

## Phytoprotection



# Plant-parasitic nematodes in Nova Scotia vineyards Nématodes phytopathogènes dans les vignobles de Nouvelle-Écosse

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### Article abstract

Plant-parasitic nematode populations were analyzed from composite soil samples collected from 62 vineyard blocks throughout Nova Scotia in 2018 and 2019. Nematode groups of potential concern that were found included ring nematodes (family Criconematidae), dagger nematodes (*Xiphinema* spp.), and root-knot nematodes (*Meloidogyne* spp.). Ring nematodes were overall the most widespread and abundant group of plant-parasitic nematodes, recovered from 79% of blocks with an overall average population density of 114 nematodes per 100 cm<sup>3</sup> soil. Ring nematodes tended to be more abundant in older blocks. DNA sequence analyses of a subset of the ring nematode populations confirmed the presence of *Mesocriconema xenoplax*, which is the species known to be damaging to and most widely associated with grapevine globally. The analyses indicated that *Criconema permistum* was also present, notably in samples with the greatest ring nematode population densities. The results indicate that ring nematodes could be affecting the health of Nova Scotia vineyards, particularly in the future as populations continue to develop in relatively young vineyards and as older blocks are replanted. Additional research is needed to delineate the distribution of *M. xenoplax* vis-à-vis other species and to experimentally assess the host-parasite relationship between *C. permistum* and grapevine.

## Plant-parasitic nematodes in Nova Scotia vineyards

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Plant-parasitic nematode populations were analyzed from composite soil samples collected from 62 vineyard blocks throughout Nova Scotia in 2018 and 2019. Nematode groups of potential concern that were found included ring nematodes (family Criconematidae), dagger nematodes (*Xiphinema* spp.), and root-knot nematodes (*Meloidogyne* spp.). Ring nematodes were overall the most widespread and abundant group of plant-parasitic nematodes, recovered from 79% of blocks with an overall average population density of 114 nematodes per 100 cm<sup>3</sup> soil. Ring nematodes tended to be more abundant in older blocks. DNA sequence analyses of a subset of the ring nematode populations confirmed the presence of *Mesocriconema xenoplax*, which is the species known to be damaging to and most widely associated with grapevine globally. The analyses indicated that *Criconema permistum* was also present, notably in samples with the greatest ring nematode population densities. The results indicate that ring nematodes could be affecting the health of Nova Scotia vineyards, particularly in the future as populations continue to develop in relatively young vineyards and as older blocks are replanted. Additional research is needed to delineate the distribution of *M. xenoplax* vis-à-vis other species and to experimentally assess the host-parasite relationship between *C. permistum* and grapevine.

Keywords: Criconematidae, nematode distribution, *Meloidogyne*, *Mesocriconema*, *Xiphinema*.

### [Nématodes phytopathogènes dans les vignobles de Nouvelle-Écosse]

Les populations de nématodes phytopathogènes ont été analysées à partir d'échantillons de sol composites prélevés dans 62 parcelles de vignobles dans toute la Nouvelle-Écosse en 2018 et en 2019. Les groupes de nématodes potentiellement préoccupants qui ont été trouvés comprenaient des nématodes annelés (famille Criconematidae), des nématodes dagues (*Xiphinema* spp.) et des nématodes à galles (*Meloidogyne* spp.). Les nématodes annelés étaient globalement le groupe de nématodes phytopathogènes le plus répandu et le plus abondant, récupérés dans 79 % des blocs avec une densité de population moyenne globale de 114 nématodes par 100 cm<sup>3</sup> de sol. Les nématodes annelés avaient tendance à être plus abondants dans les blocs plus anciens. Des analyses de séquences d'ADN d'un sous-ensemble des populations de nématodes annelés ont confirmé la présence de *Mesocriconema xenoplax*, qui est l'espèce connue pour être nuisible à la vigne et la plus largement associée à la vigne dans le monde. Les analyses ont indiqué que *Criconema permistum* était également présent, notamment dans les échantillons présentant les plus fortes densités de population de nématodes annelés. Les résultats indiquent que les nématodes annelés pourraient affecter la santé des vignobles de la Nouvelle-Écosse, en particulier à l'avenir, alors que les populations continuent de se développer dans les vignobles relativement jeunes et que les blocs plus âgés sont replantés. Des recherches supplémentaires sont nécessaires pour délimiter la distribution de *M. xenoplax* vis-à-vis d'autres espèces et pour évaluer expérimentalement la relation hôte-parasite entre *C. permistum* et la vigne.

Mots-clés : Criconematidae, distribution des nématodes, *Meloidogyne*, *Mesocriconema*, *Xiphinema*.

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## INTRODUCTION

While early European settlers likely planted some of the first grapevines in Canada in Nova Scotia in the 1600s, the commercial wine grape industry did not begin to develop in Nova Scotia until the early 1980s (Naugler *et al.* 2004). Since then, the Nova Scotia wine grape industry has undergone rapid expansion and growth, particularly in recent years (Rimmerman 2017). The wine grape growing regions within Nova Scotia have fewer growing degree days (GDDs) and shorter growing seasons than other major wine grape regions in Canada, such as those located in British Columbia, Southern Ontario and Quebec, which makes them unique.

Plant-parasitic nematodes are significant pests of grapevines in most major viticultural regions of the world, particularly those with Mediterranean climates (Kapp *et al.* 2013; Nicol *et al.* 1999). Nematode infestations are slow to spread within perennial production systems and significant infestations throughout vineyards can take years to develop. Consequently, potential nematode problems are often overlooked in relatively young viticultural regions such as Nova Scotia. As well, the role of plant-parasitic nematodes in cool-climate viticulture is generally less well known than for regions with Mediterranean climates. In Canada, Bélair *et al.* (2001) reported the presence of several groups of plant-parasitic nematodes among thirteen vineyards in Quebec, and Forge *et al.* (2021) recently reported the presence of several groups of plant-parasitic nematodes among 57 vineyards in British Columbia. The objective of this survey was to develop baseline knowledge of the types of plant-parasitic nematodes present in Nova Scotia vineyards.

## MATERIALS AND METHODS

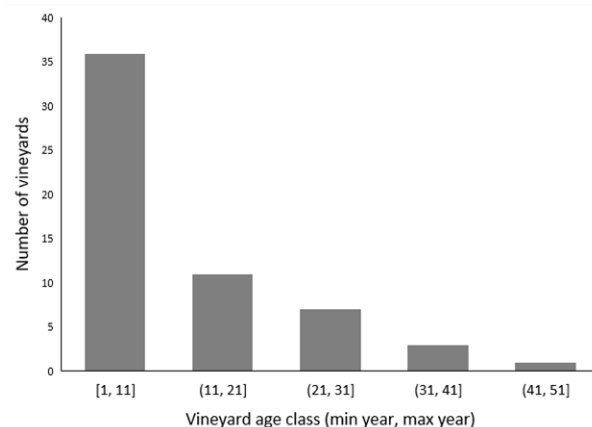
A total of 62 soil samples, each representing a distinct vineyard block, were collected in early fall of 2018 (37 samples) and 2019 (25 samples) from vineyards in Nova Scotia. Fifty-one of the total 62 samples were collected from the 126 km Annapolis Valley, which spans central Kings and Annapolis Counties and is the location of the majority of the vineyards located within Nova Scotia. The remaining samples were collected from vineyards located in the adjacent Gaspereau Valley (also Kings County) as well as Cumberland and Lunenburg Counties, in northern and southern Nova Scotia, respectively. The vineyards included in this survey were located on sandy loam to loam podzols formed on glacial till, with Kentville, Wolfville and Woodville soil type series accounting for most sites (Harlow and Whiteside 1943). The vineyard blocks ranged in age from one year (planted the year of sampling) to 50 years, with over 55% being planted within ten years of sampling (Fig. 1). The majority of the blocks were hybrid varieties including (number of blocks in parentheses): Baco Noir (5), Geisenheim (5), L'Acadie Blanc (3), Lean Millot (6), Marechal Foch (3), Marquette (6), Muscat (6), Seyval Blanc (7) and Vidal Blanc (5); with 12 blocks being varieties of *Vitis vinifera*: Riesling (6), Chardonnay (3), and Pinot Noir (2). Forty-eight of the blocks were self-rooted while fourteen were grafted on rootstocks, eleven of which were 3309C.

In each vineyard block, approximately 30 cores were taken to a depth of 30 cm across multiple rows in a manner that best represented the dimensions of the block. The cores were collected approximately 30 cm out from vine trunks and varied between 0, 45 and 90 degrees out from the vine row so that approximately ten cores came from each of the three positions relative to the row axis. The 30 cores were combined in a bucket, mixed, and a subsample was collected into a labelled polyethylene bag. The samples were kept refrigerated and

shipped to the Agriculture and Agri-Food Canada Summerland Research and Development Centre in British Columbia for nematode analyses. A wet sieving-sucrose centrifugation procedure was used to extract nematodes from triplicate 100 cm<sup>3</sup> subsamples from each composite sample (Forge and Kimpinski 2007). Plant-parasitic nematodes in each extract were identified to the genus and counted using an inverted microscope with a gridded counting dish.

After counting, samples from the five sites with the greatest population densities of nematodes in the family Criconematidae (ring nematodes) were preserved in DESS (Yoder *et al.* 2006) for subsequent species-level identification via DNA barcoding. For DNA barcode analyses, ten individual specimens were hand-picked from each of the five samples, DNA was extracted from each specimen, and a portion of the mitochondrial cytochrome oxidase subunit 1 gene (COI) was amplified using primers and procedures described in Olsen *et al.* (2017). The 719 bp product was then compared to accessions in NCBI Genbank via the BLAST procedure.

Relationships between vineyard block age and population densities of each major group of nematodes were analyzed using simple linear regression and by allocating the blocks into three age classes and then performing analysis of variance to test the effect of age class on population densities. The age classes were > 19 years (planted before 2000,  $n = 13$ ), between 19 and 9 years (planted between 2000 and 2010,  $n = 13$ ), and < 9 years (planted since 2010,  $n = 32$ ). Vineyard age information was not available for four of the vineyards sampled and they were omitted from these analyses.



**Figure 1.** Frequency distribution of Nova Scotia vineyard blocks sampled for this study in five decadal age classes

## RESULTS AND DISCUSSION

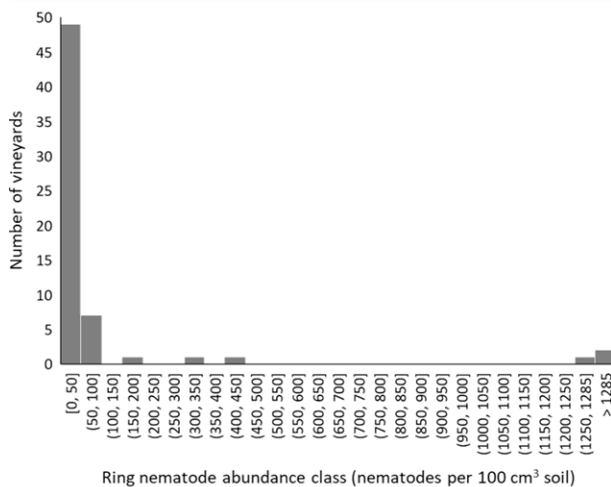
Seven groups of plant-parasitic nematodes of potential interest were recovered from the sampled vineyards: ring nematodes (family Criconematidae), dagger nematodes (*Xiphinema* spp.), root-knot nematodes (*Meloidogyne* spp.), root-lesion nematodes (*Pratylenchus* spp.), pin nematodes (*Paratylenchus* spp.), spiral nematodes (family Hoplolaimidae, genera *Helicotylenchus* and *Rotylenchus*), and stunt nematodes (Belonolaimidae, Telotylenchinae). Spiral and stunt nematodes are well-known associates of grasses and forbs that grow in vineyards and orchards, and neither group has previously been implicated in the parasitism of the grapevine. Consequently, they will not be discussed further in this report.

**Table 1. Percentage of positive vineyard blocks (out of 62 sampled), mean, median and maximum population densities (nematodes per 100 cm<sup>3</sup> soil), standard deviation and skewness, of the five major groups of plant-parasitic nematodes found in Nova Scotia vineyards. A total of 37 blocks were sampled in October 2018 and 25 blocks in October 2019.**

	Nematode groups				
	Ring	Dagger	Root-knot	Lesion	Pin
Prevalence (%)	79	57	42	82	82
Mean	114	22	11	9	19
Median	7	1	0	3	5
Maximum	2439	205	189	105	176
Standard Deviation	390	41	30	17	37
Skewness	4.72	2.56	4.17	3.77	3.04

## Ring nematodes

Members of the Criconematidae were found in 79% of the 62 blocks sampled over the two years (Table 1). It was the most abundant group of plant-parasitic nematodes, with average and maximum population densities of 114 and 2439 nematodes per 100 cm<sup>3</sup> soil, respectively, across the 62 blocks. Among positive blocks the average population density was 144 nematodes per 100 cm<sup>3</sup>. The distribution of ring nematode population densities was also more highly skewed than for other groups of plant-parasitic nematodes, with 80 and 90 percent of sites having population densities less than 50 and 100 nematodes per 100 cm<sup>3</sup> soil, respectively (Table 1; Fig. 2).



**Figure 2. Distribution of Nova Scotia vineyards among ring nematode population density size classes**

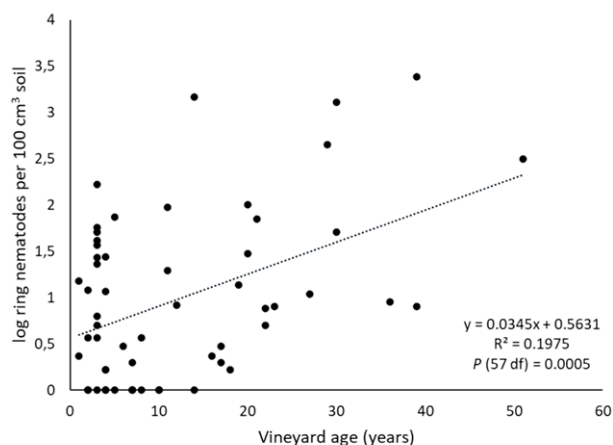
The prevalence of ring nematodes in Nova Scotia vineyards is comparable to the Okanagan Valley of British Columbia where Forge *et al.* (2021) reported ring nematodes in 82% of 57 vineyards. The prevalence of ring nematodes contrasts, however, with Quebec where *Criconemoides* spp. were reported in 38% of the thirteen vineyards surveyed, with no population

densities greater than 9 nematodes per 100 cm<sup>3</sup> soil (Bélair *et al.* 2001). The apparent differences in prevalence of ring nematodes between Nova Scotia and Quebec could be the result of different extraction methods utilized. The Quebec survey used a modified Baermann pan extraction method, which is not as efficient for extraction of ring nematodes than wet sieving-sucrose centrifugation (Viglierchio and Schmitt 1983).

Morphological features of many of the ring nematode populations were consistent with *Mesocriconema xenoplax* (Raski, 1952) Loof & De Grisse, 1989, the species most widely associated with cultivated woody perennial crops globally, but other morphotypes were observed. BLAST analyses of the 719 bp portion of the COI gene indicated that specimens from two of the sites (GenBank accession numbers OK561949-OK561958 and OK561965-OK561969) had 99.6 and 99.9% sequence homology with known *M. xenoplax* populations, confirming the presence of *M. xenoplax* in Nova Scotia. The other three populations (GenBank accession numbers OK561943-OK561948, OK561959-OK561961, OK561962-OK561964) had > 99.5% sequence homology with multiple accessions in GenBank identified as *Criconema permistum* (Raski & Golden, 1966) Raski & Luc, 1985. Given that identification of five ring nematode populations revealed the presence of at least two different species, additional research to identify more populations to species level is warranted, but was outside the scope of this initial survey.

Research on the host-parasite relationships of ring nematodes with grapevine has been limited to one species, *M. xenoplax*. Field micro-plot experiments demonstrated that *M. xenoplax* can have significant impacts on the early growth of young grapevines in other relatively cool-climate viticultural regions such as western Oregon (Pinkerton *et al.* 2004; Schreiner *et al.* 2012) and British Columbia (Forge *et al.* 2020). Population densities of 50 *M. xenoplax* per 100 cm<sup>3</sup> soil were suggested as damage thresholds for California (McKenry 1992). It is difficult, however, to extrapolate from these previous studies to estimate the impacts that *M. xenoplax*, or other ring nematode species, may be having on the vigour of established vineyards in Nova Scotia. If more thorough species-level identifications of additional populations indicates that *C. permistum*, and perhaps other species, are found to be prevalent in Nova Scotia, it will be necessary to conduct controlled-inoculation studies to confirm their feeding on grapevine roots and determine their impacts on grapevine health.

The fact that at least a few vineyard blocks had relatively high ring nematode population densities indicates that climatic constraints are not fundamentally limiting the development of ring nematode populations in Nova Scotia. We speculate that the large number of blocks with very low ring nematode population densities is a function of the relatively young age of most vineyards in Nova Scotia. Ring nematode abundance tended to increase with block age (Fig. 3; regression  $P = 0.0005$ , 57 df). The analysis of variance also indicated a significant effect of block age class on population densities of ring nematodes (ANOVA  $P = 0.001$ ), with average population densities of 17, 125 and 365 ring nematodes per 100 cm<sup>3</sup> for the vineyards planted since 2010, between 2000 and 2010, and before 2000, respectively. Although *M. xenoplax* has the potential for rapid population growth after becoming introduced to a good host under appropriate environmental conditions (Forge *et al.* 2020; Pinkerton *et al.* 2004), at the scale of entire vineyard blocks it may nonetheless take many years for point infestations to become sufficiently widespread to be manifest as high nematode counts in composite soil samples. We speculate that overall population densities of *M. xenoplax* will likely increase through time in Nova Scotia vineyards, and the species will become a more significant pest in the future, particularly as the current generation of vineyards are replanted.



**Figure 3. Relationship between vineyard age and population densities of ring nematodes**

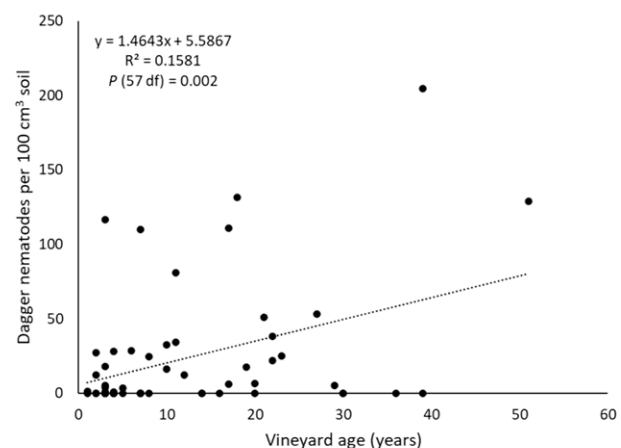
## Dagger nematodes

*Xiphinema* spp. were found in 57% of the vineyards with an overall average population density of 22 nematodes per 100 cm<sup>3</sup> soil (Table 1). Like ring nematodes, *Xiphinema* spp. abundance tended to increase with the vineyard age class (Fig. 4; regression  $P = 0.002$ , 57 df). The analysis of variance also indicated a significant effect of block age class on *Xiphinema* population densities (ANOVA  $P = 0.02$ ), with average population densities of 12, 34 and 41 *Xiphinema* per 100 cm<sup>3</sup> for the vineyards planted since 2010, between 2000 and 2010, and before 2000, respectively.

Species-level identification of the *Xiphinema* populations was outside the scope of this study. However, all dagger nematodes observed had morphological characteristics conforming to the *X. americanum*-complex of species, *X. americanum sensu lato*. Ebsary *et al.* (1984) previously reported *X. americanum* from apple in Kentville, NS. Both *X. americanum sensu stricto* and *X. rivesi*, which was previously included in the *X. americanum*

species group, have been reported from Quebec (Ebsary *et al.* 1984; Vrain and Rousselle 1980). However, no *Xiphinema* populations were found in Quebec vineyards surveyed in 1998 (Bélair *et al.* 2001). In contrast, Allen *et al.* (1988) demonstrated that *X. rivesi* was present in 21 of 27 Ontario vineyards, and was highly associated with tomato ringspot virus. Similarly, in British Columbia *Xiphinema* populations presumed to be *X. bricolensis* were found in 77% of 57 vineyards (Forge *et al.* 2021).

The direct impacts of *X. americanum*-group species on vine growth are poorly understood. They are, however, vectors of several nepoviruses of concern to viticulture in North America, including tomato ringspot virus and tobacco ringspot virus (Brown *et al.* 1994). If *X. americanum*-group species are found to be present in vineyard blocks where a nepovirus is detected, then removal of virus-infected vines would be inadequate to stop the spread of the virus. In such situations it is necessary to also treat the soil to suppress the dagger nematodes. Currently, there are no records of these viruses in Nova Scotia, but the widespread occurrence of *Xiphinema* populations will present additional management challenges for sites where any of these viruses should occur in the future. Other nepoviruses of significant concern for grape production in other major grape-growing regions include grapevine fanleaf virus and arabis mosaic virus, of which only grapevine fanleaf virus has been reported in Nova Scotia vineyards to date (Poojari *et al.* 2020). These two viruses appear to be specifically vectored by *X. index* Thorne & Allen, 1950, and *X. diversicaudatum* (Micoletzky, 1927) Thorne, 1939, respectively, neither of which exists in Canada (Robbins and Brown 1991).



**Figure 4. Relationship between vineyard age and population densities of dagger nematodes, *Xiphinema* species**

## Root-knot nematodes

*Meloidogyne* spp. were the least widespread of the nematode groups found in this survey, being present in 42% of the sites (Table 1). *Meloidogyne* spp. abundance did not vary with vineyard age in this study. Globally, *Meloidogyne* species are the most damaging group of nematodes to grapevines and are the focus of rootstock breeding programs (Ferris *et al.* 2012). The three most damaging species, *M. incognita* (Kofoid & White, 1919) Chitwood, 1949, *M. arenaria* (Neal, 1889) Chitwood, 1949, and *M. javanica* (Treub, 1885) Chitwood, 1949, are unable to persist where the soil freezes and none of them have established anywhere in Canada. The fourth most important

species is the “northern root-knot nematode”, *M. hapla* Chitwood, 1949, which is widespread on horticultural crops throughout Canada (Bélair *et al.* 2018) and was reported in 23% of Quebec vineyards (Bélair *et al.* 2001) and 25% of British Columbia vineyards (Forge *et al.* 2021). Self-rooted *V. vinifera* vines are excellent hosts for *M. hapla*, but the species appears to be less damaging to its grapevine hosts than the other species of *Meloidogyne* (Zasada *et al.* 2019). Zasada *et al.* (2019) recently demonstrated that many common grape rootstocks appear to be at least partially resistant to *M. hapla* (Zasada *et al.* 2019). As sedentary endoparasites, *Meloidogyne* spp. have more punctuated life-cycles than ectoparasites, and the presence of the infective second-stage juveniles (J2) in soil can vary considerably within a growing season. East *et al.* (2019) found that for *M. hapla* on grapevines in Washington state there was only one generation per growing season, with J2 numbers being negligible in midsummer and peaking in late fall. It is not clear if *M. hapla* population dynamics would be similar in Nova Scotia, and it is possible that the population densities recorded in this survey, which involved sampling in October, represent potentially larger infestations.

### Root-lesion nematodes

Overall, *Pratylenchus* was the most widespread genus, being found in 82% of sites (Table 1). However, the role of these populations as parasites of grapevine is questionable. The species *P. vulnus* Allen & Jensen, 1951 is widely recognized to be a serious pest of grapevines, primarily in Mediterranean regions of the world (Castillo and Vovlas 2007; Chitambar and Raski 1984). However, *P. vulnus* is not known to exist anywhere in Canada and little is known of the pathogenicity to grapevine of other species. *P. penetrans* (Cobb, 1917) Filipjev & Schuurmans Stekhoven, 1941, and *P. crenatus* Loof, 1960 have been reported from Nova Scotia, and *P. flakkensis* Seinhorst, 1968 and *P. pratensis* (de Man, 1880) Filipjev, 1936 have been reported from nearby maritime provinces (Yu 2008). Published data on the relationship between *P. penetrans* and grape are limited and equivocal (Ramsdell *et al.* 1996), and the host status of grapevine for *P. crenatus*, *P. flakkensis* and *P. crenatus* has not previously been studied. Forge *et al.* (2021) found *P. penetrans* and *P. neglectus* (Rensch, 1924) Filipjev & Schuurmans Stekhoven, 1941 in Okanagan vineyard soils, but also found that neither species was able to parasitize grapevines in greenhouse pot experiments. They speculated that *P. penetrans* and *P. neglectus* were persisting in vineyard soils by feeding on weeds and grasses in the alleyways. Similarly, we speculate that the populations of *Pratylenchus* recovered from Nova Scotia vineyards in this survey are not of significance for vine health. Future research should nonetheless identify the species extracted from grape roots and rhizosphere soil in Nova Scotia vineyards, and conduct experiments to directly assess the potential for those species to parasitize grapevine.

### Combined infestations

Previous studies on the pathogenicity of *Mesocriconema*, *Xiphinema*, *Meloidogyne* or *Pratylenchus* to perennial fruit crops have universally employed single-species inoculations. Most orchards and vineyards are, however, infested with multiple species. In this study, 15%, 44% and 21% of vineyards were infested with two, three or four of the four main groups of nematodes discussed here. The high frequency of multi-species infestations indicates that experimental analyses of the interactive effects of multiple species on grapevine health are needed to obtain a realistic understanding of the impacts of plant-parasitic nematodes in Nova Scotia vineyards.

## CONCLUSION

Of the five groups of plant-parasitic nematodes found in Nova Scotia vineyards, three are known as parasites of grapevine and could be impacting grapevine health. Ring nematodes (family Criconematidae) were present in 79% of the vineyards and the species *M. xenoplax*, which is a known pest of grapevine, was confirmed to be present. Analyses indicated that another species, potentially *C. permistum*, was also present. A high proportion of the vineyards had very low ring nematode population densities, indicating that ring nematodes are probably not currently having widespread effects on grapevine health and productivity across the region. However, the nascent ring nematode populations at such sites are expected to grow and will likely affect productivity in the future, particularly when such vineyards are replanted. Additional research is needed to reveal the distribution of *M. xenoplax* vis-à-vis other species of ring nematodes in Nova Scotia vineyards, and to examine the potential impacts of species other than *M. xenoplax* on grapevine health and productivity.

Dagger nematodes in the *X. americanum sensu lato* group of species were found in 57% of vineyards. These nematodes are generally not known to be particularly damaging on their own, but as vectors of nepoviruses the presence of these nematodes could pose significant problems if and when vines become infected with any nepoviruses. Northern root-knot nematodes (*M. hapla*) were present in 42% of the vineyards and at relatively low population densities. Northern root-knot nematodes are generally not as damaging as other major species, such as *M. incognita*, *M. arenaria* and *M. javanica*, which are limited to regions where the soil doesn't freeze. Similar to ring nematodes, these nematodes could become more problematic if populations continue to expand as the wine grape industry matures. Root-lesion nematodes (*Pratylenchus* spp.) were the most widespread group, in 82% of vineyards sampled. None of the species known to occur in Nova Scotia have been reported to be pathogenic to grapevine, but additional research on the species identities and their ability to parasitize grapevines is warranted.

While none of the populations of plant-parasitic nematodes currently appear to be posing a significant threat to the overall productivity of Nova Scotia vineyards, their presence indicates the likelihood of future impacts as the established populations grow and as complicating factors such as the introduction of nepoviruses occur.

## ACKNOWLEDGEMENTS

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