Supplement to Journal of Nematology 25(4S):876–879. 1993.

Occurrence of the Clover Cyst Nematode, *Heterodera trifolii*, in Prince Edward Island Soils[1]

J. KIMPINSKI,[2] G. PLUMAS,[3] AND M. C. MACDONALD[4]

Abstract: In a survey of potato and rotational crops on Prince Edward Island, Canada, the cyst stage of the clover cyst nematode, *Heterodera trifolii*, was found in 43 of 63 sites sampled; however, only 12% of the cysts contained eggs. The root lesion nematode, *Pratylenchus penetrans*, was the dominant plant parasitic nematode and was found in 56 sites. Extraction of cysts from soil was similar using either the Schuiling centrifuge or the Fenwick can method, although the former was more convenient to use. The modified Baermann funnel method was not efficient for detecting the clover cyst nematode in soil.

Key words: Baermann funnel, clover cyst nematode, Fenwick can, *Heterodera trifolii*, method, nematode, *Pratylenchus penetrans*, root lesion nematode, Schuiling centrifuge, survey.

The clover cyst nematode, *Heterodera trifolii*, is a common parasite of forage legumes in eastern Canada and the northeastern United States (5,12,16,17), but there is no indication that it causes economic losses. Although this nematode does not attack potato (*Solanum tuberosum*) (6), several Latin American countries that import seed potatoes from Canada have expressed concern about the possible entry of *H. trifolii* cysts with soil attached to tubers. Each cyst may contain several hundred eggs that are protected from adverse conditions for extended periods (15). Some *H. trifolii* cysts were found in soil of potato crops (8). Therefore, a survey was conducted on Prince Edward Island to determine the occurrence of *H. trifolii* cysts and juveniles in potato fields, and in forage and cereal crops that are grown in rotation with potato. Other common nematode genera and species were recorded also. In addition, the nematode extraction efficiencies of the Fenwick can (3), Schuiling centrifuge (4), and a modified Baermann funnel (14) were assessed to determine an appropriate extraction method for diagnosing grower soil samples for export certification.

MATERIALS AND METHODS

Soil samples were collected during August 1992 from 63 locations in the potato production region of Prince Edward Island. Thirty-two fields were planted to potato, 27 to clover or clover–grass mixtures, and four to barley or a mixture of oat and wheat. Soil types, as indicated by several soil tests, were mostly fine sandy loams (65% sand, 25% silt, 10% clay; pH 5.2 to 6.0; and 2.5% organic matter).

At least 30 soil cores, each 2.5-cm-d and 15–25 cm deep, were taken from each location in patterns suggested by Barker (1) for row crops and solidly planted crops. The soil cores from each site were combined to make one composite sample and stored at 4 C. Each sample was mixed thoroughly, and a subsample of ca. 150 g was air-dried for 48 hours at 22 C. Just before extraction, a 100-g portion from each air-dried subsample was soaked in 50 ml tap water to reduce the amount of organic matter on sieves (10).

The Schuiling centrifuge extraction method for cysts was used for all field samples. The methodology was similar to that described by Shepherd (13). Each presoaked soil subsample was added to water in the centrifuge, mixed at 450–500 rpm for 20 seconds, brought to a full stop, and then mixed again for 10 seconds. The overflow passed into the central drain

Received for publication 20 April 1993.

[1] Agriculture Canada Research Station, Charlottetown, Prince Edward Island, contribution no. 789.

[2] Agriculture Canada Research Station, Charlottetown, Prince Edward Island C1A 7M8, Canada.

[3] Laboratorio Central de Cuarentena, Sanidad Vegetal, Siboney, La Habana, Cuba.

[4] Acrotec Laboratories, West Royalty, Prince Edward Island C1E 1B0, Canada.

We thank C. E. Gallant, Agriculture Canada Research Station, Charlottetown, for technical assistance.

leading to the collection sieve. The material collected on the sieve was gently rinsed to one side with a hand-held shower, the sieve was inverted, and the material was washed into the water column. The material was overflowed onto filter paper (12 × 23 cm) and gently washed into an approximate 5 × 5 cm area for examination. The cysts were counted at ×10 magnification and then moved to slides with a fine-tip brush. The number of cysts containing eggs were confirmed at ×40 to ×100 magnification. A compound microscope (up to ×1000 magnification) was used for species identification (11).

Greenhouse populations of *H. trifolii*, propagated on white clover (*Trifolium repens*), were used to compare the Fenwick can and Schuiling centrifuge methods. The Fenwick can procedure was similar to that described by Shepherd (13). With the air lock closed, the water supply was turned on. When the water level in the can was above the closed air lock, the presoaked soil sample (100 g air-dried) from the greenhouse was washed into the can with a wash bottle. After 15 minutes, the air lock was opened. The sample with the overflow drained into two stacked 30-cm-d sieves (125- and 500-μm-pore sizes). The material in each sieve was washed onto 9-cm-d filter paper in a Buchner funnel. The two sieves were placed again under the collection spout and the sides of the Fenwick can were washed down. The material from each sieve was washed onto 9-cm-d filter paper. A total of four filter papers with material were examined for each sample. The cysts were examined and identified in the same fashion as cysts recovered by the Schuiling centrifuge.

The number of second-stage juveniles (J2) of *H. trifolii* was estimated in all the field samples following extraction from soil by the modified Baermann pan method (14). A 50-g subsample was removed from each field sample, placed in the pan and left for 7 days at 20–25 C. *Heterodera trifolii* J2 and the vermiform stages of other prevalent plant nematodes were counted at ×40 to ×80 magnification. Individual specimens were identified to genera and species at magnifications up to ×1,000.

Results and Discussion

Clover cyst nematode was common in the potato growing region of Prince Edward Island (Table 1). Egg-filled and empty cysts were recovered from 43 of the 63 sites, with an average of 5.2 detected in 100-g samples; however, only about 12% of the cysts (mean of 0.6 cysts per 100-g sample) contained eggs. The reason for the lack of eggs in cysts is not known. Soil samples were collected late in the growing season, and the majority of eggs may have hatched and *H. trifolii* J2 exited the cysts. The population levels of cysts were similar in potato and pasture fields. The presence of cysts in grain fields was due probably to clovers in the rotation or to volunteer clovers and weeds between rows.

Root lesion nematodes (primarily *Pratylenchus penetrans*) were the dominant plant nematodes at 56 sites in soil samples when the nematodes were extracted with the modified Baermann funnel method (Table 2). These nematodes have caused potato tuber yield losses in the region (7). Other parasites such as the northern root-knot nematode, *Meloidogyne hapla*, were recovered occasionally but were not considered numerous enough to reduce yields of either potato or forage legumes. *Heterodera trifolii* J2 were observed from only two of

Table 1. Occurrence of *Heterodera trifolii* on Prince Edward Island.

Crop	Number of sites			Number per 100 g soil†	
	Total	With cysts	With cysts containing eggs	All cysts	Cysts with eggs
Potato	32	20	9	5.1	0.4
Pasture‡	27	20	9	5.4	0.8
Grain§	4	3	2	4.5	1.3
All crops	63	43	20	5.2	0.6

† Arithmetic means based on total number of sites. "All cysts" include both those containing eggs and empty cysts.
‡ Clover or clover–grass mixture.
§ Barley or oat–wheat mixture.

TABLE 2. Vermiform nematodes found† in soils on Prince Edward Island.

Crop	Number of sites	Numbers of nematodes per kg of soil‡				
		Root lesion	Root-knot	Pin	Spiral	Stunt
Potato	31	1970 (29)§	50 (5)	180 (11)	30 (3)	240 (14)
Hay¶	23	3550 (23)	80 (4)	440 (13)	120 (6)	700 (17)
Cereals#	4	1710 (4)	0	5 (1)	190 (2)	770 (3)

† Modified Baermann funnel extraction method.
‡ Root lesion, root-knot, pin, and spiral nematodes are, respectively, *Pratylenchus penetrans, Meloidogyne hapla, Paratylenchus* spp., and *Helicotylenchus* spp. Stunt nematodes are either *Tylenchorhynchus* spp. or *Merlinius* spp.
§ Means based on number of sites; number of sites in which nematodes were detected are in parentheses.
¶ Primarily red clover and timothy mixtures.
# Barley or oat–wheat mixture.

the sites at levels of 2,770 and 580 nematodes/kg dry soil. In contrast, the Schuiling centrifuge extracted cysts from 43 of 63 sites and egg-filled cysts from 20 sites (Table 1). These results confirmed that the Schuiling centrifuge is much more effective than the modified Baermann funnel for detecting clover cyst nematodes in soil. The modified Baermann funnel is the standard extraction technique used for soil samples submitted by growers in the Maritime region because *Pratylenchus* spp. are the dominant nematodes (9); however, if information on the distribution and abundance of *H. trifolii* is needed, then the Schuiling centrifuge is recommended.

Extraction of cysts from greenhouse soils was comparable whether the Fenwick can or the Schuiling centrifuge was used. The Fenwick can and the Schuiling centrifuge extracted 124.1 ± 23.3 and 132.5 ± 20.4 cysts, respectively, from 100-g soil samples (n = 14). These results were similar to those of Clayden et al. (2) for *Globodera* spp. in potato fields in Northern Ireland. They stated that the choice between the two extraction methods depended mostly on considerations such as convenience of use and cost effectiveness, but that the Schuiling centrifuge had some advantages over the Fenwick can method. For example, an operator is required for every sample processed with the Fenwick can, while more than one sample can be processed by one person using the Schuiling method, depending on the number of centrifuges available. Other advantages of the Schuiling centrifuge over the Fenwick can method are better working conditions, less water requirements, and a cleaner residue (13).

Our study indicated that the clover cyst nematode is found in the soils of Prince Edward Island, although the occurrence of cysts containing eggs is only about 12% of the total number of cysts. Further studies are required to determine if this nematode species damages crops grown in rotation with potato.

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