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Survey of plant-parasitic and entomopathogenic nematodes in vineyards of Quebec

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A survey of plant-parasitic and entomopathogenic nematodes associated with vineyards was undertaken in the Estrie and Montérégie regions, the two major grapevine-producing areas in Quebec. Soil samples from 13 sampled vineyards were analyzed for the occurrence of plant-parasitic and entomopathogenic nematodes. Six genera of plant-parasitic nematodes were observed. The most commonly encountered plant-parasitic nematode genera were *Pratylenchus* and *Paratylenchus*, both occurring in 85% of sampled vineyards. No *Xiphinema* sp. were observed in surveyed vineyards. Entomopathogenic nematodes were recovered from 85% of the samples. Heterorhabditid and steinernematid nematodes were isolated from one and 11 vineyards respectively. Steinernematid isolates were identified as *Steinernema carpocapsae*.

[Inventaire des nématodes phytoparasites et entomopathogènes dans des vignobles du Québec]

Un inventaire des nématodes phytoparasites et entomopathogènes présents dans des vignobles du Québec a été réalisé dans les régions de l'Estrie et de la Montérégie, les deux principales régions productrices de vignes. Des échantillons de sol provenant de 13 vignobles ont été analysés pour la présence de nématodes phytoparasites et entomopathogènes. Six genres de nématodes phytoparasites ont été observés. Les genres les plus fréquemment retrouvés étaient *Pratylenchus* et *Paratylenchus*, lesquels ont été observés dans 85 % des échantillons de sol. Aucun spécimen du genre *Xiphinema* n'a été retrouvé dans les vignobles. La présence de nématodes entomopathogènes fut notée dans 85 % des vignobles échantillonnés. Des nématodes entomopathogènes de la famille des Steinernematidae ont été observés dans 11 vignobles et des Heterorhabditidae dans un seul vignoble. Tous les isolats de Steinermatidae ont été identifiés comme étant de l'espèce *Steinernema carpocapsae*.

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INTRODUCTION

Since the first commercial vineyard was planted in Quebec in 1976, the total wine grape (*Vitis vinifera* L.) area has increased to more than 125 ha supporting approximately 30 active enterprises (Dubois and Deshaies 1997). Most vineyards are located in the Estrie and Montérégie regions. An earlier nematological survey conducted in these same agricultural regions reported several plant-parasitic nematodes of economic importance (Vrain and Rousselle 1980). Among these, *Xiphinema* spp., *Pratylenchus* spp. and *Meloidogyne* spp. were frequently encountered and have been shown to cause major economic losses to wine grape in other countries (McElroy 1972). In Quebec, the occurrence of plant-parasitic and entomopathogenic nematodes in vineyards has not been investigated.

Lately, major growers in this industry have joined an integrated pest management program to reduce their reliance and use of pesticides and thus promote and increase populations of predatory entomofauna on insect pests. In this regard, the presence of entomopathogenic nematodes, such as steinernematids and heterorhabditids (which are known to kill a large number of insect larvae), could be useful in maintaining pressure on soil insect pests (Bedding and Miller 1981; Simons 1981).

Our objective was to survey the major vineyards for the occurrence of both plant-parasitic and entomopathogenic nematodes associated with grape vines in Quebec.

MATERIALS AND METHODS

From June to August 1998, a survey of 13 vineyards in the Estrie and Montérégie regions was undertaken to assess the distribution of plant-parasitic and entomopathogenic nematodes in vineyards. The 13 vineyards surveyed represent 42% of Quebec vineyards and 59% of vineyards located in the Estrie and Montérégie (Table 1). Vineyards were between 13 and 18 yr old, and vineyard size ranged from 1.4 to 11.3 ha.

A total of 142 soil samples was collected from the 13 vineyards. The number of soil samples for each vineyard ranged from 4 to 17 with approximately 1.5 kg soil collected for each sample to a depth of 0-20 cm. For each vineyard, soil samples were randomly collected beside the grapevine roots with a hand trowel (5-cm-diam x 20-cm deep). Soil samples were placed in plastic bags and stored at 4°C until the nematode extraction or baiting.

Plant-parasitic nematode numbers were estimated by processing one subsample of 100 cm^3 by the modified Baermann pan method (Townshend

Table 1. Description of the vineyards sampled in the survey

Locality	Sampling date	Vineyard's name	Production area (ha)
Dunham #1	1998-06-25	Les Côtes d'Ardoises	8.0
Dunham #2	1998-06-25	Les Arpents de Neige	9.9
Dunham #3	1998-06-25	L'Orpailleur	11.3
Grantham	1998-07-29	L'Aurore Boréale	4.0
Iberville	1998-06-23	Dietrich-Jooss	4.5
Lacolle	1998-06-23	Angell	5.0
Magog	1998-06-30	Le Cep d'Argent	11.0
Napierville #1	1998-06-23	Morou	1.8
Napierville #2	1998-08-04	Leroyer/St-Pierre	5.0
Rock-Forest	1998-06-30	Sous les Charmilles	1.4
Sabrevois	1998-06-23	Des Pins	2.2
Saint-Grégoire	1998-06-23	Clos de la Montagne	2.8
Stanbridge	1998-06-25	Domaine de l'Ardennais	2.0

1963). Plant-parasitic nematodes were counted by using a stereo-microscope and identified to the genus.

Entomopathogenic nematodes were isolated from soil by using wax moth larvae (*Galleria melonella* L.) [Lepidoptera : Galleriidae] as bait according to the method of Bedding and Akhurst (1975). Soil samples (400 g) were placed in 500-ml plastic containers covered with a lid, and five *Galleria* larvae were added. Each plastic container was incubated at room temperature (22°C) for 5 d. If no larvae died after 5 d, we considered that entomopathogenic nematodes were absent from that sample. Dead *G. melonella* larvae were removed and placed on a moistened filter paper (Whatman No. 2) in a 90-mm Petri dish at room temperature (22°C). Each Petri dish was checked every 2 d for nematodes. After 10 d, all decayed and/or filthy odoured cadavers were discarded. Emerging nematodes from well-preserved, odorless cadavers and with apparent synchronous larval stage were concentrated and checked for positive killing of healthy *Galleria* larvae in a Petri dish bioassay. Newly emerging nematodes from larvae were recorded as Steinermatidae, and those emerging from reddish cadavers were classified as Heterorhabditidae.

RESULTS AND DISCUSSION

Plant-parasitic nematodes isolated during this survey are grouped into two major biological classes : endoparasites and ectoparasites. The endoparasitic species are generally the most pathogenic species to cultivated plants (McElroy 1972). *Meloidogyne hapla* Chitwood and *Pratylenchus* spp. were the two endoparasites, and *Criconemoides* spp., *Gracilacus* spp., *Helicotylenchus* spp., and *Paratylenchus* spp. were the four ectoparasites recovered from the surveyed vineyards (Table 2).

Pratylenchus was the most commonly encountered endoparasitic genus, present in 85% of surveyed vineyards. The northern root-knot, *Meloidogyne hapla*, was observed in 23% of soil samples (Table 2). The results concerning the root-lesion nematode are in agreement with many plant-parasitic nematode surveys conducted in the province of Quebec, which have shown that in most agricultural regions, *Pratylenchus* was the most dominant genus (Meloche *et al.* 1980; Santerre and Lévesque 1982; Vrain and Dupré 1982; Vrain and Rousselle 1980; Willis *et al.* 1976).

Among the six genera of plant-parasitic nematodes, *Meloidogyne hapla* was the only species found in this survey that has been reported to cause economic loss in other grape-growing areas of the world (Bird and Ramsdell 1985; Pinkerton *et al.* 1999; Ramsdell *et al.* 1996). Fortunately, *Meloidogyne hapla* was limited to a small number of vineyards in Quebec and is not known to promote any serious disease on this crop.

When grapevines are planted in soil infested with high population densities of plant-parasitic nematodes, their establishment and growth will most likely be reduced (McKenry 1992). In Quebec vineyards found infested with *Meloidogyne hapla*, it is unlikely that establishment and productivity of grapevine plants will suffer, based on the low population densites (<100 nematodes 100 cm^{-3} soil) recorded after 10 yr of grapevine culture.

Pin nematodes, *Paratylenchus* spp., were the most frequently (85%) encountered ectoparasitic nematodes (Table 2). The ectoparasitic nematodes found in the current survey (Table 2) have been shown to occur in large numbers on the roots and the soil around the roots of grapes in many vineyards around the world (McElroy 1972). Unfortunately, the lack of information on the pathogenicity of these species does not allow us to determine their exact importance and effect on the grapevine culture in Quebec. There is a general consensus that these ectoparasitic species have a low impact on the plants (McElroy 1972).

Even though a nematological survey of Quebec orchards revealed the widespread distribution of *Xiphinema rivesi* Dalmasso in the apple producing areas (Vrain and Rousselle 1980), *Xiphinema* spp. were not recovered from Quebec's

Table 2. Frequency of occurence and density (minimum and maximum) of plant-parasitic nematodes in soil samples collected in 13 vineyards in Quebec

		Endoparasitic nematodes				Ectoparasitic nematodes							
Locality	no.[a]	*Meloidogyne hapla*		*Pratylenchus* spp.		*Criconemoides* spp.		*Gracilacus* spp.		*Helicotylenchus* spp.		*Paratylenchus* spp.	
Dunham #1	12	-		33	(2-36)	8	(1-1)	58	(4-30)	-		8	(4-4)
Dunham #2	4	-		75	(4-35)	-		50	(2-4)	-		75	(4-25)
Dunham #3	12	-		50	(1-8)	25	(2-9)	17	(2-2)	17	(2-6)	58	(2-110)
Grantham	12	33[b]	(22-174)[c]	33	(2-8)	-		8	(1-1)	25	(2-20)	58	(4-42)
Iberville	15	-		47	(2-150)	-		-		-		47	(2-220)
Lacolle	8	13	(25-25)	63	(4-35)	-		-		-		75	(2-340)
Magog	17	-		44	(2-20)	-		-		-		94	(4-440)
Napierville #1	11	-		18	(2-2)	9	(3-3)	82	(4-50)	-		55	(2-36)
Napierville #2	15	-		-		-		7	(5-5)	-		7	(5-5)
Rock-Forest	10	10	(1-1)	80	(1-35)	10	(1-1)	-		10	(2-2)	30	(1-9)
Sabrevois	12	-		67	(2-25)	-		25	(1-2)	8	(4-4)	25	(2-35)
Saint-Grégoire	7	-		14	(2-2)	-		-		-		-	
Stanbridge	7	-		-		14	(2-2)	-		-		-	
Occurrence in soil samples (%)	4			39		6		18		5		42	
Occurrence in vineyards (%)	23			85		38		54		31		85	

[a] no. = number of soil samples in each vineyard (total no. = 142).

[b] Frequency = percentage of occurrence in soil samples.

[c] Minimum and maximum numbers of nematodes per 100 cm^3 of soil.

vineyards. This was a surprise, since some vineyards in the Estrie region were planted in old apple orchards found to be infested with *Xiphinema* spp. in an earlier survey (Vrain and Rousselle 1980). In all major grape-growing regions of the world, *Xiphinema* spp. are generally present and some species have caused important economic damage as primary vectors of virulent plant viruses, such as the fanleaf virus of grape (Raski and Krusberg 1984) and the tomato ringspot virus (Allen *et al.* 1988).

Entomopathogenic nematodes were recovered from 85% of the surveyed vineyards (Table 3). Steinernematids were found in 11 vineyards and heterorhabditids in one vineyard (Table 3).

The relative prevalence of the Steinernematidae in comparison with the Heterorhabditidae agree with earlier surveys carried out by Mracek and Webster (1993) and Yoshida *et al.* (1998) in which they reported that steinernematids were more common than heterorhabditids.

It is easier to find steinernematid or heterorhabditid nematodes in soil habitats where high populations of susceptible insect hosts occur (Mracek and Webster 1993). Thus, there seems to be a strong relationship between the diversity and abundance of insect populations and the presence of entomopathogenic nematodes in the soil. This may provide some explanation as to why several vineyards were found to harbor a higher frequency (70-100%) of entomopathogenic nematodes, compared to the other vineyards. At the Grantham site, the vineyard was characterized by a large and diversified weed population covering more than 50% of the soil surface. At the Rock-Forest site, the entire area of the vineyard was covered by a mulch made of deciduous tree leaves that was used for weed control, winter protection, and organic matter supply. We hypothesize that both weeds and mulch created microhabitats favorable for many soil dwelling insects, therefore increasing the abundance of entomopathogenic nematodes.

Table 3. Occurence of entomopathogenic nematodes in soil samples collected in 13 vineyards in Quebec

		Frequency of positive soil samples (%)	
Locality	no.[a]	*Heterorhabditis* spp.	*Steinernema* spp.
Dunham #1	12	25	42
Dunham #2	4	–	25
Dunham #3	11	–	9
Grantham	12	–	100
Iberville	15	–	13
Lacolle	8	–	37[b]
Magog	17	–	71[b]
Napierville #1	10	–	70[b]
Napierville #2	14	–	-
Rock-Forest	10	–	70[b]
Sabrevois	12	–	25
Saint-Grégoire	7	–	14
Stanbridge	6	–	–
N total	138		
Positive sample (%)		2	39
Positive vineyard (%)		8	85

[a] no. = number of soil samples collected in each vineyard.
[b] Identified as *Steinernema carpocapsae.*

The entomopathogenic nematodes from the four vineyards that were successfully cultured under laboratory conditions were submitted for identification. Both morphological (Nguyen and Smart 1996) and molecular (Grenier *et al.* 1995) criteria have confirmed that the nematodes recovered from these four sites were all the same species, *Steinernema carpocapsae* (Table 3).

Further studies should be undertaken to investigate the relationship between the plant-parasitic nematode population densities and grapevine growth under Quebec's grapevine production system. Many factors such as plant age, cultivar, and soil type may affect the pathogenicity of each plant-parasitic nematode. With the implementation of an integrated pest management program, it will be most useful to know more about the factors involved in the presence and promotion of entomopathogenic nematodes in vineyards as a significant biological tool for the control of insect pests for the grapevine industry in Quebec.

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