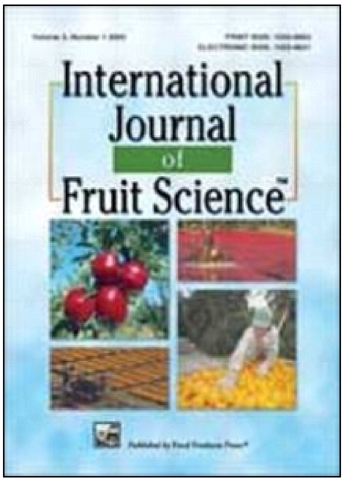

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*Blueberries (*Vaccinium *spp.) are an important agricultural crop in the Pacific Northwest of the U.S. and coastal British Columbia of Canada. Three separate surveys were conducted to elucidate the occurrence and distribution of plant-parasitic nematodes in the Pacific Northwest and British Columbia. Plant-parasitic nematodes were detected in 73% of the surveyed blueberry fields. The two most commonly encountered plant-parasitic nematodes in all geographical locations surveyed were* Paratrichodorus *spp. and* Pratylenchus *spp.* Xiphinema americanum *was also detected during the surveys, but was geographically limited to southern Washington and the Willamette Valley of western Oregon. Five other plant-parasitic nematode genera/families were detected during the surveys, but never at high frequencies.*

KEYWORDS Hemicycliophora, Paratylenchus, Helicotylenchus, Criconematidae, Vaccinium

INTRODUCTION

Blueberry production is a rapidly expanding agricultural sector in the Pacific Northwest (PNW) region of the U.S. and coastal British Columbia (BC) of

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Canada. In 2009, 5,700 and 4,500 acres of cultivated blueberries were harvested in Oregon and Washington State, respectively, with a value of US$37 and US$28 million (USDA, 2010). In recent years, blueberry production in the PNW has expanded into the more arid climate of eastern Washington. In BC, over 17,000 acres of blueberries have been planted, with over 80 million pounds harvested in 2009 (BC Blueberry Council, http://www.bcblueberry.com). The predominant species of blueberry grown in the PNW and BC is highbush blueberry (*Vaccinium corymbosum*).

The occurrence of plant-parasitic nematodes associated with blueberries has been assessed in some other blueberry-producing regions of North America. In Arkansas, five plant-parasitic nematode genera were associated with mature southern highbush blueberry with *Paratichodorous christiei* [syn. *P. minor* (Boutsika et al., 2004); here within referred to as *P. minor*] and *Xiphinema americanum* encountered at high population densities (Clark et al., 1987). A subsequent survey confirmed that *P. minor* and *X. americanum* were the major plant-parasitic nematodes associated with southern highbush blueberry in Arkansas (Clark and Robbins, 1994). Several different plant-parasitic nematodes were recovered from northern highbush blueberry roots or rhizosphere soil collected from Massachusetts, North Carolina, New Jersey, and Maryland (Goheen and Braun, 1955). Plant-parasitic nematode genera from these locations included *Tetylenchus* sp. [syn. *Merlinius* sp. (Sher, 1974); here within referred to as *Merlinius* sp.], *Meloidogyne incognita*, *Xiphinema* sp., *Trichodorus* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., and *Dolichodous* sp.; the species of *Merlinius* was found most frequently. In Massachusetts, four species of *Merlinius* and *Hemicycliophora* were widely distributed in blueberry fields (Zuckerman and Coughlin, 1960), with *Merlinius joctus* being found equally distributed in areas of poor and good blueberry growth (Hutchinson et al., 1960). In greenhouse studies, Zuckerman (1962) demonstrated that *P. minor* multiplied rapidly and significantly reduced root growth of small cuttings of northern highbush blueberry. In the 1980's, *Paratrichodorus* sp. was found causing severe damage to rooting blueberry plants in a propagation bed in Oregon (Pinkerton, personal observation).

Current knowledge of plant-parasitic nematodes associated with blueberry in the PNW and BC is limited. It is known that *X. americanum*, *Pratylenchus* spp., and *Trichodorus* sp. are present in western Oregon blueberry fields, with plant-parasitic nematodes being found in 9 of 10 fields sampled in 1982 (Converse and Ramsdell, 1982). *Xiphinema americanum* is the vector of Tomato Ringspot and Tobacco Ringspot viruses, and these viruses were also detected in the same survey. The population dynamics, distribution, and control of *Pratylenchus* sp., *Trichodorus* sp., and *X. americanum* in highbush blueberry were assessed in three Oregon fields (Schroeder, 1987). However, the impact of these nematodes on plant growth and yield was not reported. An assessment of plant-parasitic nematodes associated with blueberry in PNW regions other than Oregon, as well as in BC, has not been conducted.

The objective of this study was to determine the occurrence and distribution of plant-parasitic nematodes in blueberry fields in the PNW and BC. The results obtained are an important preliminary step in determining the potential importance of plant-parasitic nematodes to PNW and BC blueberry production and to direct future research needs.

MATERIALS AND METHODS

Soil samples were collected from established blueberry plantings during 2001, 2006, and 2008. In 2001, 76 soil samples were collected in late summer from fields that represented the geographic, site, and management diversity of blueberry production in the Willamette Valley of Oregon. Twenty soil cores (2.5 cm diameter) were collected along a "W" walk pattern in 1 to 4 acre blocks of each field. Cores were taken within 30 cm of the crown and to a depth of approximately 30 cm. Nematodes were extracted from 250 g wet soil by wet sieving-sucrose centrifugation (Jenkins, 1964). Additional site information collected in 2001 included: variety, age, vigor rating (1 to 5 with 1 being not vigorous and 5 being vigorous), bed type, mulch type, irrigation type, and ground cover.

In 2006, 53 and 44 fields were sampled in coastal BC and northern Washington, respectively. The soil samples were collected from each field in a manner similar to the 2001 survey. Nematodes were extracted from Washington soil samples by wet sieving-sucrose centrifugation (250 g wet soil). BC samples were extracted by Baermann pan method (50 g soil/pan; 7 day incubation) (Ingham, 1998). Most BC samples were also subjected to a duplicate sucrose centrifugation extraction; nematode counts from the sucrose centrifugation samples were adjusted to Baermann pan-equivalents via a correction factor and averaged with the Baermann pan counts.

In 2008, survey efforts were expanded to include the growing blueberry industry in eastern Washington as well as fields in southwestern Washington. A total of 24 fields were sampled with 76 soil samples collected. In each field a 5 row by approximately 20 plant area was selected for sampling and 20 soil cores (2.5 cm diam.) were collected. Samples were taken within 30 cm of the crown and to a depth of approximately 30 cm and bulked for nematode extraction. Nematodes were extracted from 250 g wet soil by wet sieving-Baermann funnel, with nematodes being collected from funnels after 7 days.

Plant-parasitic nematodes in each sample were identified to genus level and counted. *Paratrichodorus* spp. from a subset of samples were identified to species using morphological and molecular characteristics (Forge et al., 2009), but enumeration was done at the genus level. Nematode population densities are expressed as number/250 g soil. Plant-parasitic nematode population frequency of occurrence, average, and range for the entire PNW and BC, as well as specific geographical locations within the regions were calculated in Excel 7.0 (Microsoft, Seattle, WA, USA).

RESULTS AND DISCUSSION

Across years, plant-parasitic nematodes were detected in 72% of the blueberry fields sampled in the PNW and BC. The occurrence of plant-parasitic nematodes varied depending upon geographical location. Plant-parasitic nematodes were detected most frequently in BC blueberry fields (94%) and least frequently in eastern Washington blueberry fields (59%). A previous survey in Oregon detected plant-parasitic nematodes in 90% of sampled blueberry fields (Converse and Ramsdell, 1982); in 2001, we detected plant-parasitic nematodes in 67% of the blueberry fields surveyed in Oregon. In southern Washington, plant-parasitic nematodes were detected in 84% of surveyed blueberry fields in 2008. Plant-parasitic nematodes are known to be commonly associated with blueberry plantings in other regions, with nematodes being found in 96% to 100% of sampled blueberry fields in Arkansas (Clark et al., 1987; Clark and Robbins, 1994).

The composition of the plant-parasitic nematode community was markedly different between the geographical locations surveyed (Table 1). The most frequently encountered plant-parasitic nematode genera at all geographical locations, except southern Washington, was *Pratylenchus* spp. *Xiphinema americanum* dominated the plant-parasitic nematode community in southern Washington, with this nematode being detected in 75% of the sampled fields. *Paratrichodorus* spp. comprised a proportion of the total plant-parasitic nematode population in all geographical locations. Other plant-parasitic nematodes that were occasionally detected in PNW blueberry fields included *Paratylenchus* spp., *Meloidogyne* spp., Criconematidae, *Helicotylenchus* spp., *Hemicycliophora* spp., *X. bakeri*, and *Xiphinema* spp. The magnitude of plant-parasitic nematode community diversity in PNW and BC blueberry fields was similar to that observed in the eastern U.S. (7 nematode genera) (Goheen and Braun, 1955) and Arkansas (9 nematode genera) (Clark and Robbins, 1994) blueberry fields.

We detected two species of *Paratichodorus* in North American PNW and BC blueberry fields, *P. renifer* and *P. allius*. This was a first report for *P. renifer* on blueberry (Forge et al., 2009). *Paratrichodorus allius* was detected only in samples from eastern Washington. This nematode is commonly found parasitizing potato and is the vector of Tobacco Rattle virus, which causes corky ringspot of potato (Mojtahedi and Santo, 1999). Potato is an important crop in eastern Washington, and it is possible that the blueberry fields where this nematode was detected were cropped with potato in

TABLE 1 Mean and Range Population Densities and Frequencies of Detection of Plant-Parasitic Nematode Genera and Families Encountered in Soil Samples Collected from Pacific Northwest of the U.S. and Coastal British Columbia of Canada Blueberry Fields during 2001, 2006, and 2008[a]

Location[b]	*Paratrichodorus*	*Paratylenchus*	*Pratylenchus*	*Meloidogyne*	Criconematidae	*Helicotylenchus*	*Hemicycliophora*	*X. americanum*	*Xiphinema* spp.
Mean # nematodes/250 g soil (range # nematodes/250 g soil)									
BC	82 (0–1820)	5 (0–140)	33 (0–423)	1 (0–45)	0 (0–5)	6 (0–145)	0 (0–10)	0	1 (0–25)
OR	5 (0–153)	8 (0–393)	10 (0–87)	<0 (0–7)	2 (0–122)	2 (0–70)	0	5 (0–103)	0
E. WA	2 (0–26)	<0 (0–4)	10 (0–152)	1 (0–56)	<0 (0–2)	0	<0 (0–12)	0	0
S. WA	24 (0–32)	5 (0–152)	10 (0–48)	0	0	0	0	30 (0–100)	0
N. WA	13 (0–280)	3 (0–20)	13 (0–120)	0	<0 (0–5)	10 (0–360)	0	0	1 (0–30)
Frequency of detection relative to total number of soil samples collected									
BC	0.62	0.23	0.79	0.02	0.02	0.21	0.02	0	0.06
OR	0.14	0.18	0.43	0.01	0.05	0.09	0	0.21	0
E. WA	0.20	0.04	0.41	0.02	0.02	0	0.04	0	0.08
S. WA	0.29	0.08	0.33	0	0	0	0	0.75	0
N. WA	0.23	0.32	0.66	0	0.02	0.18	0	0	0.11

[a]Soil samples (2.5 cm diameter × 30 cm deep) were collected from within the root zone of established blueberries and extracted from soil. Plant-parasitic nematodes in each sample were identified to genus level and counted.

[b]BC = Coastal British Columbia, Canada; OR = Oregon; E. WA = eastern Washington; S. WA = southern Washington; N. WA = northern Washington.

the past. *Paratrichodorus* sp. and *Trichodorus* sp. have been reported to be commonly associated with blueberry in Arkansas (Clark and Robbins, 1994; Clark et al., 1987), Oregon (Converse and Ramsdell, 1982; Schroeder, 1987), Maryland, and North Carolina (Goheen and Braun, 1955). While the frequency of occurrence varied between geographical locations considered in our survey (Table 1), *P. renifer* was consistently detected. Population densities of *P. renifer* tended to be the highest in BC followed by southern and northern Washington (Fig. 1). Most fields had relatively low population densities; less than 10% of the blueberry fields sampled had *P. renifer* population densities >51 nematodes/250 g soil (Fig. 1). The mean population density of *Paratrichodorus* spp. in our survey was much lower than previously reported. The mean number of *Trichodorus* sp. found in three Oregon blueberry fields was 142 nematodes/250 g soil (Schroeder, 1987). In Arkansas (Clark et al., 1987), the mean population level of *P. minor* from 32 samples was 263 nematodes/250 g soil. Converse and Ramsdell (1982) detected *Paratrichodorus* spp. in three out of 10 fields sampled, but did not report population densities.

One of the most interesting findings of these surveys was the limited distribution of *X. americanum* in PNW blueberry. It is important to note that while the *Xiphinema* sp. encountered in this study was morphologically similar to *X. americanum*, and thus identified accordingly, there is taxonomic controversy in this nematode group, with many "species" possessing overlapping morphological characteristics (Robbins, 1993). Converse and Ramsdell (1982) detected *X. americanum* in 4 of 10 sampled blueberry fields in Oregon, and this nematode was detected in 21% of fields in our 2001 survey of Oregon blueberry fields. We detected *X. americanum* in southern Washington blueberry fields with the highest frequency of population density (29%) being between 26 and 50 nematode/250 g soil (Fig. 1). A very high population, >100 *X. americanum*/250 g soil, was encountered in one Oregon blueberry field. In Oregon, Converse and Ramsdell (1982) reported that *X. americanum* population densities ranged between 11 and 63 nematodes/250 g soil, and the mean number of *X. americanum* was 159 nematodes/250 g soil in Arkansas (Clark et al., 1987). The direct impact of *X. americanum* parasitism on blueberry and other perennial crops is not well understood. Grape cultivars grew larger in the presence of *X. americanun* than where the nematode was absent (McKenry et al., 2001). However, *X. americanum* did reduce yield of some grape cultivars in another study (Ramsdell et al., 1996). The impact of *X. americanum* on blueberry establishment and growth has not been studied. It is known that *X. americanum* transmits Tomato Ringspot and Tobacco Ringspot viruses to blueberry and that both viruses can cause severe damage to most blueberry cultivars (Converse and Ramsell, 1982). We rarely encountered other species of *Xiphinema* in these surveys. *Xiphinema bakeri* was detected in 6 and 11% of surveyed fields in BC and northern

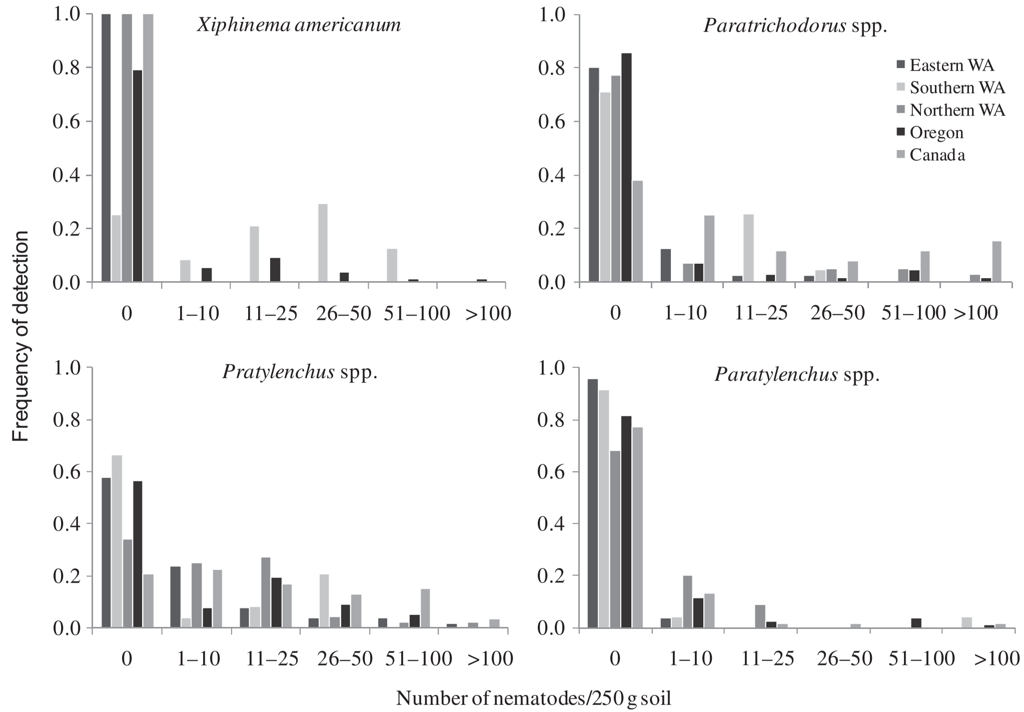

FIGURE 1 Frequencies of population densities for four genera of plant-parasitic nematodes observed in the Pacific Northwest of the U. S. and Coastal British Columbia blueberry fields. The frequency represents the proportion of soil samples in each region in which nematode populations were within the specified density classes.

Washington, respectively. This nematode is a parasite of red raspberry (*Rubus ideaus*) (McElroy, 1977), and it is possible that blueberry fields where this nematode was found had previously been cropped to raspberry.

The most perplexing question that has arisen from these surveys is the pathogenicity of *Pratylenchus* spp. on blueberry. We consistently detected *Pratylenchus* spp. across geographic locations considered in our surveys (Table 1). While this genus of nematode was commonly associated with blueberry, in most locations population densities were low (<25 nematodes/250 g soil) (Fig. 1). In the majority of previous surveys the species of *Pratylenchus* was not identified (Converse and Ramsdell, 1982; Clark et al., 1987). In the two aforementioned studies, *Pratylenchus* spp. populations varied from 42 to 253 nematodes/250 g soil (Converse and Ramsdell, 1982) and 0 to 8 nematodes/250 g soil (Clark et al., 1987). In a subsequent survey conducted in Arkansas, *P. scribneri* and *P. zeae* were identified (Clark and Robbins, 1994). While implicated only once in New Jersey to be associated with blueberry (Mai et al., 1960), the pathogenicity of *P. penetrans* on blueberry has been studied. Blueberry (unspecified species) was reported as a very poor host to *P. penetrans* (Race and Hutchinson, 1959), and lowbush blueberry (*V. ashei*) seedlings grown *in vitro* did not support penetration or reproduction of *P. penetrans* (McCrum and Hilborn, 1962). *Pratylenchus* spp. are migratory endoparasites that move between soil and roots during their life cycle. In our surveys we did not collect root samples; therefore, we cannot link the presence of *Pratylenchus* spp. found in soil samples collected from PNW and BC blueberry fields directly to the blueberry plant. It is possible that the *Pratylenchus* spp. detected in our surveys were associated with weeds or ground covers that were present in the blueberry fields.

Six other plant-parasitic nematode genera/families were detected in PNW and BC blueberry fields. In general, the frequency of detection for most of these nematodes was very low (Table 1 and Fig. 1). *Paratylenchus* spp., an ectoparasitic nematode, is commonly found associated with perennial crops, but has not been shown to result in economic damage. In all geographic locations the frequency of occurrence was low, and only a few locations had high populations (>100 nematodes/250 g soil) (Fig. 1). *Meloidogyne* spp. was found in four of the sampled blueberry fields. While *Meloidogyne* spp. are rarely found associated with blueberry, there is a blueberry root-knot nematode (*M. carolinensis*) (Eisenback, 1982). *Meloidogyne carolinensis* is not considered to be a successful agricultural pest because it has a limited host range and an isolated distribution. *Hemicycliophora* sp. was found in one blueberry field in eastern Washington, representatives of the family Criconematidae were found in several geographical locations, and one high population of *Helicotylenchus* spp. was found in northern Washington (Table 1). It is interesting to note that *M. joctus* was not found in PNW and BC blueberry fields. *Merlinius joctus* was the most commonly found plant-parasitic nematode in the eastern U.S. (Hutchinson et al., 1960; Goheen and Braun, 1955); however, in controlled experiments this nematode did not appear to be a serious pathogen of blueberry (Zuckerman, 1964).

In 2001 when many variables were considered and compared, there was no relationship between plant vigor, variety, or mulching system/ ground cover and total population densities of plant-parasitic nematodes ($p > 0.05$). There was a weak, but significant positive correlation ($r^2 = 0.20$, $p = 0.001$) between age of planting and total densities of plant-parasitic nematodes. Perennial plants may support and compensate for nematode parasitism without noticeable loss of vigor if the plants are not subjected to other biotic (pests and diseases) and abiotic (soil physic-chemical properties, water, cold) stresses.

CONCLUSIONS

Economic damage thresholds have not been developed for nematodes on blueberry, so it is difficult to relate the population densities observed in these surveys to potential damage to the plant. The most commonly found plant-parasitic nematodes in blueberries in the PNW and BC were *P. renifer* and *Pratylenchus* spp. *Xiphinema americanum* was limited in its distribution. Several questions remain regarding plant-parasitic nematodes in this region of the world including: (1) does *P. renifer* cause damage to blueberry, (2) what species of *Pratylenchus* are present in the PNW and BC and can they parasitize blueberry, and (3) do *X. americanum* and perhaps *P. renifer* routinely transmit viruses to blueberry and if so, what is the significance of these virus infections?

Based upon these findings, it is now possible to focus our research efforts to better serve the blueberry grower in the PNW of the U.S. and coastal BC, Canada. We have identified the most frequently encountered plant-parasitic nematode genera in the region and have set baseline population densities for each of them upon which to compare and make recommendations. The results of these surveys have enabled us to focus our research on the pathogenicity and economic impacts of *P. renifer* and *Pratylenchus* spp. The results of these ongoing studies will clarify the need to incorporate nematode control measures into the suite of practices used for economic blueberry production.

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