Effects of Crop Rotations and Tillage on *Pratylenchus* spp. in the Semiarid Pacific Northwest United States

Richard W. Smiley, Professor, **Stephen Machado,** Associate Professor, **Jennifer A. Gourlie,** Faculty Research Assistant, **Larry C. Pritchett,** Faculty Research Assistant, **Guiping Yan,** Research Associate, and **Erling E. Jacobsen,** Farm Manager, Oregon State University, Columbia Basin Agricultural Research Center, Pendleton 97801

Abstract

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There is interest in converting rainfed cropping systems in the Pacific Northwest from a 2-year rotation of winter wheat and cultivated fallow to direct-seed (no-till) systems that include chemical fallow, spring cereals, and food legume and brassica crops. Little information is available regarding effects of these changes on plant-parasitic nematodes. Eight cropping systems in a low-precipitation region (<330 mm) were compared over 9 years. Each phase of each rotation occurred each year. The density of *Pratylenchus* spp. was greater in cultivated than chemical fallow, became greater with increasing frequency of host crops, and was inversely associated with precipitation ($R^2 = 0.92$, $\alpha < 0.01$). Densities after harvesting mustard, spring wheat, winter wheat,

and winter pea were greater ($\alpha < 0.01$) than after harvesting spring barley or spring pea. Camelina also produced low densities. Winter wheat led to a greater density of *Pratylenchus neglectus* and spring wheat led to a greater density of *P. thornei*. Density of *Pratylenchus* spp. was correlated ($R^2 = 0.88$, $\alpha < 0.01$) but generally higher when detected by real-time polymerase chain reaction on DNA extracts from soil than when detected by a traditional method. Selection of different *Pratylenchus* spp. by different wheat cultivars or growth habit must be addressed to minimize the level of nematode risk to future plantings of intolerant crops.

The root-lesion nematodes *Pratylenchus neglectus* (Rensch) Filipjev Schuurmanns & Stekhoven and *P. thornei* Sher & Allen suppress yields of rainfed cereals in temperate regions worldwide (5,43). These species are present in 90% of fields in low-precipitation regions (150 to 300 mm) of north-central Oregon and south-central Washington, and the nematode density in soil is above a potentially damaging level in perhaps 60% of the fields (42). Rainfed wheat (*Triticum aestivum* L.) in the region is planted on 1.5 million ha annually. In all, 90% of the hectares in the <330-mm annual precipitation area are managed as a monoculture "rotation" of winter wheat (10 months) and summer fallow (14 months) because precipitation occurs mostly from late autumn (October) to early spring (April) and the amount is highly variable from year to year (31,32). Winters are cold and intervals of frozen soil are common. Warm to hot days and cool nights prevail during the dry summer period when crops are maturing. Optimal grain yield for cereal crops is dependent upon healthy root systems capable of extracting water stored deeply in the soil profile. Most fallow is managed as a mechanically prepared "dust mulch".

Direct seeding into non-tilled chemical summer fallow is of strong interest but still lags behind mechanical cultivation in the low-precipitation region because direct-seed systems have historically been less profitable than mechanical fallow systems when fuel and fertilizer expenses were not excessive (2,16,30,31). Three-year rotations of winter wheat, a spring crop, and chemical summer fallow are also of interest but have not been widely adopted in low-precipitation zones because they also have been less profitable and have had greater year-to-year economic risk compared with winter wheat–summer fallow rotations (30,32). Although practiced on only a small percentage of hectares, most direct-seeded, annually planted fields in the low-precipitation region are planted repeatedly

Corresponding author: R. Smiley, E-mail: richard.smiley@oregonstate.edu

to spring wheat or spring barley (*Hordeum vulgare* L.) because these crops have been more profitable than rotation with crops such as yellow mustard (*Sinapsis alba* L.), canola (*Brassica napus* L.), or camelina (*Camelina sativa* (L.) Crantz), albeit less profitable than winter wheat rotated with fallow (15,30). Winter field pea (*Pisum sativum* L. var. *arvense* (L.) Poir) is another crop of interest to growers in low-precipitation regions, and spring pea (*P. sativum* L.) is commonly produced in regions where higher precipitation (>450 mm) is more favorable for annual cropping systems.

Densities of *Pratylenchus* spp. following fallow are generally lower than densities immediately following wheat (19,21,42). Marley and Littler (17) associated reduced water-use efficiency in no-till wheat, as compared with wheat planted into cultivated soil, to root injury caused by elevated densities of *Pratylenchus thornei* in the direct-seed system. Ornat et al. (22) reported that density of *P. neglectus* in the spring was reduced more by a combination of tillage and fallow than by fallow alone, and that the percentage of juveniles containing spores of parasitic *Pasteuria* spp. was greater in soil that had not been cultivated. However, Thompson (50) found more *P. thornei* in no-till than in cultivated fallow and Strausbaugh et al. (47) detected fewer *Pratylenchus* spp. in chemical than in cultivated fallow. In 2 of 5 years, Paulitz et al. (24) detected fewer *P. neglectus* in irrigated winter wheat that was planted without tillage than into cultivated soil, and no differences among tillage treatments occurred in other years. Brmež et al. (4), Smiley et al. (42), and Smiley and Machado (41) did not detect differences in *Pratylenchus* spp. density between chemical and cultivated fallows.

The density of *Pratylenchus* spp. is often correlated with the frequency of cereal crops (9,29). Many wheat and winter pea cultivars appear to be particularly good hosts but barley is generally considered a poorer host than wheat (13,41,42,50,55,57). In studies of multiple cultivars of many crop species, Hollaway et al. (13) and Taylor et al. (50) reported that field pea were resistant to both *P. thornei* and *P. neglectus*, that lentil (*Lens culinaris* Medik.) was resistant to *P. thornei*, that chickpea (*Cicer arietinium* L.) was susceptible to *P. neglectus*, and that canola (*B. napus* L.) was moderately resistant to *P. thornei* but susceptible to *P. neglectus*. Owen et al. (23) also reported that densities of *P. thornei* were greatly

reduced following canola compared with wheat. Oilseed rape (*B. napus* L. subsp. *oleifera* (DC.) Metzg.) was also shown to be a good host of *Pratylenchus* spp. (1,58). Nevertheless, certain *Brassica* spp. can be used as biofumigant crops to reduce final nematode densities when the crop is macerated into soil as a green manure (25). *Brassica* spp. cultivars used as biofumigant crops contain elevated concentrations of certain glucosinolates which are not directly nematicidal (7) but release toxic isothiocyanate molecules following tissue damage, as when foliage is macerated and incorporated into soil. Potter et al. (25,26) reported that roots of lines of *B. napus* L. (canola, or oilseed rape) and *B. oxyrrhina* (Coss.) Willk. (a wild species from Morocco) contained higher concentrations of 2-phenylethyl glucosinolate than lines of *B. rapa* L. (*B. campestris* L.; field mustard), *B. nigra* (L.) W. Koch (black mustard), and *B. carinata* A. Braun (Ethiopian mustard), and intermediate levels occurred in roots of *B. juncea* (L.) Czernj. & Coss (brown mustard). Suppression of *P. neglectus* was negatively associated with root concentrations of 2-phenylethyl glucosinolate. In the semiarid regions of the Pacific Northwest, the brassica crops, mainly canola, mustard, and camelina, are grown as seed crops and are seldom, if ever, rototilled into soil as a biofumigant crop because all cropping systems must place highest consideration on availability of limited water resources. We are not aware of reports for effects of camelina on densities of *Pratylenchus* spp.

Both *P. neglectus* and *P. thornei* have reduced yields of annually cropped spring wheat by as much as 70% in Oregon (45,46). It was also demonstrated in those studies that spring wheat with roots heavily infested by *P. neglectus* and *P. thornei* had canopy temperatures elevated as much as 4°C higher than in adjacent plots, where nematode populations were suppressed by applying a nematicide. Increased plant canopy temperature is directly correlated with water stress in wheat (6,39). Root dysfunction caused by *Pratylenchus* spp. appeared to reduce the capacity of spring wheat to extract deeply stored water late in the growing season. Similarly, a greater tolerance of barley to *P. thornei* compared with wheat enabled barley to have greater efficiency in extracting and in being more responsive than wheat to stored soil moisture in a year with limited in-crop precipitation (53). Invasion of roots by *Pratylenchus* spp. also reduces the capacity of wheat to extract plant nutrients from soil (54).

A replicated multiyear experiment was established during 2003 to examine multidisciplinary aspects of eight representative cropping systems at a low-precipitation site infested with *P. neglectus* and *P. thornei*. Smiley and Machado (41) previously reported an inverse relationship between density of *P. neglectus* and yield of winter wheat during the first 5 years of this experiment, and that winter wheat treatments most affected by the nematode also extracted the least amount of water from the soil profile. This article reports associations between cropping systems and further evaluations of nematode densities over a 9-year period. During the last year of the experiment, inoculum densities for *P. neglectus* and *P. thornei* were compared using three methods, including real-time polymerase chain reaction (PCR) on DNA extracted from soil and two traditional extraction and morphological identification methods.

Materials and Methods

The experiment was performed at the Columbia Basin Agricultural Research Center, 1 km southeast of Moro, Sherman County, OR (45° 29.041′ N and 120° 43.127′ W). The site is at 575 m of elevation and receives 282 mm of mean annual precipitation, nearly all of which occurs from late autumn (October) through spring (May). Mean daily air temperature is –1°C during January and 19°C during July and August. Soil is a moderately deep (mostly >120 cm) Walla Walla silt loam; a coarse-silty, mixed, superactive, mesic Typic Haploxeroll. Daily precipitation was measured at an official U.S. National Weather Service recording site located 0.5 km from the experimental site.

Cropping systems. A uniform crop of spring wheat was planted over the intended experimental area during 2003. The experimental area was mapped into 42 plots of 15 by 105 m arranged as 14 treat-

ments of eight crop rotations, randomized within each of three blocks. Each phase of each rotation was present each year to allow data for each treatment to be collected every year. Crops and cultivars or the type of fallow are shown in Table 1. Crops were harvested in late July or early August. A strip following the centerline of each 15-m wide plot was harvested using a commercial combine with a 5.5-m header, and grain yield was measured using a weigh wagon to determine yield per treatment.

Rotation 1. Two-year rotation of winter wheat and cultivated fallow. After harvest, the field was not cultivated until mid-April of the following (fallow) year. Glyphosate was applied as needed in the fall and spring. In April, primary tillage was conducted to a depth of 15 cm using a John Deere 1600 cultivator fitted with chisel plow turning points, followed by sweep cultivation to a depth of 13 cm using the same cultivator equipped with 30-cm wide sweeps. Plots were rod-weeded at a depth of 8 to 10 cm whenever necessary to control weeds. Rod-weeding is the control of weeds by pulling a longitudinal square rod that is rotating backward below the soil surface. Plots were generally rod-weeded two or three times from May to August. In accordance with industry standards and based on soil sampling, anhydrous ammonia and gypsum were shank-applied into fallow during September to meet soil fertility requirements based upon target yields for the region. Soft-white winter wheat cultivars were planted into moist soil at 200 to 244 seeds/m^2 in mid-September using a John Deere 7616 HZ drill (Deere and Co.) with 40-cm row spacing. In-crop herbicides were applied as necessary.

Rotation 2. Two-year rotation of winter wheat and chemical fallow. Glyphosate was applied to control weeds three to four times during the chemical fallow phase. The fallowed plots were direct-seeded at a shallow depth and a rate of 244 seeds/m^2 using a Fabro Drill (Fabro Ltd.) during late September or early October. Seed was planted into moist or dry soil, depending upon weather conditions each year. Urea was banded 2.5 cm below the seed and ammonium sulfate was applied with the seed, each based upon industry standards and results of soil tests and target yields. In-crop herbicides were applied as necessary.

Rotation 3. Annual winter wheat. Plots were sprayed in late September or early October with glyphosate to control summer weeds. Plots were then direct-seeded during October or early November at a shallow depth and a rate of 220 to 240 seeds/m^2 using the Fabro disk-type drill with 30-cm row spacing. A blend of urea and ammonium sulfate was banded 2.5 cm below the seed during planting, and herbicides were applied to the crop in accordance with industry standards.

Rotations 4 and 5. Annual spring wheat and spring barley. Plots were sprayed with glyphosate in late September or early October. In April, the annual spring wheat and spring barley plots were direct-seeded at rates within the range of 222 to 322 seeds/m^2, depending upon anticipated moisture available each year. The Fabro drill was used to plant soft-white spring wheat and two-rowed spring feed barley cultivars. Fertilizer and herbicide applications were as described for the annual winter wheat treatment.

Rotation 6. Three-year rotation of winter wheat, spring barley, and chemical fallow. Management practices for winter wheat following chemical fallow and the chemical fallow were the same as in the 2-year direct-seed rotation. Practices for spring barley following winter wheat were the same as for the annual spring barley treatment.

Rotation 7. Two-year rotation of winter wheat and winter pea. Following winter wheat harvest, plots were sprayed with glyphosate during late September to early October. Plots were then seeded with feed-type winter field pea cultivars at 78 seeds/m^2 during October or early November using the Fabro drill. Granular inoculant was applied with the seed. Starter fertilizer (N at 9 kg/ha) was banded below the seed, at a depth of 7.5 cm. Herbicides were applied in accordance with standard practice. When winter pea plants succumbed to winterkill, the plots were replanted to spring field pea (Table 1). After the pea crop was harvested in late July or early August, glyphosate was applied to prepare plots for planting winter

wheat. In October or early November, winter wheat was seeded at 244 seeds/m^2 using the Fabro drill. Urea was banded 2.5 cm below the seed during planting, and starter fertilizer (ammonium sulfate) was applied with the seed. In-crop herbicides were applied as necessary.

Rotation 8. Flexible cropping sequences (Flex crop). Two direct-seed flexible cropping treatments (rotations 8A and 8B) were examined. Each treatment allowed independent and complete annual flexibility in selecting the crop species produced, with decisions based upon market prices, soil moisture available before planting, and occurrences of weeds and diseases. Cropping decisions were made by consensus of the project leader and the scientists and farmers who served on an advisory committee for this experiment. Crops grown in these rotations (Table 1) were winter wheat or spring-planted wheat, barley, yellow mustard, canola, or camelina. Management was as described for direct-seed annual crops.

Soil sampling and nematode extraction. Soil was collected each year to assess *Pratylenchus* spp. populations in individual treatments when soil was moist shortly before or after spring crops were planted, and at a time when fall-planted crops were still small following periods of frozen soil during the winter. In a similar environment, the density of *P. neglectus* during the spring was strongly correlated with the density measured in the autumn (8). Sampling dates were during late March or early April each year. Samples during 2004 to 2011 consisted of 20 cores (2.5 cm in diameter by 30 cm deep) composited for each plot; 14 treatments × 3 replicates = 42 samples. A larger volume of soil was collected during 2012 by doubling the sampling intensity. Samples were stored at field moisture level at 4°C for up to 7 days before being transported to a commercial nematode-testing laboratory for

extraction and identification of genera present. Supplemental extractions and species identifications were performed in our laboratory.

Western Laboratories. Soil samples were sent annually to Western Laboratories (Parma, ID) for nematode extraction and identification using traditional methods. The lab typically moistens soils uniformly and incubates them at ambient temperature for up to 1 week to minimize differences in nematode extraction efficiency due to differences in soil moisture content. A modified elutriation method (14) was used to extract all soil-dwelling nematodes from 250-g subsamples of soil plus root fragments (H. Kreeft, *personal communication*). Using an Oosterbrink elutriator, coarse material was collected on a 500-μm sieve and nematodes on a stack of two 38-μm and two 32-μm sieves. Nematodes were washed into 100-ml cups, suspensions were stored overnight, and settled nematodes were transferred to 50-ml centrifuge tubes and concentrated by 3,200 rpm for 5 min. Water was discarded, replaced by magnesium sulfate solution at specific gravity 1.80, mixed by spatula, and centrifuged again for 4 min. The supernatant solution containing nematodes was passed over a 20-μm sieve, transferred to another 50-ml tube, and allowed to settle for at least 2 h before counting.

Enumeration of nematodes was performed by reducing the volume of suspension in the 50-ml tubes to 10 ml, mixing the remaining suspension in a mini-vortex mixer for 5 s, removing 4 ml of suspension, placing 1 ml on a Peter's counting slide, counting and identifying all plant-parasitic nematode genera on the slide, and reporting nematodes per kilogram of oven-dry soil.

Root Disease Testing Service. During 2012, soil samples from the 42 plots were mixed thoroughly and a field-moist 500-g sub-

Table 1. Crop and management treatments over 9 years (2004 to 2012) in a long-term experiment at Moro, OR

Treatment[x]	RN[y]	Phase[z]	Crop and cultivar or fallow type during each harvest year[w]								
			2004	2005	2006	2007	2008	2009	2010	2011	2012
WW/CvF	1	1A	WW Tubbs	CvF	WW Stephens	CvF	WW ORCF101	CvF	WW ORCF102	CvF	WW ORCF101
		1B	CvF	WW Tubbs	CvF	WW Stephens	CvF	WW Tubbs '06	CvF	WW ORCF101	CvF
WW/ChF	2	2A	WW Tubbs	ChF	WW Stephens	ChF	WW ORCF101	ChF	WW ORCF102	ChF	WW ORCF101
		2B	ChF	WW Tubbs	ChF	WW Stephens	ChF	WW Tubbs '06	ChF	WW ORCF101	ChF
Annual WW	3	3	WW Tubbs	WW Tubbs	WW Stephens	WW ORCF101	WW ORCF101	WW Tubbs '06	WW ORCF102	WW ORCF101	WW ORCF101
Annual SW	4	4	SW Zak	SW Zak	SW Louise	SW Louise	SW Louise	SW Louise	SW Louise	SW Louise	SW Louise
Annual SB	5	5	SB Camas	SB Camas	SB Camas	SB Camas	SB Haxby	SB Camas	SB Camas	SB Camas	SB Camas
WW/SB/ChF	6	6A	WW Tubbs	SB Camas	ChF	WW ORCF101	SB Haxby	ChF	WW ORCF102	SB Camas	ChF
		6B	SB Camas	ChF	WW Stephens	SB Camas	ChF	WW Tubbs '06	SB Camas	ChF	WW ORCF101
		6C	ChF	WW Tubbs	SB Camas	ChF	WW ORCF101	SB Camas	ChF	WW ORCF101	SB Camas
WW/WP	7	7A	WW Tubbs	WP 706	WW Stephens	WP Specter	WW ORCF101	WP Austrian	WW ORCF102	WP Windham	WW ORCF101
		7B	WP Austrian	WW Tubbs	WP Specter	WW ORCF101	WP/SP Universal	WW Tubbs '06	WP/SP Universal	WW ORCF101	WP Windham
Flex crops	8	8A	SB Camas	SW Zak	SM Tilney	SW Louise	SC Calina	SP Universal	SW Louise	SC Calina	WW ORCF101
		8B	SW Zak	SB Camas	ChF	WW ORCF101	SP Universal	SW Louise	SP Universal	SW Louise	SC Calina

[w] ChF = chemical fallow, CvF = cultivated fallow, SB = spring barley, SC = spring camelina, SM = spring mustard, SP = spring pea, SW = spring wheat, WP = winter pea, WW = winter wheat, and Flex crop = decisions regarding rotational sequences were reexamined annually to facilitate real-time considerations of available stored water, crop prices, and occurrences of weeds or diseases. The experimental area was planted uniformly to SW in 2003. All rotational sequences were performed without tillage (no-till) except rotations 1A and 1B, which were cultivated between WW crops. All WW and SW cultivars were of the soft-white marketing classification, and all SB was two-rowed spring feed barley. Mustard 'Tilney' is a yellow (also called white) mustard (*Sinapis alba*). WP/SP is winter pea that was killed during winter and replanted to spring pea.

[x] Treatment sequence.

[y] Rotation number.

[z] Phase: for multi-year rotational sequences (rotations 1, 2, 6, and 7), each phase of the sequence was present each year to allow data to be collected every year during the experiment.

sample was submitted to Western Laboratories for extractions and reporting of total *Pratylenchus* spp., as described above. Another 500-g subsample was dried in an oven at 40°C for 48 h and submitted to the Root Disease Testing Service (RDTS), South Australia Research and Development Institute, Adelaide, Australia. The RDTS extracted DNA from soil using the PreDicta B analysis system (20; http://www.sardi.sa.gov.au/products__and__services/entomology/diagnostic_service/predicta_b) and reported numbers of individual species of *Pratylenchus*. Data included numbers of *P. neglectus*, *P. thornei*, *P. teres*, *Heterodera avenae*, and *Ditylenchus dipsaci* per gram of soil. Of these species, only *P. neglectus*, *P. thornei*, and *H. avenae* have been previously detected at the experimental site and only *P. neglectus* had been detected in densities sufficiently high to have been considered economically important at this site (44,46).

Columbia Basin Agricultural Research Center Laboratory. Suspensions of nematodes extracted and identified to genus level by Western Laboratories were returned to our laboratory at the Columbia Basin Agricultural Research Center for identification of *Pratylenchus* spp. in four to five randomly selected plots during each of the last 3 years of the experiment. The authors used PCR (61) and morphological characteristics and measurements (11) to identify these species.

During 2012, we also extracted nematodes from each replicate of rotation 3 (annual winter wheat) and rotation 4 (annual spring wheat) using the Whitehead tray method (59) for extractions of nematodes from a 200-g subsample of the same samples submitted for extraction at Western Laboratories and the RDTS. Numbers and identities of *P. neglectus* and *P. thornei* were determined using morphological characteristics. Data are presented as means of counts from three separate aliquots taken from each of three replicates for the two treatments.

Statistical analysis. Nematode data were transformed using $\ln(x + 1)$ to normalize population estimates prior to statistical analysis (27). Long-term effects of rotations were evaluated by grouping nematode density data across years for analysis, with years and rotation treatments or crops as main effect variables and replicates as blocks. The densities of *Pratylenchus* spp. during the spring following the growth of each crop were also analyzed. Analyses were performed using the generalized linear mixed-model procedure (PROC GLIMMIX) in Statistical Analysis System (SAS, version 9.2; SAS Institute), which does not require a normal distribution of data. When main effects for treatments were significant at $\alpha < 0.05$, means were separated using the Tukey's honestly significant difference test if numbers of observations were uniform among treatments or by the least significant difference test when numbers of observations differed among treatments. Logarithmic means of nematode density were back transformed to real numbers for presentation in this article. Regression analysis was used to evaluate associations between precipitation and nematode density and between nematode densities determined by traditional extraction methods and DNA extraction during 2012.

Results

The only plant-parasitic nematodes capable of affecting plant health in this experiment were *P. neglectus* and *P. thornei*. Densities of these species differed among rotations and were evaluated annually. Other plant parasites that were detected infrequently, were always in low numbers, and occurred without any relationship to crop treatment included species of *Criconemoides, Geocenamus, Helicotylenchus, Heterodera, Meloidogyne, Paratylenchus*, and *Tylenchorhynchus*. Analyses of results were restricted to species of *Pratylenchus*.

Annual precipitation over the 9-year experimental period averaged 289 mm and ranged from 181 mm in 2008 (driest year) to 396 mm in 2010 (wettest year). Precipitation during the over-winter and early-spring period (November to March) was considered more indicative of potential nematode activity. Precipitation during this presampling period averaged 112 mm over the 9-year period, including two particularly dry 5-month periods (83 and 59 mm in 2007 and 2008, respectively) and three comparatively wet 5-month periods (125, 171, and 132 mm in 2004, 2010, and 2012, respectively). When the *Pratylenchus* spp. density for all 42 plots sampled each year were averaged and plotted against the amount of precipitation from November through March during that year, there was a significantly inverse relationship between the nematode density and the precipitation (Fig. 1).

Main rotations. Main effects were significant ($\alpha < 0.0001$) for year, rotation, and the year–rotation interaction. Mean density of *Pratylenchus* spp. over the 9-year sampling period for each rotation (Table 2) was significantly higher in annual spring wheat than for five other rotations: annual spring barley, the 3-year rotation, the flexible cropping treatment, and the direct-seed winter wheat rotations with either winter pea or chemical fallow. The density did not differ between the direct-seed and the cultivated rotations of winter wheat with fallow, or between the direct-seed rotations of winter wheat with fallow or with winter pea. An evaluation of year–rotation interactions was not helpful for interpreting further differences among the data.

Rotation phases. To identify possible changes in *Pratylenchus* spp. density within phases of individual rotations, analyses were performed for each phase of the main rotations after removing data for the first year (2004) in which all crops and treatments followed spring wheat. Data were also removed for the flexible cropping sequence (rotation 8) due to the irregular pattern of crops planted and to the occurrence of only three repetitions of several crops during the last 8 years of the experiment. Data for the annual crop
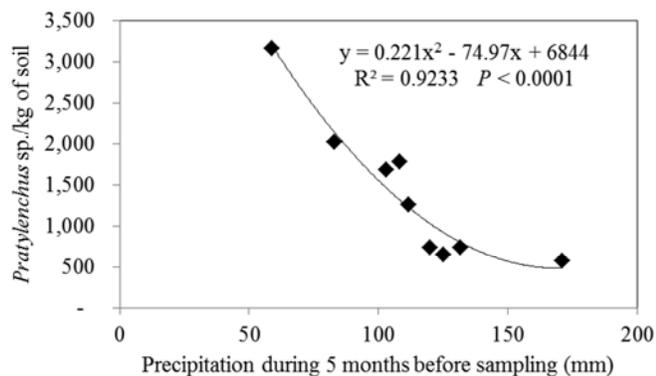

Fig. 1. Relationship between the mean density of *Pratylenchus* spp. in 42 plots during each of 9 years (2004 to 2012) and the over-winter and early-spring precipitation (November to March) that preceded collection of soil samples during those years.

Table 2. Density of *Pratylenchus* spp. in the upper 30 cm of soil during the spring for eight cropping systems during a 9-year interval (2004 to 2012) at Moro, OR

Rotation[w]	Rotation name[x]	Nematodes/kg[y]	n[z]
4	Annual SW	3,017 a	27
3	Annual WW	2,305 ab	27
1	WW/CvF	1,653 ab	54
7	WW/WP	1,486 b	54
2	WW/ChF	1,291 b	54
8	Flex crop	1,242 b	54
6	WW/SB/ChF	676 c	81
5	Annual SB	352 c	27

[w] Crops and management treatments are shown in Table 1. $P < 0.01$.

[x] ChF = chemical fallow, CvF = cultivated fallow, SB = spring barley, SW = spring wheat, WP = winter pea, WW = winter wheat, Flex crop = variable crop sequences over years.

[y] *Pratylenchus* nematodes per kilogram of soil. Data are back-transformed means of the $\ln(x + 1)$ transformation used for analysis. Numbers followed by the same letter are not significantly different at $\alpha = 0.05$ according to the least significant difference test for non-equal treatments.
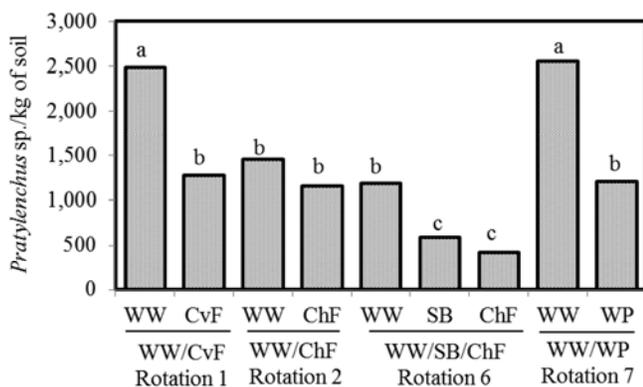
[z] Number of observations available during the 9-year interval.

sequences (rotations 3, 4, and 5) were also deleted because those data were presented in Table 2.

Nematode densities following specific 2- or 3-year crop or fallow treatment sequences in rotations 1, 2, 6, and 7 are presented in Figure 2. Main effects were significant ($\alpha < 0.0001$) for year, rotation, and the year–rotation interaction. It is important to note that Figure 3 shows nematode densities during the spring following the crop indicated within each rotational sequence. For instance, in the 2-year rotations of winter wheat and fallow, the nematode density shown is for the spring following winter wheat, which is the current fallow phase of that rotation. Therefore, the nematode density was higher during the fallow phase than during the current winter wheat phase, because the winter wheat was still small when sampling occurred during the spring following a fallow phase. The mean density of *Pratylenchus* spp. over 8 years was greater ($\alpha = 0.02$) in cultivated fallow (2,481 nematodes/kg of soil) than in chemical fallow (1,456 nematodes/kg of soil). For individual years, the density was significantly greater ($\alpha = 0.02$) in cultivated than chemical fallow during 2005 and 2006, years of "dry" and "normal" precipitation, respectively. Numerical differences ($\alpha > 0.05$) also occurred during 2007 (dry year) and 2009 (normal precipitation). No numerical differences among these treatments occurred during 2008 (a dry year) or during 2004, 2010, and 2012 (wetter-than-normal spring-time precipitation).

Because the nematode density was comparable in the young winter wheat crops following years of cultivated or chemical fallow, it appeared that the density of *Pratylenchus* spp. declined more rapidly in cultivated than in chemical fallow. In the cultivated fallow of rotation 1, the density of *Pratylenchus* spp. diminished by about 50% during the 14-month fallow period. The mean density of nematodes decreased from 2,481 *Pratylenchus* nematodes/kg of soil in fallow during the spring following the winter wheat crop to 1,278 nematodes/kg of soil during the spring of the winter wheat crop that had been planted 6 months earlier, following 14 months of fallow. In contrast, in the direct-seed treatment (rotation 2), the decrease in mean density of *Pratylenchus* spp. was 21% during the same time period; from 1,456 to 1,151 *Pratylenchus* nematodes/kg of soil. When the density of *Pratylenchus* spp. was evaluated for the current winter wheat crops following chemical or cultivated fallows in rotations 1 and 2, the difference was not significant across years or during individual years.

In the 3-year direct-seed rotation (rotation 6), the density of *Pratylenchus* spp. was very low (418 nematodes/kg of soil) at the time of sampling of young winter wheat (Fig. 3, ChF), was significantly higher when the spring barley was planted following winter wheat (1,190 nematodes/kg; Fig. 3, WW), and began diminishing again during the chemical fallow following the spring barley cycle (586 nematodes/kg; Fig. 3, SB). This cycling indicated that winter wheat led to amplification of the *Pratylenchus* spp. density and that it decreased during the years when a poor host (barley) was present and was further diminished during chemical fallow. Only a single year of winter wheat was required to amplify densities of *Pratylenchus* spp. to values comparable with those following winter wheat in the 2-year rotation with chemical fallow.

In the winter wheat/winter pea rotation (rotation 7), the density of *Pratylenchus* spp. following winter pea was similar to the density detected in comparable phases of the winter wheat rotation with chemical fallow (rotation 2). However, it was of particular interest that there was a numeric doubling of nematode density in the direct-drill winter wheat that followed winter pea, as compared with direct-drill winter wheat that followed chemical fallow.

Crop species. The influence of all crops was evaluated by analyzing data of *Pratylenchus* spp. density for 9 years on the basis of the crop during the previous year, without regard to the current crop. This analysis was for spring crops that were newly planted or winter crops that had been planted 6 months earlier but were still small following the winter months. The main effect of previous crop was significant at $\alpha < 0.0001$. Densities of *Pratylenchus* spp.
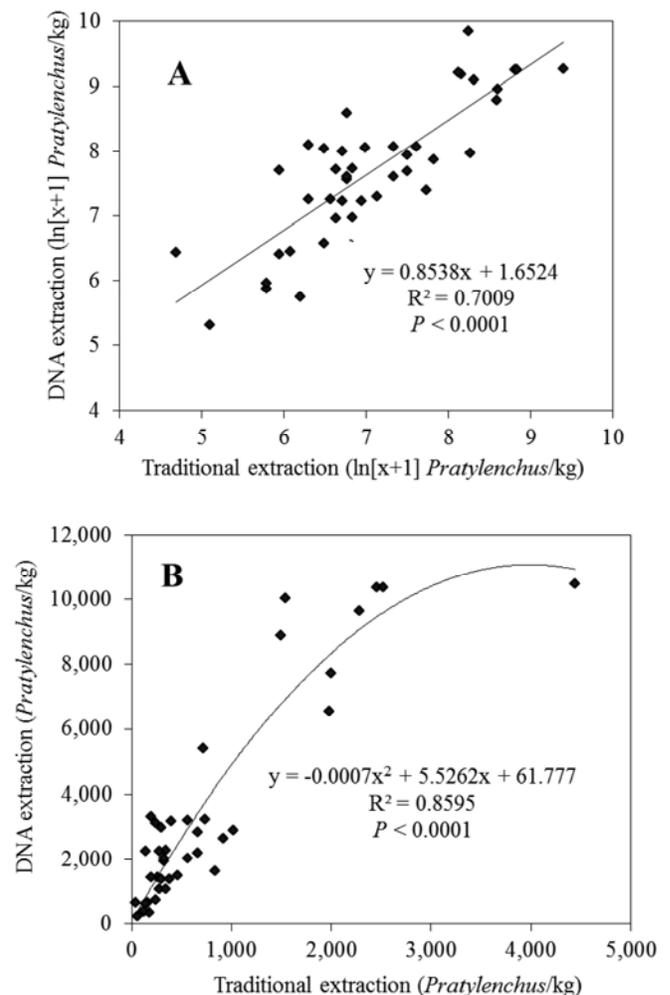
Fig. 3. Comparison of *Pratylenchus* spp. nematodes per kilogram of soil reported for 42 plots of the experiment at Moro, OR during 2012; data are based upon traditional physical extractions reported by Western Laboratories (Parma, ID) or upon real-time polymerase chain reaction on DNA from soil extracts using the PreDicta B analysis reported by the Root Disease Testing Service (Adelaide, Australia). **A,** Natural logarithmic transformations [ln (x + 1)] of data for 42 plots. **B,** Nematode densities reported by each lab for 41 of 42 plots.

Fig. 2. Density of *Pratylenchus* spp. in the upper 30 cm of soil during the spring following a specific crop or fallow treatment in different phases of 2- and 3-year rotations during the final 8 years (2005 to 2012) at Moro, OR. Crops and management treatments are shown in Table 1. WW = winter wheat, CvF = cultivated fallow, ChF = chemical fallow, SB = spring barley, WP = winter pea. Treatments (crop or fallow) in each plot alternate annually in the sequence shown for each rotation. Bars (n = 24) having the same letter do not differ significantly at α = 0.05 according to the least significant difference test. Because sampling was during the spring when WW and WP were 5 months old but still small, when SB had recently been planted, and before CvF had been cultivated, the data for each treatment phase reflects the influence of the previous crop or treatment in each rotation (e.g., data for WW in rotation 1 is currently in CvF, reflecting the influence of the preceding WW crop).

following crops of spring wheat, mustard, winter pea, and winter wheat were significantly greater than densities following camelina, spring barley, or spring pea (Table 3).

Species-specific nematode assays. Annual assessments of *Pratylenchus* spp. density were performed at Western Laboratories, which did not distinguish among individual species of *Pratylenchus*. During the final year of the experiment, the soil samples were uniformly mixed and subsamples were sent to both Western Laboratories and the RDTS. Additional soil was retained for use in our laboratory. The RDTS performs species-specific analyses on DNA extracted from soil. A strong correlation was revealed between densities of total *Pratylenchus* nematodes per kilogram of soil reported by the two labs (Fig. 3A), with greater densities reported from DNA extractions than from a traditional extraction and identification method. Estimates of density were approximately four times greater from DNA extraction than from traditional extraction when the density reported by the traditional method was less than about 2,500 *Pratylenchus* nematodes/kg of soil (Fig. 3B). Data for one replicate of the annual winter wheat treatment (rotation 3) was not included in Figure 3B. The soil was very dry in the annual winter wheat plots during the spring and the soil probes had to be vigorously hammered into the soil to collect samples of a depth equal to other treatments. Estimates of nematode density from DNA extracts from one particularly dry replicate had a reported density 13 times greater than was indicated using traditional extraction and identification procedures (*data not reported*), suggesting that intensive fracturing of soil while collecting soil from that plot likely caused an underestimate of *Pratylenchus* spp. when the density was assessed using a traditional method.

The species-specific DNA data (Table 4) indicated that winter wheat had a greater density of *P. neglectus* and that spring wheat had a greater density of *P. thornei*. These trends were relatively consistent in the annual rotations (rotations 3 and 4), in the 2-year rotations of winter wheat with fallow (rotations 1 and 2) or with winter pea (rotation 7), in the 3-year rotation that includes winter wheat as one of the two crops (rotation 6), and in the flexible cropping decision treatments (rotation 8). It was notable that, in rotation 7, there was a greater inoculum density of *P. neglectus* when the current crop was winter wheat and a greater inoculum density of *P. thornei* when the current crop was winter pea, each reflecting the influence of the crop during the previous year, yet the density of generic *Pratylenchus* spp. was equivalent in each phase of the rotation (Table 4).

Because the DNA assays revealed an apparent selection of *Pratylenchus* spp. by different cropping systems, we reevaluated that possibility by using the Whitehead tray extraction method and morphological identification. We used additional subsamples from soil collected from three plots each of annual winter wheat and annual spring wheat treatments (rotations 3 and 4) that had already been examined by the RDTS and by Western Laboratories during 2012. Data for the Whitehead-based and DNA-based assays were

consistent in that both indicated a significantly ($\alpha < 0.05$) greater prevalence of *P. neglectus* than *P. thornei* in winter wheat compared with spring wheat (Table 5). The DNA-based assay revealed a significantly greater density of *P. thornei* than *P. neglectus* in spring wheat. Only the elutriation method revealed a significant difference in total *Pratylenchus* spp. density between spring and winter wheat. Data were highly correlated ($\alpha < 0.01$) between our lab and the RDTS for densities of *P. neglectus* ($R^2 = 0.85$) and *P. thornei* ($R^2 = 0.88$). Densities reported for total *Pratylenchus* nematodes per kilogram of soil in these six plots were not significantly ($\alpha > 0.05$) correlated between labs; our lab versus RDTL ($R^2 = 0.17$), our lab versus Western Lab ($R^2 = 0.25$), and RDTL versus Western Lab ($R^2 = 0.11$).

Discussion

We previously reported relationships between *P. neglectus* density and grain yield during the first 5 years of this long-term experiment (41). We reported an increasing prevalence of *P. neglectus* as intensity of wheat was increased, a high nematode density following crops of mustard or winter pea, a low density following spring barley and spring pea, a lack of influence of tillage (chemical versus cultivated fallow) on nematode density, an inverse relationship between yield of winter wheat and in-crop density of *P. neglectus* during the spring, and a reduced capacity of wheat roots to extract water from the soil profile in plots with the highest densities of *P. neglectus*.

In this report for all 9 years of the experiment, our results support our earlier observations. Moreover, we have now also demonstrated that (i) the distribution of two *Pratylenchus* spp. in this experiment was far more complex than previously understood, (ii) apparent *Pratylenchus* spp. density was significantly correlated ($\alpha < 0.01$) and generally higher when based upon extraction of DNA from soil than when based upon a traditional extraction and identification method, (iii) sampling very dry soil sometimes provided a strong underestimate of *Pratylenchus* spp. density when using a traditional extraction method, (iv) there was an inverse relationship ($\alpha < 0.01$) between density of *Pratylenchus* spp. in the upper soil profile and the amount of precipitation during the months preceding the collection of soil samples, and (v) camelina was a poor host for *Pratylenchus* spp.

Previously, we reported a predominance of *P. neglectus* in this experiment (41). We reported that *P. thornei* was also present but occurred at very low densities. Those observations were based upon periodic determinations of species in a few selected treatments, typically of winter wheat. During the last year of the study, while employing real-time PCR to assess nematode densities, we found that there was much more *P. thornei* in certain treatments than had been previously understood. Therefore, the distribution of these two *Pratylenchus* spp. in this experiment was complex. In particular, winter wheat promoted higher densities of *P. neglectus* than *P. thornei* and the reverse was true for spring wheat. It is unknown whether the differential selection of *Pratylenchus* spp. in this experiment was associated with undetermined differences among cultivars of winter and spring wheat or whether it may be associated with different growth habits that differentially favor one species over another. More research is required to validate and to fully understand this observation because wheat cultivars have important differences in their ability to allow one or another of these species to reproduce (13,34–37,50,52). Pacific Northwest wheat cultivars also differ in their ability to tolerate invasion by these species (40). For instance, the spring wheat 'Louise' which was planted in our experiment has been shown to be moderately intolerant to invasion by both *P. neglectus* and *P. thornei* (40). Sheedy et al. (35) reported that *P. thornei* reproduced very effectively on Louise, which was among the most susceptible of spring wheat cultivars, and twice or three times as effectively as on 'Alpowa' spring wheat or 'Camas' barley, respectively. In another study, *P. thornei* reproduced three times more effectively on Alpowa than on Camas (38). Sheedy et al. (33) also showed that Alpowa was much more favorable for reproduction of *P. thornei*

Table 3. Density of *Pratylenchus* spp. in the upper 30 cm of soil during the spring following production of the indicated crop during a 9-year interval (2004 to 2012) in the cropping systems experiment at Moro, OR

Previous crop or treatment[x]	Nematodes/kg[y]	n[z]
Spring wheat	2,873 a	42
Mustard	2,791 ab	3
Winter pea	2,269 ab	18
Winter wheat	1,965 b	123
Camelina	659 c	6
Spring barley	537 c	54
Spring pea	376 c	15

[x] Crops are shown in Table 1. $P < 0.01$.
[y] *Pratylenchus* nematodes per kilogram of soil. Data are back-transformed means of the $\ln(x + 1)$ transformation used for analysis. Numbers followed by the same letter within a column are not significantly different at $\alpha = 0.05$ according to the least significant difference test for non-equal treatments.
[z] Number of observations available during the 9-year interval.

than of *P. neglectus*. Similar differences occur among cultivars of winter wheat. Sheedy et al. (36) reported that *P. thornei* reproduced twice as effectively on 'Tubbs' and 'Tubbs '06' than on 'ORCF 102', and that 'ORCF 101' was intermediate. Sheedy et al. (37) showed that *P. neglectus* reproduced prolifically on ORCF 102 and ORCF 101 and only slightly less on Tubbs and Tubbs '06. Compared with winter wheat cultivars used in our experiment, popular cultivars such as 'Stephens' and 'Madsen' were much poorer hosts of *P. neglectus* but very good to excellent hosts of *P. thornei* (36,37). The choice of wheat cultivar will clearly have an impact on the density and dominance of these two species of *Pratylenchus* in Pacific Northwest fields. This aspect of management for *Pratylenchus* spp. should be given greater consideration for minimizing the level of risk to future plantings of intolerant cultivars or crop species.

Densities of *Pratylenchus* spp. reported from a lab that extracted DNA from soil and identified species by using real-time PCR was strongly correlated ($R^2 = 0.70$, $\alpha < 0.01$) with densities reported by a lab that used traditional elutriation-based extractions and morphological identifications. However, the apparent density reported by the DNA-based method was often about four times greater than for the traditional elutriation method. This discrepancy appeared to diminish as the density based upon traditional extractions became elevated above about 3,000 *Pratylenchus* nematodes/kg of soil. The soils of various treatments in our experiment differed in moisture content at the time of sampling. Data from the RDTS would have included DNA from all nematode stages, including eggs, whereas data from Western Laboratories was based upon morphologically identifiable juvenile and adult stages. Eggs survive in an anhydrobiotic state in dry soils (5) and contribute to DNA extracted for evaluation by real-time PCR (18) but, compared with other life stages, are not easily or commonly detected when nematodes are extracted and identified using traditional methods. This likely con-

Table 4. Density of *Pratylenchus neglectus* and *P. thornei* in the upper 30 cm of soil during the spring for 14 rotational phases of eight main cropping systems; back-transformed means for each rotation during the final year (2012) at Moro, OR

| | | Crop or treatment[x] | | *Pratylenchus* nematodes per kilogram of soil[w] | | | |
| | | | | Root Disease Testing Service | | | Western |
RN[y]	Treatment[z]	Current	Previous	*P. neglectus*	*P. thornei*	Total	Total
1	WW/CvF	WW	CvF	1,790 ± 387 bcd	331 ± 154 c	2,121 ± 363 cd	1,526 ± 350 c
1	WW/CvF	CvF	WW	1,996 ± 278 bc	774 ± 419 bc	2,769 ± 267 cd	1,757 ± 330 c
2	WW/ChF	WW	ChF	1,178 ± 179 cde	524 ± 170 bc	1,702 ± 257 cd	694 ± 122 de
2	WW/ChF	ChF	WW	1,163 ± 642 cde	1,228 ± 520 bc	2,391 ± 539 cd	1,731 ± 259 c
3	Annual WW	WW	WW	10,913 ± 2,402 a	1,874 ± 609 b	12,787 ± 2,983 a	3,546 ± 481 b
4	Annual SW	SW	SW	1,110 ± 542 cde	7,959 ± 1,814 a	9,066 ± 1,277 b	6,269 ± 571 a
5	Annual SB	SB	SB	316 ± 22 e	35 ± 35 c	351 ± 22 d	372 ± 20 de
6	WW/SB/ChF	WW	SB	743 ± 299 de	276 ± 245 c	1,019 ± 214 cd	645 ± 44 de
6	WW/SB/ChF	SB	ChF	183 ± 74 e	295 ± 162 c	478 ± 139 d	188 ± 31 e
6	WW/SB/ChF	ChF	WW	1,002 ± 254 cde	566 ± 189 bc	1,568 ± 426 cd	951 ± 96 de
7	WW/WP	WW	WP	2,460 ± 275 b	607 ± 276 bc	3,067 ± 59 c	832 ± 76 de
7	WW/WP	WP	WW	1,356 ± 873 bcde	1,909 ± 188 b	3,264 ± 1,061 c	1,058 ± 121 cd
8	Flex crop	WW	Cam	418 ± 42 e	1,503 ± 734 bc	1,921 ± 694 cd	648 ± 53 de
8	Flex crop	Cam	SW	1,006 ± 40 c de	7,997 ± 823 a	9,003 ± 803 b	6,443 ± 908 a
	Mean	…	…	1,831	1,848	3,679	1,903
	P > F	…	…	<0.01	0.01	<0.01	<0.01

[w] Densities of *Pratylenchus* spp. per kilogram of soil were based upon DNA extractions using the PreDicta B analysis procedure at the Root Disease Testing Service, Adelaide, Australia, or using traditional physical extraction procedures at Western Laboratories, Parma, ID (Western). Numbers followed by the same letter within a column and within a group are not significantly different at $\alpha = 0.05$ according to the Tukey's honestly significant difference test.

[x] Cam = camelina, ChF = chemical fallow, CvF = cultivated fallow, SB = spring barley, SW = spring wheat, WP = winter pea, and WW = winter wheat.

[y] Rotation number. Crops and treatments in each rotation are shown in Table 1.

[z] Treatment sequence. Flex crop = variable crop sequences over years.

Table 5. Relationship between densities of *Pratylenchus neglectus, P. thornei*, and total *Pratylenchus* spp. using three different methods to extract nematodes from soil collected from annual winter wheat (rotation 3) and annual spring wheat (rotation 4) during 2012 in the long-term experiment at Moro, OR (mean ± standard error of the mean)[x]

Nematodes and extraction method[y]	Winter wheat	Spring wheat	*P > F*	W/S ratio[z]
Total *Pratylenchus* nematodes/kg of soil				
Whitehead	7,322 ± 973	8,936 ± 2,204	0.51	0.8
DNA	12,787 ± 2,983	9,066 ± 1,277	0.28	1.4
Elutriation	3,546 ± 481 b	6,269 ± 571 a	<0.01	0.6
P. neglectus nematodes/kg of soil				
Whitehead	6,631 ± 439 a	1,747 ± 706 b	0.04	3.8
DNA	10,913 ± 2,402 a	1,110 ± 542 b	0.03	9.8
P. thornei nematodes/kg of soil				
Whitehead	1,691 ± 541	7,189 ± 2,854	0.19	0.2
DNA	1,874 ± 609 b	7,959 ± 1,814 a	0.05	0.2
P. neglectus/P. thornei ratio				
Whitehead	3.9 ± 0.8 a	0.2 ± 0.0 b	0.04	…
DNA	5.8 ± 1.2 a	0.2 ± 0.2 b	0.03	…

[x] Numbers followed by the same letter within a column and within a group are not significantly different at $\alpha = 0.05$ according to the Tukey's honestly significant difference test.

[y] Subsamples of well-mixed soil from each of three replicates for each crop type were used to extract *Pratylenchus* spp. from soil. Whitehead = 200-g subsamples were extracted using the Whitehead Tray method and nematodes were identified to species level based upon morphological characteristics at the author's laboratory at Pendleton, OR. DNA = DNA was extracted from 500-g subsamples of the aforementioned samples and nematode counts were derived from species-specific real-time polymerase chain reaction analyses and standard curves at the Root Disease Testing Service at Adelaide, Australia. Elutriation = 250-g subsamples were extracted using an elutriation method and nematodes were identified to genus level based upon morphological characteristics at Western Laboratories at Parma, ID.

[z] Winter wheat/spring wheat ratio.

tributes to greater estimates of nematode density commonly encountered in real-time PCR assays than in traditional assays (12,18,20,60). However, soil handling can also affect the vigor and integrity of nematodes extracted using traditional methods. Hollaway et al. (12) reported that the PreDicta B method using DNA extracted from soil, as used at the RDTS, was not influenced by soil water content at the time of sampling or of drying the soil after sampling. In dry soils, the DNA method revealed apparent densities of *P. neglectus* and *P. thornei* two to four times greater than were revealed by the Whitehead extraction method, which extracts mostly active nematodes. The differences between methods were minimal when extractions were made from the same soils that were sampled while wet. The Whitehead tray also extracted fewer *Pratylenchus* nematodes from soils that had been dried before storage (12). The magnitudes of reduction varied depending upon soil type and differences in soil moisture content. For very dry soils infested by *P. neglectus* and *P. thornei*, the DNA method revealed apparent nematode densities as much as eight times higher than were revealed by the Whitehead method.

One of the 42 data points used to construct Figure 3A was 13 times higher from the DNA extraction than from the traditional extraction and, therefore, was not included in Figure 3B. That datum point represented one replicate of the direct-seed annual winter wheat rotation, for which the soil was very dry and compact during the spring, and for which the soil probes had to be vigorously hammered into the soil to collect samples of a depth equal to other treatments. Collection of those three samples required as many as 20 jarring blows with a heavy hammer to the top of the sampling probe to attain an adequate sampling depth due to very dry soils and an impervious tillage pan. The resulting DNA analyses for one replicate indicated densities much higher than the approximately fourfold differences indicated in the other 41 plots. We suspect that cuticles of *Pratylenchus* spp. were ruptured at much greater frequency when probes were hammered into the soil than when the probes were manually pressed into soil without the use of a hammer. Taylor and Evans (49) reported that fewer *Pratylenchus* spp. could be extracted from dry soils relative to wet soils if the nematodes were physically impaired during sampling. *Pratylenchus* spp. survive dry periods in the anhydrobiotic state (10,56), at which time they are brittle and more easily damaged. Sampling procedures that disrupt soil physical structure reduce the recovery of nematodes using traditional extraction methods (3). In our experiment, the hammer had also been used to sample soils during earlier years when some of the annual winter wheat plots were also hard and dry compared with plots of other treatments. It appears likely that *Pratylenchus* spp. densities were underestimated for annual winter wheat by the elutriation method during 2012 and were likely also underestimated during the very dry springs of 2007 and 2008. However, it is also possible that eggs were the most prevalent life stage in the driest soils sampled, making it difficult to distinguish between potential effects of physical injury to the anhydrobiotic vermiform stages and differences among proportions of life stages caused by differences in soil moisture.

We previously reported that higher numbers of *Pratylenchus* spp. were typically detected in fallow following wheat than during the spring of the winter wheat phase of the rotation (44). In this study, we found twice as many *Pratylenchus* spp. in the cultivated fallow (2,481 nematodes/kg of soil) than in the chemical fallow (1,456 nematodes/kg of soil) treatment nearly 8 months after harvest. The density of nematodes in the young winter wheat crop following these fallow types were very similar: 1,456 versus 1,151 *Pratylenchus* nematodes/kg of soil, respectively. Because wheat cultivars were the same for each phase of rotations 1 and 2, it was assumed that winter wheat in each treatment had a uniform capacity to amplify the density of these nematodes. The least numerical differences in nematode density between types of fallow occurred during 3 years of greater-than-normal spring-time precipitation. Numerical differences were generally greatest during years of normal spring-time precipitation or of drier-than-normal years. These differences suggest that either the rate of *Pratylenchus* nematode multiplication was lower in wheat following chemical fallow than cultivated fallow or the mortality was lower in cultivated soil than in no-till soil. Strausbaugh et al. (47) and Paulitz et al. (24) also reported that fewer *Pratylenchus* spp. were detected in chemical than in cultivated fallow but several reports indicated that densities of *Pratylenchus* spp. were greater in no-till than in cultivated fallows (22,51). Brmež et al. (4), Smiley et al. (42), and Smiley and Machado (41) did not detect a significant difference in density of *Pratylenchus* spp. between chemical and cultivated fallows. However, in our earlier report from the current experiment (41), we did detect nonsignificantly greater numbers of *Pratylenchus* nematodes at all depth intervals in cultivated than in chemical fallow, and the difference in density was greatest (2,194 versus 770 *Pratylenchus* nematodes/kg of soil) in the 30- to 45-cm depth interval. Moreover, in the profile depth sampling we reported earlier (41), the density of *Pratylenchus* spp. was very low (<200 nematodes/kg of soil) in the surface 15 cm and was much greater (about 3,000 nematodes/kg of soil) in the 15- to 30-cm profile segment for each of the fallow systems. In this study, mechanical tillage operations in the cultivated fallow (rotation 1) never disrupted soil to a depth greater than 15 cm. Data reported in this article were from samples routinely collected to a depth of 30 cm, presumably reflecting mostly differences in *Pratylenchus* spp. densities in the 15- to 30-cm depth interval. Because seasonal differences in densities of *Pratylenchus* spp. in the two fallow management systems were detected in our experiment, it is likely that soil moisture, biological antagonism or predation (5,51,55), or other factors differentially influenced the production or survival of *Pratylenchus* spp. in cultivated and uncultivated cropping systems. It is possible that biological interactions led to lower densities of *Pratylenchus* spp. in the uncultivated than cultivated fallow system because these contrasting systems often have important differences in microbial and faunal properties (48). However, it is also important to consider that most microbial differences among tillage systems occur within the tillage zone, which coincided with the depth at which we previously reported the lowest density of *Pratylenchus* spp. in this experiment (41).

We also noted that the density of *Pratylenchus* spp. declined more rapidly during cultivated (50%) than chemical fallow (21%) treatments. Likewise, Ornat et al. (22) reported that density of *P. neglectus* in the spring was reduced more by a combination of tillage and fallow than by fallow alone. It is possible that multiple tillage operations while the soil was very dry, as is standard practice for weed control in the semiarid Pacific Northwest (32), caused mechanical damage to bodies of *Pratylenchus* spp. while they were in the anhydrobiotic state (10,49,56).

When the densities of *Pratylenchus* spp. were averaged over the 42 plots for each year of this experiment, there was an inverse relationship ($R^2 = 0.92$; $\alpha < 0.01$) between mean density of nematodes in the upper soil profile and the amount of precipitation that occurred during the 5 months (November through March) which preceded the time when soil samples were collected. However, this relationship did not occur when compared with amounts of precipitation during shorter time periods immediately preceding sampling, as for January through March ($R^2 = 0.32$) or January through April ($R^2 = 0.47$). There was also no relationship between densities of nematodes and precipitation over the winter wheat crop year (September through August; $R^2 = 0.08$). Although the cause for this relationship is not understood, we hypothesize that, compared with drier soil, the wetter soil during the winter months reduced survival of nematodes produced on crop roots the previous year. It is likely that the number of wetting and drying cycles during the winter months are fewer during the driest winters. If so, the drier periods during late autumn and early winter could favor longer periods of anhydrobiosis induced by cold temperature (56) or they could reduce mortality from parasitism (22). Another possibility is that drier autumn and winter months could be less favorable for vertical migration downward into the soil profile (44), or that soil temperatures were more buffered against surface freezing during wet winters. Further research will be required to understand this observation.

Lowest densities of *Pratylenchus* spp. were detected following spring barley, spring pea, or camelina and higher densities occurred following winter wheat, spring wheat, spring yellow mustard, or winter pea. We are unaware of previous reports of the susceptibility of camelina cultivars to *Pratylenchus* spp. Barley is generally considered more tolerant and more resistant than wheat to *P. neglectus* and *P. thornei* (40,50,55,57). Our findings are also in agreement with previous observations that *Pratylenchus* nematodes become more numerous as host-crop frequency is increased (9,29). Smiley (40) and Smiley and Machado (41) reported a high density of *P. neglectus* following either yellow mustard or wheat. Field pea is considered resistant to *P. neglectus* in Australia (57) but has been associated with high populations of *Pratylenchus* spp. in the Pacific Northwest (28). The hosting abilities of field pea require further investigation, including the spring versus winter growth habit (e.g., the length of the growing season) and differences in cultivars or subspecies. In particular, the apparently greater multiplication of *Pratylenchus* spp. in winter wheat that followed winter pea than in winter wheat that followed chemical fallow requires further investigation because the greater amplification of nematode density following winter pea could be expected to substantially increase the level of root injury in subsequent plantings of intolerant winter wheat cultivars.

Camelina, barley, and spring pea were identified as poor hosts that could be used to reduce the density of *P. neglectus* and *P. thornei* in heavily infested wheat fields, particularly if used in 3-year rotations. Selection of different *Pratylenchus* spp. by wheat of different growth habit or cultivar selection requires further study, pending development of cultivars with dual-species resistance. This finding also underscores the importance for developing species-specific diagnostic methods that can be easily and fully adopted into routine use by nematode diagnostic laboratories. This is critical in view of spring wheat cultivars that differentially vary in tolerance to *P. neglectus* or *P. thornei* (40).

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