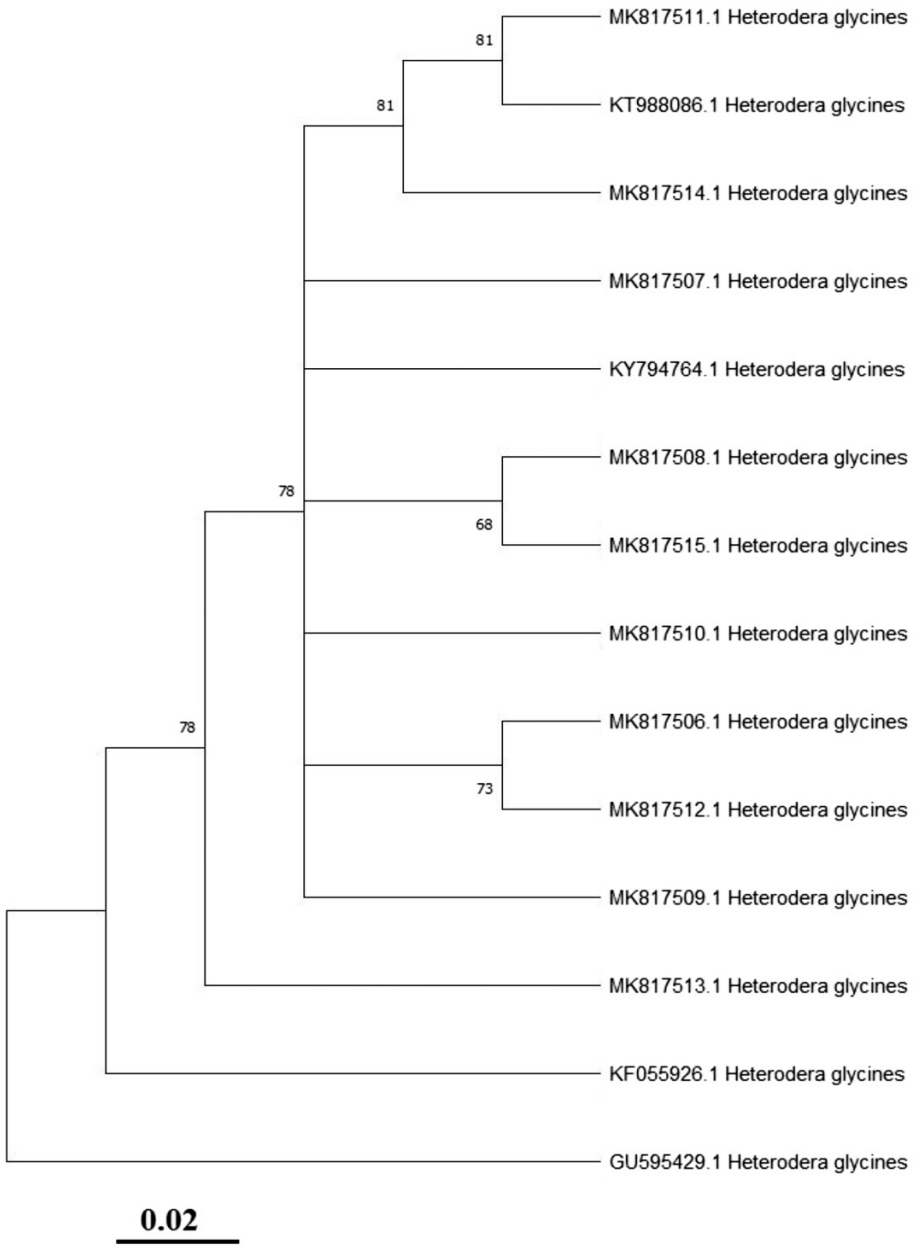


2. Materials and methods

Plant samples were collected from the stunted area as well as six other locations in the same field with no obvious aboveground symptoms. White to light brown cysts similar to those of SCN were observed on the roots of each plant sampled. Methods for cyst and egg collection from soil and plant samples as well as pathogenicity experiments were described previously (Poromarto and Nelson 2009). In brief, three replicates of soil from each sampled area was sieved to remove the cysts and used to determine the number of cysts and eggs 100 g^{-1} of soil. A minimum of five plants were selected from the stunted area as well as four of the six other locations in the field and the number of cysts per plant were determined. Pathogenicity experiments used the red kidney bean cultivars "Pink Panther", "Red Hawk", and "Dynasty", each in four replicates, which were inoculated with 4000 eggs per plant, using eggs harvested from cysts at the site. These cultivars were chosen, as previous research found the kidney cultivars "Red Hawk" and "Dynasty" had high cyst counts similar to the SCN-susceptible soybean cultivar "Lee 74" (Zhang 2018; Katsande 2019), which agreed with published research (Poromarto and Nelson 2009). Plants were placed in a growth chamber for 30 days at 27 ± 0.5 °C under 700 μmol supplemental light with 16-h photoperiod (Poromarto and Nelson 2009).

To confirm the nematode species, a total of 10 cysts and 30 second-stage juveniles were selected randomly to study morphological traits, using a compound ocular microscope at 10× and 20× magnification, respectively. Furthermore, ribosomal DNA from 10 cysts containing ITS1-5.8S-ITS2 regions was amplified and sequenced using primers TW81 (5′-GTTTCCGTAGGTGAACCTGC-3′) (Joyce et al. 1994) and rDNA2 (5′-TTTCACTCGCCGTTACTAAGG-3′) (Vrain et al. 1992) by Laboratory Services, University of Guelph to obtain a 915–986 bp amplicon of each cyst. The 10 cysts' sequences were identified as *H. glycines* by BLASTN search. These and another

Fig. 2. Neighbour-joining tree constructed with sequences from 10 cysts from a commercial dry bean field in Bruce County, Ontario, Canada, in 2018 and identified as *H. glycines* and four sequences of *H. glycines* from the NCBI database. The scale bar shows 0.02 changes, and bootstrap support values from 1000 replicates are shown at the nodes.



four sequences identified as *H. glycines* selected from the NCBI database (Table S1) were aligned by MUSCLE (https://www.ebi.ac.uk/Tools/msa/muscle/). The alignments were extracted and used to create a maximum-likelihood phylogram using MEGA-X software (https://www.megasoftware.net/) with 1000 bootstrap replications displayed as percentages on the branches.

To further confirm SCN, a species-specific PCR targeting the sequence-characterized amplified region (SCAR) primers SCNF1/SCNR1 was also completed by Laboratory Services, University of Guelph using the method of Ou et al. (2008).

3. Results and discussion

The population density from samples collected in the stunted field area was 72 cysts root^{-1}, 929 cysts 100 g^{-1} of soil, and 37 991 eggs 100 g^{-1} of soil (Table S2). Population densities from locations with no aboveground symptoms ranged from 7 to 30 cysts root^{-1}, 182 to 269 cysts 100 g^{-1} of soil, and 3744 to 7017 eggs 100 g^{-1} of soil. A SCN density of 10 000 eggs 100 g^{-1} of soil is considered very high in soybean, with the potential to cause economic damage in any soil type on susceptible as well as on resistant cultivars. Lower egg densities of more than 1000 and 2000 eggs 100 g^{-1} of soil are considered high risk on coarse sandy soil and fine-texture silt or clay soils, re-

spectively (OMAFRA 2021). The high density of SCN eggs in soil in the stunted area of the field suggests SCN may be associated with economic damage in dry bean. Economic thresholds for SCN egg density in soil for dry bean have not been established.

In the pathogenicity study, the mean number of cysts per experimental unit (one plant in approximately 100 cm^3 of soil) for "Pink Panther", "Red Hawk", and "Dynasty" were 383 ± 31.9, 334 ± 92.0, and 323 ± 45.9, respectively, demonstrating the ability of *H. glycines* from the commercial field to support cyst development. These cyst numbers are similar to values for "Red Hawk" and "Dynasty" and the SCN susceptible soybean cultivar "Lee 74" in two growth cabinet studies (Zhang 2018; Katsande 2019). In those studies, the cyst numbers for "Zorro" black bean were 50%–90% lower than the kidney bean cultivars. Poromarto and Nelson (2009) found similar results in a comparison of kidney and black bean cultivars to Lee 74 in North Dakota. The mean SCN reproduction was 787 cysts $plant^{-1}$ on a root system of four kidney bean cultivars, which was statistically similar ($P > 0.05$) to the 715 cysts $plant^{-1}$ on the roots of Lee 74. Four black bean cultivars had significantly ($P < 0.001$) less females, averaging 84% lower than Lee 74.

Morphological observations found that cysts were lemon-shaped (length 511 ± 37.3 μm, width 347 ± 38.0 μm) with a protruding neck and vulva cone (slit length 50 ± 2.3 μm), ambifenestrate (length 52 ± 2.2 μm, width 39 ± 1.7 μm) and underbridged with bullae. Second-stage juveniles were vermiform (length 464 ± 32.2 μm) with a distinct stylet (length 24 ± 1.2 μm) and hyaline region (length 25 ± 3.0 μm) at the tail terminal with a tail length of 45 ± 4.0 μm (see Figs. 1*b* and 1*c*). These physical traits are consistent with *H. glycines* (Subbotin et al. 2010).

For ITS sequence results, there were a significant number of successful matches in the sequence alignment (Fig. S1). The relatedness of the 10 *H. glycines* sequences from the commercial dry bean field and the four *H. glycines* selected from the NCBI database were highly similar, clustered together in a single clade showing simple differences between each other because of point mutations (Fig. S1 and Fig. 2). These relative relationships are in an agreement with the status of *H. glycines* as conspecific varieties (Kang et al. 2016; Powers et al. 2019). All the *H. glycines* members were highly relative to each other in this study. The species-specific PCR-targeting SCAR primers SCNF1/SCNR1 also confirmed the identity of all 10 cysts from the commercial dry bean field as *H. glycines* (Fig. 1*d*).

Previous reports (Zhang 2018; Katsande 2019) found SCN was able to infect dry bean at infested research sites in Canada. However, this is the first report of naturally occurring infection associated with damage at a commercial field. Dry bean production in Canada has an export value of over 300 million CAD and the presence of SCN in commercial fields is a potential threat to the industry. Further research is required to determine the impact of SCN on dry bean growth and yield.

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