

EVALUATION OF ALTERNATIVE CROPS FOR MANAGEMENT OF  
*PRATYLENCHUS NEGLECTUS* IN MONTANA  
WINTER WHEAT PRODUCTION

by

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## ABSTRACT

A series of crop rotations were evaluated for their impacts on soil-borne populations of root lesion nematodes, *Pratylenchus neglectus*. Population changes, measured as the ratio of *P. neglectus* adults counted at harvest to those counted at planting time, was recorded under a series of two-year rotations alternating winter wheat with either fallow, barley, pea, lentil, canola, and camelina. Fallow, barley, pea, and camelina were found to have a neutral effect on nematode populations ( $p < 0.001$ ). Winter wheat and canola caused significant increases in populations, while lentils caused significant decreases ( $p < 0.001$ ). Populations were sustained through winter following winter wheat and barley, but not canola, camelina, pea, lentil, or fallow treatments ( $p < 0.001$ ). In addition to the rotation study, cultivars of barley and canola were evaluated for their resistance to *P. neglectus*. Greenhouse trials for barley showed significant differences among 19 cultivars tested ( $p < 0.001$ ), with a 5-fold difference in *P. neglectus* multiplication separating the least- from the most resistant. Separation among cultivars was not found with canola ( $p = 0.20$ ). The information gathered in this study will help Montana wheat growers better understand the impacts of their crop selections on this important pest.

## CHAPTER 1

## INTRODUCTION

Root lesion nematodes (*Pratylenchus neglectus*, (Rensch, 1924) Filipjev and Schuurmanns Stekhoven, and *P. thornei*, Sher and Allen 1953) are important pests of wheat in the semi-arid regions of Australia, Iran, Israel, Mexico, and the Pacific Northwestern United States (Thompson, et al., 2008, Fatemy et al., 2006, Orion et al., 1984, Van Gundy et al., 1974, Smiley, et al., 2004, Strausbaugh, et al., 2004). The feeding of root lesion nematodes (RLN) destroys lateral roots, limiting their ability to draw water and nutrients from the soil (McGawley et al., 1998). This results in increased late season water stress, and reductions of plant biomass, kernel weight, and grain yield (Smiley, et al., 2004). In addition to directly damaging crop roots, the feeding of RLN creates infection courts for other root pathogens that further reduce root function (Castillo 1998). As growers add fertilizers to offset poor growth caused by the nematodes, they may exacerbate the situation because nitrogen enhances nematode reproduction and creates higher inoculum levels for successive crops (Thompson, et al., 1995). Generally, the extent of the damage is population dependent with field studies showing a linear correlation between the soil-borne population densities of *Pratylenchus* spp. and yield losses for wheat and other crops (Hollaway et al., 2003). Typical yield losses due to RLN range from 10-30%, but losses as high as 70% have been reported for intolerant wheat cultivars grown under semi-arid conditions (Thompson et al., 2008).

Root lesion nematodes are especially important in dryland production because they hinder the plants' uptake of moisture and plant nutrients by pruning lateral roots and inhibiting root function (Agrios, 2005). Compounding the problem is that *Pratylenchus* spp. are particularly well suited to semi-arid growing conditions. As endoparasites, RLN complete their entire life cycle within the root cortex of the host plant (Duncan and Moens, 2006). Inside the roots, they are protected from many of the hazards of the living soil, such as antagonistic fungi and bacteria and limited soil moisture. (Lambert and Bekal, 2002). In wheat-fallow systems, which are typical of semi-arid growing regions, RLN may survive drought conditions and the lack of suitable host plants by entering a quiescent state called anhydrobiosis (Van Gundy, 1965, Talavera et al., 1998). This desiccated state allows the nematode to survive in dormancy for extended periods of drought and extreme temperatures (Glazer and Orion, 1983). This adaptability combined with the protection RLN receive from their host plants allows them to thrive under a broad range of environmental conditions.

Controlling RLN has been difficult, as biological and chemical controls have little effect and cultural controls such as tillage run counter to the water conservation needs of semi-arid wheat growing regions of the world. With these facts in mind, management of RLN has consisted of the incorporation of limited resistances into locally adapted wheat varieties (Taylor et al., 2000) and rotation to crops that are non-host or resistant hosts to these pests (LaMondia 2006, Taylor et al., 1999, Ferris et al., 1994). For resistance to *P. neglectus*, the *Rlnn1* gene has been identified in the Australian spring wheat variety 'Excalibur'

(Williams et al., 2002). Additional resistance genes have since been identified for both *P. neglectus* and *P. thornei* (Zwart et al., 2005).

While these resistance genes have been identified, none have yet been bred into wheat varieties adapted to Montana. Because resistant wheat lines take several years to develop, Montana wheat producers must rely on rotation crops for RLN management in the short term. In Montana, hard red bread wheat is at the center of dryland agricultural production with annual production occurring on about 2 million hectares (USDA). The wheat is typically produced in a wheat-on-wheat or wheat- summer fallow rotation but more recently rotations to barley, legumes (pea, lentil and chick peas), and mustards (canola and camelina) have gained interest. In 2005, RLN were first identified in poorly producing wheat fields within Montana. Subsequent surveys in 2006 and 2007 showed damaging populations of the root lesion nematode, *P. neglectus* were common in the north-central portion of the state (Johnson et al., 2008). These populations were highest in fields under winter wheat production and annual losses due to the nematode were considerable. Because this is a newly identified problem for the state, RLN resistance has not yet been incorporated into locally adapted wheat cultivars. With losses mounting, growers within the state are demanding control options for this pest. Without resistant wheat, focus has shifted to the possible incorporation of resistant rotational crops. Unfortunately, consensus on the value of Montana's rotational crops as controls remains unclear. There are conflicting reports on the relative resistance of both peas and barley, which may relate to initial pathogen population sizes or genetic variability among the

cultivars used (Riga, et al., 2008, Smiley et al., 2004, Smiley et al., 2009). The information on resistance in lentil and camelina is sparse. Several report canola as being a good host for *P. neglectus* (Taylor et al., 2000) but there are also reports that the decomposition of canola residues is lethal to these nematodes (Potter et al., 1998, Kirkegaard and Sarwar 1999). Given these inconsistencies, the lack of information about locally adapted cultivars and the newly discovered importance of *P. neglectus* to wheat production in Montana, investigations into the utility of Montana's common rotations were necessary.

This study was conducted to understand the potential of common rotational crops to controlling losses due to *P. neglectus*. Its focus was on the impacts of peas, lentils, camelina, barley, canola, and fallow periods on RLN population and subsequent winter wheat yields. In addition, it explored locally adapted barley and canola varieties to identify any potentially useful resistance that may exist among them. The three principle objectives for the study were: 1) Evaluate a series of two-year rotations for their efficacy in controlling populations of *P. neglectus* in winter wheat rotations, 2) Measure the impact of these rotations on winter wheat yields, and 3) Determine variability in resistance to *P. neglectus*, if any, among locally adapted cultivars of barley and canola.

## CHAPTER 2

## LITERATURE REVIEW

Root lesion nematodes (*Pratylenchus neglectus*, (Rensch, 1924) Filipjev & Schuurmanns Stekhoven) cause widespread damage in the semi-arid grain producing regions of Australia, Iran, Israel, Mexico, and the United States (Thompson, et al. 2008, Fatemy et al. 2006, Orion et al. 1984, Van Gundy et al. 1974, Smiley, et al. 2004, Strausbaugh, et al. 2004). As with most pathogens, damage caused by RLN is largely dependent on the size of its population. Studies have drawn a linear correlation between the soil-borne population of *Pratylenchus* spp. and yield losses in wheat and other crops (Hollaway et al. 2003). The population level of RLN at planting time will indicate the degree of damage to be expected for a crop in the coming growing season (Kimpinski et al. 1992). It has also been shown that *Pratylenchus* population levels measured after harvest will indicate the degree of damage to be expected in the following year's crop (Hollaway et al. 2003, Kimpinski et al. 1992).

The feeding of root lesion nematodes (RLN) destroys lateral roots, limiting their ability to draw water and nutrients from the soil (McGawley et al. 1998). In dryland wheat, this characteristic damage hinders the plants' ability to take up the soil moisture stored by fallowing, stubble retention, and no-till practices (Thompson, et al. 1995,). Adding nitrogen fertilizer to offset yield losses often increases RLN populations, thereby creating higher initial inoculum levels for successive crops (Thompson, et al. 1995). No-till management compounds the

problem because nematodes multiply more successfully in undisturbed soil (Lopez-Fando and Bello 1995). In these ways, *P. neglectus* presents a tough dilemma for dryland wheat farmers; many of their best management practices for moisture and topsoil retention are creating a more favorable environment for a damaging endemic pest. To make matters worse, the harsh environmental conditions that often slow the proliferation of pathogens in semi-arid climates do not affect these endoparasites because they feed and reproduce inside living roots. (Lambert & Bekal, 2002).

In addition to directly damaging crop roots, the feeding of RLN can create infection courts for other plant pathogens. Colonization of *Fusarium oxysporum* has been shown to increase in chickpeas with increasing populations of RLNs (Castillo 1998). Increased root lesioning can occur when *P. neglectus* infested wheat plants are co-infected with various fungal pathogens, including *Pythium irregulare*, which causes damping off, and *Gaumannomyces graminis*, the agent responsible for take-all disease (Taheri, et al. 1994).

Because of the nature of *Pratylenchus*'s life cycle and survival strategies, control by any means has been difficult to obtain. Fumigation is minimally effective because it only kills the nematodes in the top stratum of the soil profile; root lesion nematodes are known to feed on roots several feet deep in soil (Edwards, et al. 1997). Fumigating is also problematic because it kills indiscriminately. Many beneficial organisms are lost, perhaps to the disadvantage of crop health (Taylor et al. 1999). Fumigants can also be phytotoxic to crops (Johnson, et al. 2007). In low input systems like the high-

acreage dryland grain farms found in Montana, fumigation is prohibitively expensive. The land area is simply too great and the yield per acre is too low to offset the high cost of treatment.

Tillage is often considered as an option for control of soil borne nematodes. The assumption is that tillage disturbs the soil such that it no longer provides a favorable environment for the pest. It has been found that more than 50% of *P. neglectus* populations in dryland fields are found in the top 30cm of the soil profile (Smiley, et al. 2008), where they would be most affected by plowing. However, numerous studies on tillage have resulted in no real consensus as to the efficacy of tillage for control of *P. neglectus*. While there is abundant proof that tillage can reduce nematode populations in the short term (Lopez-Fando and Bello 1995, Lenz and Eisenbois 2000), it has also been shown that tillage can shuffle the trophic relationships among soil fauna such that plant parasites become the dominant nematode group (Lopez-Fando and Bello 1995).

Biological controls have shown some promise, as there are fungi and bacteria which are demonstrably pathogenic to root lesion nematodes (Kerry 1997). Bacteria of the genus *Pasteuria* are the most well known parasites of soil-borne nematodes, but a protocol for establishing a functioning population of this agent in true field conditions has not yet been developed (Sikora 1992). The same is true for the known nematode-antagonistic fungi. It is a common refrain for many proposed biological control agents: When exposed to the elements in the field, they rarely work as well as they do in the Petri dish (Kerry 1997).

Since eliminating the pest directly is not a serviceable option in the case of endemic endoparasites in low-input crop systems, our best hope for reducing the economic impact of RLN is to plant crops and varieties which are either resistant to the pest or are non-hosts of them (Kratochvil et al. 2004, Taylor et al. 2000). Using resistant or non-host crops and cultivars in rotation can be as effective as nematicides at controlling RLN populations (Taylor et al. 1999). In areas where a host crop is of particular economic importance, rotations out of the host crop must be explored in an attempt to reduce populations of the nematodes below yield-reducing thresholds.

Wheat is widely known to be an excellent host for *Pratylenchus* spp. (Smiley, et al. 2004, Thompson, et al. 2008). The resulting difficulties faced by wheat include reductions of plant biomass, grain yield, test weight and kernel weight, and increased late season water stress (Smiley, et al. 2004). The end result is yield losses of 10-30% under typical circumstances, and up to 70% in extreme cases (Thompson et al. 2008). While wheat in general is highly susceptible to *P. neglectus*, resistance genes have been identified for this crop. The most well studied gene is *Rlnn1*, which was isolated from the Australian cultivars 'Excalibur' (spring wheat) and 'Krichauff' (winter wheat) (Williams, et al. 2002). Other quantitative trait loci (QTL) for resistance to *P. neglectus* have also been discovered (Zwart, et al. 2005), but none of these genes are currently represented in cultivars adapted for Montana. Since we are years away from the possibility of having resistant wheat lines available in this region, we must look to our rotation crops for cultural control of *P. neglectus*.

While there are not many crops that are economically viable for dryland farmers in Montana, there are a few that show promise with respect to controlling *P. neglectus* populations. Peas (*Pisum sativum*) are increasing in popularity as a shallow-rooted alternative crop in Montana. They fix nitrogen for improved soil fertility, consume less water than most other crops grown in the region, and provide a break for disease cycles common among cereals. Peas generally have been shown to be among the most resistant crops to *P. neglectus* (Taylor, et al. 2000). However, recent data collected in dryland regions of the Pacific Northwest suggest that peas can indeed increase *P. neglectus* populations in rotation with winter wheat (Smiley, et al. 2009). Further examination has shown that *P. neglectus* can cause 50-70% height reduction in peas grown under greenhouse conditions (Riga, et al. 2008). Peas were included in this study to evaluate their effectiveness as a break crop for *P. neglectus*.

Brassica crops present an interesting case. Canola (*Brassica napus*) is known to be among the best hosts of *P. neglectus* (Taylor 2000), but like all brassicas, canola cultivars produce varying levels of volatile glucosinolates. These compounds themselves do not affect *P. neglectus*, but their breakdown products do. Upon plant death, 2-phenylethyl glucosinolate forms 2-phenylethyl isothiocyanate (2-PITC), a compound known to reduce *P. neglectus* populations (Potter et al 1998). 2-PITC suppresses the hatching of nematode eggs, and this effect has been found to be particularly strong on *P. neglectus* (Yu 2005). The principal question with canola is whether the chemical antagonist characteristics of the plant can counteract its strong hosting ability. The secondary question is

whether or not there are canola cultivars that exhibit resistance to *P. neglectus*. Research supports that there is considerable variation in resistance among cultivars (Taylor, et al. 2000).

Camelina (*Camelina sativa*) is another brassica crop that is gaining popularity as a rotation crop in Montana, with 50,000 acres planted in Montana in 2007 (McVay, et al. 2007). Currently, there is no information published on camelina's relative susceptibility to *P. neglectus*. We do know that camelina is generally lower in glucosinolate content than canola, and that 2-phenylethyl glucosinolate is not among the glucosinolates reported to be present in camelina (Schuster et al. 1998).

Barley shows excellent potential as a break crop for *P. neglectus* for two reasons: 1. It is already a popular, economically viable rotation crop for Montana grain producers, and 2. It has exhibited resistance to *P. neglectus* in numerous field studies (Taylor et al. 2000, Smiley et al. 2004). Because of this resistance, barley is able to make better use of plant-available water in RLN-infested soils as compared to wheat (Thompson 1995). Despite the studies that have suggested barley is resistant to RLNs, there is evidence that there is a high degree of genetic variability among cultivars with respect to RLN resistance (Smiley et al. 2004, Taylor et al. 2000). In fact, barley has been shown to sustain high populations of *P. neglectus* in intensive cereal culture (Gair 1969). A recent survey of 565 barley cultivars found a broad range of susceptibility, and thus concluded that RLN resistance in barley is likely a multiple gene scenario (Keil, et al. 2009).

There is little published information on the relationship between *P. neglectus* and lentils. In southeastern Australia, lentils were reported to be resistant to *P. thornei* (Taylor, et al. 2000), but this does not mean that they are also resistant to *P. neglectus*. Recently, high populations of *P. neglectus*, *P. thornei*, and the pin nematode, *Paratylenchus hamatus*, were discovered in Idaho in pea and lentil fields exhibiting large patches of yellowing plants. This prompted a greenhouse study that found *P. neglectus* reduced plant height by 50-70% in lentils, and that the RLN populations increased in these experimental pots (Riga, et al. 2008). It is the first report of *P. neglectus* or *P. thornei* causing damage in lentils, and raises the question of whether or not lentils would be a satisfactory rotation crop in RLN infested grain fields.

Past research has yielded valuable information on the relationship between *P. neglectus* and several rotation crops that are profitable for Montana growers. However, there are sufficient gaps in our knowledge to warrant further examination of these crops in rotation with winter wheat. Furthermore, the variability observed among cultivars of barley and canola with respect to *P. neglectus* resistance suggests that area growers will benefit from cultivar trials with these crops using locally adapted varieties.

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## CHAPTER 3

## MATERIALS AND METHODS

Field Trials

All field experiments were conducted in 2008 and 2009 at the Central Agricultural Research Center (CARC) in Moccasin, Montana (elev. 1275 m). This site was previously identified as having substantial populations of *P. neglectus*. Precipitation totaled 31.8 cm and 27.48 cm for the 2008 and 2009 crop years, respectively, with 23.2 cm falling between Apr. and Aug. 2008 and 16.92 cm falling during the same period in 2009. Mean high temperatures for July and Aug. were 28.6°C and 26.9°C for 2008 and 2009, respectively. Soil at the CARC is a shallow, sandy clay loam with a plant-limiting hardpan layer at around 60cm in depth.

Rotation Experiment

The dryland rotation study was a fully phased, randomized block design consisting of twelve, two-year rotations with four replications. Each rotation treatment featured winter wheat (cv. 'Yellowstone') in alternating years with one of six alternative crops: fallow, barley (cv. 'Haxby'), canola (cv. 'Hyola 357 Magnum'), camelina (cv. 'Blaine Creek'), lentil (cv. 'Red Chief'), or pea (cv. 'Baronesse') (*table 1*). Each rotation was run both ways, with winter wheat either preceding or following the alternate crop, for a total of twelve treatments per block. Plots measured 8m x 20m, and all crops in the rotation study were planted on 30cm row spacing. All plots were managed with no-till practices. The field

encompassing all of the rotation study plots was previously no-till winter wheat-fallow, with winter wheat in 2006.

For the rotation trials, crops were seeded at the following rates: Winter wheat: 27.2 kg/acre; barley: 29.5 kg/acre; canola: 1.8 kg/acre; camelina: 1.4 kg/acre; lentils: 20.4 kg/acre; peas: 70.3 kg/acre. A 45-0-0 fertilizer was applied to all non-legume crops at rates of 40.8 kg N/acre for winter wheat, 20.4 kg N/acre for barley and canola, and 12.2 kg N/acre for camelina. A 20-20-20-10 NPKS fertilizer was added to the canola and camelina plots at a rate of 45.4 kg/acre. Lentils and peas were not fertilized. Roundup ® (glyphosate, Scotts Company, LLC, Marysville, OH) and 2,4-D herbicides were applied to all plots at a rate of 650 and 355 mL/acre, respectively, for seedbed preparation. Bronate ® herbicide (MCPA + bromoxnil, Bayer Cropscience AG, Monheim am Rhein, Germany), at 379 mL/acre was applied to winter wheat and barley for weed control during the growing season. Prowl ® (Pendimethalin, BASF, Research Triangle Park, NC) and Assure II ® (Quizalofop P-Ethyl, DuPont, Wilmington, DE) herbicides were applied as needed at 1.42 L/acre and 296 mL/acre, respectively, to all broadleaf crops in the rotation. Peas were treated with Sevin XLR ® (Carbaryl, Bayer) at 1.18 L/acre for insect pests.

The winter wheat was seeded 18 Sept. 2007 and 23 Sept. 2008 and harvested 14 Aug. 2008 and 17 Aug. 2009. Canola and camelina were seeded 28 Mar. 2008 and 20 Apr. 2009, and harvested 1-14 Aug. 2008, and 5-17 Aug. 2009. Barley, peas, and lentils were planted 4 Apr. 2008 and 14 Apr. 2009. Barley was harvested 14 Aug. 2008 and 28 Aug. 2009. Peas were harvested 1 Aug. 2008 and 4

Aug. 2009. Lentils were harvested 5 Aug. 2008 and 17 Aug. 2009. The winter wheat plots of the rotation study were measured for yield and grain quality parameters following harvest each year.

### Barley Cultivar Trials

The dryland barley trials consisted of 20 cultivars with three replicaitons in a randomized block design. Nine of these cultivars were present in both 2008 and 2009. Plots measured 2m x 4m with 30cm row spacing. Seed was planted at a rate of 23kg per acre with a double disk seeder with saturated edge. A 10-10-10-5 NPKS fertilizer was applied with the seed and the plots were top dressed with 20kg urea N/ac. 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 (MCPA + bromoxynil) at 946 mL/ac. Barley was seeded on 18 Apr. 2008, and 10 Apr. 2009, and harvested on 25 June, 2008 and 6 July, 2009, respectively.

### Canola Cultivar Trials

The dryland canola nursery plot was planted into fallow in 2008 and into spring wheat stubble in 2009. The canola trials consisted of 14 cultivars with four replications in a randomized block design. Plots measured 3m x 6m with row spacing of 30 cm. Canola was seeded at a rate of 1.8 kg/ac. The plots were seeded 14 Apr. 2008 and 22 Apr. 2009, and harvested 12 Aug. 2008 and 17 Aug. 2009. Fertilizer and herbicide were applied as described above for the canola plots in the rotation study. For both years, the canola plots were managed with no-till practices.

### Field Soil Sampling for RLN

Nematodes were extracted from soil samples taken with manually operated 2.5cm diameter probes to a depth of 30cm. Global positioning (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 soil-borne nematodes (Taylor, et al 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 around 15% water content. Samples for the rotation experiment were taken on 15 May and 23 Oct. for 2008 and on 27 May and 3 Sept. for 2009. Samples from the barley trials were taken on 15 May and 23 Oct. 2008 and on 21 May and 19 Sept. 2009. Samples from the canola trials were taken on 20 May and 27 Oct. 2008 and on 21 May and 19 Sept. 2009.

Six soil cores were taken 150 cm apart from each plot for the rotation experiment as well as the barley and canola 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 4wk (Hollaway 2003).

### Processing Soil Samples for RLN

For all greenhouse and field experiments a modified Whitehead tray method was used to extract *P. neglectus* (Whitehead and Hemming, 1965). For this, 200g of soil was removed from the sample for nematode extraction. The

200g sample was spread evenly about 1cm deep and wrapped in tissue, then placed on a nylon screen in a wire tray. The wire tray was placed in a plastic tub, which was then filled with approximately 2L of water, enough to wet the soil sample without completely submerging it. This allowed the nematodes to migrate from the soil into the water. After soaking for 48h, the water in the tubs was strained through a 20-micron sieve to collect the extracted nematodes. The resulting nematode suspension was collected in 40mL bottles and stored at 4°C until the RLN could be counted. Stored samples were processed within 4wk of refrigeration such that nematode viability would not be affected (Hollaway 2003).

Concurrent with the processing of the 200g sample, a second 100g of soil was removed. The 100g samples were dried at 40°C for 72h and then weighed again. The percent soil mass was recorded for the computation of the nematode population, which was expressed as RLN/kg dry soil.

In order to count RLN, 2mL of the nematode suspension was placed on a Chalex counting slide. The slide is etched with a grid, which encompasses 1mL of solution. Adult *P. neglectus* were counted within the entire 1mL grid, giving the number of RLN/mL of solution. After counting, the suspension on the slide was returned to the sample bottle and the process repeated such that each sample was counted three times. The RLN was identified by its sclerotized labium, thick stylet, overlapping intestine, and vulva position at 75-80% down the length of the body (Ryss, 2002). Juveniles were not counted. The mean of the

three counts was then used to calculate the number of nematodes per kg of dry soil using the equation:

$$[N*1000/(2*S)] * V$$

Where N = number of nematodes/mL, S = percent dry soil of the sample, and V = volume of the sample nematode suspension.

### Greenhouse RLN Resistance Trials

#### Inoculum

The Arthur H. Post Agronomy Farm 6 miles west of Bozeman, MT was previously identified as having high populations of the *P. neglectus*. To establish working populations of *P. neglectus*, field soil was collected at the Post Farm and mixed with equal parts coarse sand, placed in 20cm pots, and planted with 6 seeds of the RLN susceptible wheat cultivar 'Machete' (Taylor 2000). The resulting pot cultures were maintained for 8-12wk at diurnal temperatures of 22°C by day and 18°C by night. Day length was supplemented to 14h with 430-Watt sodium halide lamps during winter months. Root lesion nematodes were extracted from the pot culture soil using the modified Whitehead tray method as described above. Nematode population counts were then determined for the extracted suspension. Immediately after extraction, these nematodes were used as inoculum for all greenhouse experiments.

### Barley Greenhouse Cultivar Trials

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 non-porous plastic bag to prevent nematode leaching. The pots were filled with 800g 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 seeds. Each experimental pot was inoculated with 1000 *P. neglectus* adults. 200g of the same soil mix was then added to the pots to cover the seeds and nematodes. 3g of Osmocote® 14-14-14 slow release fertilizer pellets (Scotts Company, LLC, Marysville, OH) were sprinkled on top of the soil. The pots were then watered to maintain field capacity. 14 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 (*table 5*). Each block included known resistant (cv. 'Excalibur') and susceptible (cv. 'Machete') wheat cultivars as controls (Taylor 2000). The experimental area was surrounded with barley plants (cv. 'Haxby') to reduce edge effects. The plants were grown for a period of 12wk 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 2cm above the soil surface after 12wk 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 4wk of harvest to minimize losses of nematodes

during storage (Hollaway 2003). Soil samples were processed using the Whitehead tray method as described above for the field experiments. A concurrent 100g subsample was also removed to determine percent soil mass.

### Canola Greenhouse Cultivar Trials

14 canola varieties were tested for susceptibility to *P. neglectus*. Plants were grown on greenhouse benches in a randomized complete block design consisting of 4 blocks. The blocks also contained a resistant crop, pea (cv. 'Baronesse') and susceptible crop, spring wheat (cv. 'Machete') (Taylor 2000) as controls. Canola was planted in 20 cm pots using a steam-pasteurized mixture of one part loamy field soil, one part sand, and one part sphagnum peat. Each pot was first lined with a non-porous plastic bag to prevent nematode leaching. The pots were then filled with 1800g of the soil mixture. Five canola seeds were placed on the soil surface. The nematode extraction was then pipetted on the soil surface among the seeds. Each experimental pot was inoculated with 1000 *P. neglectus* adults. 200g of soil mix was then placed on top of the seeds and nematodes. Pots were then arranged in 4 x 4 blocks on 4cm spacing. 6g of Osmocote® 14-14-14 slow release fertilizer pellets were sprinkled on top of the soil. The pots were watered to maintain field capacity over the duration of the trial. Fourteen days after emergence, the pots were thinned to one plant per pot. Canola plants (cv. '7145RR') were placed around the perimeter of the experimental area to reduce edge effects. Canola plants were maintained and processed in the same manner as previously described for the barley trials, but received foliar treatments of fluvalinate at 1.3 mL/L for aphids as needed. After

12wk, all canola plants were cut 2cm above the soil surface. The soil bags were removed from the pots, sealed and stored at 4°C. The nematodes were extracted from the soil samples within 4wk of harvest to minimize losses of nematodes during storage (Hollaway 2003). Soil samples were processed using the Whitehead tray method as described above for the field experiments. A concurrent 100g subsample was also removed to determine percent soil mass.

### Statistical Analysis

Analysis of variance was performed on all data with the log-transformed multiplication rate ( $\text{Log}(R_f)$ ) as the response variable. Least significant differences were used to separate 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 two-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 canola and barley field trials, cultivar means were separated and assigned ranks to the varieties with respect to *P. neglectus* multiplication rate ( $R_f$ ).  $R_f$  for the rotation study and barley and canola cultivar trials were expressed as  $P_f/P_i$ , where  $P_f$  = final population as measured in fall, and  $P_i$  = initial population as measured in spring. All  $R_f$  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  $\text{Log } R_f = \text{Block} + \text{Year} + \text{Cultivar}$ . Treatments with  $R_f$  values not significantly different from  $R_f=1$  were considered to have a

“neutral” effect on RLN multiplication. Treatments with Rf values less than Rf=1 were considered to have a “negative” effect on RLN multiplication, and treatments with Rf values significantly greater than Rf=1 were considered to have a “positive” effect. For experiments that were repeated, an F-max test was used to assess homogeneity of variance between like experiments. Experiments that were determined to have homogeneous variances were combined and analyzed as a single data set.

## CHAPTER 3

## RESULTS

Rotation Experiment

The mean initial nematode population for the 2008 rotation study was 624 nematodes/ kg of soil. At the Aug. 18<sup>th</sup> post-harvest sampling date, these populations had increased to an average of 1688 nematodes/kg of soil. Among the crops studied there was significant difference in their effects on *P. neglectus* populations (*table 1*; ANOVA,  $p < 0.001$ ). In particular, winter wheat acted as the best host for *P. neglectus*, increasing nematode populations by 8.6 times their starting population during the growing season. This increase was significantly greater than that of all other treatments in the study (ANOVA,  $p < 0.001$ ). Winter wheat was followed by canola, which increased populations 3.8-fold. Of the remaining treatments, none were significantly different from neutral in their effects on nematode populations although there was significant separation among them. In particular, fallow treatments increased populations 2.8-fold, and RLN populations averaged a negligible change under camelina. Nematode populations declined on plots of pea, lentil and barley, although these crops were not significantly different from camelina or a neutral effect on nematode populations.

For the rotational study in 2009, there was significant difference in the effects crops had on *P. neglectus* populations (*table 1*;  $p < 0.001$ ). In 2009, spring nematode populations in plots following winter wheat averaged 3600/kg soil.

Initial population under the 2009 winter wheat plots, which followed the 2008 alternative crops averaged only 173 nematodes/kg soil. Again, winter wheat had the greatest positive effect on nematode populations, causing an 11.3-fold increase in populations. This was statistically similar to canola ( $\alpha = 0.05$ ), which was associated with a 4.8-fold increase in nematode populations. Similar to 2008, nematodes populations in plots of peas, barley and camelina were relatively unchanged from their spring populations. In 2009, the only crop that appeared to have a negative effect on nematode populations was lentil, which reduced nematode populations to 27% of their original population. Fallow period also reduced populations to 40% of their original population, but this result was not significantly different from barley and camelina, which produced neutral Rf of 1.02 and 1.11, respectively.

Table 1. *P. neglectus* multiplication rates for rotation crops in 2008 and 2009. Rates expressed as  $Rf = Pf/Pi$ , where Pf = final population and Pi = initial population. Rf were log transformed to normalize variances. Overall means highlighted in bold. <sup>a</sup>Significant differences (F test,  $p < .0001$ ) among crops. <sup>b</sup>LSD = .344. <sup>c</sup>LSD = .459. <sup>d</sup>Numbers followed by the same letter are not significantly different.

<i>Treatment</i>	n	2008	2008	2008	2008	2009	2009	2009	2009
		Mean Pi	Mean Pf	Mean Rf	Mean Log(Rf) <sup>abd</sup>	Mean Pi	Mean Pf	Mean Rf	Mean Log(Rf) <sup>acd</sup>
W. Wheat	24	328	2376	8.56	0.835a	173	1121	11.82	0.787 a
Canola	4	942	2393	3.80	0.444 b	6178	11290	4.86	0.416 ab
Pea	4	1094	567	0.65	-0.244 d	3839	5136	1.38	0.134 bc
Barley	4	1051	474	0.56	-0.343 d	2159	2229	1.02	0.001 bcd
Camelina	4	833	594	1.02	-0.041 cd	3868	2611	1.11	-0.072 cd
Fallow	4	839	1447	2.76	0.211 bc	3252	1254	0.40	-0.438 de
Lentil	4	768	522	0.84	-0.11 cd	2303	330	0.27	-0.777 e
<b>Means</b>		<b>837</b>	<b>1196</b>	<b>2.60</b>		<b>3110</b>	<b>3425</b>	<b>2.98</b>	

<sup>a</sup>Significant differences (F test,  $p < .0001$ ) among crops. <sup>b</sup>LSD = .344. <sup>c</sup>LSD = .459. <sup>d</sup>Numbers followed by the same letter are not significantly different.

Between fall 2008 and spring 2009, *P. neglectus* Rf following winter wheat and barley were neutral (*table 2*). All other treatments in the study had overwinter Rf that were significantly negative. ( $\alpha = 0.05$ ). Analysis of the area under the population curve (AUPC) showed that rotations starting with winter wheat generally produced higher populations than those starting with the alternative crops (*table 3*). The greatest areas were produced by canola and pea following winter wheat ( $\alpha = 0.05$ ). These two rotations were similar to each other, but significantly different from all other rotations in the study. The rotations of winter wheat following barley, lentil, camelina, and peas produced the lowest areas under the curve and were statistically similar to each other in this regard ( $\alpha = 0.05$ ).

Yield data for the rotation experiment showed significant differences in 2009 winter wheat yield dependent on the previous year's crop (*table 4*, ANOVA,  $p = .035$ ). Winter wheat yield was the same following fallow, pea, lentil, and canola, and yields following these crops were significantly higher than yields following barley. Yields following barley, camelina, and lentil were not significantly different. Winter wheat following fallow had the highest yield.

Table 2. Overwinter *P. neglectus* multiplication rates between fall 2008 and spring 2009. <sup>a</sup>Pf 2008 = nematode population as counted post-harvest in fall 2008. <sup>b</sup>Pi 2009 = nematode population as counted post-emergence in spring 2009. <sup>c</sup>Rf = Overwinter multiplication rate, computed as Pi 2009/Pf 2008. <sup>d</sup>Log transformation of ORf as recommended by Box-Cox analysis. <sup>e</sup>Significant differences among crop treatments (F-test,  $p < .0001$ ). <sup>f</sup>LSD = .6215. <sup>g</sup>Numbers followed by the same letter are not significantly different.

2008 Crop	n	Pf 2008 <sup>a</sup>	Pi 2009 <sup>b</sup>	Rf <sup>c</sup>	Log(Rf) <sup>defg</sup>
W. Wheat	24	2382	3600	1.96	0.1695 a
Barley	4	474	226	0.81	-0.3986 ab
Fallow	4	1447	304	0.47	-0.6438 bc
Camelina	4	594	91	0.19	-0.8078 bc
Canola	4	2393	230	0.09	-1.0389 c
Pea	4	567	48	0.08	-1.0560 c
Lentil	4	522	32	0.05	-1.1855 c

Table 3. Area under the population curve (AUPC) for two-year rotations. AUPC calculated across four observations taken May 15<sup>th</sup> and October 23<sup>rd</sup>, 2008, and May 27<sup>th</sup> and September 3<sup>rd</sup>, 2009. ANOVA was calculated using Log transformation of AUPC to normalize variances. <sup>a</sup>Significant differences among rotation treatments (F-test,  $p < .0001$ ). <sup>b</sup>LSD = .318. <sup>c</sup>Numbers followed by the same letter are not significantly different.

Crop year 1	Crop year 2	Mean AUPC	Mean Log(AUPC) <sup>abc</sup>
W. Wheat	Canola	15508.88	4.17 a
W. Wheat	Pea	8961.25	3.93 a
W. Wheat	Fallow	6450.63	3.79 b
W. Wheat	Camelina	6879.88	3.74 bc
W. Wheat	Barley	5959.25	3.73 bc
Canola	W. Wheat	3630.25	3.53 bcd
W. Wheat	Lentil	4522.00	3.51 bcd
Fallow	W. Wheat	2940.88	3.44 cde
Barley	W. Wheat	2653.50	3.39 def
Lentil	W. Wheat	2208.75	3.27 def
Camelina	W. Wheat	1540.63	3.16 ef
Pea	W. Wheat	1331.88	3.1 f

Table 4. 2009 winter wheat yields following alternative crops. Yields are expressed in lb./ acre. Significant differences were found among previous crops ( $p = .035$ ). <sup>a</sup>Numbers followed by the same letter are not significantly different from each other. <sup>b</sup>LSD = 391.28

2008 Crop	Winter Wheat Yield 2009
Fallow	1624.51 a
Pea	1506.39 ab
Lentil	1386.14 abc
Canola	1249.35 abc
Camelina	1179.83 bc
Barley	1110.72 c

#### Barley Field Cultivar Trials

The mean initial *P. neglectus* populations for 2008 and 2009 were 1339 and 711 nematodes/kg soil, respectively. Across all cultivars tested in 2008, Rf was 1.22, and for 2009 it was 0.92. An F-max test (Hartley, 1950) for the two years of the barley trial revealed that variances were homogeneous between the two years, so they were combined and analyzed as a single data set. No significant differences were found among cultivars across years ( $p = 0.92$ ). The overall mean Rf across both years was 1.09, which was not significantly different from 1.

#### Barley Greenhouse Cultivar Trials

The two barley greenhouse trials were combined and analyzed as a single data set based on the F-max test for homogeneity of variance. Significant differences were found among cultivars (table 5,  $p = 0.0006$ ). The Pi for all

cultivars was 1000. The mean Rf for the first greenhouse barley trial was 0.66 across the 19 cultivars tested, indicating that populations declined on average. The mean Rf for the second greenhouse barley trial was 0.4, again indicating an overall decline in *P. neglectus* populations. None of the cultivars produced a net increase in *P. neglectus* populations during the second trial as they decreased populations to 45% of their original populations. The overall mean Rf over both trials was 0.53. The susceptible wheat cultivar 'Machete' had a mean Rf of 0.91, and the resistant wheat cultivar 'Excalibur' had a mean Rf of 0.39. Despite the difference in means between these two controls, they were not significantly different from each other. Analysis of the combined data set showed that 'Stellar ND' and 'Tradition' were the only two cultivars with a mean Pf greater than the Pi of 1000. However, they were statistically similar to six other cultivars ('Drummond', 'Haxby', 'Boulder', 'Merit', and 'Legacy') and the increase in population was not significantly different from neutral (Rf = 1). Fifteen of the barley cultivars, as well as the resistant wheat cultivar 'Excalibur', had a decrease in *P. neglectus* populations that was significantly different from neutral. Barley had a neutral Rf across all cultivars.

Table 5. Barley greenhouse cultivar trials. Cultivars are ranked by Log Rf (Rf = Pf/1000). Significant differences were found among cultivars ( $p = .00057$ ). <sup>a</sup>Numbers followed by the same letter are not significantly different from each other. <sup>b</sup>LSD = .37.

Cultivar	Mean Pf	Mean Rf	Mean Log (Rf)
Stellar ND	1184	1.18	-.10 a
Haxby	924	0.92	-0.24 ab
Machete	910	0.91	-0.25 ab
Drummond	970	0.97	-0.27ab
Tradition	1014	1.01	-0.27ab
Boulder	742	0.74	-0.37 abc
Merit	553	0.55	-0.43 abcd
Legacy	470	0.47	-0.43 abcd
Excalibur	392	0.39	-0.50 bcde
Conrad	431	0.43	-0.50 bcde
Eslick	495	0.50	-0.51 bcdef
MT960225	402	0.40	-0.53 bcdef
Craft	406	0.41	-0.56 bcdef
Hockett	363	0.36	-0.58 bcdef
Metcalfe	481	0.48	-0.63 cdef
MTLB32	244	0.24	-0.74 cdef
MT010158	236	0.24	-0.76 def
WPB Xena	221	0.22	-0.79 def
Geraldine	215	0.22	-0.83 ef
Baronesse	211	0.21	-0.89 f
Harrington	247	0.25	-0.90 f

\*Susceptible wheat cultivar

†Resistant wheat cultivar

### Canola Field Cultivar Trials

The initial *P. neglectus* populations for canola plots were 843/kg soil in 2008 and only 135/kg soil in 2009 (data not provided). Multiplication rates ranged from 2.82 to 13.56 in 2008, and from 3.85 to 18.03 in 2009. An F-max test indicated homogeneity of variance for the two years, so they were combined and analyzed as a single data set. Two year mean Rf ranged from 3.33 for 'Invigor 5630' to 11.77 for 'Invigor 5440'. Despite this broad range, no significant differences were found among cultivars ( $p = .17$ ). The overall mean Rf over both years combined was 7.52.

### Canola Greenhouse Cultivar Trials

For the first greenhouse trial, the mean Rf was 0.28 (*table 5*). Trial 2 generated higher Rf, with a mean Rf of 1.25. Variances for both trials were found to be homogeneous by F-max test, so they were combined for analysis. Rf ranged from 0.68 for 'Invigor 5440' to 1.06 for 'Interstate 3057RR'. No significant differences were found among cultivars ( $p = 0.20$ ). To confirm that the experiments ran properly, the susceptible wheat cultivar 'Machete' had a mean Rf of 1.29 while the resistant pea cultivar 'Baronesse' had a mean Rf of .08.

## CHAPTER 4

## DISCUSSION

Surveys conducted in 2006 and 2007 (Johnson et al., 2007) had indicated that the root lesion nematode, *P. neglectus*, was causing significant losses to wheat production in Montana (Johnson et al., 2007). Because there are no locally adapted wheat varieties resistant to this pest within the state, management options are limited. This study examined several of Montana's rotational crops for their ability to control this root lesion nematode. Based on its results, the effects of most of these rotational crops on nematode populations can be described as either neutral, defined as having a Rf that is not significantly different from one, or positive, defined as having a multiplication factor significantly greater than one. The neutral treatments included fallow, peas, camelina and barley. Of these treatments, fallow, pea, and barley had been suggested as good control options by previous research conducted in Australia and the Northwestern United States (Taylor et al., 2000, Smiley et al., 2004). Unfortunately the data from this study shows that these crops are not significantly different from neutral in their effects on *P. neglectus* populations and therefore do not act as effective trap crops for these pests in wheat dominated rotations. Given that the fallow period had a similar effect on the *P. neglectus* populations to these crops suggests that these neutral crops may not be sustaining the *P. neglectus* populations but that the pest is able to survive without hosts for the short durations tested by this study. While not acting as trap crops,

peas and camelina may be valuable controls when employed for more than a single year. This is supported by the observation that *P. neglectus* populations significantly declined over the winter of 2008-9 following these crops (*table 2*).

Data from this study agree with others in that winter wheat and canola appear to be good hosts for *P. neglectus* populations (Taylor et al., 2000). In fact, canola following winter wheat produced the highest *P. neglectus* populations. The strong host ability seen with canola is consistent with past research (Fatemy et al., 2006, Taylor et al., 2000). The only crop tested that appears to have a consistently negative effect on *P. neglectus* populations is lentils. This effect was primarily seen in the second year when starting populations of the pest were high.

Ultimately the effects rotational crops are measured with respect to their effects on each other's yield and not to their effects on a single pest species. For this study, the effects of rotational crops on winter wheat yield did not relate to their relative effects on nematode populations. This was not surprising because the only non-wheat crop species that significantly affected the first year's nematode populations was canola. In the rotations, wheat yielded more following peas and fallow periods than it did following barley. This was expected given that barley uses more water than peas or fallow periods (Anderson et al., 2003), and also has many more pathogens in common with wheat than any other rotational crop tested (Smiley et al., 1994). Given that yields were compared for only one year and the effects of treatments on nematode populations were minimal over that time span, testing over additional

years may lead to more dramatic changes in nematode populations and consequent wheat yields and may warrant additional research.

Barley was included in this study because it is already a well established, profitable rotation on Montana grain farms, and thus it would be of great benefit to growers to find cultivars that exhibit strong resistance to *P. neglectus*. From the greenhouse trials, significant differences among cultivars were indeed identified. In particular, the cultivar 'Harrington' decreased nematode populations to about a quarter of their original populations while the cultivar 'Stellar' supported a small increase in the populations. Given the differences in relative resistance among these cultivars, it may warrant replacing 'Haxby' in the rotational trial, one of the least resistant barley cultivars tested, with one like Harrington, that is more resistant. Results from the field trials suggest that the controlled environment of the greenhouse is more appropriate for detecting cultivar separation with respect to nematode resistance. This lack of significant separation of cultivars in the field trials was not unexpected as field trials have significantly more uncontrolled and confounding factors than greenhouse trials. These include but are not limited to variability in starting nematode populations, differences in soil moisture levels (Lopez-Fando and Bello, 1995, Govaerts et al., 2007), cultural factors (Lenz & Eisenbois 2000, Thomas 1978), and sampling error. Considering that past research is conflicted on the impact of barley on these nematodes (Gair and Harvey 1969, Smiley et al., 2008) and our greenhouse trials show significant differences, barley as a nematode control warrants more study

as significant variability in resistance probably exists that could be actively exploited in wheat-barley rotations.

Past research has demonstrated that while canola cultivars are good hosts (Fatemy et al., 2006, Taylor et al., 2000), they exhibit a significant range of susceptibility (Potter et al., 1999, Taylor et al., 2000). As with barley, the objective of our canola trials was to find cultivars that may prove useful in reducing *P. neglectus* populations. Unfortunately, despite being given larger containers, canola did not grow well under the experimental conditions. This was reflected in very low nematode Rf, which were undoubtedly the result of unthrifty canola plants. The results of the greenhouse trials are not reflective of what is expected from the canola-nematode interactions as demonstrated by our field observations. As had happened in barley, canola field trials showed considerable variability across cultivars with respect to nematode susceptibility with the analysis yielding no significant differences among canola cultivars. Again, confounding factors undoubtedly overwhelmed this system. The high mean Rf seen among the canola cultivars in the field trial did confirm the observation from the rotational trial that canola is a significant host of *P. neglectus*.

The majority of analyses conducted for this study used comparisons between spring and fall populations of the nematode. While this provides information on the multiplicity of nematodes on a host during the growing season, it is important to consider the dynamics of nematode populations between rotation crops. It is established that nematode damage to crops

correlates strongly to spring population numbers, which indicate the inoculum level that will be imposed on the developing plant (Smiley et al., 2004, 2005, Vanstone et al., 1998). While this trial has only one overwintering observation, peas, camelina, and canola displayed significant drops in nematode populations during the winter, or non-crop, period. This suggests that measuring Rf as the ratio of the fall, or final, population, to spring, or initial, population may not be giving the best representation of how these crops truly affect this pest.

While speculative at this point, the observation of overwinter population dynamics suggests that peas and camelina are non-sustaining crops (populations crashed over winter). Barley would be considered a neutral and sustaining crop for RLN. Canola would function as a strong host during the growing season but be a non-sustaining, possibly biofumigant crop during the winter season. The biofumigant concept is supported by previous studies (Kirkegaard et al., 1999a, Potter et al., 1998) that showed breakdown products of canola's glucosinolates to be lethal to RLN. The canola data also suggest that, despite canola's strong host ability, it may be beneficial for winter wheat to follow canola because RLN populations drop to non-damaging levels by the time the subsequent wheat crop is planted. Unlike the situation observed with canola, peas, and camelina, nematode populations following winter wheat remained at damaging levels from fall to spring. While it is unknown why this occurred, it adds strength to the argument for conducting fall to spring comparisons in the future.

In summary, it was found that wheat and canola were strong hosts of *P. neglectus*, although questions remain as to whether population increases brought

on by canola persist into the following spring. Lentils were the only crop in this study that significantly reduced *P. neglectus* populations. Peas and fallow periods were found to have an overall neutral effect and thus did not have the negative impact on *P. neglectus* that existing literature suggests they would. Peas may in fact increase RLN multiplication if spring populations are sufficiently high, but more data are needed to confirm this observation (Smiley et al., 2009). Camelina, which had not been studied to this point, had a neutral effect on *P. neglectus* and could thus be considered a better management option than its close relative, canola. Barley in general was found to be a neutral host of *P. neglectus*, however greenhouse cultivar trials demonstrated that some barley varieties are considerably more resistant than others. Field trials for barley and canola were not successful in separating cultivars by degree of resistance, but the trials reinforced our findings regarding the relative susceptibility of these two crops in the rotation study. Continued and perhaps more frequent observations should be made to draw more definitive conclusions about the impact of the rotations on the population dynamics of *P. neglectus*. Understanding the hosting ability of crops during the growing season is important, but we must also evaluate the ability of RLN to sustain their populations in the field following these crops if we are to properly assess rotation crops for their potential in managing *P. neglectus*.

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APPENDICES

APPENDIX A

MOCCASIN 2008 ROTATION STUDY RLN POPULATION DATA

Appendix A. Moccasin 2008 Rotation Study Nematode Population Data: Data are organized by two-year crop rotation. Initial population ( $P_i$ ) was recorded from soil collected 15 May, 2008. Final population ( $P_f$ ) was recorded from soil collected 23 Oct., 2008.

## MOCCASIN 2008 ROTATION STUDY RLN POPULATION DATA

Crop 2008	Rep	Pi 2008	Pf 2008
Winter wheat	1	144	2000
Winter wheat	2	282	2304
Winter wheat	3	460	1042
Winter wheat	4	103	1440
Winter wheat	5	757	4272
Winter wheat	6	258	2342
Winter wheat	7	499	1724
Winter wheat	8	319	4487
Winter wheat	9	816	2861
Winter wheat	10	299	2094
Winter wheat	11	137	2876
Winter wheat	12	190	2823
Winter wheat	13	491	5477
Winter wheat	14	250	2216
Winter wheat	15	214	1890
Winter wheat	16	411	1661
Winter wheat	17	249	2501
Winter wheat	18	197	2669
Winter wheat	19	144	447
Winter wheat	20	104	1260
Winter wheat	21	0	239
Winter wheat	22	774	4229
Winter wheat	23	728	4159
Winter wheat	24	0	10
Camelina	1	1581	756
Camelina	2	1230	878
Camelina	3	236	410
Camelina	4	286	332

Crop 2008	Rep	Pi 2008	Pf 2008
Pea	1	639	359
Pea	2	554	606
Pea	3	2027	513
Pea	4	1157	789
Fallow	1	1406	947
Fallow	2	1048	2265
Fallow	3	289	2191
Fallow	4	614	387
Barley	1	1949	301
Barley	2	1114	846
Barley	3	560	527
Barley	4	580	223
Canola	1	1312	4324
Canola	2	1568	1209
Canola	3	279	2307
Canola	4	609	1733
Lentil	1	1412	944
Lentil	2	977	460
Lentil	3	206	281
Lentil	4	477	404

APPENDIX B

MOCCASIN 2009 ROTATION STUDY RLN POPULATION DATA

Appendix B. Moccasin 2009 Rotation Study Nematode Population Data: Data are organized by two-year crop rotation. Initial population ( $P_i$ ) was recorded from soil collected 27 May, 2009. Final population ( $P_f$ ) was recorded from soil collected 3 Sept., 2009.

## MOCCASIN 2009 ROTATION STUDY RLN POPULATION DATA

Crop 2009	Rep	Pi 2009	Pf 2009
Winter Wheat	1	23	620
Winter Wheat	2	108	565
Winter Wheat	3	139	999
Winter Wheat	4	110	1794
Winter Wheat	5	108	965
Winter Wheat	6	99	893
Winter Wheat	7	100	1321
Winter Wheat	8	30	266
Winter Wheat	9	221	1319
Winter Wheat	10	80	4945
Winter Wheat	11	290	1375
Winter Wheat	12	3177	39
Winter Wheat	13	180	954
Winter Wheat	14	54	177
Winter Wheat	15	270	3243
Winter Wheat	16	155	922
Winter Wheat	17	409	1347
Winter Wheat	18	0	2604
Winter Wheat	19	163	413
Winter Wheat	20	0	152
Winter Wheat	21	585	599
Winter Wheat	22	557	3766
Winter Wheat	23	114	599
Winter Wheat	24	21	90
Fallow	1	3211	1859
Fallow	2	2608	1340
Fallow	3	3554	1148
Fallow	4	3634	670
Barley	1	2294	2278
Barley	2	2857	2230

Crop 2009	Rep	Pi 2009	Pf 2009
Barley	3	2765	3713
Barley	4	718	696
Camelina	1	6377	3131
Camelina	2	4542	4173
Camelina	3	4022	1754
Camelina	4	530	1385
Pea	1	2371	4032
Pea	2	4404	4988
Pea	3	3094	4056
Pea	4	5487	7467
Canola	1	11723	6417
Canola	2	2138	9058
Canola	3	1141	15003
Canola	4	9709	14684
Lentil	1	4759	139
Lentil	2	2914	422
Lentil	3	1061	606
Lentil	4	477	154

APPENDIX C

MOCCASIN 2008 BARLEY CULTIVAR TRIAL RLN POPULATION DATA

Appendix C. Moccasin 2008 Barley Cultivar Trial RLN Population Data: Data are organized by cultivar. Initial population (Pi) was recorded from soil collected 15 May, 2008. Final population (Pf) was recorded from soil collected 23 Oct., 2008.

## MOCCASIN 2008 BARLEY CULTIVAR TRIAL RLN POPULATION DATA

Cultivar	Rep	RLN Pi	RLN Pf	RLN Pf/Pi
Baronesse	1	613	1464	2.39
Baronesse	2	705	828	0.43
Baronesse	3	2003	839	0.46
Boulder	1	1782	2522	1.42
Boulder	3	3186	136	1.63
Boulder	1	1788	769	2.39
Challenger	3	263	1326	5.04
Challenger	1	1670	493	0.94
Challenger	2	653	235	0.55
Conrad	3	1445	2276	1.57
Conrad	1	1492	1804	0.41
Conrad	2	1455	556	1.50
Craft	3	1028	2308	2.24
Craft	1	1048	2040	0.25
Craft	2	1098	1396	2.48
Drummond	3	919	1737	1.89
Drummond	1	1843	749	0.30
Drummond	2	1458	1164	2.79
Eslick	3	855	1274	0.71
Eslick	1	978	1407	1.44
Eslick	2	1824	461	1.16
Geralidine	1	1142	417	0.37
Geraldine	2	2346	1532	0.09
Geraldine	3	1140	1304	1.14
Harrington	1	1623	1489	0.92
Harrington	2	1428	1345	1.21
Harrington	3	859	1288	0.36
Haxby	1	986	3879	3.93
Haxby	2	986	1009	1.95
Haxby	3	1991	1375	0.98

Cultivar	Rep	RLN Pi	RLN Pf	RLN Pf/Pi
Hockett	1	1341	740	0.55
Hockett	2	1956	173	0.68
Hockett	3	1102	511	0.80
Legacy	1	1024	1749	1.71
Legacy	2	1356	1372	1.17
Legacy	3	1388	750	1.49
Merit	1	732	2174	2.97
Merit	2	674	1607	1.01
Merit	3	1454	1040	1.27
Metcalf	1	253	872	3.44
Metcalf	2	1402	758	0.65
Metcalf	3	791	2206	0.69
MT010158	1	1169	1882	1.61
MT010158	2	829	563	1.86
MT010158	3	519	1287	0.04
MT020155	1	1589	470	0.30
MT020155	2	1667	604	0.54
MT020155	3	0	854	0.54
MT020204	1	507	448	0.88
MT020204	2	647	787	1.22
MT020204	3	603	267	0.89
Stellar-ND	1	1731	2480	1.43
Stellar-ND	2	1704	422	1.09
Stellar-ND	3	1932	1066	0.42
Tradition	1	1946	1064	0.55
Tradition	2	655	762	0.36
Tradition	3	650	1059	0.38
WPB Xena	1	1044	1954	1.87
WPB Xena	2	1571	1717	0.25
WPB Xena	3	1248	1106	0.44

APPENDIX D

MOCCASIN 2009 BARLEY CULTIVAR TRIAL RLN POPULATION DATA

Appendix D. Moccasin 2009 Barley Cultivar Trial RLN Population Data: Data are organized by cultivar. Initial population ( $P_i$ ) was recorded from soil collected 21 May, 2009. Final population ( $P_f$ ) was recorded from soil collected 19 Sept., 2009.

## MOCCASIN 2009 BARLEY CULTIVAR TRIAL RLN POPULATION DATA

Cultivar	Rep	Pf	Pi	Pf/Pi
Baronesse	1	1651	653	2.53
Baronesse	2	652	850	0.77
Baronesse	3	1127	449	2.51
Conrad	1	1325	4583	0.29
Conrad	2	903	835	1.08
Crat	1	635	1362	0.47
Craft	2	272	242	1.13
Geraldine	1	748	413	1.81
Geraldine	2	426	859	0.50
Geraldine	3	277	453	0.61
Harrington	1	592	1653	0.36
Harrington	2	317	1013	0.31
Harrington	3	392	327	1.20
Haxby	1	695	2725	0.26
Haxby	2	348	1464	0.24
Haxby	3	104	223	0.47
Hockett	1	1949	1131	1.72
Hockett	2	251	531	0.47
Hockett	3	253	216	1.17
Metcalfe	1	722	385	1.88
Metcalfe	2	307	814	0.38
Metcalfe	3	333	618	0.54
MT010158	1	2146	1277	1.68
MT010158	2	634	757	0.84
MT010158	3	587	2160	0.27

APPENDIX E

MOCCASIN 2008 CANOLA CULTIVAR TRIAL RLN POPULATION DATA

Appendix E. Moccasin 2008 Canola Cultivar Trial RLN Population Data: Data are organized by cultivar. Initial population (Pi) was recorded from soil collected 20 May, 2008. Final population (Pf) was recorded from soil collected 27 Oct., 2008.

## MOCCASIN 2008 CANOLA CULTIVAR TRIAL RLN POPULATION DATA

Cultivar	Rep	Pf	Pi	Pf/Pi
940	1	5754	751	7.66
940	2	1607	156	10.28
940	3	2359	654	3.61
940	4	5178	581	8.90
Crosby	1	3180	520	6.11
Crosby	2	875	782	1.12
Crosby	3	2259	772	2.92
Crosby	4	4257	1607	2.65
DKL 30-42	1	2282	87	26.26
DKL 30-43	2	1969	582	3.38
DKL 30-44	3	1344	750	1.79
DKL 30-45	4	2339	557	4.20
DKL52-41	1	3286	1181	2.78
DKL52-41	2	1227	230	5.33
DKL52-41	3	2414	641	3.77
DKL52-41	4	4604	1873	2.46
Hyclass 924RR	1	4822	329	14.66
Hyclass 924RR	2	1835	438	4.19
Hyclass 924RR	3	1496	645	2.32
Hyclass 924RR	4	1976	647	3.06
Hyola 357 Mag.	1	2438	164	14.91
Hyola 357 Mag.	2	1639	439	3.73
Hyola 357 Mag.	3	1238	1463	0.85
Hyola 357 Mag.	4	4205	2092	2.01
Interstate 3057	1	5272	501	10.52
Interstate 3057	2	2211	384	5.75
Interstate 3057	3	5694	2476	2.30
Interstate 3057	4	4463	1293	3.45
Interstate 7145	1	1896	448	4.24
Interstate 7145	2	5650	927	6.10

Cultivar	Rep	Pf	Pi	Pf/Pi
Interstate 7145	3	1066	43	24.67
Interstate 7145	4	1930	919	2.10
Invigor 5440	1	5159	909	5.68
Invigor 5440	2	1471	760	1.94
Invigor 5440	3	1360	618	2.20
Invigor 5440	4	2390	1644	1.45
Invigor 5550	1	1271	96	13.19
Invigor 5550	2	3102	1678	1.85
Invigor 5550	3	727	323	2.25
Invigor 5550	4	9373	2133	4.39
Invigor 5630	1	1852	139	13.30
Invigor 5630	2	3683	380	9.69
Invigor 5630	3	2100	306	6.87
Invigor 5630	4	1617	667	2.43
Invigor 8440	1	3151	315	10.00
Invigor 8440	2	2781	1071	2.60
Invigor 8440	3	3855	682	5.65
Invigor 8440	4	3684	578	6.37
Minot	1	3141	1062	2.96
Minot	2	926	80	11.56
Minot	3	3104	327	9.50
Minot	4	3958	822	4.82
Oscar	1	2785	3962	0.70
Oscar	2	1864	88	21.28
Oscar	3	3176	187	17.01
Oscar	4	2444	1020	2.40

APPENDIX F

MOCCASIN 2009 CANOLA CULTIVAR TRIAL RLN POPULATION DATA

Appendix F. Moccasin 200 Canola Cultivar Trial RLN Population Data: Data are organized by cultivar. Initial population ( $P_i$ ) was recorded from soil collected 21 May, 2009. Final population ( $P_f$ ) was recorded from soil collected 19 Sept., 2009.

## MOCCASIN 2009 CANOLA CULTIVAR TRIAL RLN POPULATION DATA

Cultivar	Rep	Pi RLN 2009	Pf RLN 2009	Pf/Pi RLN 2009
DKL 30-42	1	232	322	1.39
DKL 30-43	2	107	1060	9.90
DKL 30-44	3	75	432	5.74
DKL 30-45	4	50	563	11.30
DKL52-41	1	201	2174	10.81
DKL52-41	2	107	2015	18.83
DKL52-41	3	24	706	29.11
DKL52-41	4	179	914	5.10
Hyola 357 Mag.	1	204	86	0.42
Hyola 357 Mag.	2	99	1061	10.74
Hyola 357 Mag.	3	168	510	3.04
Hyola 357 Mag.	4	77	541	7.03
Hyclass 924RR	1	79	463	5.87
Hyclass 924RR	2	80	955	11.99
Hyclass 924RR	3	77	1153	14.97
Hyclass 924RR	4	98	1161	11.86
Invigor 5440	1	158	1401	8.87
Invigor 5440	2	50	1266	25.53
Invigor 5440	3	59	1774	30.00
Invigor 5440	4	120	922	7.70
Invigor 5550	1	158	811	5.13
Invigor 5550	2	120	117	0.98
Invigor 5550	3	71	1453	20.36
Invigor 5550	4	35	326	9.28
Invigor 5630	1	88	44	0.50
Invigor 5630	2	101	83	0.82
Invigor 5630	3	121	839	6.95
Invigor 5630	4	131	934	7.13

Cultivar	Rep	Pi RLN 2009	Pf RLN 2009	Pf/Pi RLN 2009
Invigor 8440	1	31	137	4.37
Invigor 8440	2	204	272	1.33
Invigor 8440	3	77	689	8.95
Invigor 8440	4	133	85	0.64
Interstate 3057	1	200	829	4.14
Interstate 3057	2	262	1442	5.50
Interstate 3057	3	166	1434	8.61
Interstate 3057	4	112	645	5.76
Interstate 7145	1	166	450	2.70
Interstate 7145	2	289	1395	4.82
Interstate 7145	3	83	1294	15.57
Interstate 7145	4	500	2779	5.56
Oscar	1	87	879	10.16
Oscar	2	30	288	9.56
Oscar	3	85	961	11.26
Oscar	4	83	583	7.02

