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Plant-parasitic Nematodes Associated with Grasses Grown for Seed in the Willamette Valley of Oregon

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Abstract

Plant-parasitic nematodes (PPN) are an understudied pathogen group in the Oregon cool-season grass seed cropping system. In this survey, the PPN associated with annual ryegrass, bentgrass, fine fescue, orchardgrass, perennial ryegrass, and tall fescue were determined. Thirty-seven fields were sampled in the 2022 or 2023 growing season by collecting 10 soil cores in each of six 100-m transects for nematode extraction and visual identification. PerMANOVA testing indicated significant differences in PPN community composition across grass host and sampling time. *Pratylenchus* and *Meloidogyne* were the most commonly encountered nematodes, with maximum population densities of 1,984 and 2,496 nematodes/100 g soil, respectively. Sequencing of the COX1 gene region indicated the presence of *P. crenatus, P. fallax, P. neglectus, P. penetrans,* and *P. thornei,* with some of these species being detected for the first time on these grass hosts. The only *Meloidogyne* sp. found in these grasses was *M. nassi*, based upon sequencing of the ITS gene region. This first-of-its-kind survey indicates the need for further assessment of the impact of these PPNs on yield and stand longevity in cool-season grass seed fields in Oregon.

Keywords

grass for seed, *Meloidogyne*, molecular identification, Oregon, *Pratylenchus*, survey

Oregon produces 70% of the world's cool-season grass seeds used for forage and turfgrass. U.S. commercial production of annual ryegrass (aka Italian ryegrass *Lolium multiflorum* Lam, forage grass), perennial ryegrass (*L. perenne* L., turfgrass), bentgrass (*Agrostis* spp. L., turfgrass), fine fescue (*Festuca* spp. Tourn ex. L., turfgrass), orchardgrass (aka Cocksfoot; *Dactylis glomerata* L., forage grass), and tall fescue (*Schedonorus phoenix (Scop.) Holub Note; Schedonorus phoenix*) is centered in Oregon's Willamette Valley, on approximately 162,000 ha per year (USDA National Agricultural Statistics Service, 2017). These cool-season grasses are produced primarily in perennial production with stands of the same grass species in the ground for on average three to five years (Chastain et al., 2000), with some fields in production for greater than 10 years (i.e., orchardgrass, Pfender and Alderman, 1999). This type of production system can lead to challenges managing pests of all kinds and can lead to pest buildup in the soil. One group of pests that has been understudied in cool-season grass seed production is plant-parasitic nematodes (PPNs).

Most of the cool-season grasses grown for seed in Oregon have been reported to have associated PPNs. The primary focus of nematology research in cool-season grasses in Oregon has been *Anguina* spp. (Alderman, 1991; Alderman et al., 2005; Rivedal et al., 2024). This group of nematodes causes both foliar and seed galling in grasses grown for seed in Oregon. *Anguina funesta* and other *Anguina* spp.

can cause significant export rejections of forage grasses, including annual ryegrass, bentgrass, and orchardgrass (Pinkerton and Alderman, 1994; Alderman et al., 2005; Murray et al., 2017). In addition, *A. funesta* can vector the bacterium, *Rathayibacter toxicus*, which causes ryegrass toxicity (RGT) that can kill grazing animals like cattle, horses, and sheep (Price et al., 1979). *Rathayibacter toxicus* has been categorized as a national priority pest (Murray et al., 2014). Since 2019, the state's seed growers have been dealing with seed lot rejections due to the presence of *Anguina*, with rejections of almost 500,000 kg of seed. This detection comes at considerable cost to Oregon grass seed producers, though no *R. toxicus* has ever been detected in Oregon grass seed production.

The other PPN associated with grasses grown for seed in Oregon, and their impact, have not been considered. There is information from other geographic locations on the PPNs that have either been found associated with grasses or have been evaluated for host status and pathogenicity. A diversity of *Meloidogyne* spp. have been found to be associated with cool-season grasses, including the temperate species *M. chitwoodi, M. fallax,* and *M. minor* (Michell et al., 1973; Griffin et al., 1984; Person-Dedryver and Fischer, 1987; Den Nijs et al., 2004; Thoden et al., 2012; Nischwitz et al., 2013). Other common genera found associated with these grasses include *Pratylenchus, Paratylenchus, Tylenchorhynchus, Mesocriconema, Xiphinema,* and *Heterodera* (Ferris, 2023). So, while there is information available for the host status of various grasses for different genera of PPNs, to our knowledge, a survey of PPNs has not been conducted in the Oregon grass seed industry. Therefore, the objectives of this study were to (i) determine the occurrence and diversity of PPN in annual ryegrass, bentgrass, fine fescue, orchardgrass, perennial ryegrass, and tall fescue grass seed fields; (ii) identify the most commonly encountered PPN species using molecular techniques; and (iii) compare temporal shifts in the PPN community of annual ryegrass and orchardgrass fields in spring and autumn of a growing season.

Materials and Methods

Sample collection. During the 2022 and 2023 growing seasons, grass seed fields were selected for sampling after soliciting interested growers and field scouts in western Oregon. In 2022, 14 annual ryegrass and seven orchardgrass fields were identified and sampled in early spring (March 2022) and in late autumn (November 2022); these fields had previous *Anguina* detections (Rivedal et al., 2024). In 2023, three bentgrass, three fine fescue, five tall fescue, and five perennial ryegrass fields were identified and sampled in early spring (April 2023); these fields had no known nematode problems previously. Fields were in the Willamette Valley between 44.1415˚ S and 45.1858˚ N and –122.6316˚ E and –123.3233˚ W. Field boundaries were drawn in ArcGIS Online (Esri, Redlands, CA) and uploaded to the FieldMaps (Esri) phone application. To ensure uniform sampling, the fields were separated into thirds and two transects per third were walked per field. In total, six 100-m transects were walked across the field, collecting a 7.62-cm soil core every 10 m. The 10 soil cores per transect were bulked and homogenized in the field and placed in a 1-gallon resealable zip top bag on ice until processing in the lab.

Nematode extraction from soil. PPNs were extracted from soil using the Baermann funnel method (Ayoub, 1980). From each sample, nematodes were extracted from 50 g soil for five d. Extracted nematodes were collected in a 50-ml tube and identified to genus and enumerated on an inverted microscope (Leica Microsystems, Wetzlar, Germany). Nematode population densities are expressed as number of nematodes/100 g soil.

Molecular identification. When *Pratylenchus* or *Meloidogyne* spp. were found in a sample, up to 10 individuals were lysed using previously reported techniques (Peetz and Zasada, 2016). A single nematode was transferred to 10 µl of worm lysis buffer (WLB;10 mM Tris pH 8.2; 2.5 mM $MgCl_2$; 50 mM KCl; 0.45% Tween 20; 0.05% gelatin; 60 µg/ml proteinase K) on a sterile depression slide and subsequently dissected. Nematode segments were then mixed gently using pipet action with an additional 10 µl of WLB, transferred to a 0.2-ml tube and incubated at –80°C for at least 30 min. The samples were thawed and 1.2 µl of 60 µg/ml proteinase K (Qiagen, Hilden, Germany) was added. The samples were then incubated at 60°C for 60 min followed by a 15 min incubation at 95°C to denature the proteinase K. Finally, samples were stored at –20°C until PCR was performed.

The PCR components included 6.5-µl sterile DNase free molecular grade water, 12.5-µl AccuStart II PCR ToughMix (Quantabio, Beverly, MA, USA), 2.0 µl of each 10-µM primer, and 2 µl of lysed nematodes. Primer set JB3/JB4.5 was used to amplify the COX1 mitochondrial region of *Pratylenchus* (Bowles et al., 1992). Primer set TW81/AB28 was used to amplify the ITS region of *Meloidogyne* (Howlett et al., 1992; Joyce et al., 1994). Cycling conditions included an

initial denaturation step of 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 45 sec, and finished with one cycle at 72°C for 7 min. PCR reactions were performed using a Veriti 96-well thermal cycler (Life Technologies, Grand Island, NY, USA). A 5-µl aliquot of each PCR reaction was analyzed by electrophoresis on 1% agarose/Tris-acetate-EDTA (TAE) gels stained with ethidium bromide (1 µg/ml) and visualized by UV illumination.

PCR products were enzymatically prepared for direct sequencing using ExoSAP-IT PCR (Applied Biosystems, Carlsbad, CA, USA) following the manufacturer's protocol. Cleaned PCR products (10.8 µl) were mixed with 1.2-µl primer and sequenced unidirectionally using standard methods with Eurofins Genomics LLC (Louisville, KY, USA) using the AB28 or one of the JB primer pair for *Meloidogyne* or *Pratylenchus* samples, respectively. DNA sequence data were trimmed using Geneious Prime® 2022.1.1 (Biomatters Inc. Auckland, New Zealand). Trimmed *Pratylenchus* and *Meloidogyne* sequences were roughly 400 and 490 bp in length, respectively. Sequences obtained in this study were used as queries in a nucleotide blast search of GenBank to identify each associated sample to species. Criteria for identification of species included ≥ 95% pairwise identity and query coverage, as well as an E-value of 0. Representative sequences of each detected species were then submitted to GenBank and are available under accession numbers PP002286-PP002291 (see also Supplementary File 1).

Community analysis. PPN community data were analyzed according to recommended procedures from McCune and Grace (2002). For each field, species counts from all six transects were averaged for a final PPN species composition per field. Any average that was not a whole number was rounded up to the next whole number. There was high variability in counts between PPN species (range from 2 nematodes/100g soil to 2,496 nematodes/100g soil); therefore, these average PPN counts per field were relativized by the total average number of PPNs in that field and expressed as a proportion (i.e., relative abundance) for all subsequent analyses. Since there was a low number of total genera recovered (six), rare species were retained.

The data were analyzed using two main sets of sample information: (1) spring samples for all grass species, and (2) the difference between spring and autumn samples for annual ryegrass and orchardgrass samples. In each of these sample sets, alpha, beta, and gamma diversity were calculated according to Whittaker (1972) for each grass species. Alpha diversity (species richness) is expressed as the average number of PPN species per grass species. Beta diversity (community heterogeneity) is calculated from the ratio of gamma to alpha diversity minus 1 (β_w, Wittaker, 1972). Gamma diversity (landscape-level diversity) is expressed as the total estimated number of PPN species per grass species.

Using the "vegan" package (Oksanen et al., 2022) in R (R Core Team, 2023), permutational multivariate analysis of variance (PerMANOVA) was conducted on both data sets. First, for all spring samples (i.e., evaluating all six grass species), a Bray–Curtis distance matrix was calculated and, using the "adonis2" function, species diversity was compared across grass species. Second, for the paired sample of annual ryegrass and orchardgrass fields at either spring or autumn time points, the change in relative abundance for each field was calculated by subtracting March abundances from November abundances. Using Euclidian distances, the adonis2 function was used to compare the change in relative species abundance across grass species (only annual ryegrass or orchardgrass) and sample time. Data visualization of species diversity was conducted using the "ggplot2" package (Wickham, 2016) in R.

Results and Discussion

This is the first comprehensive survey of PPNs associated with grass seed crops in the Willamette Valley of Oregon, the area of the world where 70% of cool-season grass seed is grown. Across the sampling dates, a total of six genera of PPNs were recovered. PPNs were found in 91% of the fields, regardless of the grass host or the time of year that samples were collected. The nematode genera found in Oregon grass seed fields included *Pratylenchus, Meloidogyne, Helicotylenchus, Paratylenchus, Tylenchorynchus,* and *Xiphinema* (Figs. 1 and 2). All of these PPN genera have been reported to be parasites of at least one of the grass seed crops considered in this survey.

It is important to note that *Anguina* spp., a foliar PPN, was not found in soil samples at either sampling date (spring or autumn), in either year of the survey, or in any of the highly susceptible grass species (annual ryegrass, bentgrass, or orchardgrass). An in-depth survey evaluating appropriate sample materials indicated that tiller and seed head samples are most appropriate for targeting *Anguina* spp., since the soil stage of their life cycle is very short (Rivedal et al., 2024). Above-ground plant samples collected in the early spring and at harvest provided the best timing for detecting *Anguina* spp. in Oregon grass seed fields (Rivedal et al., 2024).

In samples collected in the spring of both 2022 and 2023, the diversity of PPNs varied significantly

across grass hosts according to PerMANOVA testing (F = 2.88, P = 0.01). Pairwise comparisons between grass species were not significant when adjusted for multiple comparisons (15 comparisons) using the Benjamini–Hochberg method (data not shown), likely due to the relatively small sample sizes present in this data set. Orchardgrass had the most numerically diverse nematode communities, with an average species richness of 2.1 species, and detections of five of the six detected nematode species (Table 1, Fig. 1). Orchardgrass fields are typically kept in production for many seasons–the average stand age in the orchardgrass fields sampled in 2022 was 7 yr. The soils in these fields were likely minimally disturbed over those years, leading to a diverse soil and rhizosphere community of microbes and PPNs (Berkelmans et al., 2003; McSorley, 2011). Perennial ryegrass fields had the next highest level of species richness (alpha diversity = 2.0), though there was also a perennial ryegrass field with no detection of PPN (Table 1; Fig. 1). While perennial ryegrass was a host to multiple genera of PPNs, densities were low (<35 nematodes/100 g soil), suggesting perennial ryegrass is a less fecund host than the other grass species. Annual ryegrass and tall fescue both had gamma diversity estimated at three genera (Table 1). However, annual ryegrass had higher average species richness (alpha diversity) and lower heterogeneity (beta diversity; Table 1), suggesting more consistency and fecundity of particular PPNs (*Pratylenchus* and *Meloidogyne*) than in tall fescue. Bentgrass and fine fescue fields had the lowest gamma diversity of all the grass species considered (two and one genera of PPNs, respectively), but bentgrass supported higher densities of the encountered genera (Table 1). Fine fescue had only one genus detected, *Pratylenchus* spp., but at low densities (an average of 3 nematodes/100g soil) (Table 1).

When evaluating the presence of PPNs in annual ryegrass and orchardgrass at different sampling dates (spring versus autumn), there were significant changes in PPN diversity. Utilizing PerMANOVA, the interaction between grass species and time was not

Table 1: Average plant-parasitic nematode species densities and diversity measures for all grass species sampled in 2022 and 2023.

			Average Density (#/100 g soil)						Diversity Measures[a]		
Grass species	N[b]	Sample time	*Pratylenchus*	*Meloidogyne*	*Helicotylenchus*	*Paratylenchus*	*Tylenchorhynchus*	*Xiphinema*	Alpha	Beta	Gamma
Annual ryegrass	14	Spring 2022	90	206	0	1	0	0	1.9	0.6	3
		Autumn 2022	0	34	0	0	0	0	0.7	0.4	1
Bentgrass	3	Spring 2023	169	38	0	0	0	0	1.7	0.2	2
Fine fescue	3	Spring 2023	3	0	0	0	0	0	1.0	0.0	1
Orchardgrass	7	Spring 2022	227	58	22	14	1	0	2.1	1.4	5
		Autumn 2022	0	20	7	0	0	1	1.1	1.7	3
Perennial ryegrass	5	Spring 2023	3	6	1	1	0	0	2.0	1.0	4
Tall fescue	5	Spring 2023	166	4	0	0	0	1	1.6	0.9	3

[a]Diversity measures calculated as described by Whittaker (1972). Alpha diversity is expressed as the average number of plant-parasitic nematode species per grass species, or species richness; beta diversity is expressed as the ratio of gamma diversity over alpha diversity minus 1, or heterogeneity; gamma diversity is expressed as the total estimated number of plant-parasitic nematode species per grass species, or the landscape level diversity.
[b]Total number of fields sampled for each grass species. Fields consisted of six total transects with 10 three-inch cores of soil. Each transect was placed on a Baermann funnel separately, and total counts were generated by summing the findings across all six transects.

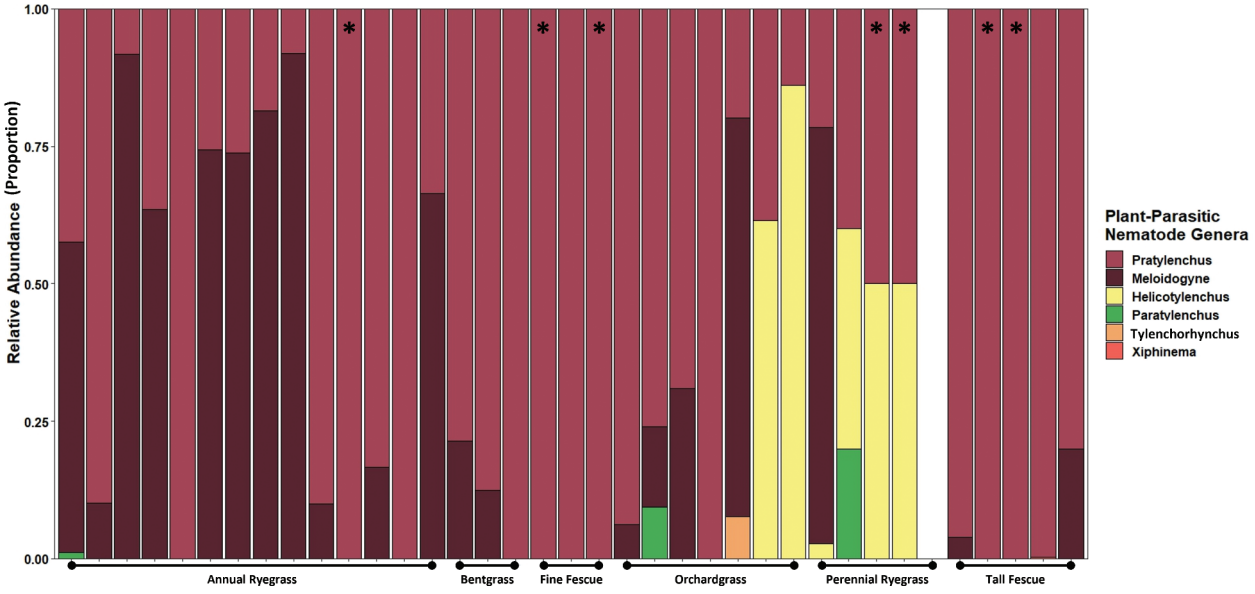

Figure 1: Relative abundance (proportion of total field PPN population) for each sampled field in the Spring of 2022 or 2023. Each column represents one sampled field and the total community of PPNs found in that field. Fields are organized by grass species and colored by the proportion of each PPN genus detected in the field. A column with no color (i.e., white) had undetectable populations of PPN. * indicates a field with fewer than four individual nematodes for the total population count.

significant. However, the PPN community differed significantly across grass species ($F = 3.86$, $P = 0.03$) and sample time ($F = 13.46$, $P = 0.001$). In both grass hosts, *Pratylenchus* detections dropped to zero in the autumn sample, while in spring, they were the most encountered PPN genera (Table 1, Fig. 2). All PPN diversity measures in annual ryegrass were reduced when comparing autumn to spring samples (Table 1), with detections of only *Meloidogyne* spp. across all 14 fields. Nematode alpha and gamma diversity was reduced in orchardgrass, though beta diversity increased slightly (1.7 in autumn versus 1.4 in spring), indicating greater variability in nematode community membership in the fall (Table 1). *Meloidogyne, Helicotylenchus*, and a single *Xiphinema* individual were detected in autumn samples from orchardgrass (Fig. 2). These trends suggest that temporally, PPNs in grass seed fields may cycle through population peaks before spring harvest and then drop off in the dry summer season into autumn. However, for *Pratylenchus*, a migratory endoparasite, it is possible that nematodes migrated into roots after spring and were therefore undetectable in autumn soil samples. A future survey in which plant roots are sampled in addition to soil would be beneficial for the grass seed industry.

Pratylenchus was the most prevalent PPN found in the surveyed grass seed fields (Table 2; Fig. 1). It was also one of the most abundant PPNs found in grass seed fields, with the second highest mean (117 nematodes/100 g soil) and second highest maximum (1,984 nematodes/100g soil) population densities (Table 2). Based upon molecular identification results, there is a diversity of *Pratylenchus* spp. parasitizing grass seed crops in Oregon. A total of 258 *Pratylenchus* individuals from 24 fields were sequenced for identification. Five species were found, including *P. neglectus, P. crenatus, P. penetrans, P. fallax,* and *P. thornei* (Table 3). *Pratylenchus neglectus* and *P. crenatus* were the most commonly encountered species, representing 47% and 42% of sequenced individuals, respectively. Nine percent of individuals were identified as *P. penetrans* and only 1% of sequenced individuals were identified as *P. fallax* or *P. thornei*. In our survey, *P. thornei* was only found associated with orchardgrass, which to our knowledge is the only report of *P. thornei* associated with this host. *Pratylenchus fallax* has been identified in association with Kentucky bluegrass in Canada (Yu et al., 1997), but in this survey, *P. fallax* was only found to be associated with annual ryegrass, which, to our knowledge, is the first report of this nematode

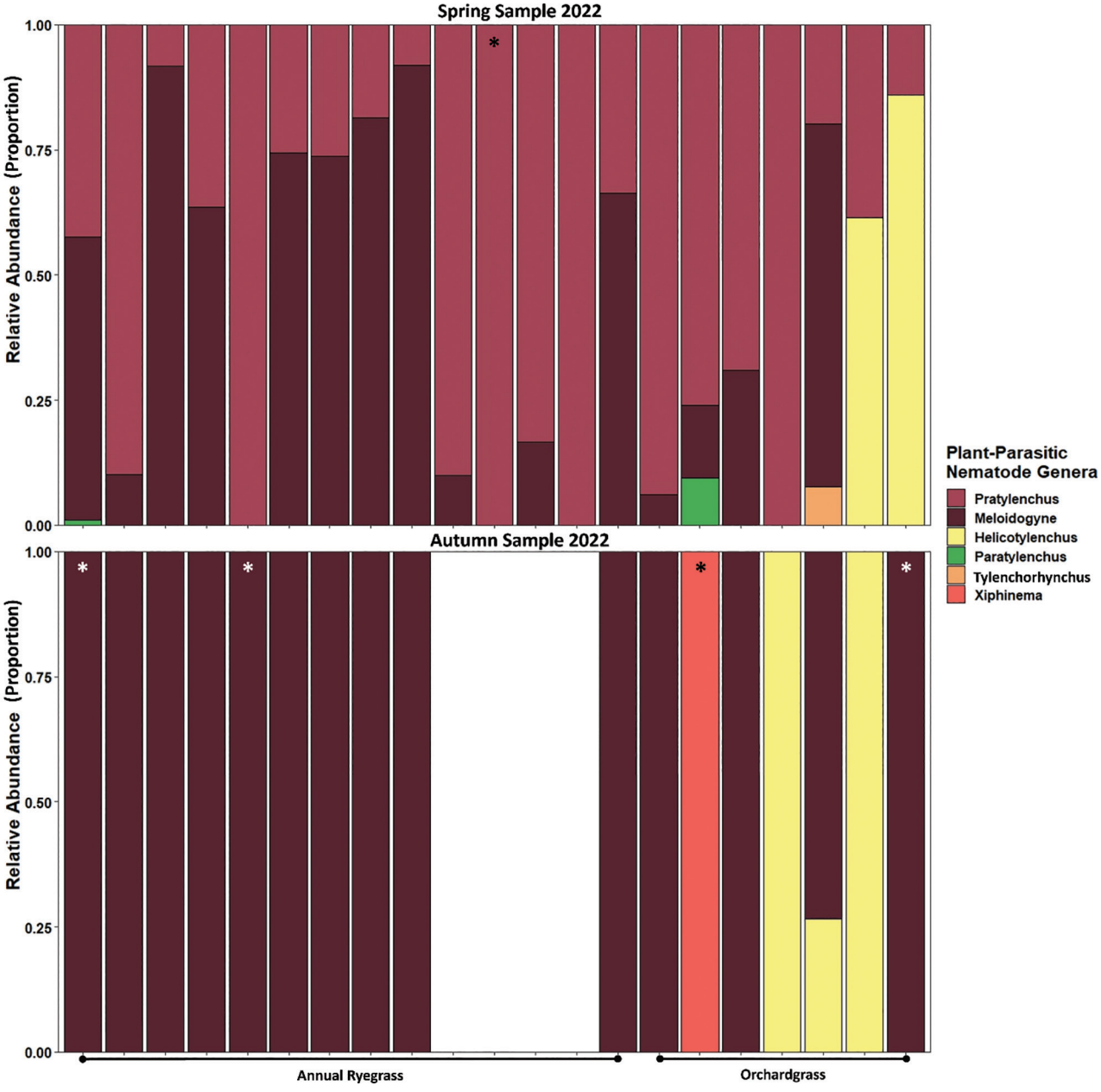

Figure 2: Comparative PPN relative abundance (proportion of total field PPN population) in annual ryegrass and orchardgrass seed fields sampled in spring (top) and autumn (bottom) 2022. Each column represents one sampled field at either time point. Fields are organized by grass species and colored by the proportion of each PPN genus detected in a field. A column with no color (i.e., white) had undetectable populations of PPN. * indicates a field with fewer than four individual nematodes for the total population count.

in association with this host in the Pacific Northwest (Zasada et al., 2018; Kantor et al., 2022). A mix of *Pratylenchus* spp. were found in 58% of fields in which *Pratylenchus* individuals were identified. *Pratylenchus* spp. were molecularly identified from three bentgrass fields and one tall fescue field; in all of these fields, only *P. crenatus* was detected. No *Pratylenchus* spp. could be recovered for sequencing from perennial ryegrass or fine fescue fields, due to low (< 8 individuals in average population) or undetected populations.

The reports of host status and pathogenicity of *Pratylenchus* spp. have been variable across grass

Table 2: Percentage frequency of occurrence (%FO), mean population densities when present (# nematodes/100 g soil), and maximum population densities (# nematodes/100 g soil) of plant-parasitic nematodes associated with grass for seed crops in Oregon sampled in Spring 2022 or 2023.

	Pratylenchus	*Meloidogyne*	*Helicotylenchus*	*Paratylenchus*	*Tylenchorhynchus*	*Xiphinema*
%FO[a]	97	54	16	8	3	3
Mean[b]	117 (30.2)	172 (61.7)	26 (18.2)	32 (26.4)	7	1
Max[c]	1,984	2,496	288	556	24	2

[a]Percent frequency of occurrence during the spring timepoint of sampling, calculated as the number of fields with detections of a particular nematode species.
[b]Mean population density for a given nematode species during the spring collection time point based on average population density in fields with detectable populations (i.e., fields with zero counts were not included in the calculation of the mean). Standard errors of the means are presented in parentheses. For those with no value in parentheses, only one sample had a detection of the individual nematode, and thus a standard error could not be calculated.
[c]Maximum population density expressed as the highest count found in an individual transect (10 three-inch soil cores bulked).

species and locations. *Pratylenchus crenatus* has been reported to be associated with annual ryegrass in Spain (Abrantes et al., 1987) and with perennial ryegrass (Goodey et al., 1965). Field and greenhouse experiments in Prince Edward Island, Canada demonstrated that perennial ryegrass, orchardgrass, and annual ryegrass were poor hosts for *P. penetrans* and *P. crenatus* compared to other grass species like timothy (*Phleum pretense*, Kimpinski et al., 1984). Pathogenicity of *P. penetrans* alone and in combination with other PPNs was demonstrated on creeping bentgrass in a greenhouse experiment (Sikora et al., 1972); however, the reproductive potential of *P. penetrans* on creeping bentgrass was lower than by *M. nassi* and *Tylenchorhynchus agri.* Bentgrass, tall fescue, and perennial ryegrass supported 2 to 3 times fewer *P. penetrans* than *Poa trivialis* (Townshend et al., 1984). In grass alleyway field experiments conducted by Townshend et al. (1984), perennial ryegrass, orchardgrass, and tall fescue were all good hosts for *P. penetrans.* In Ontario, Canada, orchardgrass was an excellent host for *P. neglectus* (Townshend and Potter, 1973, 1976). Other than in this survey, there are no reports of *P. neglectus* associated with perennial ryegrass.

The only *Meloidogyne* spp. identified in Oregon grass for seed fields was *M. nassi*. A total of 58 individuals from seven fields were identified to species. *Meloidogyne nassi* was the second most encountered PPN and had the highest mean (172 nematodes/100 g soil) and highest maximum (2,496 nematodes/100 g soil) population densities (Table 2). Most of the grasses considered in our survey have been shown to be hosts for *M. nassi* populations from Europe and the U.S., including orchardgrass, perennial ryegrass, bentgrass (two different species), fescue, and annual ryegrass (Michell et al., 1973). Additionally, Person-Dedryver et al., (1987) demonstrated the host status of orchardgrass, perennial ryegrass, and annual ryegrass for *M. nassi. Meloidogyne nassi* alone and in combination with other PPNs reduced creeping bentgrass dry root weight by 65% to 75% compared to a noninoculated control (Sikora et al., 1972). There are other *Meloidogyne* spp. that are commonly found in Oregon agriculture systems, including *M. hapla* and *M. chitwoodi* (Zasada et al., 2018); neither of these nematodes was found in our survey. However, orchardgrass has been shown to be a host for *M. chitwoodi* in greenhouse host assays (Griffin et al., 1984). In the Columbia Basin of Washington, orchardgrass has been reported to support large populations of *M. naasi* and is also a host for *M. chitwoodi* (Kugler, 2006).

The four other species of PPN (*Paratylenchus, Helicotylenchus, Tylenchorynchus*, and *Xiphinema*) were each found in less than 16% of grass seed fields sampled (Table 2). Mean population densities of these nematodes in grass seed fields were lower than the mean population densities reported in Oregon from a diversity of other crops based on a summary of 5 yr of diagnostic laboratory data (Zasada et al., 2019). There is information on the host status and damage potential of some of these migratory ectoparasites in grass for seed crops. Orchardgrass was demonstrated as a host for *Paratylenchus projectus* in test plots in Ontario, Canada

Table 3: *Pratylenchus* spp. identified on grass for seed crops in Oregon.[a]

Grass type	*P. crenatus*	*P. fallax*	*P. neglectus*	*P. penetrans*	*P. thornei*
Annual ryegrass	+ (11)[b]	+ (2)	+ (9)	+ (5)	–[c]
Perennial ryegrass	nd[d]	nd	nd	nd	nd
Bentgrass	+ (3)	–	–	–	–
Tall fescue	+ (1)	–	–	–	–
Fine fescue	nd	nd	nd	nd	nd
Orchardgrass	+ (1)	–	+ (6)	+ (2)	+ (2)

[a]Species was determined by amplifying COX1 mitochondrial region with the primer set JB3/JB4.5 followed by sequencing.
[b]+ indicates a positive PCR and sequencing result for a *Pratylenchus* spp. The number of fields from which the species was identified is indicated in parentheses.
[c]– indicates a *Pratylenchus* spp. was not identified from a grass species that did have individuals available for sequencing.
[d]nd = could not be determined due to small (fewer than eight individuals on average per field) or undetected populations.

(Townshend and Potter, 1973). In an orchard cover crop trial, perennial ryegrass, orchardgrass, annual ryegrass and tall fescue were all good hosts for *P. projectus* (Townshend et al., 1984). In an annual ryegrass cultivar trial on Prince Edward Island, Canada, all of the evaluated cultivars were excellent hosts for *Tylenchorhynchus* and *Paratylenchus* spp. *Tylenchorhynchus agri* was demonstrated to be pathogenic to creeping bentgrass (Sikora et al., 1972). *Tylenchorhynchus dubius* was pathogenetic on creeping bentgrass, suppressing secondary stolon formation and resulting in shortened internodes and premature inflorescence initiation at inoculation densities of 500 and 1,000 nematodes/pot (Laughlin and Vargas, 1972). In an evaluation of the host status of 15 pasture species for *Helicotylenchus pseudorobustus*, perennial ryegrass, annual ryegrass, and orchardgrass were poor hosts for the nematodes, with reproduction factor (final population density/initial population density) values less than 1 (Davis et al., 2004). In the same study, two cultivars of tall fescue were good hosts for *H. pseudorobustus* with reproduction factors greater than 1. The overall impact of these lesser-encountered nematodes on the Oregon grass for seed cropping system is yet to be determined. Greenhouse studies are warranted to further relate their presence to plant damage and yield loss.

Conclusion

The grass seed industry of Oregon is currently not controlling PPNs in their production system. These pathogens are now clearly proven to be associated with the major grass seed species in Oregon based on this survey effort, and in some cases, are present at very high densities. These nematodes are feeding on the grass species present in the field, so it is possible that they are contributing to yield loss or reduced stand longevity. With this survey as a starting point, future studies to identify the grass seed crop loss associated with PPNs are necessary.

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Supplementary File 1. Sequence data for *Pratylenchus* species identified in this study via COX1 gene amplification. Sequences are currently under review for submission to NCBI GenBank.

>Pcrenatus_1-4, host = annual ryegrass

ggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgttta

caattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctg

ttccaactggaataaaagtttttttcttgaatattaaccatacaaggttcaatatttaaac

tcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgt

ctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttg

ttgcccattttcattatgttcttttcttta

>Pcrenatus_2-3, host = orchardgrass

tttaaggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatat

gtttacaattggaatggatggggattcccgggcttattttagagcagcaacaatgttaat

tgctgttccaactggaataaaagtttttttcttgaatattaaccatacaaggttcaatatt

taaactcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtgg

tgtgtctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatatta

cgttgttgcccattttcattatgttcttttcttta

>Pcrenatus_3-6, host = orchardgrass

ggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgttta

caattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctg

ttccaactggaataaaagtttttttcttgaatattaaccatacaaggttcaatatttaaac

tcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgt

ctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttg

ttgcccattttcattatgttcttttcttta

Pcrenatus_4-9, host = annual ryegrass

ggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgttta

caattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctg

Continued

Supplementary File 1: Continued

ttccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatttaaac

tcaggattttagagttctgatctctaggttttatttttatgtttagcttgggtggtgtgt

ctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttg

ttgcccattttcattatgttcttttcttta

Pcrenatus_5-8, host = annual ryegrass

tttaaggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatat

gtttacaattggaatggatggggattcccgggcttattttagagcagcaacaatgttaat

tgctgttccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatt

taaactcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtgg

tgtgtctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatatta

cgttgttgcccattttcattatgttcttttcttta

Pcrenatus_6-9, host = orchardgrass

gtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgtttac

aattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctgt

tccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatttaaact

caggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgtc

tggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttgt

tgcccattttcattatgttctttcttta

Pcrenatus_7-10, host = annual ryegrass

cataaaaataaaacctagagatcaaaactctaaaatcctgagtttaaatattgaaccttg

tatggttaatattcaagaaaaaactttttattccagttggaacagcaattaacattgttgc

tgctctaaaataagcccgggaatccccatccattccaattgtaaacatatgatgagccca

aaccatacaacccacataaccaatagcttgcaccgcagaccttaaagcctttacactaaa

aagcacctttttgaagataccaactgacaggccattcctaccagcccgaaagctggagc

aatcaataca

Continued

Supplementary File 1: Continued

Pcrenatus_8-1, host = orchardgrass

ggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgttta

caattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctg

ttccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatttaaac

tcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgt

ctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttg

ttgcccattttcattatgttctttcttta

Pcrenatus_14-9, host = annual ryegrass

tttaaggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatat

gtttacaattggaatggatggggattcccgggcttattttagagcagcaacaatgttaat

tgctgttccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatt

taaactcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtgg

tgtgtctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatatta

cgttgttgcccattttcattatgttcttttcttta

Pcrenatus_15-9, host = annual ryegrass

gtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgtttac

aattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctgt

tccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatttaaact

caggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgtc

tggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttgt

tgcccattttcattat

Pcrenatus_16-7, host = annual ryegrass

ggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgttta

caattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctg

ttccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatttaaac

tcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgt

Continued

Supplementary File 1: Continued

ctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttg

ttgcccattttcattatgttctttcttta

Pcrenatus_17-9, host = annual ryegrass

gtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgtttac

aattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctgt

tccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatttaaact

caggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgtc

tggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttgt

tgcccattttcattatgttctttcttta

Pcrenatus_19-1, host = annual ryegrass

gtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatatgtttac

aattggaatggatggggattcccgggcttattttagagcagcaacaatgttaattgctgt

tccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatttaaact

caggattttagagttttgatctctaggttttatttttatgtttagcttgggtggtgtgtc

tggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatattacgttgt

tgcccattttcattatgttctttcttta

Pcrenatus_20-8, host = annual ryegrass

tttaaggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatat

gtttacaattggaatggatggggattcccgggcttattttagagcagcaacaatgttaat

tgctgttccaactggaataaaagttttttcttgaatattaaccatacaaggttcaatatt

taaactcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtgg

tgtgtctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatatta

cgttgttgcccattttcattatgttcttttcttta

Pcrenatus_21-10, host = annual ryegrass

tttaaggtctgcggtgcaagctattggttatgtgggttgtatggtttgggctcatcatat

gtttacaattggaatggatggggattcccgggcttattttagagcagcaacaatgttaat

Continued

Supplementary File 1: Continued

tgctgttccaactggaataaaagtttttttcttgaatattaaccatacaaggttcaatatt

taaactcaggattttagagttttgatctctaggttttatttttatgtttagcttgggtgg

tgtgtctggggtggttttaaggcatgctagtttagatgtgtttattcatgatacatatta

cgttgttgcccattttcattatgttcttttcttta

Pcrenatus_44-3, host = annual ryegrass

aaactagcatgccttaaaaccaccccagacacaccacccaagctaaacataaaaataaaa

cctagagatcaaaactctaaaatcctgagtttaaatattgaaccttgtatggttaatatt

caagaaaaaactttattccagttggaacagcaattaacattgttgctgctctaaaataa

gcccgggaatccccatccattccaattgtaaacatatgatgagcccaaaccatacaaccc

acataaccaatagcttgcaccgcagaccttaaagcctttacactaaaaagcacctttttt

gaagataccaactgacaggccattcctaccagcccgaaagctggagcaatcaataca

Pfallax_19-9, host = annual ryegrass

tgtaggaatagatttagattctcggggttattttagggtagctactatagttattgctat

tcctactggtataaaagtttttagttgaataataactttatttagttctaattatacaga

acatttaattatggattgggttttagggtttatttttatatttactttaggcggattatc

tggtttggttttaaggaatgctagattagatatttttttacatgatacttattatgtagt

tgctcattttcattatgttcttttct

Pfallax_21-2, host = annual ryegrass

aagatgtgctataaaaagtattggttttgtagggtctttagtttgggctcatcatatgtt

tactgtaggaatagatttagattctcggggttattttagggtagctactatagttattgc

tattcctactggtataaaagtttttagttgaataataactttatttagttctaattatac

agaacatttaattatggattgggttttagggtttatttttatatttactttaggcggatt

atctggtttggttttaaggaatgctagattagatatttttttacatgatacttattatgt

agttgctcattttcattatgttctttcttta