Assessment and Management of Root Lesion Nematodes in Montana Wheat Production

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Abstract

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Root lesion nematodes (*Pratylenchus* spp.) hinder dryland wheat production worldwide. Montana, a leading U.S. wheat production region, has climatic conditions favorable for *Pratylenchus* spp. A 2006-2007 statewide soil survey revealed damaging populations of *Pratylenchus neglectus*, primarily in winter wheat production areas of Montana, whereas *P. thornei* was not found. Analyses of wheat yields in infested fields revealed negative correlations between yields and spring nematode populations (all $P < 0.05$ and all $R^2 > 0.2$). Statewide yield losses due to root lesion nematodes were an estimated 12 and 15% for winter wheat in 2006 and 2007, respectively. A subsequent study conducted in 2008 to 2009 revealed significant

differences in reproductive success of *P. neglectus* among seven rotation treatments ($P < 0.001$). Nematode populations persisted from spring to fall under fallow, barley, pea, and camelina; increased under winter wheat and canola; and decreased under lentil. Populations were sustained through winter following winter wheat and barley but declined following canola, camelina, pea, lentil, and fallow. A screening of 19 barley lines for resistance to *P. neglectus* revealed significant variation in resistance among entries ($P < 0.001$), with 'Harrington' barley displaying promising levels of resistance. Development of resistant wheat cultivars remains a principal goal in managing this nematode.

Root lesion nematodes (*Pratylenchus thornei* Sher & Allen and *P. neglectus* (Rensch) Filipjev Schuurmans & Stekhoven) present a serious challenge to dryland wheat production worldwide (Smiley et al. 2004a; Thompson et al. 2008b). Of the two, *P. thornei* is reportedly more destructive, causing estimated yield losses of up to 85% in Australia, 70% in Israel, 37% in Mexico, and 50% in the United States (Armstrong et al. 1993; Nicol and Ortiz-Monasterio 2004; Smiley et al. 2005b). Estimated yield losses due to *P. neglectus* of up to 30% have been reported in Australia (Vanstone et al. 2008) and up to 37% in the Pacific Northwest region of the United States (Smiley et al. 2005c). Whether due to an increased awareness or spread of the nematode, reports of crop damage have been growing over recent years in the United States (Appel et al. 2012; Johnson et al. 2007).

Both *P. thornei* and *P. neglectus* infest wheat roots, resulting in reduced root growth, along with the formation of dark necrotic lesions that can serve as secondary infection sites for root-rotting fungi (Jones et al. 2013). The root damage that results from *Pratylenchus* infestation hinders a plant's absorption of water and nutrients. Crops infested while growing under moisture stress experience the greatest yield losses (Nicol and Ortiz-Monasterio 2004). Aboveground symptoms are nonspecific and often mistaken for nutrient deficiencies or drought stress (Doyle et al. 1987; Smiley et al. 2005b,c; Thompson et al. 1995; Van Gundy et al. 1974). These include stunted plant growth, chlorosis, and early death of lower leaves, as well as reductions in plant vigor, grain yield, grain quality, and tiller count (Smiley and Nicol 2009).

Root lesion nematodes are endoparasites capable of completing their entire lifecycle within the root systems of host plants (Williams et al. 2002). This ability to multiply within host tissue allows *Pratylenchus* spp. to thrive in semiarid growing regions where the absence

of free moisture can limit the activity of free-living nematodes (Vanstone et al. 1998). Like many wheat pathogens, greater population densities of *Pratylenchus* nematodes build under annual wheat cropping systems as opposed to wheat-fallow rotations (Smiley et al. 2004b). Given that spring population densities of root lesion nematodes negatively correlate with yield for both *P. neglectus* and *P. thornei*, spring survey data for these nematodes have been invaluable in the assessment of their damage (Smiley et al. 2005b,c).

Tools available for the practical management of root lesion nematodes in wheat are limited. For research studies, the nematicide Aldicarb (Temik 15G) has shown efficacy in early protection of root development but, due to its persistence, toxicity, and cost, is not currently labeled for commercial cereal production (Kimpinski et al. 1987). There are reports that tillage can reduce nematode population density in the short term (Lenz and Eisenbeis 2000; López-Fando and Bello 1995) but it has also been shown that tillage can shuffle the trophic relationships among soil fauna such that plant parasites become the dominant nematode group (López-Fando and Bello 1995). This leaves the incorporation of fallow periods or resistant rotational crops (Kratochvil et al. 2004; Taylor et al. 2000) and the use of resistance incorporated into locally adapted wheat cultivars as the primary sources of control for root lesion nematodes.

In areas where wheat is of particular economic importance, alternative resistant rotational crops must be identified and deployed in an attempt to reduce population densities of the nematodes below yield-reducing levels. Use of resistant or nonhost cultivars and crops in rotation have been shown to be as effective as nematicides in controlling *Pratylenchus* spp. (Taylor et al. 1999). A small number of rotational crops have been identified that are both suitable for low-moisture environments and economically viable. Reports of root lesion nematode control exist for several rotational crops, including field pea, lentil, canola, and barley (Smiley et al. 2004a, 2005a; Taylor et al. 2000). Unfortunately, the existing literature is conflicted on their efficacy. For crops such as pea and barley, conflicts are perhaps related to the nematode species involved (Gair et al. 1969; Riga et al. 2008; Smiley and Machado 2009; Smiley et al. 2004a; Taylor et al. 2000) or to the variability in resistance among cultivars each crop presents (Keil et al. 2009). Other crops such as cruciferous oilseeds (*Brassica* spp.) are documented hosts of root lesion nematodes

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(Taylor et al. 2000). However, *Brassica* spp. also produce varying levels of volatile glucosinolates that form 2-phenylethyl isothiocyanate, a compound known to reduce *P. neglectus* population density, upon degradation (Potter et al. 1998; Yu et al. 2005). Assessment of the overall impact of *Brassica* crops on root lesion nematodes has not been fully explored.

Development of wheat cultivars with resistance, a term describing a plant's ability to inhibit a pathogen's reproductive rate, is the most important and economical tool for long-term management of root lesion nematodes. Resistance to *Pratylenchus* spp. in wheat has been shown to be largely species specific, meaning that resistance genes that are effective for *P. thornei* are not necessarily effective for *P. neglectus*, and vice versa (Farsi et al. 1995). Problematically, mixed populations of both species are often found in the same field (Smiley et al. 2004a; Thompson et al. 2008a). Therefore, the development of cultivars with dual resistance would greatly simplify selection of an appropriate resistant cultivar for growers experiencing yield loss due to *Pratylenchus* nematodes. Middle Eastern landrace lines and ancient wheat progenitors have been identified as the most effective sources of root lesion nematode resistance (Hollaway et al. 2000; Nicol et al. 1999, 2001, 2003; Nombela and Romero 1999; Sheedy et al. 2008, 2012; Thompson and Haak 1997; Thompson et al. 1999; Toktay et al. 2006; Zwart et al. 2004, 2005). However, these lines lack the essential adaptation and agronomic traits to be utilized in large-scale production. Resistance genetics from these sources will need to be incorporated into adapted cultivars using backcross breeding regimes; however, the low throughput of phenotypic screenings (Zwart et al. 2005) and a lack of reliable marker data (May 2015) have slowed breeding efforts toward the development of resistant wheat lines such that only a few resistant cultivars are currently available worldwide.

In 2005, *P. neglectus* was detected in soil samples from a poorly performing wheat field in central Montana. Although root lesion nematodes had been found in Montana before, this was the first association between root lesion nematodes and reduced wheat yield in the state. Subsequently, three projects were carried out: to survey Montana field soils for populations of *Pratylenchus*, identify resistant rotation crops, and screen resistant barley germplasm.

Materials and Methods

Soil survey project. *Survey methodology.* Nematode surveys were conducted in 2006 and 2007 to identify the species of *Pratylenchus* associated with wheat production in Montana, to define their

distribution both spatially and by cropping system, and to assess the damage caused by these nematodes in terms of reduced wheat yield. Seventeen Montana counties were chosen for the survey based on wheat acreage (NASS 2004), with those selected accounting for 82% of total wheat acreage for the state. All soil samples for the survey were taken after spring planting from April through late June in 2006 and 2007. In 2006, 148 bulked soil samples were collected for processing. Ten fields were sampled in 11 counties, and five to eight fields were sampled in six other counties (Table 1). In all, 117 bulked soil samples were collected for processing in 2007: 11 fields were sampled in one county, 10 fields in seven counties, and two to eight fields in seven other counties (Table 2).

For each field sampled, county agents took nine soil cores in a "W" pattern. The cores were collected starting 30 m from the edge of the field, with 8 m separating each core from its neighbor. Cores were taken to a 23-cm depth using a 2.5-by-30-cm soil core sampler. The nine soil cores were then combined and mixed thoroughly to make a bulked soil sample. The bulked samples were then placed in a soil collection bag labeled with the following information: grower, county, previous crop, and cropping system; either no-till or conventional tillage; and annually cropped or wheat-fallowed.

Soil sample processing. Once soil samples were received, they were placed in cold storage at 4°C until processing. The Whitehead tray method was modified to extract nematodes from the soil samples (Whitehead and Hemming 1965). For each soil sample, nematodes were extracted from 200 g of fresh soil over 48 h at 20°C using 1 liter of tap water, after which the extraction water was passed through a 20-µm mesh sieve. The nematodes were stored in water at 4°C until microscopic examination could be conducted. Extracted nematode populations were examined for the presence of both *Pratylenchus* spp. known to infect wheat (*P. neglectus* and *P. thornei*) and stunt nematodes (*Tylenchorhynchus* spp.). Concurrent with nematode extractions, percent soil moisture was determined by drying 100 g of fresh soil at 70°C for 48 h. Time from receiving to processing samples varied from 1 day to 2 weeks.

Nematode extraction and quantification. The nematode suspension (2 ml) was placed into a McMaster Counting Slide (Chalex Corporation, Wallowa, OR) and the nematodes were counted using 10× magnification on a Nikon Eclipse 50i microscope (Kent, WA). Resulting counts from the 200-g soil samples were converted to nematodes per kilogram of dry soil. Nematode identifications were conducted based on length, width, and vulva position in relation to percent body length. *P. neglectus* is distinguishable from *P. thornei*

Table 1. Mean population density and incidence of root lesion (*Pratylenchus neglectus*) and stunt (*Tylenchorhynchus* spp.) nematodes for 17 counties sampled in Montana in 2006

County	N ^a	Root lesion (nematodes/kg of soil) ^b	Root lesion (%) ^c	Stunt (nematodes/kg of soil) ^b	Stunt (%) ^c	Wheat area (ha) ^d
Chouteau	10	3,844	60	1,036	90	215,700
Fergus	10	3,375	100	2,310	100	76,700
Cascade	10	3,252	80	667	90	57,700
McCone	10	1,440	70	2,485	100	95,500
Hill	10	880	60	3,005	100	181,000
Pondera	10	679	40	1,410	100	72,400
Toole	10	565	40	1,860	100	96,100
Yellowstone	6	301	83	2,311	100	40,900
Glacier	10	89	20	2,544	90	54,000
Phillips	6	73	50	420	83	52,500
Roosevelt	8	61	13	1,672	100	138,500
Valley	10	5	10	1,460	90	117,900
Liberty	10	0	0	2,970	100	100,700
Dawson	5	0	0	1,900	100	66,500
Sheridan	7	0	0	202	28	132,400
Richland	6	0	0	303	100	61,800
Daniels	10	0	0	1,895	100	90,800

^a Number of fields sampled.

^b Mean root lesion or stunt nematode population density indicates the mean of all samples taken from a county in 2006. All soil samples taken after spring planting, April to June 2006. Nematodes were extracted from a 200-g subsample from each bulked field soil sample using the 48-h tray method described by Whitehead and Hemming (1965). Nematodes were enumerated on the microscope and resulting counts were converted to nematodes per kilogram of dry soil.

^c Root lesion (*P. neglectus*) or stunt (*Tylenchorhynchus* spp.) incidence as a percentage of field samples examined from each county.

^d Based on 2002-2003 county estimates (NASS 2004).

by being notably shorter and wider and having a more pointed tail than the dorsally flattened tail of *P. thornei*; diagnostic vulva position for *P. neglectus* is between 80 and 87% of the body length versus 73 to 80% of body length for *P. thornei* (Handoo and Golden 1989). *Tylenchorhynchus* spp. were identified to genus level by having a strong stylet, nonoverlapping esophagus, didelphic vulva position, and conical tail shape (Mai and Mullin 1960). In total, 12 representative samples were sent to the Oregon State University nematode diagnostic laboratory and 10 samples sent to Columbia Basin Agricultural Research Station (Pendleton, OR) for confirmation of results.

In addition to the above statewide soil survey, two field sites were selected in 2006 and 2007. The 2006 field site was a winter wheat field ('Genou') infested with *P. neglectus* in Ulm, MT, while the 2007 site was an infested spring wheat field ('Vida') at the Arthur H. Post Farm in Bozeman, MT. Both studies utilized randomly selected 3-m segments of row to analyze the relationship between spring population densities of *P. neglectus* and subsequent fall grain yield. In spring 2006 and 2007, three soil cores were removed from the center 1 m of each selected row, bulked, and subjected to the nematode extraction and quantification protocols described above. Selected rows were flagged and the global positioning (GPS) coordinates were recorded for each. In fall 2006 and 2007, grain was harvested and weighed from 1 m of row on either side of the sampled center portion. The resultant grain yield in grams was converted to kilograms per hectare. Overall, 20 and 24 randomly selected 3-m segments were collected in 2006 and 2007, respectively.

Crop rotation project. *Experimental design.* A field crop rotation experiment was conducted in 2008 and 2009 at the Central Agricultural Research Center (CARC) in Moccasin, MT. This site was previously identified as having substantial populations of *P. neglectus*. Precipitation totaled 32 and 28 cm for the 2008 and 2009 crop years, respectively, with 23 cm falling between April and August 2008 and 17 cm falling during the same period in 2009. Average long-term precipitation for this site is 39 cm for crop years (September to August), with 29 cm falling between April and September (CARC 2016). Mean high temperatures for July and August were 29 and 26°C for 2008 and 2009, respectively. Soil at CARC is a shallow clay loam with a plant-limiting hardpan layer at around 60 cm in depth.

The crop rotations included fallow-winter wheat, barley-winter wheat, canola-winter wheat, camelina-winter wheat, pea-winter wheat, and lentil-winter wheat. The dryland crop rotation study was fully phased, and the rotation treatments were replicated four times. All rotation treatments—fallow, winter wheat ('Yellowstone'), barley ('Haxby'), canola ('Hyola 357 Magnum'), camelina ('Blaine Creek'), lentil ('Red Chief'), and pea ('Delta')—appeared each year in 2008 and

2009. Plots measured 8 by 20 m, and all crops in the rotation study were planted on 30-cm row spacing. All plots were managed with no-till practices. Crops were managed following the local production guides (McVay and Lamb 2008; McVay et al. 2009, 2010, 2013).

Field soil sampling for root lesion nematodes. Nematodes were extracted from soil samples taken with manually operated probes (2.5 cm in diameter) to a depth of 30 cm. GPS coordinates were used to record the location where each soil core was taken. This enabled a more accurate resampling of locations for each sampling date, thereby reducing the variability associated with the horizontal distribution of soilborne nematodes (Taylor and Evans 1998). Spring sampling was conducted shortly after emergence to ensure that the nematodes were active. Within these spring and fall periods, samples were taken from the field when soil moisture content was optimum for probing, or approximately 15% water content. Samples for the rotation experiment were taken on 15 May and 23 October 2008 and 27 May and 3 September 2009. Samples from the barley trials were taken on 15 May and 23 October 2008 and 21 May and 19 September 2009.

Six soil cores were taken 150 cm apart from each plot for the rotation experiment as well as the barley cultivar trials. These six cores were then combined and mixed in a plastic bag for a single bulked sample. The bulked samples were stored at 4°C while awaiting processing. Storage times never exceeded 4 weeks (Hollaway et al. 2003). Nematode extraction, identification, and quantification methodology for the 2008-2009 survey was identical to that utilized in the 2006-2007 statewide survey.

Barley greenhouse and field studies. *Barley greenhouse cultivar trials.* The Arthur H. Post Agronomy Farm located in Bozeman, MT was previously identified as having high populations of *P. neglectus*. To establish working populations of the pathogen, field soil was collected at the Post Farm and mixed with equal parts coarse sand, placed in 20-cm pots, and planted with six seeds of susceptible 'Machete' wheat (Taylor et al. 2000). The resulting pot cultures were maintained for 8 to 12 weeks at diurnal temperatures of 22°C by day and 18°C by night. Day length was supplemented to 14 h with 430-W sodium halide lamps during winter months. Root lesion nematodes were extracted from the pot culture soil using the modified Whitehead tray method, as described for the 2006-2007 survey. Nematode population counts were then determined for the extracted suspension. Immediately after extraction, these nematodes were used as inoculum for all greenhouse experiments.

Barley was planted in a steam-pasteurized mixture of 50% loamy field soil and 50% coarse sand. Each 15-cm pot was first lined with a nonporous plastic bag to prevent nematode leaching. The pots were

Table 2. Mean population density and incidence of root lesion (*Pratylenchus neglectus*) and stunt (*Tylenchorhynchus* spp.) nematodes for 14 counties sampled in Montana, 2007

County	N ^a	Root lesion (nematodes/kg of soil) ^b	Root lesion (%) ^c	Stunt (nematodes/kg of soil) ^b	Stunt (%) ^c	Wheat area (ha) ^d
Chouteau	10	3,306	80	5,895	90	215,700
Fergus	10	2,400	90	756	90	76,700
Cascade	6	2,670	100	1,220	50	57,700
McCone	10	1,285	50	3,437	100	95,500
Hill	10	953	50	1,330	100	181,000
Pondera	11	811	45	725	45	72,400
Toole	10	2,375	20	1,001	80	96,100
Glacier	10	100	30	1,825	90	54,000
Phillips	6	0	0	538	83	52,500
Roosevelt	8	24	13	1,306	88	138,500
Valley	2	29	50	1,337	100	117,900
Liberty	10	385	20	4,254	100	100,700
Sheridan	7	0	0	751	86	132,400
Daniels	5	0	0	1,020	60	90,800

^a Number of fields sampled.

^b Mean root lesion or stunt nematode population density indicates the mean of all samples taken from a county in 2007. All soil samples taken after spring planting, April to June 2007. Nematodes were extracted from a 200-g subsample from each bulked field soil sample using the 48-h tray method described by Whitehead and Hemming (1965). Nematodes were enumerated on the microscope and resulting counts were converted to nematodes per kilogram of dry soil.

^c Root lesion (*P. neglectus*) or stunt (*Tylenchorhynchus* spp.) incidence as a percentage of field samples examined from each county.

^d Based on 2002-2003 county estimates (NASS 2004).

filled with 800 g of the soil mixture. Five seeds were then placed on top of the soil. The nematode suspension was pipetted onto the surface of the soil in the area of the seed. Each experimental pot was inoculated with 1,000 *P. neglectus* adults. The same soil mix (200 g) was then added to the pots to cover the seed and nematodes. Osmocote 14-14-14 slow-release fertilizer pellets (3 g; Scotts Company, LLC, Marysville, OH) were sprinkled on top of the soil. The pots were then watered to maintain field capacity. Fourteen days after emergence, the pots were thinned to one plant per pot.

For each variety trial, 19 barley varieties were planted in a randomized complete block design consisting of four blocks. Each block included known resistant ('Excalibur') and susceptible ('Machete') wheat cultivars as controls (Taylor et al. 2000). The experimental area was surrounded with barley plants ('Haxby') to reduce edge effects. The plants were grown for a period of 12 weeks under conditions described above for the pot cultures, then processed for counting *P. neglectus* populations.

To count final *P. neglectus* populations, all greenhouse barley plants were cut 2 cm above the soil surface after 12 weeks of growth. Soil bags were removed from the pots, sealed, and stored at 4°C. The nematodes were then extracted from the soil samples within 4 weeks of harvest to minimize losses of nematodes during storage (Hollaway et al. 2003). Soil samples were processed using the Whitehead tray method and nematodes were identified and quantified as described above for the 2006-2007 survey. A concurrent 100-g subsample was also removed to determine percent soil mass.

Barley field cultivar trials. The dryland barley trials consisted of 20 cultivars replicated three times each in a randomized block design. Nine of these cultivars were present in both 2008 and 2009. Plots measured 2 by 4 m with 30-cm row spacing. Seed was planted at a rate of 57 kg/ha with a double disk seeder. A 10-10-10-5 NPKS fertilizer was applied with the seed and the plots were later top dressed with urea N at 49 kg/ha. The 2008 barley nursery was planted into winter wheat stubble, and the 2009 nursery was planted into untilled camelina stubble. Plots in both years were managed with no-till and treated as needed with Bronate herbicide (2-methyl-4-chlorophenoxyacetic

acid + bromoxynil) at 2.3 liters/ha. Barley was seeded on 18 April 2008 and 10 April 2009, and harvested on 25 June 2008 and 6 July 2009. Soil samples were taken and nematodes were extracted following the same procedure described in the dryland crop rotation study.

Statistical analysis. The survey study utilized a *t* test analysis to perform statistical comparisons of mean *Pratylenchus* population densities based on information reported along with sample material, including the previous year's crop (spring or winter wheat), wheat-fallow versus annual cropping systems, and conventional versus no-till field management. Simple linear regression was used to test for correlation of mean root lesion nematode population densities for counties between the 2006 and 2007 survey years. To test for a relationship between spring soil *P. neglectus* population density and grain yield from the 2006 and 2007 field studies, a simple linear regression model was fit using spring *P. neglectus* population density as the explanatory variable and grain yield as the response variable.

Analysis of variance (ANOVA) was performed on all rotation study data, with the log-transformed reproductive factor log(Rf) as the response variable. Reproductive factors (Rf) for the rotation study and barley cultivar trials were calculated as Pf/Pi, where Pf = final *P. neglectus* population density as measured in fall and Pi = initial population density as measured in spring. Additionally, overwinter reproductive factors (ORf) for the rotation study were calculated as a ratio of postemergence *P. neglectus* population densities in 2009 over the postharvest *P. neglectus* population densities measured in 2008. If the *P* value obtained from the overall ANOVA provided evidence (*P* < 0.05) of at least one difference in mean Rf among the treatments, a protected Fisher's least significant difference (LSD) test was used to distinguish treatment means. For the rotation experiments, the treatments were the different crop species in the individual years. Aside from being analyzed for individual year effects, the 2-year rotations were analyzed using area under the population curve (AUPC) as the response variable, with the four population measurements making up the curve. For the barley field trials, cultivar means were separated and assigned ranks to the varieties with respect to *P. neglectus* Rf. All Rf were log-transformed in order to stabilize variances. For all experiments, a block factor was included to account for variance resulting from differences in environmental conditions across the experimental area. The general linear model used was log Rf = block + year + cultivar. For experiments that were repeated, an *F*-max test was used to assess homogeneity of variance between like experiments (Kuehl 2000). Experiments that were determined to have homogeneous variances were combined and analyzed as a single data set. All statistical procedures were conducted using SAS statistical software (v. 9.3; SAS Institute Inc., Cary, NC).

Results

Soil survey project. *Root lesion nematodes* (*Pratylenchus* spp.). In 2006, *P. neglectus* was identified in 57 of the 148 samples examined, comprising 12 of the 17 counties surveyed (Fig. 1A). Populations of *P. neglectus* were prominent in north-central Montana. Field samples taken from Chouteau, Fergus, and Cascade Counties had mean spring population densities of *P. neglectus* at 3,844, 3,375, and 3,252 nematodes/kg of soil, respectively (Table 1). *Pratylenchus* spp. were not found in fields of the northeastern-most portion of the state, including Daniels, Sheridan, Richland, and Dawson Counties.

Specimens of *P. neglectus* were identified in 47 of the 117 samples examined in 2007, involving 11 of the 15 counties surveyed in that year. Due to issues with sample quality, data for Richland County was omitted from further statistical analysis in 2007. The greatest population densities of *P. neglectus* were predominantly in the north-central counties of Chouteau ($\bar{x}$ = 3,306 nematodes/kg of soil), Cascade ($\bar{x}$ = 2,670 nematodes/kg of soil), and Fergus ($\bar{x}$ = 2,400 nematodes/kg of soil, Table 2). *Pratylenchus* spp. were not found in Phillips County during 2007, although the nematode was detected in soil samples from that county in the previous year. Samples from the far northeast corner of Montana once again contained no *Pratylenchus* spp. (Fig. 1B).

Statistical comparisons of samples containing *P. neglectus* in 2006 provided strong evidence of a difference in mean spring nematode

Fig. 1. Montana counties surveyed for root lesion nematode (*Pratylenchus* spp.) in **A**, 2006 and **B**, 2007. Counties are ranked based on average *Pratylenchus neglectus* spring population densities (*P. thornei* was not found in the survey). Number 1 indicates the county with greatest mean *P. neglectus* population density and number 12 the smallest; x represents counties sampled where no *Pratylenchus* sp. was found.

population density between fields previously planted to winter wheat and fields previously planted to spring wheat ($P = 0.02$; Fig. 2A). The mean 2006 *P. neglectus* population density for fields planted to winter wheat in the prior growing season was 3,390 nematodes/kg of dry soil, compared with 1,275 nematodes/kg of dry soil for fields previously planted with spring wheat. The results of statistical tests indicated no significant difference ($P = 0.14$) in mean spring nematode population density between fields managed with conventional tillage ($\bar{x} = 1,672$ nematodes/kg of soil) and fields managed as no-till ($\bar{x} = 2,886$ nematodes/kg of soil). The difference in mean 2006 spring *P. neglectus* population density between annually cropped and wheat-fallowed fields was also not significant ($P = 0.51$). The mean spring 2006 root lesion nematode population density was 2,800 nematodes/kg of soil for annually cropped fields and 2,134 nematodes/kg of soil for wheat-fallowed fields.

In 2007, analysis of soil samples infested with *P. neglectus* revealed that fields planted previously to winter wheat had larger mean spring nematode population densities ($\bar{x} = 4,045$ nematodes/kg of soil) than fields previously planted to spring wheat ($\bar{x} = 1,921$ nematodes/kg of soil); however, the difference between these means was not significant ($P = 0.15$; Fig. 2B). As in 2006, further comparisons of 2007 mean field *Pratylenchus* population densities in terms of management yielded no evidence of a difference between no-till and conventional till production fields ($P = 0.42$) and between annually

cropped and wheat-fallowed fields ($P = 0.95$). The mean spring 2007 nematode population density for fields managed as no-till was 3,235 nematodes/kg of dry soil, as opposed to 1,699 nematodes/kg of dry soil for fields managed with conventional tillage. Annually cropped fields exhibited a mean spring 2007 population density of 2,974 nematodes/kg of dry soil compared with the 2007 mean of 3,072 nematodes/kg of dry soil for fields that were wheat fallowed.

Among the 17 counties surveyed in 2006, 15 were resampled in the 2007 survey. Simple linear regression analysis for 14 of the 15 resampled counties (Richland County samples were omitted) indicated a strong correlation between the first and second year's mean root lesion nematode population ($R^2 = 0.81$, $P < 0.01$). *P. thornei* was not detected in any of the samples examined. An additional, unknown species of *Pratylenchus* was detected; however, this species was encountered too infrequently in samples to conduct any meaningful statistical comparison.

Stunt nematodes (*Tylenchorhynchus* spp.). Stunt nematodes were recovered from 92% of all samples examined and in all counties surveyed in 2006. The mean spring 2006 *Tylenchorhynchus* population density for fields harboring stunt nematodes was found to be 1,674 nematodes/kg of dry soil. The mean spring 2006 stunt nematode population density was significantly greater in annually cropped fields ($\bar{x} = 2,082$ nematodes/kg of soil) than wheat-fallowed fields ($\bar{x} = 1,357$ nematodes/kg of dry soil) ($P = 0.01$; Fig. 3A). Although

Fig. 2. Comparison of root lesion nematode (*Pratylenchus neglectus*) spring population densities in no-till versus conventional, annual crop versus wheat-fallow, and spring wheat versus winter wheat in A, 2006 and B, 2007. Hash marks represent standard error and an asterisk (*) indicates a significant difference between treatments ($P = 0.02$).

Fig. 3. Comparison of stunt nematode (*Tylenchorhynchus* spp.) spring population densities in no-till versus conventional, annual crop versus wheat-fallow, and spring wheat versus winter wheat in A, 2006 and B, 2007. Hash marks represent standard error and an asterisk (*) indicates a significant difference between treatments ($P = 0.01$).

the difference in mean *Tylenchorhynchus* population density between the tillage system (no-till or conventional) and growth habit (spring or winter) treatments was not significant (both $P > 0.05$), the mean spring 2006 stunt nematode population density was greater for no-till fields ($\bar{x} = 1,920$ nematodes/kg of dry soil) when compared with conventionally tilled fields ($\bar{x} = 1,504$ nematodes/kg of dry soil); a greater mean 2006 stunt nematode population density was observed in fields with previous spring wheat crops ($\bar{x} = 1,778$ nematodes/kg of dry soil) compared with fields with previous winter wheat crops ($\bar{x} = 1,348$ nematodes/kg of dry soil).

For 2007, stunt nematode populations were present in 83% of all samples examined and in all counties involved. Fields containing *Tylenchorhynchus* spp. had a mean spring 2007 population density

of 2,358 nematodes/kg of dry soil. No significant differences in mean spring 2007 stunt nematode population densities were observed, based on statistical comparisons between cropping system (annual or wheat-fallow), tillage system (no-till or conventional), or growth habit (spring or winter) treatments (all $P > 0.05$; Fig. 3B). Mean 2007 population densities were similar in annually cropped fields and in fallow fields ($\bar{x} = 2,054$ and 1,858 nematodes/kg of dry soil, respectively), no-till systems and conventional systems ($\bar{x} = 2,409$ and 2,551 nematodes/kg of dry soil, respectively), as well as spring wheat and winter wheat fields ($\bar{x} = 2,276$ and 3,002 nematodes/kg of dry soil, respectively).

Regression analysis of wheat yield versus *P. neglectus* population. A linear relationship between grain yield and spring *P. neglectus* population density is evident from the scatter plots of the 2006 winter wheat and 2007 spring wheat fields (Fig. 4). In both years, the analysis provided evidence that an increase in initial *P. neglectus* population density was associated with a lower grain yield (2006 winter wheat trial: $P = 0.032$, $R^2 = 0.231$; 2007 spring wheat trial: $P = 0.016$, $R^2 = 0.235$). An increase in mean spring *P. neglectus* population density by 2,000 nematodes/kg of soil was associated with an estimated 7.6% loss in grain yield for winter wheat, while the same increase in initial pathogen density was associated with an estimated 6.8% loss in grain yield for spring wheat. Using the estimated mean spring statewide *P. neglectus* population densities for winter and spring wheat fields from the 2006 and 2007 surveys, along with the linear models we obtained, estimated statewide yield reductions due to root lesion nematodes in 2006 would have been 12.9 and 4.3% for winter and spring wheat, respectively. The estimated statewide loss in yield would have been 15.4 and 6.5% for winter and spring wheat fields in 2007.

Crop rotation project. The mean initial nematode population density for the 2008 rotation study was 625 nematodes/kg of soil. At the 23 October postharvest sampling date, these populations had increased to an average of 1,688 nematodes/kg of soil. Based on ANOVA, significant differences in mean final Rf occurred among the crops evaluated in the 2008 experiment ($P < 0.001$; Table 3). In particular, winter wheat was the best host for *P. neglectus*, with a mean Rf of 8.6. A Fisher's LSD test indicated that the mean Rf of winter wheat was significantly different from that of any other entry ($P < 0.001$). Canola was the second-best host for *P. neglectus* in the 2008 study, with mean Rf of 3.8. Of the remaining treatments, Fisher's LSD test showed no evidence that any were significantly different from neutral (Rf = 1) in terms of their mean nematode reproductive rates, although there were significant differences in mean Rf among them ($\alpha = 0.05$). In particular, the *P. neglectus* population density in fallow plots increased by a mean factor of 2.8; weeds and volunteer crop growth were controlled under the fallow treatments in the trial. *P. neglectus* populations had a negligible change under camelina. Nematode populations declined, on average, in plots of pea, lentil, and barley, although evidence from the post hoc

Fig. 4. Simple linear regression analyses between spring field plot *Pratylenchus neglectus* populations and corresponding fall field plot yields for A, winter wheat (Genou) in Ulm, MT in 2006 and B, spring wheat (Vida) in Bozeman, MT in 2007.

Table 3. *Pratylenchus neglectus* reproductive factors (Rf) for rotation crops in 2008 and 2009^a

Crop	n	Mean 2008				Mean 2009			
		Pi ^b	Pf ^c	Rf	log(Rf) ^d	Pi ^e	Pf ^f	Rf	log(Rf) ^d
Winter wheat	24	328	2376	8.6	0.835	173	1121	11.8	0.787
Canola	4	942	2393	3.8	0.444	6178	11290	4.9	0.416
Pea	4	1094	567	0.7	-0.244	3839	5136	1.4	0.134
Barley	4	1051	474	0.6	-0.343	2159	2229	1.0	0.001
Camelina	4	833	594	1.0	-0.041	3868	2611	1.1	-0.072
Fallow	4	839	1447	2.8	0.211	3252	1254	0.4	-0.438
Lentil	4	768	522	0.8	-0.110	2303	330	0.3	-0.777
LSD _{0.05} ^g	...	...	...	...	0.344	...	...	...	0.459

^a Rf = Pf/Pi, where Pf = final *P. neglectus* and Pi = initial *P. neglectus* population density in nematodes per kilogram of soil.

^b Samples taken 15 May 2008.

^c Samples taken 23 October 2008.

^d Significant differences (F test, $P < 0.0001$) in mean Rf among crops.

^e Samples taken 27 May 2009.

^f Samples taken 3 September 2009.

^g Least significant difference (LSD, $\alpha = 0.05$) values for mean log(Rf).

tests indicated that these crops had a mean Rf that was significantly different from neither camelina nor an overall neutral effect (Rf = 1) on *P. neglectus* populations.

In 2009, spring nematode population densities in plots following winter wheat averaged 3,600 nematodes/kg of soil. Conversely, initial population densities under the 2009 winter wheat plots, which followed the 2008 alternative crops, averaged 173 nematodes/kg of soil. For the rotational study in 2009, there were significant differences in mean Rf among the crops, based on the ANOVA ($P < 0.001$; Table 3). Again, winter wheat plots sustained the greatest overall increase in *P. neglectus* populations, with a mean Rf of 11.8. There was no evidence from Fisher's LSD test that mean Rf of winter wheat was significantly different from that of canola, which exhibited mean Rf of 4.9. Similar to 2008, *P. neglectus* population densities in plots of pea, barley, and camelina remained relatively unchanged from their spring levels. In 2009, the only crop for which an overall reduction in nematode populations was observed was in lentil plots, where nematode populations declined to 27% of their original level. On average, populations of *P. neglectus* in fallow treatment plots declined to 40% of their original population but the mean Rf of fallow was not significantly different from those of barley and camelina, which exhibited approximately neutral mean Rf values of 1.0 and 1.1, respectively.

Results obtained from a second ANOVA, comparing mean *P. neglectus* ORf between fall 2008 and spring 2009 under treatment plots, indicated that there were significant differences in mean ORf among the 2008 rotation crops ($P < 0.0001$, Table 4). Although the mean winter wheat ORf value was greater than 1 and mean ORf for barley was less than 1, the two means were not significantly different, according to Fisher's protected LSD test. All other treatments in the study had mean ORf values that were significantly less than 1. Estimated mean ORf values for crops with significantly negative effects on *P. neglectus* population density were 0.19 for camelina, 0.09 for canola, 0.08 for pea, and 0.05 for lentil.

Analysis of the AUPC showed that rotations starting with winter wheat generally had higher populations than those starting with the alternative crops (Table 5). The greatest increases occurred when canola and pea followed winter wheat, and the mean AUPC of both of these treatments were found to be significantly different from all others based on Fisher's LSD test ($P < 0.05$). The rotations of winter wheat following barley, lentil, camelina, and pea resulted in the lowest AUPC, and significant differences among these means were not inferred from the LSD test ($P > 0.05$).

For the 2009 study, an ANOVA F test indicated significant differences ($P = 0.035$) in mean winter wheat yield among the previous year's crop treatments (Table 6). Based on the protected Fisher's LSD test, there were no significant differences among the mean winter wheat yields following fallow, pea, lentil, and canola plantings in

the previous year, but there was a significant difference in mean 2009 winter wheat yield between fallow and camelina, as well as between fallow and barley and pea and barley. No evidence was provided from the post hoc tests that 2009 mean yields following barley, camelina, canola, and lentil were significantly different. Winter wheat following fallow had the highest 2009 mean yield.

Greenhouse and field barley trials. *Barley field cultivar trials.* Mean initial *P. neglectus* population densities for 2008 and 2009 were 1,339 and 711 nematodes/kg of soil, respectively. Across all cultivars tested in 2008, mean Rf was 1.22, and for 2009 it was 0.92. Data from the 2008 and 2009 trials were combined and analyzed as a single data set, based on an F -max test for homogeneity of variance. Evidence obtained from an ANOVA F test indicated no significant differences in mean *P. neglectus* Rf among the cultivars across years ($P = 0.92$). The overall mean Rf across both years' trials was 1.09, and no evidence was acquired from the statistical tests to suggest the overall mean Rf to be significantly different from neutral.

Barley greenhouse cultivar trials. Data from the 2008 and 2009 greenhouse resistance screenings were combined and analyzed as a single data set, based on the F -max test for homogeneity of variance. Results from the ANOVA F test indicated significant differences in mean final *P. neglectus* Rf among the cultivars evaluated ($P < 0.01$; Table 7). Initial inoculum for the combined experiments averaged 1,000 nematodes/plant. Mean Rf across all entries were less than 1 in both trials, indicating that *P. neglectus* populations declined on average. Mean Rf across both trials was 0.53. No cultivar sustained a net increase in *P. neglectus* populations during the second trial. Susceptible 'Machete' wheat exhibited a mean Rf of 0.91 over both trials, while resistant 'Excalibur' wheat had a mean Rf of 0.39. However, despite this observed difference in means, the difference was not significant ($P > 0.05$). Analysis of the combined data set showed that only 'Stellar ND' and 'Tradition' exhibited a mean Rf greater than 1; however, there was no evidence that the mean Rf of either 'Stellar ND' and 'Tradition' was significantly different from neutral (Rf = 1), or from that of five of the other cultivars evaluated (Drummond, Haxby, Boulder, Merit, and Legacy). Furthermore, results of the post hoc tests provided evidence that the mean Rf of 15 of the barley cultivars, along with that of resistant wheat cultivar Excalibur, were significantly different from 1.

Discussion

Results of the 2006-2007 statewide survey make up the first assessment of root lesion nematodes (*P. neglectus* and *P. thornei*) affecting wheat in the state of Montana. The primary finding from the survey was that, although *P. neglectus* was found to be widely distributed throughout the state, *P. thornei* was not detected. The current known distribution of *P. thornei* in North America now extends from the state

Table 4. *Pratylenchus neglectus* overwinter reproductive factors (ORf) between fall 2008 and spring 2009

2008 Crop	Mean population (nematodes/kg of soil)		Mean ORf ^c	Mean log(ORf) ^d
	Fall 2008 ^a	Spring 2009 ^b		
Winter wheat	2382	3600	1.96	0.1695
Barley	474	226	0.81	-0.3986
Fallow	1447	304	0.47	-0.6438
Camelina	594	91	0.19	-0.8078
Canola	2393	230	0.09	-1.0389
Pea	567	48	0.08	-1.0560
Lentil	522	32	0.05	-1.1855
LSD _{0.05} ^e	...	...	...	0.6215

^a Pf 2008 = *P. neglectus* population density as determined postharvest in fall 2008.

^b Pi 2009 = *P. neglectus* population density as determined postemergence in spring 2009.

^c ORf computed as Pi 2009/Pf 2008.

^d Significant differences in mean ORf among crop treatments (F test, $P < 0.0001$).

^e Least significant difference (LSD, $\alpha = 0.05$) value for mean log(ORf).

Table 5. Area under the population curve (AUPC) for 2-year rotations^a

Crop year 1	Crop year 2	Mean AUPC	Mean log(AUPC) ^b
Winter wheat	Canola	15,509	4.17
Winter wheat	Pea	8,961	3.93
Winter wheat	Fallow	6,451	3.79
Winter wheat	Camelina	6,880	3.74
Winter wheat	Barley	5,959	3.73
Canola	Winter wheat	3,630	3.53
Winter wheat	Lentil	4,522	3.51
Fallow	Winter wheat	2,941	3.44
Barley	Winter wheat	2,654	3.39
Lentil	Winter wheat	2,209	3.27
Camelina	Winter wheat	1,541	3.16
Pea	Winter wheat	1,332	3.10
LSD _{0.05} ^c	...	...	0.32

^a AUPC calculated across four observations taken 15 May and 23 October 2008, and 27 May and 3 September 2009. Analysis of variance was performed using log transformation of AUPC to normalize variances.

^b Significant differences in mean AUPC among rotation treatments (F test, $P < 0.0001$).

^c Least significant difference (LSD, $\alpha = 0.05$) value for mean log(AUPC).

of California, north to Washington state, east to Colorado, and farther northeast into southern Ontario (Yu 1997). The absence of *P. thornei* is likely due to having not been introduced into Montana at this time. The reproductive capabilities of neither *P. neglectus* nor *P. thornei* are greatly inhibited by soil type or moisture, because both species have been found in clay loams, silt loams, irrigated sandy loams, and dryland wheat fields averaging as little as 25 cm of annual precipitation (Smiley 2015). Although conditions in Montana are cooler than those of the Pacific Northwest that favor *P. thornei* over *P. neglectus*, most other factors (including, but not limited to, soil type and moisture levels) are conducive to the presence of this species. The lack of *P. thornei* has important control implications, because host resistances to the two species are independently inherited traits (Farsi et al. 1995) and their respective host ranges vary. This finding greatly simplifies the management challenge facing wheat production in Montana.

P. neglectus was found primarily within the central winter-wheat-growing regions of Montana. Finding greater population densities of *P. neglectus* in fields following a winter wheat crop than a spring wheat crop in 2006 was unexpected, because there were fewer reports of injury in winter wheat than spring wheat at the time (Mojtahedi and Santo 1992). Greater nematode population densities in winter wheat are likely due to a longer growing season and the fact that this crop is less conducive to rotations. Winter wheat crop cycles in Montana typically last a period of 9 to 10 months, compared with 4 to 5 months for spring wheat. In the northeast corner of Montana where low or no populations of root lesion nematodes were detected, spring wheat is typically grown in rotation with safflower, flax, and field pea, crops that are generally reported to be poor hosts of *P. neglectus* (Smiley et al. 2005c). Furthermore, studies have shown the minimum root temperature for development of populations of *P. neglectus* is 7°C (Umesh and Ferris 1992), which may suggest that this nematode has the ability to survive and reproduce within host roots even during the course of inclement Montana winters. Taken together, these observations, along with our findings, indicate that winter wheat host plants provide a more favorable opportunity for amplification of *P. neglectus* populations than spring wheat hosts. This tendency has been supported by a more recent study conducted in the region, which showed that annually cropped winter wheat resulted in significantly greater population densities of *P. neglectus* than annually cropped spring wheat (Smiley et al. 2013).

It is established that nematode damage to crops correlates strongly with spring population numbers, which indicate the inoculum density that will be imposed on the developing plant (Smiley et al. 2004a, 2005c; Vanstone et al. 1998). Studies in Oregon have shown negative correlations between grain yield and spring population densities of *P. neglectus* for spring wheat. For these studies, yield losses of 36% were reported (Smiley et al. 2005b). Relationships between spring nematode population densities and yield losses have been confirmed for both spring and winter wheat in studies conducted in Montana. Based on these correlations and the 2006-2007 survey data, estimated losses due to root lesion nematodes in 2006 would have been 13 and 4% for winter wheat and spring wheat, respectively; estimated losses due to root lesion nematodes would have been 15 and 7% for winter and spring wheat fields, respectively, in 2007. This places *P. neglectus* firmly among the most economically damaging pests

for wheat in Montana. County data from the 2006 and 2007 surveys of *Pratylenchus* populations are strongly correlated. This emphasizes that samples sizes were adequate for determining the distribution of nematodes throughout the state. Generally, cropping systems are fairly homogeneous within counties and reflect not only grower behavior but also common environmental conditions.

Stunt nematode populations were consistent during both years. These results are similar to those of another survey of plant-parasitic nematodes which concluded that factors such as tillage, crop type, and watering had no effect on stunt nematode populations (Strausbaugh et al. 2004). For fields not containing root lesion nematodes, the high incidence of *Tylenchorhynchus* nematodes among soil samples acted as a positive control, indicating that soil samples were properly handled and the nematode extraction method was effective. The substantial population densities and wide distribution of stunt nematode suggest that these nematodes may be an additional cause for concern for Montana's wheat producers. Occurrence of stunt nematode was extensive but its importance is not well understood. Given its prevalence, its interaction and pathogenicity with wheat as a pest should be investigated further.

The 2006-2007 survey established that the predominant species of root lesion nematode, *P. neglectus*, is present in the state of Montana and is of concern to growers. Due to a lack of resistant wheat lines, rotations to nonhost crops will become essential components in grower management practices. The 2008-2009 rotation study examined several viable alternative crops for their ability to control root lesion nematodes. The absence of *P. thornei* simplified this process. The resulting spring to fall population dynamics followed one of three patterns among the rotational crops tested. For wheat and canola, *P. neglectus* population densities significantly increased, indicating that these crops act as good hosts for the parasite, allowing it to not only persist but also reproduce. For fallow, pea, camelina, and barley, population densities of *P. neglectus* did not significantly change. For these crops, the established populations were able to sustain their numbers over the 3 months separating the spring and fall soil assessments. The only crop tested that demonstrated a consistently negative effect on *P. neglectus* population density was lentil. This effect was primarily seen in the second year, when initial populations of the pest were high.

Table 6. Winter wheat yields in 2009 following alternative crops

2008 Crop	Yield 2009 (kg/ha) ^a
Fallow	1,625
Pea	1,506
Lentil	1,386
Canola	1,249
Camelina	1,180
Barley	1,111
LSD _{0.05} ^b	391

^a Significant differences in mean 2009 winter wheat yield among previous crops ($P = 0.035$).

^b Least significant difference (LSD, $\alpha = 0.05$) value for mean 2009 winter wheat yields.

Table 7. Barley greenhouse cultivar trials^a

Cultivar ^b	Mean Pf (nematodes/kg of soil)	Mean log Pf ^c
Stellar ND	1,184	2.90
Haxby	924	2.76
Machete (S)	910	2.75
Drummond	970	2.73
Tradition	1,014	2.73
Boulder	742	2.63
Merit	553	2.57
Legacy	470	2.57
Excalibur (R)	392	2.50
Conrad	431	2.50
Eslick	495	2.49
MT960225	402	2.47
Craft	406	2.44
Hockett	363	2.42
Metcalfe	481	2.37
MTLB32	244	2.27
MT010158	236	2.24
WPB Xena	221	2.21
Geraldine	215	2.17
Baronesse	211	2.11
Harrington	247	2.10
LSD _{0.05} ^d	...	0.39

^a Cultivars are ranked by log Pf (Pf = final *Pratylenchus neglectus* population density as determined 12 weeks after inoculation). Each experimental pot was inoculated with 1000 *P. neglectus* adults.

^b Resistant (R) and susceptible (S) control lines are indicated.

^c Significant differences in mean Pf among cultivars (F test, $P < 0.001$).

^d Least significant difference (LSD, $\alpha = 0.05$) value for mean log(Pf).

Initial assessments of population data were disappointing because fallow, pea, and barley have all been reported as good control options by previous research conducted in Australia and the Pacific Northwest (Smiley et al. 2004a; Taylor et al. 2000). It was suspected that these dynamics were misleading because nematodes present in soil samples from these rotational crops appeared listless, were not producing eggs, and had clear intestinal regions, indicating a lack of recent feeding (data not presented). For fallow treatments, the observed increase in *P. neglectus* population density between spring and fall 2008 was thought to have been a chance occurrence stemming from the low initial levels of inoculum, and was unrelated to growth of volunteer plants and weeds, which were controlled for the growing season in these plots. These observations were prescient because *P. neglectus* populations declined, on average, over the winter of 2008-2009 following fallow, pea, and barley. Unfortunately, the experimental design did not include observations in spring 2010 to allow for a repeated observation of this dynamic. However, a recent report from Oregon has demonstrated that winter survival plays an important role in the population dynamics of *P. neglectus* (Smiley et al. 2013). Montana has a particularly short crop season that may make these effects even more pronounced, making it more important that crop and treatment evaluations for this pest involve overwinter survival assessments. When reevaluated using overwintering data, fallow, pea, camelina, and canola displayed the most substantial declines in nematode populations during the winter period.

Data from this study agree with others in that winter wheat and canola appear to be good hosts for *P. neglectus* (Fatemy et al. 2006; Taylor et al. 2000). Plots of canola following winter wheat exhibited the highest mean *P. neglectus* population density. However, overwintering populations following canola dramatically declined, possibly due to biofumigation. The biofumigant concept is supported by previous studies (Kirkegaard and Sarwar 1999; Potter et al. 1998) that showed breakdown products of canola glucosinolates to be lethal to root lesion nematodes. The canola data also suggest that, despite canola's perceived strong hosting ability, it may, in fact, have an overall neutral effect on root lesion nematodes, because populations of the pathogen dropped back to nondamaging levels by the time the subsequent wheat crop was planted. Unlike the situation observed with canola, pea, and camelina, *P. neglectus* population density following winter wheat continued to increase from fall to spring despite fields being fallowed at that time. Because volunteer and weed control did not occur in these plots until spring 2009, the increase in *P. neglectus* population density may be at least partially attributable to growth of volunteer and weed host plants. However, this observation may also suggest that dead wheat root tissues continue to provide food and refuge for populations of *P. neglectus* even after harvest in no-till systems.

Barley cultivars resistant to root lesion nematodes would be beneficial to regional growers, because barley is already a well-established, profitable rotation on Montana grain farms. Resistance screenings were conducted in both the field and the greenhouse. Data from the greenhouse cultivar resistance screening indicated significant differences in mean final *P. neglectus* population density among entries. 'Harrington' barley showed considerable potential in terms of its resistance; *P. neglectus* populations decreased to an estimated one-quarter of their original populations in pots planted with this cultivar. Conversely, 'Stellar ND' supported a modest increase in nematode populations. The differences observed in mean Rf among these cultivars may warrant replacing 'Haxby', one of the least resistant barley cultivars tested in the greenhouse trial, with a more resistant variety such as 'Harrington' in future rotational studies. Although past research has been conflicted on the impact of barley on root lesion nematodes (Gair et al. 1969; Smiley et al. 2008), more recent studies have identified moderately resistant phenotypes in wild and cultivated barley accessions, and five quantitative trait loci associated with resistance to *P. neglectus* have been mapped in the barley genome (Kiel et al. 2009; Sharma et al. 2011). Further screening of adapted barley germplasm could prove useful for Montana small grain producers seeking to actively exploit readily available resistance in their wheat-barley rotations.

Results from the field resistance screening suggest that the controlled environment of the greenhouse is more appropriate for detecting

cultivar separation with respect to nematode resistance. The lack of separation in mean Rf for cultivars in the field trials was not surprising, because such studies have considerably more uncontrolled and confounding factors than greenhouse trials. These include but are not limited to variability in initial nematode populations, differences in soil moisture levels (Govaerts et al. 2007; López-Fando and Bello 1995), cultural factors (Lenz and Eisenbeis 2000; Thomas 1978), and sampling error.

In summary, the 2006-2007 survey showed significant populations of *P. neglectus* and an absence of *P. thornei* within Montana. Populations of *P. neglectus* were primarily found in soils planted with winter wheat, likely due to its longer duration of growth as well as spring wheat being more amenable to rotations. The subsequent rotational study showed that both winter wheat and canola were good hosts of *P. neglectus* whereas pea, lentil, camelina, and barley were not. Differences among crops were more apparent when spring-to-spring rather than spring-to-fall population comparisons were used. The 1 year of spring-to-spring comparisons available indicated that canola plots had substantial postharvest declines, which may be attributable to the decomposition of glucosinolates into compounds toxic to root lesion nematodes. More recently, a Pacific Northwest study found *Pratylenchus* sp.- and cultivar-specific effects on root lesion nematode populations for pea, chickpea, lentil, and *Brassica* spp., whereas safflower, sunflower, and flax were all found to be minor or poor hosts of both *P. neglectus* and *P. thornei* (Smiley et al. 2014). However, the economic potential of safflower, sunflower, and flax may be lacking and growers may find little benefit in utilizing these crops in rotation with wheat. Greenhouse resistance screenings of barley cultivars demonstrated that useful variation in resistance to *P. neglectus* exists among commercial cultivars that could be exploited in the future. Addressing the yield losses caused by *P. neglectus* is a continuing critical challenge for Montana's wheat production, one that will hopefully be addressed in the future through the rotations to nonhost crops and the development of resistant wheat cultivars.

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