Research

Shifting Prevalence of Plant-Parasitic Nematodes in Orchards and Vineyards of the Okanagan Valley, British Columbia

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Abstract

Fruit production in the Okanagan Valley of British Columbia is dominated by apple, sweet cherry, and wine grape. The relative importance of sweet cherry and grape has increased in recent decades, but little was known of the plant-parasitic nematodes associated with those crops. Soil samples analyzed for plant-parasitic nematodes were collected from a total of 39 apple orchards, 61 cherry orchards, and 57 vineyards; most were collected in 2018, but 36 cherry orchards were sampled in 2012. Soil properties were also assessed and related to nematode population densities. Nematode genera of potential significance were, in order of prevalence, *Pratylenchus*, *Mesocriconema*, *Xiphinema*, *Paratylenchus*, *Paratrichodorus*, *Hemicycliophora*, and *Meloidogyne*. *Pratylenchus* were found in 79, 98, and 81% of the apple, cherry, and grape plantings, respectively; *Mesocriconema* were found in 51, 79, and 82%; and *Xiphinema* were found in 59, 51, and 77%. Population densities of the three dominant genera were influenced more by soil texture than any other soil characteristics, with *Pratylenchus* being negatively correlated with percentage clay, *Mesocriconema* positively correlated with percentage sand, and *Xiphinema* positively correlated with percentage silt. The high prevalence of *Mesocriconema* in cherry orchards and vineyards in this region is significant because *Mesocriconema* is known to be an important pest of other *Prunus* crop species and grapevines in other regions. This study therefore provides a rationale for increasing grower awareness and research efforts on the impacts and management of *Mesocriconema* and other plant-parasitic nematodes in orchards and vineyards in the region.

Keywords: *Pratylenchus*, *Mesocriconema xenoplax*, *Xiphinema*, apple, sweet cherry, wine grape

The Okanagan Valley of British Columbia hosts production of high-value perennial fruit crops, with apple, sweet cherry, and wine grape being the three most important crops. Apple orchards were first planted in the early 1900s, and by the mid-1970s there was an estimated 10,200 ha of apple orchards complemented by approximately 200 ha of sweet cherry and wine grapes each. In the early 1990s, planting of sweet cherry and wine grape began to increase dramatically at the expense of apple acreage. Current estimates for the region are 3,500 ha of apple, 2,050 ha of sweet cherry, and 3,500 ha of wine grapes, with farm gate values for 2018 of $60, $89, and $73 million dollars, respectively (British Columbia Ministry of Agriculture and Lands 2020).

Surveys conducted in the 1950s (Mulvey 1955) targeted root-lesion nematodes (*Pratylenchus* spp.) infecting roots of apple, because root-lesion nematodes had already been associated with poor growth of apple trees (Ark and Thomas 1936). These surveys indicated that root-lesion nematodes were widespread in apple orchards in the region, whereas a few other taxa including a species of ring nematode, *Criconemoides* Raski, 1952, were recorded in a few soil samples only. In 1984, Vrain and Yorston (1987) sampled 58 apple orchards and 52 orchards of *Prunus* sp. fruit crops (32 peach, 11 cherry, five apricot, and four plum) in the region. Consistent with the 1955 survey, root-lesion nematodes were the most prevalent group of plant parasites, being found in 78% of the orchards sampled. The ring nematode, *Criconemella xenoplax* (Raski 1952) Luc & Raski 1981 (=*Mesocriconema xenoplax* [Raski 1952] Loof and De Grisse 1989), was present in 2% of orchards, but the data presented for *M. xenoplax* did not differentiate between apple and *Prunus* sp. orchards. Dagger nematodes (*Xiphinema* spp.) identified as *Xiphinema occiduum* (Ebsary et al. 1984) were reported from 1.5% of the orchards. Vineyards in the Okanagan Valley were sampled in 1987 using extraction methods targeting *Xiphinema* spp. only (Graham et al. 1988). That survey indicated that *X. pacificum* and *X. bricolensis* were found in 95% of the 79 vineyard blocks sampled (Graham et al. 1988). No data on other nematode species were reported.

Sweet cherry and grape host a number of nematode species that apple does not (Nyczepir and Becker 1998). Of particular note is the ring nematode. This species is a well-known pest of other cultivated

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Prunus species, notably peach (*P. persica*) and prune plum (*P. domestica*), but little is known of its potential impacts on sweet cherry (*P. avium*). The ring nematode is also a recognized pathogen of wine grape (Pinkerton et al. 2004).

In relation to the relatively recent shift in the prevalence of sweet cherry and wine grape acreage in the Okanagan Valley, it became evident that a comprehensive reassessment of plant-parasitic nematodes associated with all three crops was needed. The previous surveys (Mulvey 1955; Vrain and Yorston 1987) of nematodes in orchards used Baermann funnel and Baermann pan extraction methods, which are notoriously inefficient for extraction of ring nematodes (Viglierchio and Schmitt 1983). Consequently, the study we report here utilized a wet sieving-sucrose centrifugation method, which has been demonstrated to be more effective for extraction of ring nematodes (Viglierchio and Schmitt 1983).

Survey Design for Plant-Parasitic Nematodes in Apple and Sweet Cherry Orchards and Wine Grape Vineyards

The primary survey was conducted in 2018, as part of a broader study of the influences of horticultural production practices on soil organic matter content and spatial distribution (Midwood et al. 2020). Sites were selected based on the range of soil types most commonly found in orchards and vineyards in the Okanagan Valley, with no a priori indication of whether nematodes were affecting the crops in question. We first identified five broad groups of soils with different surficial deposit classifications or parent materials, representing a range of soil textures (Table 1). The five groups of soils are hereafter referred to by the name of the predominant soil series (Wittneben 1986) within each group, namely, Armstrong, Westbank, Rutland, Penticton, and Osoyoos (Table 1). A geographic information systems database for the region was then used to identify three to five sites of each of the following four cropping systems within each soil group: (i) drip-irrigated apple orchards, (ii) understory sprinkler-irrigated apple orchards, (iii) understory sprinkler-irrigated cherry orchards, and (iv) drip-irrigated vineyards. All orchards and vineyards were at least 10 years old and were fruit-bearing commercial plantings at the time of sampling. As much as possible, the three to five sites of each soil × cropping system combination were selected to span the north-south extent of the valley (49.00° to 50.21° latitude).

All sites were sampled between April and August of 2018. At each site, a relatively uniform and representative area of approximately 0.5 ha was selected for sampling. Within this sampling area, soil cores (0 to 15 cm and 15 to 30 cm depth intervals) were collected from 10 to 20 randomly chosen locations using a 5-cm-diameter auger. Individual cores were combined to form two composite samples representing the two depth intervals for each site. All soil cores were taken from within an approximately 25- to 50-cm radius around the bases of vines or tree trunks, alternating between 0, 45, and 90° out from the axis of the tree or vine row, to ensure that the composite sample represented the root zone. In most vineyards and orchards in the region, herbicides are used to maintain a relatively weed-free strip extending approximately 50 cm out from both sides of vine or tree rows. As well, drip irrigation systems in the region typically maintain soil moisture in a band of approximately the same width, and sampling was limited to this zone of moist and relatively weed-free soil. Composite samples were placed in plastics bags and transported in coolers to the laboratory, where they were stored in a cold room (4°C) prior to nematode extractions.

All composite samples were passed through a sieve (6 mm) over a wash basin to remove rocks and organic debris. Immediately after sieving, a 25-cm^3 aliquot of each sample was frozen for subsequent microbial analyses. For each sample site, 200-cm^3 subsamples from the 0 to 15 cm and 15 to 30 cm depth intervals were then recombined to form a composite sample of 400 cm^3 representing the entire 0 to 30 cm depth prior to nematode extraction. The remaining soils were retained as separate samples by depth increment and air dried in preparation for physicochemical analyses. Soil texture was determined using the hydrometer method (Kroetsch and Wang 2008). Soil chemical analyses were conducted at ActLabs commercial laboratory (https://actlabs.com). These analyses included pH, organic matter, Olsen-extractable P, and extractable K, Ca, Mg, Na, S, Zn, Mn, B, Cu, Fe, and cation exchange capacity. Total extractable community DNA, a measure of soil molecular microbial biomass (Dequiedt et al. 2011), was extracted in duplicate from 0.3-g subsamples of each soil, using Qiagen PowerSoil DNA Isolation kits and an updated protocol based on Earth Microbiome methods (Thompson et al. 2017). Extraction reproducibility and DNA quality were verified by spectrophotometer (NanoDrop One), duplicate DNA extractions were pooled to produce one DNA sample per soil sample, and pooled DNA samples were quantified in duplicate using fluorescence-based assays (Quant-iT dsDNA BR kits; Life Technologies).

Nematodes were extracted from three replicate 100-cm^3 subsamples of each nematode sample using the centrifugal-floatation technique (Jenkins 1964). After collecting the nematodes on a 25-μm sieve and transferring with water to 20-ml glass scintillation vials, nematode samples were stored at 4°C for a maximum of 4 weeks prior to counting. Nematode samples were viewed in a gridded counting dish under an inverted compound microscope (Meiji Techno TC5100, Meiji Techno America, San Jose, CA) at 400×, and the abundance of plant-parasitic nematodes in each sample was determined at the genus level of resolution. Immediately after nematode count data were obtained, the triplicate subsamples were pooled and then transferred to DESS preservative (Yoder et al. 2006) for subsequent molecular analyses. Prior to statistical analyses, counts for each genus from the three subsamples were averaged and expressed as nematodes/100 cm^3 of soil.

In addition to the 2018 primary survey samples, data were collected from an additional 36 cherry orchards (sampled in 2012) and 32 vineyard blocks (sampled in 2018) as part of other studies. These data were obtained using similar sample collection and nematode extraction methods as those described above. However, sampling sites were chosen in consultation with growers, with the aim of characterizing the distribution of nematodes across the north-south extent of the Okanagan Valley regardless of soil type, and auxiliary soil texture and soil chemistry data were not available for these samples. Because the selection of these sample sites could have been biased toward sites already suspected of having nematode problems, data from these samples were initially subjected to statistical analyses separately from the core 2018 survey data.

For each genus, the frequency of occurrence in each of the three crops was determined and analyzed using χ^2 tests. χ^2 tests of the frequencies of occurrence of the most prominent genera (*Pratylenchus*, *Mesocriconema*, and *Xiphinema*) did not indicate significant differences between the 2012 and 2018 samplings of cherry orchards (P = 0.23, 0.83, and 0.16, respectively), so data from the 2012 and 2018 cherry orchards were combined for further analyses and presentation (Table 2). Population densities of *Pratylenchus*, *Mesocriconema*, and *Xiphinema* were compared between crops, and between drip and sprinkler-irrigated apple orchards, using one-way analysis of variance (Proc ANOVA; SAS, Cary, NC).

Dominant Genera of Plant-Parasitic Nematodes in the Okanagan Valley

A total of seven genera of plant-parasitic nematodes of potential interest to tree-fruit and grape production were recovered from the sampled orchards and vineyards: *Pratylenchus*, *Mesocriconema*, *Xiphinema*, *Meloidogyne*, *Paratylenchus*, *Paratrichodorus*, and *Hemicycliophora*. In addition, *Helicotylenchus*, *Tylenchorhynchus* (and related genera in the Tylenchidae), and second-stage juveniles of *Heterodera* were also recovered infrequently. Because no species of these latter three groups of nematodes have been reported as pests of woody perennial crops, they will not be discussed further here.

***Pratylenchus* (root-lesion nematodes).** Overall, *Pratylenchus* was the most common genus, being found in 79, 98, and 81% of apple, cherry, and grape sites, respectively (Table 2). χ^2 analyses indicated significant differences in frequency of occurrence among crop groups, with *Pratylenchus* being present in a significantly greater proportion of cherry orchards than in apple orchards and vineyards ($P < 0.001$). The percentage of apple and cherry orchards with *Pratylenchus* was slightly greater than the 78% reported by Vrain and Yorston (1987) for apple and cherry orchards combined. *Pratylenchus* were also more abundant in cherry orchards than in apple orchards and vineyards, which did not differ from each other (ANOVA $P < 0.001$; Table 2). Within apple orchards, *Pratylenchus* abundance did not differ by irrigation type, with means of 22 and 23 *Pratylenchus*/100 cm^3 of soil for drip and sprinkler irrigation, respectively. It is important to note that sampling of the drip-irrigated apple orchards was limited to the band of soil kept moist by the irrigation. A more detailed approach to sampling that separately analyzed wetted and nonwetted areas of drip-irrigated orchard floors could yield different results.

TABLE 1
Soil texture groupings and dominant named soil series (Wittneben 1986) within each soil group, crop system, and number of sites for each combination of soil group and crop system sampled during the primary survey conducted in 2018[z]

Soil texture group/dominant soil series	Crop system	Sites
Sandy to silty loams (eolian veneer over morainal deposits)	Apple – drip	4
Armstrong, Kelowna, Harrland	Apple – sprinkler	5
	Cherry	4
	Grape	5
Clays (glaciolacustrine sediments)	Apple – drip	3
Westbank, Glenmore, Summerland	Apple – sprinkler	5
	Cherry	4
	Grape	5
Loamy sands (fluvioglacial deposits)	Apple – drip	3
Osoyoos, Parkill, Trewitt	Apple – sprinkler	3
	Cherry	4
	Grape	5
Silts and silty to sandy loams (glaciolacustrine sediments)	Apple – drip	4
Penticton, Olhausen, Munson	Apple – sprinkler	3
	Cherry	4
	Grape	5
Gravelly; fluvioglacial deposits	Apple – drip	3
Rutland, Gammil, Debeck	Apple – sprinkler	3
	Cherry	5
	Grape	5
Total		82

[z] The dominant soil series in each soil group (bold font) is used as the label for the soil group in presentation of data.

To determine the species composition of the *Pratylenchus* populations, the approach of Peetz and Zasada (2016) was used. Ten individual *Pratylenchus* nematodes from each of three sites of each crop were sampled in 2018; the three sites of each crop were selected to represent north, central, and southern areas of the Okanagan Valley (Table 3). Briefly, the approach uses four different sets of primers amplifying sections of the β-1,4-endoglucanase gene that are specific for the four most common species of *Pratylenchus* in the Pacific Northwest: *P. penetrans*, *P. neglectus*, *P. thornei*, and *P. crenatus*. Our analyses were performed as described in Peetz and Zasada (2016) with the exception that DNA was extracted using the method described by Kumari and Subbotin (2012). Individual nematodes were placed in a 0.5-ml sterile centrifuge tube containing 10× PCR buffer (New England Biolabs, Ipswich, MA), 2 µl of Proteinase K (600 µg/ml), and 6 µl of sterile double-distilled molecular-grade water. Tubes were kept at −20°C for at least 30 min and then incubated at 65°C for 1 h followed by 95°C for 15 min. The DNA suspension was stored at −20°C until PCR.

The PCR products were initially identified to species level based on band size (Peetz and Zasada 2016). To confirm identification, each amplicon was directly sequenced with appropriate primers using the ABI BigDye Terminator version 3.1 kit in an ABI DNA Analyzer (Applied Biosystems, Foster City, CA). Forward and reverse DNA sequence data were assembled using BioEdit 7.0. Assembled sequences for each population were aligned with GenBank accessions using the ClustalW algorithm to verify that expected amplicons were obtained.

Based on earlier surveys (Mulvey 1955; Vrain and Yorston 1987), it has been assumed that the populations of *Pratylenchus* in Okanagan orchards are predominantly *P. penetrans*. Vrain and Yorston (1987) confirmed the presence of *P. penetrans* on the basis of morphological observations, but they did not report the proportion of sites or specimens that were identified as *P. penetrans*. Our molecular analyses of individual nematodes indicate, however, that a significant proportion of the *Pratylenchus* populations in Okanagan orchards and vineyards are currently *P. neglectus* or mixtures of *P. penetrans* and *P. neglectus* (Table 3). No nematodes tested positive for *P. thornei* or *P. crenatus*. A supplementary DNA barcode analysis of individual nematodes hand-picked from three additional cherry sites, using a COI mitochondrial gene region as reported in Ozbayrak et al. (2019), revealed the presence of a single *P. vulnus* specimen from a single cherry site, whereas six other specimens from the three additional cherry sites were identified as *P. penetrans*.

Although the *Pratylenchus* species identification presented here is not sufficiently comprehensive to accurately represent the entirety of Okanagan Valley orchards and vineyards, it clearly indicates that Okanagan orchards and vineyards are infested by more species than *P. penetrans*. Additional research is needed to confirm the presence of *P. vulnus* and to more comprehensively document the prevalence of *Pratylenchus* species across a greater number of sites in the region.

The high frequency of occurrence of *P. neglectus* is potentially significant because little is known of the pathogenicity to apple or cherry of species other than *P. penetrans*. Diagnostic laboratories

serving the region over the past three decades only report nematode counts at the genus level, but orchard managers would most likely have interpreted the *Pratylenchus* counts as being *P. penetrans* when making decisions about whether to implement nematode management treatments such as fumigation or nematicide application.

One study of seedlings in greenhouse pots indicated that *P. neglectus* reproduced on both Antonovka apple and Mazzard cherry rootstock plants. The initial population densities above 50 nematodes/100 cm^3 of soil resulted in measurable reductions in growth of both tree species over 3 months (Zepp and Szczygiel 1985). Although this one study suggests that *P. neglectus* may be as damaging as *P. penetrans*, it is important to note that this study was conducted on very small seedlings (three- to four-leaf stage) under greenhouse conditions. More research is needed to confirm and compare the damage potential of *P. neglectus* and *P. penetrans* on apple and cherry rootstocks under Okanagan field conditions.

To our knowledge, grape has never been reported as a host for *P. neglectus*, although *P. neglectus* has previously been reported from vineyard soils (Deimi and Mitkowski 2010). Published data on the relationship between *P. penetrans* and grape are limited and equivocal (Ramsdell et al. 1996). The Mediterranean species *P. vulnus* is, however, a serious pest on grapevine (Chitambar and Raski 1984), indicating the need for improved understanding of the relationships between different species of *Pratylenchus* and grapevine health. Furthermore, preliminary greenhouse trials indicated that neither *P. penetrans* nor *P. neglectus* effectively parasitize self-rooted *Vitis vinifera* grapevines (Forge et al. 2019). If these preliminary data are substantiated with further experimentation, then we may conclude that the *Pratylenchus* populations currently found in most Okanagan vineyards are predominantly *P. neglectus*, likely persisting in the sites on grasses and weeds, and represent no threat to vineyard productivity. This idea is already partially supported by a study of nematode distributions in Washington vineyards (Howland et al. 2014), in which *P. neglectus* was found to be aggregated not in the vine row but rather in the bordering alleyway where weeds were growing.

***Mesocriconema* (ring nematodes).** *Mesocriconema* was the second-most prominent genus of plant-parasitic nematode, being found in 51, 79, and 82% of apple, cherry, and grape blocks, respectively (Table 2). χ^2 analyses indicated that the frequency of occurrence of *Mesocriconema* in apple orchards was significantly lower than in cherry orchards and vineyards ($P < 0.001$), which did not differ from each other. *Mesocriconema* nematodes were also more abundant in cherry orchards and vineyards than in apple orchards (ANOVA $P < 0.001$; Table 2). Within apple orchards, *Mesocriconema* abundance did not differ by irrigation type, with means of six and 12 *Mesocriconema*/100 cm^3 of soil for drip and sprinkler irrigation, respectively.

The high frequency of occurrence of *Mesocriconema* in Okanagan vineyards contrasts with a recent survey of vineyards in nearby eastern Washington and Idaho in which *Mesocriconema* was found in only 14 and 38% of sites, respectively (Zasada et al. 2012). Reasons for such differences between these adjacent wine grape production areas are not clear, given that sucrose-centrifugation extraction methods were used for both this and the Zasada et al. (2012) study. A significant portion of the Washington acreage is planted with self-rooted varieties of *V. vinifera*, whereas the majority of vines in the Okanagan Valley are on rootstocks. However, self-rooted vines are known to be better hosts for *M. xenoplax* than most rootstocks (Forge et al. 2020; Pinkerton et al. 2005); thus, the greater prevalence of *M. xenoplax* in the Okanagan Valley cannot be explained by differences in the use of rootstocks. Morphological features of the *Mesocriconema* populations were consistent with *M. xenoplax* (https://nematode.unl.edu/mexenob.htm). To confirm species identification, two to seven individual specimens from each

TABLE 3
Number of individual *Pratylenchus* nematodes identified as *P. neglectus* or *P. penetrans*, from each of nine sites in the Okanagan Valley[z]

Crop	Subregion	*P. neglectus*	*P. penetrans*
Grape	South	10	0
Grape	Central	10	0
Grape	North	10	0
Cherry	South	8	2
Cherry	Central	7	3
Cherry	North	4	6
Apple	South	10	0
Apple	Central	10	0
Apple	North	10	0

[z] Ten individual *Pratylenchus* nematodes were hand-picked and analyzed individually from each site using species-specific primers for the β-1,4-endoglucanase gene as described by Peetz and Zasada (2016) and subsequent sequencing of the amplicons.

TABLE 2
Percentage of sites positive and mean, standard error of mean (in parentheses), and maximum population densities (nematodes/100 cm^3 of soil) of seven genera of plant-parasitic nematodes in apple and cherry orchards and wine grape vineyards in the Okanagan Valley of British Columbia[y]

Host[z]	Parameter	*Pratylenchus*	*Mesocriconema*	*Xiphinema*	*Paratylenchus*	*Paratrichodorus*	*Hemicycliophora*	*Meloidogyne*
Apple, $n = 39$	% Positive	79	51	59	59	10	10	3
	Mean	22 (5.2) b	9 (3.1) b	13 (3.8) b	38 (15)	1 (0.3)	1 (1.0)	0 (0)
	Maximum	166	102	107	394	10	39	1
Cherry, $n = 61$	% Positive	98	79	51	44	15	7	7
	Mean	59 (14) a	81 (17) a	11 (2.7) b	3 (1.3)	1 (0.3)	1 (0.7)	0 (0)
	Maximum	804	626	114	26	13	40	2
Grape, $n = 57$	% Positive	81	82	77	54	23	5	25
	Mean	26 (7) b	103 (23) a	34 (10) a	36 (14)	2 (0.9)	0 (0)	9 (3.4)
	Maximum	346	815	434	627	46	2	159

[y] Values for means within a column labeled with different letters are significantly different according to Fisher's least significant difference after one-way analysis of variance of log-transformed data.

[z] Cherry data are compilation of data collected in 2012 ($n = 25$) and 2018 ($n = 36$) surveys; apple and grape data were collected in 2018 only.

of three cherry orchards and three vineyards, for a total of 26 specimens, were hand-picked and subjected to DNA barcode analysis using the COI mitochondrial gene region (Powers et al. 2014). All specimens fell within the *M. xenoplax* species clade (haplotype groups 8 to 14 in Powers et al. 2014), with 23 of the 26 specimens belonging to a single genetic lineage (haplotype group 11) that is largely composed of specimens from native plant communities within the Great Smoky Mountains (Powers et al. 2014). Population subdivision within this lineage suggests that some geographic substructure of *M. xenoplax* haplotype group 11 exists within the Okanagan Valley. The three remaining specimens, all from one of the three vineyards, belonged to a lineage that predominately includes specimens from peach orchards in the southeastern United States. The presence of distinct lineages suggests the potential for variation in the aggressiveness of different Okanagan populations of *M. xenoplax*, as has been observed for geographically distinct populations from Oregon and California inoculated onto grape rootstocks (Pinkerton et al. 2005). Future research comparing the aggressiveness of different Okanagan populations could provide important insight on the overall impact of *M. xenoplax* on cherry and grape production in the region.

The high percentage of cherry orchards and vineyards with *M. xenoplax* is indicative of potentially significant current or future impacts on cherry and wine grape production in the region. Field microplot studies, which simulate replanting of trees or vines into already-infested soil, have demonstrated the significant impacts of *M. xenoplax* on early growth (i.e., 4 years) of grapevines in Oregon (Pinkerton et al. 2004; Schreiner et al. 2012) and British Columbia (Forge et al. 2020). The field microplot approach has also been used to demonstrate the impacts of *M. xenoplax* on peach in other regions (Cao et al. 2006), and nonfumigant nematicides were used to demonstrate impacts of *M. xenoplax* on young plum trees in California (Ferris et al. 2004). However, the direct effects of *M. xenoplax* on sweet cherry, specifically, have not been determined. Given the wide distribution of *M. xenoplax* in the region, microplot studies to assess its impacts on sweet cherry trees are warranted. *Mesocriconema* have not often been reported from apple orchards, and no controlled-inoculation studies are known to demonstrate the host status and tolerance of apple to this nematode.

The apparently low prevalence of *Mesocriconema* in surveys that preceded planting of most cherry and vineyard blocks in the region (Mulvey 1955; Vrain and Yorston 1987) leads us to speculate that most of the *Mesocriconema* infestations developed since the current cohort of cherry orchards and vineyards were planted. It is not possible to determine from these initial survey data if the current infestations all developed from the original populations detected at low frequency in earlier surveys, or if new populations were introduced with planting material. Future research on phylogenetic relationships among populations of *M. xenoplax* may provide more information. Because the impacts of *Mesocriconema* would be cumulative over the ages of the infestations, and other factors such as winter injury, viruses, and canker diseases would have developed along with the *Mesocriconema* infestations, it would be difficult to relate current *Mesocriconema* population densities to the health of existing, mature cherry orchards and vineyards.

***Xiphinema* (dagger nematodes).** *Xiphinema* was overall the third-most prominent genus of plant-parasitic nematode, being found in 59, 51, and 77% of apple, cherry, and wine grape blocks, respectively (Table 2). χ^2 analyses indicated that the frequency of occurrence of *Xiphinema* in vineyards was significantly greater (P = 0.004) than in apple and cherry orchards, which did not differ from each other. Similarly, *Xiphinema* was more abundant in vineyards than in apple and cherry orchards, which did not differ from each other (ANOVA P = 0.005; Table 2). Within apple orchards, *Xiphinema* population densities were greater under drip irrigation than under sprinkler irrigation (P = 0.03), with means of 20 and five *Xiphinema*/100 cm^3 of soil, respectively.

The frequency of occurrence of *Xiphinema* in Okanagan vineyards is comparable to the earlier survey of Graham et al. (1988), which reported *Xiphinema* in 80% of vineyard samples, and a recent survey of vineyards in nearby eastern Washington and Idaho in which *Xiphinema* was found in 59 and 75% of sites, respectively (Zasada et al. 2012).

All *Xiphinema* species identified in the Okanagan Valley to date belong to the *X. americanum* complex of species. Vrain and Yorston (1987) identified *Xiphinema* populations from Okanagan orchards as *X. occiduum*, which was later found to be a new species, *X. bricolensis* (Ebsary et al. 1989). The 1987 survey of Okanagan vineyards (Graham et al. 1988) indicated that *X. bricolensis* was present in 95% of the 79 vineyards sampled, and *X. pacificum* was identified in two of the 79 vineyards. In the context of the extensive species-level identifications that were performed in 1987, and limited resources, additional species-level identifications of *Xiphinema* specimens were not conducted as part of this survey study. The direct impacts of *X. bricolensis* and *X. pacificum* on health of fruit trees and grapevines are poorly understood. They are, however, potential vectors of several nepoviruses of concern including tomato ringspot virus, tobacco ringspot virus, and cherry rasp leaf virus. Currently, these viruses only occur sporadically in Okanagan vineyards and orchards, 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 genera. *Paratylenchus* (pin nematodes) were relatively widespread, present in 59, 44, and 54% percent of apple orchards, cherry orchards, and vineyards, respectively (Table 2). *Paratylenchus* is commonly found in the root zone of tree fruit and vineyard crops (Vrain and Yorston 1987), although there are no studies demonstrating that it can cause significant damage to fruit trees or grapevines.

Paratrichodorus (stubby root nematodes) were present in 10, 15, and 23% of apple orchards, cherry orchards, and vineyards, respectively (Table 2). *Paratrichodorus* has been found at modest frequencies and population densities in orchards and vineyards in other regions (Hugo and Storey 2017; Storey et al. 2017), and *P. minor* was found to have significant negative impacts on growth of Thompson seedless grape (Hafez et al. 1981). This nematode would warrant additional research attention if its prevalence was found to increase in the future. *Hemicycliophora* (sheath nematodes) were present in less than 10% of sites, and little is known of their potential impacts on any of these crops.

Meloidogyne (root-knot nematodes) were highly localized, being found in 25% of vineyard sites but less than 5% of apple and cherry orchards. Based on earlier diagnostic analyses (unpublished data), all *Meloidogyne* populations reported in the Okanagan Valley to date have been identified as the northern root-knot nematode, *M. hapla*. This species is known to parasitize self-rooted varieties of *V. vinifera*, but most grape rootstocks used in the Okanagan Valley appear to be at least partially resistant to *M. hapla* (Zasada et al. 2019).

Combined infestations. Previous studies on the pathogenicity of *Pratylenchus*, *Mesocriconema*, or *Xiphinema* to perennial fruit crops have universally employed single-species inoculations. Most orchards and vineyards are, however, infested with multiple species. In this study, 76 and 22% of apple orchards were infested with two or three of the three main genera of nematodes discussed here,

respectively. Such two- and three-species infestations occurred in 93 and 34% of cherry orchards, respectively, and 88 and 53% of vineyards, respectively. The high frequency of two- and three-species infestations indicates that experimental analyses of the interactive effects of two or three species on fruit tree or grapevine health are needed to obtain a realistic understanding of the impacts of plant-parasitic nematodes in these production systems.

Relationships Between Soil Properties and Population Densities of *Pratylenchus*, *Mesocriconema*, and *Xiphinema*

Relationships between nematode population densities and soil properties were analyzed for the three genera with the greatest potential economic impacts: *Pratylenchus*, *Mesocriconema*, and *Xiphinema*. The remaining genera were generally not found sufficiently frequently to facilitate reliable statistical analyses of such relationships. These analyses were also limited to data from the 2018 primary survey only (n = 39 apple, n = 25 cherry, n = 25 grape).

Analysis of variance (ANOVA, Proc Mixed; SAS) was used to assess effects of soil type groupings (Armstrong, Westbank, Rutland, Penticton, and Osoyoos), crop (cherry, apple, and grape), and soil type × crop interaction on population densities of *Pratylenchus*, *Mesocriconema*, and *Xiphinema*. For all three genera, log-transformed population data provided the best fit to the ANOVA models. There were significant main-factor effects of crop type for all three genera, as discussed above, and hereafter only the soil and soil × crop interaction effects are discussed.

Stepwise multiple regression was used to assess relationships between nematode population densities and the following soil properties: percentage sand, silt, and clay, organic matter content, total soil DNA, pH, and cation exchange capacity. To minimize the overarching effect of host species on population densities of these obligate parasites, these regressions were performed using subsets of data from the crops known to be good hosts for each of the three nematode genera: for *Pratylenchus*, regressions were conducted with data from apple and cherry sites; for *Mesocriconema*, regressions were conducted with data from cherry and grape sites; and for *Xiphinema*, regressions were conducted with data from all three crops. The regression analyses were complemented by calculation of Pearson's correlation coefficients for relationships among the predictor variables.

***Pratylenchus*.** For *Pratylenchus*, the main-factor effect of soil type was marginally significant (P = 0.052), and there was no soil × crop interaction (Table 4). *Pratylenchus* population densities were lower in the clayey Westbank soil than in all other soils.

Across the apple and cherry sites, the stepwise multiple regression identified percentage clay (P = 0.0004) and total soil DNA (P = 0.026) as significant predictors of *Pratylenchus* population densities (Table 5). The strong negative relationship with clay content (Fig. 1) supports the observed differences between soil type groupings. Previous experimental (Griffin 1996) and survey (Chałańska et al. 2016) studies have similarly reported greater *Pratylenchus* population densities in sandy and sandy loam soils than in more fine-textured soils.

Soil management practices that enhance soil microbial activity and food web structure have been hypothesized to promote the suppression of plant-parasitic nematodes (Stirling 2014). The negative relationship between total soil DNA, an indicator of microbial biomass, and *Pratylenchus* population densities is consistent with this hypothesis. However, the influence of total soil

TABLE 5
Summary of stepwise multiple regression analyses of relationships between key soil properties and population densities of *Pratylenchus*, *Mesocriconema*, and *Xiphinema*[z]

Nematodes	Model terms	*P* value
Pratylenchus	1.87086 intercept	<0.0001
(apple + cherry)	–0.022Clay	0.0004
N = 57	–0.00009DNA	0.026
Mesocriconema	–0.80 intercept	0.24
(cherry + grape)	+ 0.024Sand	0.001
N = 46		
Xiphinema	–0.55 intercept	0.38
(all crops)	+ 0.013Silt	0.02
N = 82	+ 0.23pH	0.009
	–0.00012DNA	0.006

[z] Soil properties included in the analyses were percentage sand, silt and clay, organic matter content, total soil DNA, soil pH, and cation exchange capacity. For each genus, analyses were performed using data from combinations of crops known to be hosts for relevant species in the genus.

TABLE 4
Population densities (nematodes/100 cm^3 of soil) of *Pratylenchus*, *Mesocriconema*, and *Xiphinema* in the five soil texture groups sampled during the primary survey conducted in 2018[z]

Soil type	*Pratylenchus*	*Mesocriconema*	*Xiphinema*
Westbank (clays)	6 b	20 b	10 b
Penticton (silts, silty loams)	18 a	18 bc	63 a
Armstrong (sandy-silty loams)	23 a	38 b	16 b
Osoyoos (loamy sands)	21 a	68 ab	28 b
Rutland (gravelly sands)	16 ab	120 a	6 b
Standard error	7	23	13
ANOVA summary			
Soil	0.052	0.005	0.009
Crop	0.003	0.008	0.004
Soil × crop interaction	0.290	0.996	0.069

[z] Data were analyzed using mixed-model ANOVA of log-transformed data, and separations of least-squares means were conducted using PDIFF procedure. Least-squares means and standard errors presented in the table are from analysis of nontransformed data.

DNA in the regression model was weak relative to percentage clay, with a very small negative slope coefficient (–0.00009). As well, total soil DNA was also positively correlated with clay content (r = 0.41, $P < 0.01$), suggesting that the relationship was at least partly a spurious result of the overarching influences that clay content has on both *Pratylenchus* and soil microbial biomass.

Mesocriconema. For *Mesocriconema*, the main-factor effect of soil type was highly significant, and there was no significant soil × crop interaction (Table 4). *Mesocriconema* were more abundant in the Rutland gravelly sands than in the more fine-textured Armstrong, Penticton, and Westbank soils, with the Osoyoos loamy sand soil type being intermediate and not different from either group (Table 4).

Among cherry and grape sites, percentage sand was the only significant predictor of *Mesocriconema* population densities (Table 5), with *Mesocriconema* population densities increasing with sand content (Fig. 1). These observations are consistent with previous experimental and observational research indicating that *M. xenoplax* tends to reach larger population densities in sandy soils than in more fine-textured soils (Seshadri 1964).

Xiphinema. The main-factor effect of soil type was also significant for *Xiphinema*, and there was no soil × crop interaction (Table 4). *Xiphinema* differed from *Pratylenchus* and *Mesocriconema* in that they were most abundant in the Penticton group of silt and silt loam soils (Table 4). This affinity for silty-type soils was reflected in the stepwise regression analyses in which percentage silt, pH, and total soil DNA were identified in order as significant predictors of *Xiphinema* population densities (Table 5). Unlike the relationship between *Pratylenchus* population densities, percentage clay, and total soil DNA, there were no correlations between percentage silt and total soil DNA or pH (r = 0.16 and 0.07, and P = 0.16 and 0.54, respectively; 80 degrees of freedom).

Conclusions and Future Directions

Systematic sampling and analyses of plant-parasitic nematode populations in cherry orchards in 2012 and in apple and cherry orchards and vineyards in 2018 revealed the widespread occurrence of *M. xenoplax* in cherry orchards and vineyards in the region. Although little is known of the impacts of *M. xenoplax* on sweet cherry, specifically, the known impacts of *M. xenoplax* on other *Prunus* species strongly suggest that *M. xenoplax* could be emerging as a significant pest on this highly lucrative crop. This points to a need for research to understand the impacts of *M. xenoplax* on sweet cherry tree performance.

Identification of individual *Pratylenchus* from multiple sites indicates that *P. neglectus* is more widespread than originally assumed, and it may be more widespread than *P. penetrans*, which was previously believed to be the primary species of *Pratylenchus* in orchards in the region. The majority of previous research on relationships between *Pratylenchus* and tree fruit production has been conducted with *P. penetrans*, and there are insufficient data on *P. neglectus* to assume that it is as damaging as *P. penetrans* and warrants the same level of concern. Our results indicate that research comparing the damage potential of regional populations of both *P. neglectus* and *P. penetrans* on rootstocks used in the region is needed.

The vast majority of sites (85%) were infested with two of the three taxa of primary concern (*Pratylenchus*, *Mesocriconema*, and *Xiphinema*), and 36% of sites were infested with all three taxa, indicating that research is needed to understand the interactive effects of multiple species on health of fruit trees and grapevines. Soil texture was the strongest determinant of population densities of *Pratylenchus*, *Mesocriconema*, and *Xiphinema*, but with different aspects of soil texture being of primary importance for each genus.

Rootstocks were not considered as variables in this survey but need to be considered in future research. The most widely used grape rootstocks in the Okanagan Valley, including SO-4, 3309C, and Riparia Gloire (Reynolds and Wardle 2001), are all good hosts for *M. xenoplax* (Ferris et al. 2012; Forge et al. 2020; Pinkerton et al. 2005). However, a few rarely used genotypes (420A, UCD-GRN1, and UCD-GRN5) appear to be poor hosts (Ferris et al. 2012; Pinkerton et al. 2005), indicating that rootstock choice could factor into future vineyard nematode management programs. Little is known of the differential susceptibility of cherry or apple rootstocks to either *M. xenoplax* or *Pratylenchus* species, and future microplot-

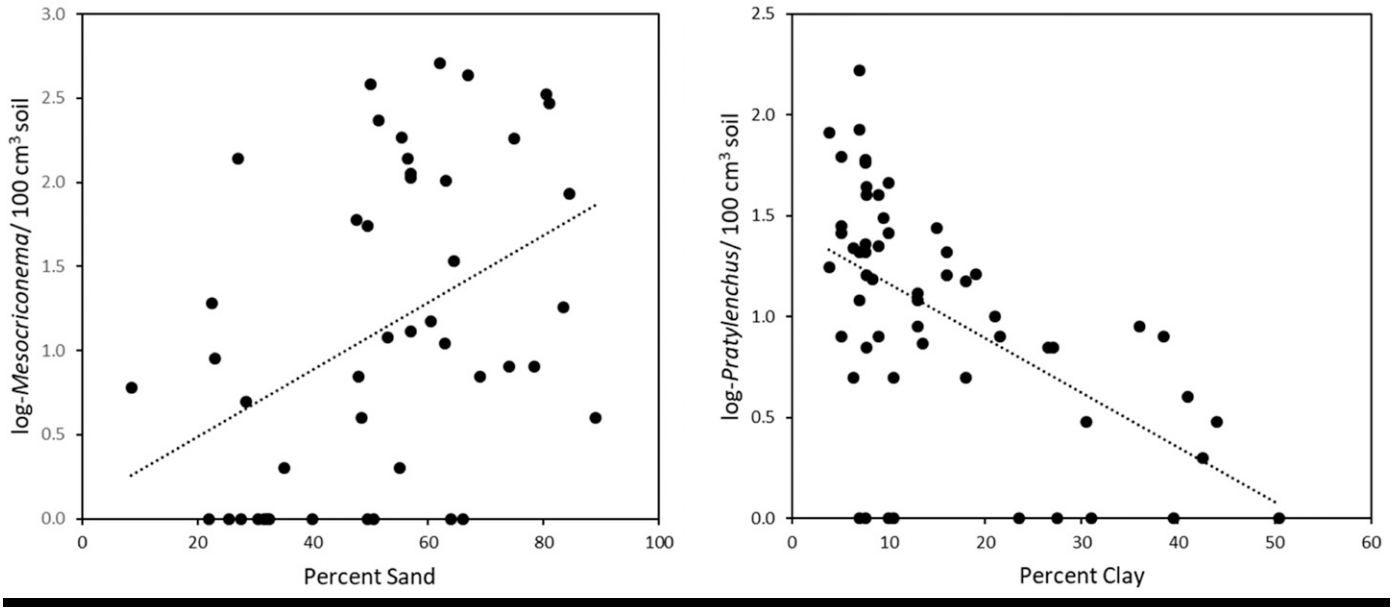

FIGURE 1

Relationships between soil percentage sand and the logarithm of population densities of *Mesocriconema* (left) and between soil percentage clay and the logarithm of population densities of *Pratylenchus* (right) in samples collected during the primary survey conducted in 2018.

type studies should include the range of commercially available rootstocks.

Due to limited availability and high cost of climatically suitable land in the Okanagan Valley, it is expected that renewal of orchards and vineyards will increasingly involve replanting into previous orchard or vineyard sites. In the case of cherry orchards and vineyards, we anticipate that the *M. xenoplax* populations now established in most such sites will have significant impacts on establishment of the replanted cherry orchards and vineyards. Previous research on replant management of tree fruit crops in the region has focused primarily on *P. penetrans* and the broader replant disease complex to which *P. penetrans* contributes (Forge et al. 2016). Future research on orchard and vineyard replant management in the region will need to refocus on the identification of nematode-tolerant rootstocks and environmentally benign soil treatments that are effective for suppressing both *M. xenoplax* and *Pratylenchus* species prior to replanting or during the first few years after replanting cherry orchards and vineyards.

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