

OCCURRENCE AND POPULATION DENSITIES OF NEMATODES ASSOCIATED WITH FORAGE CROPS IN EASTERN CANADA

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Abstract

Nine genera of plant parasitic nematodes were associated with forage crops in 560 fields sampled in eastern Canada during 1967-72. Root-lesion, pin, spiral, and root-knot nematodes were present in 961, 74%, 711, and 51%, respectively, of all fields sampled; stunt, ring, cyst, dagger, and needle nematodes were found in smaller proportions of the fields. Root-lesion nematodes had the greatest population densities in soils. Population densities of root-lesion and root-knot nematodes were greater in red clover (*Trifolium pratense*) roots than in alfalfa (*Medicago sativa*) roots except in New Brunswick. Densities of root-lesion nematodes in roots of birdsfoot trefoil (*Lotus corniculatus*) were highest in Prince Edward Island. Root-knot nematodes were not recovered from roots of forage grasses from Ontario and Quebec. Cyst nematodes were recovered only from roots of red clover and white clover (*Trifolium repens*).

Résumé

Durant la période de 1967-72, notre enquête a révélé la présence de neuf genres de nematodes parasites des plantes associés à des cultures de plantes fourragères, dans 560 champs échantillonnés dans les provinces de l'est du Canada. Des *Pratylenchus*, *Paratylenchus*, *Helicotylenchus*, et *Meloidogyne* étaient présents dans 96, 74, 71, et 51 pour-cent de tous les champs échantillonnés; des *Tylenchorhynchus*, *Criconemoides*, *Heterodera*, *Xiphinema*, et *Longidorus*, dans une plus petite proportion des champs. Les populations de *Pratylenchus* étaient les plus denses dans tous les sols. Les populations de *Pratylenchus* et de *Meloidogyne* étaient partout plus denses dans les racines de *Trifolium pratense* que dans celles de *Medicago sativa*, sauf au Nouveau-Brunswick. La densité des populations de *Pratylenchus* dans les racines de *Lotus corniculatus* était plus grande à l'île du Prince-Edouard. Les *Meloidogyne* étaient absents des racines de graminées fourragères au Québec et en Ontario, tandis que les *Heterodera* n'étaient présents que dans les racines de *T. pratense* et *T. repens*.

Introduction

The potential threat of plant parasitic nematodes to forage production in eastern Canada was recognized by Willis and Thompson (16, 17) when they reported the presence and abundance of root-lesion nematodes, *Pratylenchus* spp., and eight other genera associated with forage legumes in the Maritime Provinces. Earlier, numerous pin nematodes, *Paratylenchus* spp., were recovered from the root zone of red clover in Quebec along with species of five other genera (3).

Subsequently, Willis and his associates reported, in 1971, the presence and abundance of eight plant parasitic nematode genera associated with three forage legumes in Nova Scotia (18). Potter and Townshend (10) have observed the presence of eight genera of nematodes in soil samples from forage crop fields throughout Ontario. During these years and earlier, the nematology group in the Entomology Research Institute, Ottawa, have noted in the annual report of the Canadian Plant Disease Survey and in the Canadian Insect Pest Review isolated incidences of nematodes associated with forage legumes and grasses in Canada. Coordinated surveys of forage crops were conducted in 1967 and 1968 in southwestern Ontario and in 1971 and 1972 in eastern Ontario, Quebec, Prince Edward Island (P.E.I.), and New Brunswick (N.B.). Though these were carried out over several years, the data are compiled in a single paper to provide a broad impression of the occurrence, distribution, and numbers of plant parasitic nematodes associated with forage crops in eastern Ontario.

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Materials and methods

All counties of southwestern Ontario were sampled in 1967 and 1968. Sampling routes radiated from the western tip of Lake Ontario through these counties, and samples were taken at 5-mile intervals where possible. Forage fields in eastern Ontario were sampled in 1971. At approximately 10-mile intervals samples were taken along two parallel routes through the counties along the northern shore of Lake Ontario and the St. Lawrence River, and in the Ottawa Valley. In the province of Quebec, forage fields selected at random were sampled in 1971 and 1972 in counties south and east of Montreal and in counties along the north shore of the St. Lawrence River west and east of Quebec City. Forage fields in P.E.I. were sampled in the province's three counties in 1971. Forage fields sampled in N.B. in 1972 were also selected at random in counties with significant forage production.

Samples were taken during September and October in P.E.I. and N. B., during July and August in Quebec, and during May-August in Ontario. In Quebec, N.B., and P.E.I., soil cores were taken to a depth of 20-25 cm through the root zone of randomly selected plants of the predominant forage legume, whereas in Ontario cores were taken through the root zone of all the legume and grass species present. The soil cores from each field were bulked to provide a 1-2 kg sample. Root samples were collected from all fields except those sampled in southwestern Ontario during 1967 and 1968. Ten root systems of each legume and grass species were taken from Ontario fields. In the other provinces, up to 25 root systems of only the predominant legume were taken.

In 1971-72 plant stands were determined in all fields: in southwestern Ontario in 1967-68, the percentage of expected plant stand was determined as based on the knowledge of the local industry and past performance.

The Baermann pan technique (14) was the basic method for the extraction of migratory nematodes from all soil samples collected in eastern Canada. Each soil sample was mixed and passed through a 2 mm screen to remove large roots. Migratory nematodes were extracted from 50-g subsamples for 1 week.

In eastern Ontario and Quebec, cyst and root-knot nematodes were also detected in the samples of heavy soils with a Fenwick can (4) and a bioassay, respectively. These methods supplemented data obtained by Baermann extraction on the frequency of occurrence of both nematodes and provided cysts for species identification. With the Fenwick can cysts were recovered from 50-g air-dried subsamples. For the bioassay, a celery seedling was grown in the remainder of the original soil sample in a 8-cm clay pot held

in a greenhouse at 22 C. After 8 weeks the soil was allowed to dry; roots were then shaken free of soil, and 5g of root were placed in a Baermann pan for 2 weeks.

Root samples of legumes and grasses were washed free of soil and the feeder roots were trimmed from the main roots. Migratory nematodes in the Ontario and Quebec samples were extracted from 5g of feeder roots in Baermann pans for 2 weeks, and from 10g of feeder roots in a mistifier for 1 week for the samples from N.B. and P.E.I.

All nematode counts and generic identifications were done by using a dissecting microscope. The population density of each genus of plant parasitic nematode was recorded as the number per 0.45 kg of soil and as the number per g of dry root. After counting, all nematode specimens were killed and fixed for species identification.

All soil and root samples collected in Ontario and Quebec were processed at Vineland Station, Ontario, while those from the Maritime provinces were processed at Charlottetown, P.E.I.

Results

Soils from Ontario and Quebec were generally heavy loams or clays, while those from New Brunswick and Prince Edward Island were generally sandy loams.

Frequency of occurrence

Nine plant parasitic nematode genera were associated with forage crops in eastern Canada (Table 1). Root lesion (*Pratylenchus*), pin (*Paratylenchus*), spiral (*Helicotylenchus*), and root-knot (*Meloidogyne*) nematodes occurred in more than 50% of all fields sampled. The frequency of occurrence of the root-lesion nematode varied from province to province by 7% and of the root-knot nematode by 45%.

Root-knot nematode was detected more frequently in soils of Quebec and eastern Ontario when the bioassay was used in addition to the Baermann pan method. Extraction with the Baermann pan indicated that only 22% and 37% of the soils from Quebec and eastern Ontario, respectively, were infested with root-knot nematode. The bioassay detected root-knot nematodes in an additional 14% and 34% of the fields, respectively. Examination of forage legume roots from eastern Ontario indicated another 8% of fields infested. In southwestern Ontario, without a bioassay only 36% of sampled fields were found to be infested in 1967-68.

In P.E.I., three other nematode genera also occurred in more than 50% of the fields

Table 1. Percentage of forage crop fields infested with plant parasitic nematodes in eastern Canada

Nematode	Percentage of fields infested ¹ in indicated areas and year						
	S.W. Ontario 1967-68	E. Ontario 1971	Quebec 1971-72	New Brunswick 1972	Prince Edward Island 1971	Eastern Canada 1967-72	
Root-lesion	94	100	93	100	100	96	
Pin	85	90	63	61	87	74	
Spiral	75	80	66	59	65	71	
Root-knot	36	79	36	57	81	51	
stunt	43	51	6	24	73	30	
cyst	38	37	20	22	71	34	
Ring	10	10	11	69	73	26	
Dagger	15	3	4	4	5	6	
Needle	0	0	0	0	3	0.5	
Fields sampled	99	71	248	51	91	560	

¹ % Infestation as determined from soil and from roots of forage legumes (Prince Edward Island, New Brunswick) or forage legumes and grasses (Ontario, Quebec). A celery bioassay was also used to detect root-knot nematodes in eastern Ontario and Quebec.

Table 2. Population density of plant parasitic nematodes in infested samples of soil from forage crop fields in eastern Canada

Province	Mean and range in number of nematodes per 0.45 kg (1b) of soil								Total ²
	Root-lesion	Pin	Spiral	Root-knot	Stunt	cyst ¹	Ring	Dagger	
Southwestern Ontario	810 20- 6,500	770 40- 4,200	830 20-7,660	390 20-4,900	280 20-3,600	450 10- 7,300	170 10- 650	130 20-500	2,420
Eastern Ontario	1,620 50-11,700	2,040 50-13,600	1,290 50-6,300	560 100-2,100	340 50-1,250	530 50- 1,700	80 10- 150	40 20- 50	4,810
Quebec	820 50- 8,300	470 20- 3,900	550 50-5,400	940 20-5,200	110 50- 300	190 20- 1,200	90 50- 250	70 10-100	1,490
New Brunswick	2,100 200- 7,160	2,360 110-18,050	510 40-1,990	1,500 50-7,850	420 50-1,330	910 40- 3,750	320 40-2,130	330 40-630	4,880
Prince Edward Island	2,800 310-18,370	1,370 100-11,860	250 50-1,350	1,090 100-8,750	300 50-2,100	1,440 100-10,000	150 50- 770	30 10- 50	5,930
All ²	1,450	1,100	670	890	300	760	180	100	3,130 ²

¹ Cyst larvae.

² The means in the "Total" column and the "All" row, and the grand mean were determined from the original data and not from averaging the means in the table.

sampled: stunt (*Tylenchorhynchus*) and ring (*Criconemoides*) nematodes occurred in 73% of the fields, and cyst larvae (*Heterodera*) in 71%. The needle (*Longidorus*) nematode occurred in 69% of the fields sampled in N.B.

Population density

Soil samples - In eastern Canada, although the various provinces were sampled in different years and at different times in a season, root-lesion nematodes were present in the greatest numbers (1,450/0.45 kg) followed by pin (1,100/0.45 kg), root-knot (890/0.45 kg), cyst (760/0.45 kg), and spiral nematodes (670/0.45 kg) (Table 2). In each province, one of the four nematode genera occurring most frequently in eastern Canada (Table 1) was always dominant in terms of soil population density, although not always the same nematode was dominant in every province. The mean of the total numbers of all nematode genera per 0.45 kg of soil for each province (Total column, Table 2) probably reflects the differences in sampling times, season, and year. The position of the mean relative to the extremes of the range

for each nematode (Table 2) indicates a skewness approximating a Poisson distribution.

Root samples - Rootlets of forage legumes were infested with root-lesion, root-knot, and cyst nematodes (Table 3). Of the legumes sampled in eastern Canada, red clover was the most severely infested except in N.B., where alfalfa was more heavily infested. Root-lesion nematodes were most numerous in red clover roots in each province regardless of sampling time and method of extraction. Red clover roots also contained the most root-knot nematodes in each province except N.B. Cyst nematodes were found only in roots of red clover in all provinces except in eastern Ontario, where these nematodes were recovered also from white clover roots; none were found in either alfalfa or birdsfoot trefoil. Only root-lesion nematodes were recovered from grasses sampled in eastern Ontario and Quebec.

The mean population density relative to the extremes of the range again approximated the Poisson distribution.

Table 3. Population density of plant parasitic nematodes in infested roots of forage legumes and grasses in eastern Canada

Location	Crop and number of samples	Mean and range in number of nematodes per g dry rootlets						
		Root-lesion	Root-knot	Cyst ¹	Total ²			
Eastern Ontario	Alfalfa -61	1,070	10-13,400	2,260	30-16,100	0	0	1,570
	Red clover -31	2,500	10-15,700	9,500	40-39,200	280	30- 770	5,380
	Birdsfoot trefoil -4	150	20- 370	0	0	0	0	150
	White clover -13	1,100	80-15,700	2,050	2,050	440	80- 770	1,350
	Brome grass -33	1,300	10- 7,400	0	0	0	0	1,300
	Timothy -33	1,300	30- 8,000	0	0	0	0	1,300
	Orchard grass -12	3,380	70-18,500	0	0	0	0	3,380
	Reed canarygrass -2	290	290	0	0	0	0	290
Quebec	Alfalfa -122	660	10- 1,100	2,090	30-12,050	0	0	1,340
	Red clover -95	1,250	10- 1,900	4,640	10-54,600	260	10-1,000	3,550
	Birdsfoot trefoil -3	230	20- 6,670	2,560	2,560	0	0	3,150
	Brome grass -10	530	100- 1,200	0	0	0	0	530
	Timothy -10	630	300- 1,000	0	0	0	0	630
	Orchardgrass -4	50	50	0	0	0	0	50
New Brunswick	Alfalfa -24	1,640	90-13,300	5,900	300-23,400	0	0	3,540
	Red clover -22	1,700	60- 6,700	1,540	180- 5,400	80	20- 120	2,390
	Birdsfoot trefoil -5	460	70- 950	1,840	260- 4,700	0	0	1,810
Prince Edward Island	Alfalfa -22	1,840	10-15,400	300	50- 670	0	0	1,950
	Red clover -61	7,450	170-46,700	7,300	80-38,400	300	30-1,200	12,900
	Birdsfoot trefoil -8	7,400	60-33,000	760	20- 2,700	0	0	8,010
All ²	Alfalfa -229	1,060		2,410		0		1,760
	Red clover -209	3,440		5,930		270		6,540
	Birdsfoot trefoil -20	3,310		1,320		0		4,370

¹ cyst larvae.

² The means in the "Total" column and the "All" row, and the three grand means were determined from the original data and not from averaging the means in the table.

Correlation of nematode populations and plant stands

Total populations of plant parasitic nematodes (all genera) associated with forage crops in southwestern Ontario in 1968 were negatively correlated with the percentage of expected plant stand ($r=-0.2440$;

$P(0.1)=0.2306$). Populations of root-lesion and root-knot nematodes were also negatively correlated with plant stand ($r=-0.4503$; $P(0.1)=0.2306$ and $r=-0.5287$; $P(0.1)=0.4124$, respectively). In contrast, populations of the other genera were positively correlated with plant stand, as were some comparisons using data from 1971 sampling sites.

Discussion

The genera of plant parasitic nematodes reported in this study to be associated with forage crops are similar to those previously reported from forage crops in Nova Scotia (18), Ontario (10), New Jersey (6), Maryland (5), North Carolina (7), Texas (8), Kentucky (2), and Minnesota (12). Tentative identifications of nematode species indicate that, although essentially the same genera are involved, many species occurring in the southern United States are different from those present in eastern Canada.

The frequencies of occurrence and the population densities of root-lesion nematodes in this study are similar to those reported from forage legume fields in Nova Scotia (18). High population densities and high frequencies of occurrence of root-lesion nematodes together with the previous demonstration of increases in forage yield (13) when these nematodes are controlled indicate that they are an economically important factor in forage production in eastern Canada.

In N.B., more root-knot larvae were recovered from alfalfa roots than from either red clover or birdsfoot trefoil, whereas the converse was true in the other areas. Since essentially the same varieties of alfalfa and red clover are being grown throughout eastern Canada, the reasons for these differences in densities of nematodes in roots of alfalfa and red clover between regions are unknown. They may be coincidental or may result from climatic factors or different species or strains of the nematode. The root-knot nematode has been shown to reduce yields of alfalfa (11) and white clover (1). Because population densities in infested alfalfa root samples were high in this survey, it is likely that root-knot nematodes are economically important in eastern Canada. The cyst nematode has also been shown to reduce yields of forage legumes (9). Although forage yields were not measured, the pin nematode increased to high populations under birdsfoot trefoil (15).

The frequency of occurrence and population densities of pin and spiral nematodes in soil samples from all geographical regions suggest that these nematodes may also affect forage crops.

The survey data showed that root-lesion, root-knot, and total nematode population densities were negatively correlated with the percentage of expected plant stand. However, the wide scatter of points found when nematode populations and plant stands were plotted graphically suggests that such correlations are meaningless. For example, correlations between existing nematode populations and plant stand are based on plants that have survived nematode attack and may be tolerant. To be meaningful,

correlations should be based on nematode populations that existed at the time a forage species failed. Thus, controlled field plot experiments offer an alternate method of determining the potential economic loss caused by individual nematode species on individual forage species. In such experiments the range of population densities selected for a nematode species might be determined by the mean population density observed in eastern Canada.

The present survey results emphasize the need for intensive research on all aspects of nematode damage in forage crops in eastern Canada. *Note: see Addendum, p. 136.*

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Addendum

Since this paper was submitted for publication, an unpublished thesis (Ward 1960) on forage nematodes in New York State has come to the authors' attention. The incidence of Pratylenchus and Paratylenchus in New York State was almost identical to that in Ontario. Helicotylenchus, Meloidogyne and Tylenchorhynchus were found less frequently than in Ontario but they were ranked in the same order of occurrence. In this thesis Ward concluded, as do the present

authors, that plant parasitic nematodes are likely a limiting factor in forage production.

Ward, C.N. 1960. Occurrence, distribution and populations of plant parasitic nematodes associated with forage crops in New York State. Ph.D. Thesis, Cornell University. Diss. Abstr. 21:1702. 1961.