Host Preference of *Pratylenchus neglectus* to Major Crops of the Prairie Provinces of Canada

By

Priscillar Wenyika

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ABSTRACT

Wenyika Priscillar, M.Sc., The University of Manitoba, November 2019. Host Preference of *Pratylenchus* spp. to Major Crops Grown in the Prairie Provinces of Canada. Advisor: Dr. M. Tenuta.

Root lesion nematodes of the genus *Pratylenchus* Filipjev, 1936 are pests of economic importance worldwide. *Pratylenchus* spp. have recently been identified in soils from commercial fields in the Canadian Prairie Provinces, and there is a lack of knowledge about the host preferences of these nematodes. This research was conducted to determine: a) the crop hosts preferred by the *Pratylenchus* spp., b) the effects of selected pulse and non-pulse crops mainly grown in the Canadian Prairies in building-up densities of the nematode under growth chamber conditions, c) the effect of the nematode and population density over several crop growth cycles on performance of the plants, and d) the species identity of the *Pratylenchus* spp. Host suitability to *Pratylenchus* spp. was evaluated on the most widely grown varieties of selected pulse and non-pulse crops available in Canadian Prairies including canola, chickpea, lentil, pinto bean, soybean, Canada Western Red Spring Wheat, and yellow pea. Host status was assessed using the reproductive factor (R_f = final/ initial density) and plant growth parameters (plant height, above-ground, and root biomass) were measured at the end of each cycle. Nematodes recovered from the test soils and roots of host crops were identified using morphological identification and molecular assays. The suitable hosts for *Pratylenchus* (R_f >1) were canola, chickpea, pinto bean, soybean, and spring wheat. Soybean was the most preferred host for these nematodes with a mean above the threshold level for *Pratylenchus* spp. (>1000 nematodes per kg^{-1} of soil) in the final cycle. The population of *Pratylenchus* spp. in pots planted to canola, chickpea, pinto bean, soybean, and wheat significantly increased across the three growth cycles. Lentil was a poor host and yellow pea was a non-host for *Pratylenchus* spp. High densities of *Pratylenchus* reduced plant height, above-ground and root biomasses of canola, lentil, pinto bean, spring wheat, and yellow pea. Plant height and biomass of chickpea and soybean were not reduced by increasing *Pratylenchus* densities. The *Pratylenchus* spp. was identified as *P. neglectus* using morphometric characters, PCR with species-specific primers and DNA sequencing. Most of the crops mainly grown in the Canadian Prairies are hosts for *P. neglectus.*

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FOREWORD

This thesis is organized into three chapters. Chapter 1 contains the introduction, literature review and research objectives. The second chapter will discuss the host preference of *Pratylenchus* spp. on the selected pulse and non-pulse crops in three replicate trials of a single growth cycle under growth chamber conditions. The chapter also covers the effects of host crops on *Pratylenchus* abundance following three crop growth cycles. The third chapter discusses the effect of *Pratylenchus* spp. on crop performance, endomigratory nature of the nematodes and the species identification of recovered nematodes. The thesis is organized in a "Sandwich" style as specified by the Faculty of Graduate Studies, and Department of Soil Science, University of Manitoba. The data chapters follow a manuscript format of the Journal of Nematology which they are intended to be published. The thesis closes with a general discussion of conclusions of the findings in the research work conducted and recommendations of future studies.

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LIST OF ABBREVIATIONS

BLAST	Basic Local Alignment Search Tool
bp	base pair(s)
^{o}C	degrees Celsius
cm	centimetre
cm^3	cubic centimetre
cv.	Cultivar
d	Day(s)
df	Degrees of freedom
dH_20	distilled water
diam.	Diameter
DNA	Deoxyribonucleic Acid
e.g.	Exempli Gratia
ed.	Editors
Fig.	Figure
g	Gram
h	Height
hr	Hour(s)
ITS	Internal Transcribed Spacer
J2	Second-stage nematode
J3	Third-stage nematode
J4	Fourth-stage nematode
kg	Kilogram
min	Minute(s)
mL	Millilitre
mm	Millimetre
mM	Millimolar
ng	Nanogram
PCR	Polymerase Chain Reaction

Pf	Final Nematode Population Density
Pi	Initial Nematode Population Density
Ppm	Parts per million
PPN	Plant-parasitic nematodes
RAPD	Random Amplified Polymorphic DNA
R_f	Reproduction Factor
RFLP	Restriction Fragment Length Polymorphism
RLN	Root-lesion nematode
RNA	Ribonucleic Acid
rpm	Resolutions per minute
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
sec	Second(s)
µm	micrometre
V	volts
var.	cultivar
wk.	Week(s)
5.8S	Non-coding component of the large ribosomal subunit
18S	Small nuclear ribosomal subunit
28S	Large nuclear ribosomal subunit

1 GENERAL INTRODUCTION

1.1. Major crop production in Canadian Prairies

Agricultural land in Canada is relatively small and it equates to about 7% of the total land area. The Prairie Provinces (Alberta, Manitoba, and Saskatchewan) constitute about 85% of the total farmland of Canada. The prairies are Canada's most important agricultural region (Campbell et al. 2002). Canada's agricultural crops that are grown mainly in the prairies include canola, wheat, oats, barley, rye, mustard and sunflowers (Morrison 2018). Among the major field crops grown in Canada, wheat production covers the most area followed by canola. About 50% of Canada's wheat is produced in Saskatchewan followed by Alberta and Manitoba, and the crop accounts for about 60% of the total grain crops produced in Canada (Campbell 2013). Canada is among the world's biggest producers and exporters of wheat, with its main export markets being Brazil, Iran, the USA, and Asia. Wheat is mainly used for the production of flour, pasta and livestock feed (Campbell 2013). Canola is an important crop for Canada mainly for the production of healthy vegetable oil. The crop contributes over $26 billion per year to the economy of Canada. Residues from canola oil extraction, canola meal or press cake are useful as aquaculture and livestock feed due to their nutritional value. The residues are rich in protein and vitamins B and E (Rempel et al. 2014).

Soybean is mainly produced in Manitoba, Ontario, and Quebec but is also now produced in Southern Alberta and Saskatchewan (Soy Canada 2018). Soybean ranks fourth in acreage in Canadian crop production with its export value estimating over $1 billion per year (Agriculture and Agri-Food 2015). Approximately 50-70% of the total soybean produced is exported mostly to Asia, U.S.A, Italy, and the Netherlands. Soybean is exported in raw or processed form. It is an excellent source of quality protein, fat, calcium, and iron. The soybean seed is processed into oil, meal and end-use products such as flour and care products. Soybean also contributes to the Canadian biodiesel fuel, which has been commercially available since 2001 (Agriculture and Agri-Food 2015).

Canada is among the world's leading exporter of pulses with exports to over 150 countries. The biggest pulse export markets for Canada are India, China, and Turkey. Pulses are referred to as edible dry seeds of legumes (Bekkering 2015). The pulses mainly produced in the prairie provinces are dry beans, dry peas, chickpeas, and lentils (Bekkering 2015). Alberta, Manitoba, and Saskatchewan are the main producing regions of pulses and Ontario and Quebec produce most of the beans (Pulse Canada 2018). Pulses significantly contribute to

human nutrition because they contain high amounts of protein and fibre and they are low in fat. They also play an important role in biological nitrogen fixation, which enables most legume crops to be grown without the application of nitrogen fertilizer (Bekkering 2015).

The most limiting factors to crop production in the prairies are moisture and short growing seasons (Grise and Kulshreshtha 2016). Crop production in any agricultural region of the world is also limited by diverse diseases, most of which are caused by pathogenic bacterial species, fungal pathogens, viruses, and plant-parasitic nematodes. All the major crops being grown in Canada are hosts for at least one genus of plant-parasitic nematodes (PPNs). Plant-feeding nematodes are important parasites that cause significant crop yield losses that threaten food security (Nicol et al. 2011, Mokrini et al. 2016). There are at least 4,100 species of plant-parasitic nematodes and damage associated with these parasites is estimated at $80 billion per year (Decraemer and Hunt 2006; Nicol et al. 2011). Root-knot nematodes (*Meloidogyne* spp.), cyst nematodes *(Heterodera* and *Globodera* spp.), root-lesion nematodes (*Pratylenchus* spp.), the burrowing nematode (*Radopholus similis*) and the stem and bulb nematode (*Ditylenchus dispaci*) Filipjev (1936), are considered the top five economically important plant-parasitic nematodes because of their large impact on crops (Jones et al. 2013).

1.2. The genus *Pratylenchus*

Among the plant parasites of economic importance, *Pratylenchus* species, also known as root-lesion nematodes (RLNs) rank third after root-knot and cyst nematodes (Castillo and Vovlas 2007, Jones et al. 2013). The genus *Pratylenchus* Filipjev 1936 is widely distributed, comprising of up to 98 species (Geraert 2013). Castillo and Vovlas (2007) recognized 75 species and the number of species differentiated in this genus is increasing over the years. The members of this genus survive in almost all environments including the tropics, cool and temperate regions worldwide (Castillo and Vovlas 2007, Troccoli et al. 2008, Wang et al. 2015). Many species in this genus are recognized as economically important plant parasites due to the severe crop losses they cause all over the world (Goulart 2008). *Pratylenchus* spp. are well known for their wide host range, infecting up to 350 hosts (Castillo and Vovlas 2007, Troccoli et al. 2008). The members of this genus are migratory endoparasites that target the cortical regions of roots, tubers, and bulbs resulting in severe damage to roots of host crops (Castillo and Vovlas 2007). Infected roots develop brown lesions and they become necrotic leading to yield losses and loss of marketable quality (Duncan and Moens 2006).

1.2.1. Morphology

The *Pratylenchus* genus is made up of small, stout nematodes with elongate-slender bodies and bluntly rounded tails (Eisenback 1998). Their body length ranges from 0.4 to 0.7 mm and they are between 20 to 25 µm in diameter (Eisenback 1998, Agrios 2005). They possess a short (14-19 µm) and very prominent stylet with massive basal knobs, which they use for feeding and piercing roots of host plants. They possess a well-developed, roundish to oval-shaped median esophageal bulb (Mai et al. 1996, Makete et al. 2011). Their pharyngeal glands overlap the intestines ventrally (Perry and Moens 2006). The females and males of *Pratylenchus* species are both vermiform and can move into and out of the roots. The females possess a transverse, slit-like vulva, which is located at the posterior end of the body (Mai et al. 1996, Eisenback 1998). Males can be common in some species, but they are very rare in most of the species (Eisenback 1998). Males are rare among species including *Pratylenchus neglectus*, *P. thornei*, *P. brachyurus* and *P. zeae* but common in *P. penetrans*, *P. coffeae*, *P. goodeyi* and *P. vulnus* (Yu 2008). The males are more slender and possess small, bluntly rounded bursae (Loof 1991, Brzeski 1998, Eisenback 1998).

1.2.2. Hosts of *Pratylenchus* species

Pratylenchus species are polyphagous and they infect roots of many plant species, both monocots, and dicots. The species belonging to this genus may have common host preferences, but they generally attack different host plants. *Pratylenchus neglectus* and *P. thornei* have some common hosts, and they may occur as a mixed population (Taylor et al. 2000). *Pratylenchus* species parasitize cereals including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and pulse crops (Perry and Moens 2006, Vanstone 2007, Smiley et al. 2014). *Pratylenchus* species also parasitize other crops, such as coffee plants (*Coffea arabica* L.), potato (*Solanum tuberosum* L.), banana (*Musa x paradisiaca* L.), sugarcane (*Saccharum officinarum* L.), legumes, ornamentals, rangeland grasses, fruit trees and corn (Umesh and Ferris 1992, Castillo and Vovlas 2007, Qiu et al. 2016). Potato is also seriously targeted by several *Pratylenchus* species including *P. penetrans* (Cobb 1917) Chitwood and Oteifa 1952, *P. alleni* Ferris 1961, *P. thornei* Sher and Allen 1953, *P. brachyurus* (Godfrey 1929), *P. neglectus* (Rensch 1924) Filipjev and Schur. Stet. 1941, *P. coffeae* (Zimmermann) Sher and Allen 1953, and *P. scribneri* Steiner *in* Sherbakoff and Stanley 1943. The primary hosts for *P. neglectus* are pasture and grain crops (Cook and Yeates 1993). It also parasitizes fruit crops, alfalfa (*Medicago sativa* L.), peppermint (*Mentha* x *piperita* L.), pasture legumes,

and natural grassland plants. In Australia, *P. neglectus* mainly infects wheat, causing significant yield losses in many regions growing this crop (Vanstone et al. 1998, Taylor et al. 1999, Taylor et al. 2000). Canola (*Brassica napus* L.), chickpea (*Cicer arietinum* L.), and sorghum (*Sorghum bicolor* L.) are also good hosts of *P. neglectus* (Smiley et al. 2014). Of all the *Pratylenchus* species, *P. penetrans* exhibits greatest economic importance parasitizing nearly 400 species (Perry and Moens 2006). *P. thornei* parasitizes grasses, wheat, peas (*Pisum sativum* L.), sunflower (*Helianthus annuus* L.), alfalfa (Kleynhans et al. 1996).

1.2.3. Importance of *Pratylenchus* species

Pratylenchus species cause significant losses, ranging from about 20-85% depending on the species and the nematode populations (Vanstone et al. 1998, Taylor et al. 1999, Smiley 2010). Severe economic losses associated with root-lesion nematodes (RLNs) were recorded in Australia, Europe, and the USA (Lasserre et al. 1994, Thompson et al. 1995, Smiley et al. 2004). In Australia, *P. neglectus* causes substantial yield losses in wheat, barley, potato, and rangeland grasses (Yu 2008). In Southern Australia and USA, losses to *P. neglectus* in wheat amount up to 20% and 37%, respectively (Taylor et al. 1999, Smiley et al. 2005) and losses attributed to *P. thornei* are around 40% in cereals in the same region (Thompson et al. 1995, Vanstone et al. 1998).

1.2.4. *Pratylenchus* species in Canada

In Canada, 12 *Pratylenchus* species were identified and listed from specimens provided by the Canadian National Collection of Nematodes (CNCN). The species include; *P. neglectus, P. penetrans, P. thornei, P. zeae, P. crenatus, P. fallax, P. flakkensis, P. hexincisus, P. pratensis, P. macrostylus* and *P. sensillatus* (Table 1.2) (Yu 2008). All the species listed, except for *P. flakkensis* are found in Ontario. *Pratylenchus flakkensis* was identified in Prince Edward Island and Quebec (Yu 2008). The species that were most commonly found are *P. penetrans, P. neglectus, P. scribneri, P. crenatus* and *P. hexincisus*. Among the *Pratylenchus* spp. found in Canada, *P. penetrans* is considered a parasite with the most economic importance infecting roots of many crop species (Potter and McKeown 2003). Additionally, *P. penetrans* dominates most of the Eastern part of Canada (Prince Edward Island, New Brunswick, Quebec, Ontario) and it is found mostly in potato fields of this region. *P. neglectus* and *P. penetrans* were first reported around 1982 (Olthof et al. 1982). The earliest reports of species occurring in Canada showed the presence of *P. pratensis* in British Columbia and Quebec*; P. penetrans* in British Columbia, Nova Scotia and Ontario and *P. neglectus* in British Columbia and Ontario

(Baker 1956). *P. thornei* was first identified in 1973 (Potter and Townshend 1973) and *P. zeae* was reported in 1997 (Potter and Yu 1997).

In 1994 and from 2002 to 2004, surveys were conducted in Manitoba, a province where most of Canada's potato production occurs and results showed that *Pratylenchus* species were present in 33% of a total of 135 fields that were sampled. The populations of *Pratylenchus* ranged between 4 and 5, 300 nematodes per kg^{-1} soil (Mahran et al. 2010). Out of 21 potato fields, 6 had RLNs in population ranges of 45 up to 631 nematodes per kg^{-1} of soil (Mahran et al. 2010). According to the analyses using morphometrics, the only species present was *P. neglectus* (Mahran et al. 2010). In another survey for plant-parasitic nematodes conducted by Gouveia-Pereira (2018) in Western Canada (Alberta, Manitoba, and Saskatchewan), *Pratylenchus* species occurred in 20 of the 93 fields that were sampled. The species was identified as *P. neglectus* but there was a possibility of other species because some of the DNA sequence results did not give a reliable match to *P. neglectus*.

1.2.5. Symptoms caused by *Pratylenchus* species

The feeding action by *Pratylenchus* species causes root damage as the nematodes thrust cells using their stylets. Damage also occurs through degradation by enzymes released in saliva. As implied by their common name, root-lesion nematodes cause brownish, necrotic lesions on roots of infected plants (Davis and MacGuidwin 2000). Damaged roots fail to explore water and nutrients (Smiley 2015). Moreover, the roots become more prone to water and nutrient stresses, especially in dryer seasons and in areas where water is scarce (Thompson et al. 2008). Infection by *Pratylenchus* species also results in cracking, internal rotting of tubers, discoloration, and death of root tissues (Castillo and Vovlas 2007). Plants that have been severely infected are easily pulled from the soil due to a poor root system (Agrios 2005). *Pratylenchus* spp. cause reduction in root hairs, number and length of lateral roots (Taylor et al. 1999). Roots attacked by *Pratylenchus* are much more susceptible to infection by fungi and bacteria, which can enter through pores created by the nematodes during feeding and penetration (Castillo and Vovlas 2007). The above-ground symptoms caused by *Pratylenchus* species are not specific and can be overlooked or mistaken for damage caused by other soil pathogens and symptoms caused by nutrient deficiency or lack of water (Thompson et al. 2008). Leaf number and size are reduced in heavily infected plants. In fields, affected areas appear as chlorotic (yellowing) patches, stunted plants, and reduced tillering in wheat (Agrios 2005). Crops infected by *Pratylenchus* species yield poorly due to their inability to extract water and nutrients from soil via their roots. Additionally, RLNs reduce the marketable quality

of crops such as carrot and peanuts. However, in some cases, extremely high populations of the nematodes may not kill the host plant.

1.2.6. Life cycle and reproduction

The life cycle of *Pratylenchus* species takes about 3 to 9 weeks and the length depends on the species, host crop, moisture and temperature (Jones and Fosu-Nyarko 2014, Vos and Kazan 2016). The length of the cycle can be shortened, especially at the presence of susceptible hosts and exposure to favourable environmental conditions. In the tropical species, the life cycle lasts for about 3-4 weeks and in temperate species, it takes 5-7 weeks (Perry and Moens 2006). The *Pratylenchus* life cycle begins with the egg, which develops into four juvenile stages (J1 to J4) and adults. The eggs are laid inside the root tissues of host plants or in the soil. The eggs are laid in small groups or singularly (Agrios 2005). The females of RLNs can lay 1 to 2 eggs per day and the total eggs laid per generation range from 16-35 (Perry and Moens 2006, Castillo and Vovlas 2007). Egg deposition can take about 2.5 min to be completed. The J1 moults inside the egg and it moults again into the second juvenile stage (J2). The J2 then hatches from the egg and moults into the third (J3) and fourth (J4) juveniles, and finally the adult stage (Castillo and Vovlas 2007). Both juveniles and adults are vermiform and mobile. They can migrate into and out of the roots into the soil. The hatching process occurs in three phases: i) changes in egg-shell permeability ii) the juvenile becomes metabolically active and iii) eclosion or hatching from the egg (Perry 2002). The J2 hatches by creating a slit on the eggshell through which it forces the head out of the egg. Hatching of the J2 is dependent on the temperature, moisture, soil texture, age, and type of host (Jones and Devine 2001, Perry 2002, Pudasaini et al. 2008).

In *Pratylenchus* species, the nematode uses its stylet to poke the eggshell and creates a tear. The tear becomes enlarged as the nematode pushes its head out of the egg. The nematode will immediately begin to look for a host to infect once it is hatched (Perry and Moens 2006). The nematodes penetrate roots for feeding and reproduction. *Pratylenchus* species survive mostly inside the roots of host plants, but they can also migrate to root surfaces and the soil. When the host is dead or has senesced, the nematodes will migrate into the soil and can look for a new host nearby. Reproduction of *Pratylenchus* species occurs in one of two ways; parthenogenically or amphimictically, with more than half of the species reproducing by parthenogenesis (Perry and Moens 2006). Most *Pratylenchus* spp. reproduce by parthenogenesis that is, without fertilization of eggs by males. This is especially common for *P. neglectus, P. brachyurus, P. thornei,* and *P. zeae* and among these species, the males are

rarely found. In other species, males are abundant, and reproduction occurs amphimictically and this is true for species such as *P. penetrans, P. coffeae, P. vulnus* and *P. goodeyi.* Multiple males can fertilise a single female and this results in genetically diverse offspring (Jones et al. 2011).

1.2.7. Pathogenicity of *Pratylenchus* species.

In nematology, pathogenicity is defined as the capacity of the nematode to initiate a host-parasite interaction as well as its ability to cause damage (Castillo and Vovlas 2007). Pathogenicity of *Pratylenchus* species is influenced by two main factors, reproductive fitness, and virulence (Shaner et al. 1992). Plant nutrition also has a role to play in pathogenesis, with high nutrition favoring high nematode numbers and vice versa. At low nutrition, *P. penetrans* occurred in lower populations in soil and roots of cherry (Melakeberhan 1998). However, nutrition may promote plant growth and negatively affect nematodes. Plants infected by *Pratylenchus* species have low nutrient uptake. *Pratylenchus coffeae* reduced uptake assimilation of nitrates and ammonium in coffee plants (Vaast et al. 1998). Pathogenicity is also affected by the interaction of the RLN with other microorganisms such as bacteria and fungi (Endo 1975). When *P. coffeae* occurred together with arbuscular mycorrhizal (AM) fungi, the development of necrotic lesions on roots of coffee plants due to feeding and migration was limited to a considerable extent (Vaast et al. 1998). Nematodes that have high reproductive fitness are known to infect host plants to a greater extent. Susceptible host plants suffer more damage when infected by a nematode species with high than low reproductive fitness (Castillo et al. 1998).

Edaphic factors, including soil texture, moisture and temperature, are also known to influence the pathogenicity of *Pratylenchus* species (Wallace 1983, Castillo and Vovlas 2007). These edaphic factors affect the population densities of RLNs; for example, *P. brachyurus* had higher pathogenicity at lower soil temperatures compared to high temperatures (Lindsey and Cairns 1971). Generally, severity and yield reduction increase significantly with high temperatures, dry conditions, and low soil fertility. Plant vigour is compromised, and infected plants generally become more susceptible to winter injury and infection by other diseases (Castillo and Vovlas 2007). Nematodes may react differently when exposed to edaphic factors. For *P. neglectus,* soil texture and moisture had no effect on the pathogenicity and reproduction of the nematode (McDonald and Van den Berg 1993). This suggested that pathogenicity of

Pratylenchus spp. is not influenced by a single factor such as water stress, but by a combination of factors (McDonald and Van den Berg 1993). Plants tolerate RLNs differently and some are damaged at low nematode populations while others can withstand high populations without showing any effects of infection. The infection of plants by *Pratylenchus* results in visible symptoms both above and below-ground. Above-ground symptoms include; stunted growth and chlorosis, while below-ground symptoms include; root discoloration, brownish necrotic lesions, reduced number of feeder roots.

1.2.8. Economic thresholds of *Pratylenchus* species

Economic thresholds are determined by assessing the initial soil population levels of nematodes that will multiply over the growing season leading to economic damage to the crop. Damage thresholds for *Pratylenchus* species differ depending on the crop, geographic location and interaction with other microorganisms in disease complexes. Root-lesion nematodes can reduce crop growth with populations as low as 1 nematode per g of soil. The damage threshold for *Pratylenchus* species is 1,000 kg^{-1} soil (Fleming et al. 2016). It is often difficult to estimate the economic damage thresholds for *Pratylenchus* species because they are dependent upon different factors such as climate, nematode virulence, host genotype, and cropping system (Smiley and Nicol 2009). The threshold of *P. neglectus* in the soil before it can cause disease is greater than that of *P. penetrans*. Additionally, in causing the potato early dying (PED) syndrome, *P. neglectus* does not interact with *Verticillium dahliae* to the same extent as *P. penetrans* (Mahran et al. 2010). The PED disease occurs primarily at the synergistic interaction of *P. penetrans* and *V. dahliae* and the mechanisms involved in pathogenesis are said to be species-specific (Bowers et al. 1996).

In a field experiment conducted in Oregon, *P. neglectus* reduced wheat grain yield by an initial population of 2,000 nematodes kg^{-1} soil (Smiley et al. 2005). *P. thornei* was reported with damage thresholds ranging from 420 to 30, 000 nematodes kg^{-1} soil for different locations in Australia, France, and Mexico (Castillo and Vovlas 2007). Damage thresholds of *Pratylenchus* differ within the species-host plant combinations and range from 0.05 to 30 nematodes per cm^3 (Castillo and Vovlas 2007). High threshold levels from 1,000 root-lesion nematodes kg^{-1} soil for most vegetables to lower levels of 500 nematodes kg^{-1} soil in strawberry can significantly reduce yields. If population densities of *Pratylenchus* exceed the threshold upon soil analysis, management tactics should be put in place to control the nematodes.

Table 1.1 Threshold for root-lesion nematodes (Sharma et al. 2001)

Nematodes ml^{-1} soil	**Nematodes g^{-1} dry root**	**Effect**
0- 0.2	0- 1,000	No significant effect on cereal yields
0.2-1.0	1,000- 10, 000	No visual effects. Yield loss up to 15%
1.0- 2.0	10, 000- 100, 000	Patches in crop. 15-30% yield loss in patches
> 2.0	>100, 000	Poor crop growth. 30-50% yield loss

1.2.9. Survival strategies

Generally, nematode populations in the soil decline when there is no host to parasitize. Nematodes suffer water loss when exposed to desiccation in the soil and will become inactive as soon as they sense dryness. Nematodes can prevent water loss using their impermeable cuticle (Jones and Fosu-Nyarko 2014). Many plant-parasitic nematodes survive through the egg stage (Perry 2002). Root-lesion nematodes overwinter inside roots or in the soil and they can do this in the egg, larval and adult stages. Nematodes enter quiescence in response to dryness (Lee 2002, Ravichandra 2013). The length of the quiescent period depends on how much food reserves are available and the environmental conditions prevailing. In the absence of host crops, *Pratylenchus* species can survive in roots of volunteer crops or weed species and in the soil thereby escaping starvation and death (Smiley et al. 2004). Root-lesion nematodes can also survive from one crop to the next in root residues and they may also be harboured by weeds enabling them to survive until the next host crop (Duncan and Noling 1998, Smiley 2010).

Additionally, *Pratylenchus* species enter anhydrobiosis and can survive in this state for many years (Castillo and Vovlas 2007). Anhydrobiosis is referred to as “a life without water” which is a strategic survival mechanism employed to escape unfavourable dry conditions (Wharton 2015). In the absence of host plants or during fallowing, root-lesion nematodes also enter anyhydrobiosis. Nematodes in the anhydrobiotic state minimize metabolism and reduce the surface area that is exposed to the environment by coiling their bodies (Ravichandra 2013, Wharton 2015). Root-lesion nematodes can also become anhydrobiotic at the egg stage and can survive in this way for up to a year (Castillo and Vovlas 2007). However, egg-producing females do not survive the winter (Agrios 2005).

Nematodes survive in dormant states during fallow periods, and they can also escape dryness by migrating to the subsoil (Smiley and Nicol 2009). *Pratylenchus* species survive in high numbers during fallow periods (Talavera and Vanstone 2001). They survive in the soil or root residues. The length of these survival states depends on the availability of food reserves and environmental conditions in the soil. *Pratylenchus penetrans* was able to survive a summer fallow in the anhydrobiotic state with maintaining densities of 78% and 85% in soil and roots, respectively (Talavera and Vanstone 2001). Nematodes in the anhydrobiotic state are resistant to high temperatures, surviving temperatures of up to 40^{o}C (Glazer and Orion 1983). When water becomes available, nematodes in anhydrobiosis can rehydrate, and they will resume their regular activity (Barret 1991). Nematodes recovering from anhydrobiosis can readily multiply once inside the host more than nematodes that were not anhydrobiotic (Smiley 2010, Jones and Fosu-Nyarko 2014).

Table 1.2 Morphometric measurements and presence/ absence of males in *Pratylenchus* species found in Canada (Yu 2008)

Species	***P. crenatus***	***P. fallax***	***P. flakkensis***	***P. hexicincisus***	***P. macrostylus***	***P. neglectus***	***P. penetrans***	***P. pratensis***	***P. sensillatus***	***P. thornei***	***P. zeae***
Body length (µm)	550±73.1	475 ±52.2	495 ±50.7	475 ±49.6	660 ±70.1	385± 45.5	544 ±52.0	481± 48.3	517 ±71.7	645± 69.2	475 ±49.1
Body width (middle body)	20± 2.1	20 ±2.0	22± 1.9	18± 2.0	25± 2.3	19± 1.7	21± 2.2	20± 2.0	18 ±1.9	17 ±1.6	17 ±1.8
Excretory pore to head end (µm)	69 ±6.1	74 ±7.2	74 ±7.4	76± 7.0	113 ±8.8	60 ±5.4	77± 6.5	72 ±7.1	85± 7.8	91± 8.8	70 ±7.1
Stylet length (µm)	16 ±1.1	17 ±1.0	16± 1.2	16 ±1.2	25 ±1.5	15 ±1.3	16± 1.4	16± 1.3	17± 1.2	17 ±1.1	16± 1.0
Pharyngeal overlap (µm)	40 ±4.1	51± 4.2	50 4.2	47 ±4.3	66 ±4.5	45± 4.8	50± 3.6	40± 5.1	57 ±4.9	64 ±5.4	40± 4.7
Vulva to anus distance (µm)	59± 5.6	66 ±5.9	63± 5.7	78± 6.7	51± 5.5	44± 4.8	79± 8.1	47± 4.3	103± 9.1	93± 8.4	109± 8.6
Posterior uterine sac (µm)	30 ±3.0	21 ±2.2	18 ±1.8	15± 1.6	28 ±2.2	16 ±1.7	20 ±2.1	27± 2.5	29± 2.8	26 ±2.4	22 ±2.2
Tail length (µm)	26 ±2.4	22 ±2.1	28 ±2.5	22± 2.1	30 ±3.3	17 ±1.8	29± 2.6	21± 2.3	26 ±2.5	25± 3.4	28± 2.7
a	28± 2.7	24 ±2.5	23± 2.2	26± 2.4	26 ±2.5	20 ±2.0	26± 2.8	24± 2.3	28± 2.7	38± 2.9	29 ±3.0
b	8 ±0.7	6 ±0.5	7 ±0.7	6± 0.5	6 ±0.6	6 ±0.7	7 ±0.7	7± 0.8	6 ±0.6	7 ±0.5	7± 0.6
c	21 ±1.6	22 ±1.9	18± 1.7	22± 1.8	22± 1.6	23± 1.9	19± 1.7	23± 1.8	20± 1.6	26 ±1.9	17± 1.7
V	93 ±1.5	81± 1.1	82 ±1.6	79± 1.0	88 ±1.5	84 ±1.3	80± 1.1	86± 1.6	75 ±1.4	82± 1.7	71± 1.2
Males	Extremely rare	yes	unknown	unknown	yes	Unknown-rare	yes	yes	unknown	rare	unknown

Note: Values are provided as mean ± SD

Table 1.3 Hosts and distribution of *Pratylenchus* species in Canada (Yu 2008)

***Pratylenchus* species**	**Distribution**	**Hosts**
P. crenatus	AB, BC, SK, NS, ON, PE, QC	Fruit trees, blueberry, tobacco, oat, strawberry, potato, turnip, carrot, hay, alfalfa, apple, prune, Douglas-fir, red clover, rhubarb, corn, Timothy, sour cherry, rose, cedar, barley, nursery stock, white clover
P. fallax	SK, ON, QC,	Turfgrass
P. flakkensis	AB, ON	Unknown
P. hexicinsus	AB, ON	Fescue and pea
P. macrosylus	BC, ON	White spruce, white birch, Douglas-fir
P. neglectus	AB, BC, MB, ON	Tobacco, asparagus, oat, strawberry, cherry, barley, peach, corn, tomato, pear, red clover, apple, alfalfa, Timothy, African violet, rye, rose, prune, apricot, rhubarb, wheat, cedar, begonia
P. penetrans	AB, MB, SK, NB, NL, NS, ON, PE, QC	Apple, tobacco, peach, strawberry, bluegrass, bent grass rose, cherry, daffodil, corn, tulip, African violet, potato, red clover, soybean, raspberry, potato, oat, begonia, pine, aspen, rye, Timothy, chrysanthemum, black spruce, petunia, gingko, grape, *Poa*, carrot, pea, buckwheat, plum, onion, pear, turnip, barley, wheat
P. pratensis	BC, MB, NB, ON	English holly, oat, corn, alfalfa, strawberry, clover
P. sensillatus	ON	Timothy
P. thornei	ON	Wheat and turfgrass
P. zeae	ON	Corn

1.2.10. Dispersal

Pratylenchus species can be dispersed via propagation materials of infected plants such as rootstocks and, seed tubers. They can also be dispersed within fields in root residues or plant materials via run-off. Dispersal can also occur through soil attached to farm equipment or machinery. These nematodes can also be dispersed in the dust by wind and this can occur during the anhydrobiotic state (Perry and Moens 2006). Nematodes in this state are prone to drift by air currents (Marban-Mendoza and Viglierchio 1980, Glazer and Orion 1983). *Pratylenchus* species were observed in dust and this suggested that the nematodes could be dispersed during windstorms (Gaur 1988, Baujard and Martiny 1994).

1.3. Factors affecting nematode population densities

Reproduction and population build-up of nematodes are affected by several factors, including plant host status, cultural practices, environmental conditions, and the reproductive capacity of the nematode. Nematode populations of PPNs are affected by the host more than they are by any other factors such as environmental conditions (Norton 1989). Populations of RLNs decline sharply at the absence of host plants (Castillo and Vovlas 2007). Hosts of *Pratylenchus* spp. include wheat, lentil, chickpea, corn, canola (Taylor et al. 2000, Smiley et al. 2014) and such crops will allow the nematode to reproduce effectively, thereby leading to an increase in populations. The population growth of nematodes is mostly affected by resistance or tolerance of host plants. Resistant crops such as asparagus can suppress the reproduction of nematodes, thereby resulting in low nematode numbers. At the presence of a host crop, nematode populations can increase by a 1000-fold during a crop cycle (Gowen 2002). The population of nematodes is considered density-dependent in situations where there is competition for feeding sites because of limited food resources (Taylor 2000). This suggests that nematode numbers will multiply only to certain threshold levels. In contrast, when there is no competition for feeding sites, that is, at low initial densities, nematode population dependency is said to be density-independent (Taylor 2000).

Once the nematodes begin to associate with a suitable host, the environmental factors become the primary influence of nematode populations. Population densities of *Pratylenchus* are affected by edaphic factors including moisture, soil temperature and texture (Wallace 1983, Duncan and Moens 2006). The type of interaction that occurs between *Pratylenchus* spp. and other

nematode species and other microorganisms such as bacteria and fungi also affect the growth of nematode population (Norton 1989). Some nematodes or other microorganisms may compete with *Pratylenchus* for sites of invasion on the roots of plants. Different soil textures may allow different nematode species to survive within them and as such, a soil may favour population increase of one nematode species and not the other. Where heterogeneous soils occur, for example, in a field, the nematodes will likely have patchy distribution (Alby et al. 1983). The reproductive stage of the nematode is the critical time at which environmental factors affect population densities and, in some cases, nematode survival is compromised by unfavourable conditions. Environmental conditions such as moisture and temperature are subject to change at any time and they can do so before the nematode completes its life cycle (Norton 1989). Moisture and temperature also affect the growth of the host. Nematode populations indicate the potential amount of damage likely to be caused by the nematode (Trudgill and Phillips 1997). On susceptible hosts, populations of nematodes readily increase, and competition for feeding sites becomes very high, thereby influencing nematode infectivity, reproduction, and high final population densities.

1.4. Assessing host suitability to *Pratylenchus* species

In general, *Pratylenchus* species attack a wide range of plant species (Jones and Fosu-Nyarko 2014). Host suitability of crops to plant-parasitic nematodes, including *Pratylenchus* species can be assessed by measuring the reproduction capacity of the nematode on the crops (Lewis 1987). A plant is regarded as resistant or susceptible based on the extent to which nematode reproduction occurs (Thompson et al. 2008). A resistant crop restricts nematode multiplication and conversely susceptible crops allow nematodes to multiply readily. In susceptible crops or cultivars, nematode reproduction is relatively high, resulting in the build-up of high population densities (Thompson et al. 2008). Tolerant crops can grow and still produce good yields at even heavy nematode infestations (Thompson et al. 2008). Hosting abilities of crops can be assessed using final nematode densities (P_f) and the reproductive factor (R_f). The R_f is also known as the multiplication rate (MR) obtained by dividing the final nematode population density by the initial population density (P_f /P_i) within a plant growth cycle (Fernandez et al. 1994, Alcañiz et al. 1996). When using the P_f, comparisons are made between final nematode densities of the test crops with that of previously identified susceptible cultivars (Smiley et al. 2014). The most commonly used approach for determining plant host status is the reproductive factor (R_f) and it is used as an indicator of any

nematode-host relationship developed (Bélair and Benoit 1996, McKenry and Anwar 2006). Multiplication rates greater than 1 (MR > 1) mean a plant is a good host, poor hosts have an MR of less than 1 (MR < 1) and non-hosts have an MR of less than 0.1 (Alcañiz et al. 1996, Taylor et al. 2000). However, there are many variations in the use of MR or R_f values (Seinhorst 1967, Zhang and Schmitt 1994, Hajihassani et al. 2016, Bellé et al. 2017). For example, Hajihassani et al (2016) used the following category; R_f < 1 = non-host, 1 to 2 = poor host, 2 to 4 = good host, and > 4 = excellent host. Assessment of MR enables one to compare between nematode species and where unequal initial densities occur. Additionally, the use of MR also enables one to compare between different experiments.

Multiplication rate of a nematode is influenced by several factors such as; temperature, soil moisture, soil texture and nutrient status (Duncan and El-Morshedy 1996, Delaville et al. 1996, Mani et al. 1997). These factors often affect both the nematode and the host. Synergistic and antagonistic soil biota may influence the severity of damage as well as the multiplication rate of nematodes within a host (Taheri 1996). The MR is affected by initial densities and lower initial densities cause higher nematode multiplication (Seinhorst 1966, Fisher and Hancock 1991). Multiplication rate is, therefore, usually used in greenhouse experiments where temperature, moisture, and soil and shoot biota are more easily controlled. It is essential to validate greenhouse tests with field experiments to understand better how nematode densities may change under different environmental conditions (Romero and Nombela 1999).

1.5. Plant disease complexes involving *Pratylenchus* species

A disease complex is formed when two organisms interact synergistically, thereby inflicting much damage on infected crops. The extent of crop damage is governed by many factors such as nematode pathotype and population, crop species, management practices, environmental and climatic conditions (Seinhorst 1970, Barker and Noe 1987, Schouten and Beniers 1997). A disease complex may be very severe even with a low incidence of fungi or nematodes (Back et al. 2002); therefore, it is essential to know and understand the prevailing species and the role of the pathogens involved in the disease complex. Plant-parasitic nematodes, including *Pratylenchus* species, play several roles in disease complexes and these include: vectors of fungal pathogens, wounding crops, host modifying agents, modifying the rhizosphere, and breaking resistance to infection.

Synergistic interactions occur when the nematode and pathogen interact to cause more plant damage than the total of individual damage caused by the nematode and the pathogen (1 + 1 > 2). On the other hand, the nematode-fungus interaction can be antagonistic (1 + 1 < 2), whereby plant damage caused by the two parasites is less than the sum of the damage caused by the two organisms, acting independently (Back et al. 2002). A neutral association is whereby the nematode and the fungus cause the same amount of damage they would cause individually (1 + 1 = 2) (Back et al. 2002). Species in the same genus may not behave the same in forming disease complexes with other pathogens.

Pratylenchus species are commonly reported to interact with fungal pathogens in causing disease complexes to crop plants. They interact with *Fusarium* and *Verticillium* wilt fungal species (Back et al. 2002). Nematode feeding and lesion formation disrupt the structural barriers, thereby making it easy for the fungus to invade the root cortex (Perry and Evert 1983). The common synergistic interaction is that of *Pratylenchus penetrans* and the wilt fungi *Verticillium dahliae* in causing the potato early dying (PED) disease (Back et al. 2002, Forge et al. 2015). The PED syndrome refers to the premature vine death, which leads to a decline in yields of potato (Wheeler et al. 1992, Agrios 2005). The fungus penetrates through the root tips (Zunke 1990). Other species that interact with *V. dahliae* include *P. neglectus* and *P. thornei*. However, the interaction of *P. neglectus* and the fungus is not as obvious and common as that of *P. penetrans* (Mahran et al. 2010). In a study by Hafez et al. (1999), synergism was observed between isolates of *P. neglectus* from collected Ontario, Canada and *V. dahliae*. However, the nematode populations from Idaho, USA did not show any interaction with the fungus.

Additionally, the incidence and severity of wilt disease caused by *Verticillium dahliae* f. *menthae* on peppermint can be aggravated by *P. minyus* (syn. *P. neglectus*). In infested plants, the incubation period for *Verticillium* was shortened and the reproduction of *P. minyus* increased (Faulkner et al. 1970). It is also suggested that all *Pratylenchus* species do not interact equally with the fungi on PED in potato and variations may occur between species originating from different geographic locations (Wheeler and Riedel 1994, Hafez et al. 1999, Mahran et al. 2010). *P. crenatus* does not show synergism with *V. dahliae* (Riedel et al. 1985, Duncan and Moens 2006, Mahran et al. 2010) and *P. scribneri* can interact with the fungus provided the temperatures are above 32^{o}C

(Riedel et al. 1985). *Pratylenchus* spp. also interact with *V. albo-atrum* to facilitate early senescence or death in plants (Martin et al. 1981).

Rhizoctonia infection and disease may be increased with high densities of *P. neglectus* in wheat (Benedict and Mountain 1956). *Pratylenchus* spp. also interact with *Fusarium* wilt fungi and the root-rot pathogens including *Pythium*, *Phytophthora* and *Rhizoctonia* species. *Pratylenchus neglectus* infects tobacco and it is known as the primary disease-causing agent for the root rot of tobacco (Mountain 1954). The disease causes extreme stunting, wilting, necrosis of lower leaves, and cessation of growth. In southern Ontario, the interaction of *P. neglectus* with *R. solani* in wheat roots to cause root rot was additive (Benedict and Mountain 1956). *Pratylenchus* species promote plant attacks by bacterial pathogens and they do so in different ways, including creating entry channels, restricting plant defenses or resistance to bacterial microbes, and increasing incidence and severity of the bacterial diseases (Hackenberg et al. 2000). They also form disease complexes with certain pathogenic bacteria. Some examples of disease complexes involving *Pratylenchus* were reported for *Pseudomonas* species, such as *P. viridiflava* (Burkholder 1930). *Pratylenchus* spp. also form antagonistic interactions with bacterial species, e.g., *Pseudomonas, Rhizobium* spp. (Hackenberg et al. 2000). Since *Pratylenchus* spp. interact differently with other microorganisms, it is, therefore, essential to correctly identify the nematode species present. Additionally, it is important to understand the potential of the nematode species prevalent to cause crop damage and economic losses.

1.6. Identification of *Pratylenchus* species

Accurate identification of nematodes is the basis for any successful nematological research, and it is particularly important when developing control strategies. Over the years, nematologists have developed new and better methods of nematode identification. Although molecular tools are effective and promising for future diagnostics, they cannot be used without classical taxonomy. Molecular methods must be used to supplement classical taxonomy to obtain accurate results (Yu 2008). *Pratylenchus* species differ in their host preferences; it is, therefore, important to ensure that species are identified correctly. Accurate as well as rapid identification is critical for quarantine inspection of imported plant materials.

1.6.1. Morphological identification

Morphological identification has always been the principal method for nematode identification. It involves the use of light microscopy to observe the anatomy and morphology of the nematodes being studied (Inserra et al. 2001). New technologies for nematode identification have emerged over the years, but the use of morphological characters still plays an important role in nematological research. Morphological identification of plant-parasitic nematodes to the genus is solely based on observing mature females, but in some instances, juveniles and males are used to confirm the results obtained (Mai et al. 1996; Yan et al. 2008). The females, compared to males, have more characters that can be used in diagnostics and identification of the species (Loof 1991). Nematodes are distinguished and classified based on features that include; stylet length, body length, body width, vulva position (Loof 1991, Troccoli et al. 2008, Yu et al. 2012). For *Pratylenchus* species, certain characters are easily observed under the dissecting microscope and these include; short, prominent stylet, an overlap of the intestines in the esophagus and position of the vulva at the posterior end of the nematode (Mai et al. 1996). The other morphometric characters used for identification of *Pratylenchus* species include; the number of lip annuli (two to four), presence or absence of males, spermatheca in females (presence or absence), number of line-markings within the lateral field and tail shape (Handoo and Golden 1989, Loof 1991).

The nematodes in this genus exhibit very small morphological differences thereby making it difficult to distinguish between species (Perry and Moens 2006). Some of the ranges of morphometric characters overlap between species and some species exhibit intraspecific variations. To ensure accurate identification, one should use multiple specimens before concluding the species (Mai et al. 1996, Perry and Moens 2006). When using morphological characters, several specimens (at least 10 or 20) are examined to get a reliable description of the nematode species (Fortuner 1984, Perry and Moens 2006). Orui and Mizukubo (1999) suggested that at least 25 adult females should be observed per sample. The use of morphometric characters to identify *Pratylenchus* to species level is difficult and may not give substantial distinction because some of the characters may overlap among related species (Castillo and Vovlas 2007).

Morphological identification involves mounting of specimens onto microscope slides and preparation of specimens may involve any of the following; addition of sucrose or glycerine and heating. During specimen preparation, nematode morphology may be altered due to shrinkage or

rotting and this causes the loss of some of the useful external and internal structures used during morphological identification (Mai et al. 1996, Inserra et al. 2001, Oliveira et al. 2011). These drawbacks reveal that the nematologists should ensure that specimens are correctly preserved because when using light microscopy, it is challenging to observe individual morphological characters (Inserra et al. 2001). The distinction of detailed characters requires high magnification (Castillo and Vovlas 2007). Moreover, the taxonomist needs to have the expertise and reliable taxonomic publications for the specific genus being studied as they contain illustrations and descriptions of characteristics of importance (Mai et al. 1996, Inserra et al. 2001). This method is time-consuming and it requires careful examination to distinguish between nematode species (Subbotin et al. 2000, Tanha-Maafi et al. 2003). Another problem with morphological identification is that it is subjective and as such, can lead to erroneous results.

However, the use of morphological identification has low costs and it enables nematologists to produce results fast and to relate nematode morphometric characters to their respective functions. Additionally, morphological identification can also be used in surveys of plant-parasitic nematodes present in farmlands and even forests. The difficulties faced by nematologists during identification have prompted them to develop new methods of identification, such as scanning electron microscopy, four-dimensional imaging, confocal microscopy, and the use of polytomous keys (Corbett and Clark 1983, Chen et al. 1997, Castillo and Vovlas 2007). It is essential to achieve proper nematode diagnostics, and this can be achieved by integrating morphological, molecular and biochemical identification methods.

1.6.2. Molecular identification

Molecular methods are now being widely adopted for use in identifying plant-parasitic nematodes due to their high efficiency and reliability (Waeyenberge et al. 2000, Al-Banna et al. 2004). Unlike morphological identification, molecular tools can be used without expertise in nematode nomenclature and morphology. Molecular methods are relatively cheap, fast, accurate, reliable and straightforward to conduct (Yan et al. 2008, Blok and Powers 2009, Abebe et al. 2011, Castagnone-Sereno et al. 2011). Moreover, DNA-based tools are applicable for use on a variety of sample types including eggs, egg masses, host tissues, and soil extracts. Advancement in these tools now allows direct extraction and detection of DNA from soil samples as small as 1 g to large samples of up to 500 g or more (Yan et al. 2008, Tan 2012).

Molecular methods include polymerase chain reaction (PCR) based tools, random amplified polymorphic DNA (RAPDs) as well as restriction fragment length polymorphism (RFLPs) (Perry et al. 2007). Identification of RLNs has been achieved by the use of the 28S D2 and D3 expansion regions on the ribosomal DNA (rDNA) (Al-Banna et al. 1997, Duncan et al. 1999, Subbotin et al. 2008). The rDNA is comprised of three coding genes (18S, 5.8S, and 28S) and between them are two internal transcribed spacers, that is, the ITS1 and ITS2 and these genes are widely used in diagnostic studies (De Luca et al. 2004). *Pratylenchus* spp. differ in the sequence size of the ITS region of the rDNA and they be distinguished from each other using this region. The ITS evolves faster than the coding genes and it shows a great deal of molecular variability, thereby making it useful and applicable in distinguishing closely related species or subspecies from each other (Palomares-Rius et al. 2017).

Another protein-coding mitochondrial gene, cytochrome c oxidase subunit 1, shortened as (*cox1*) is now being used for molecular identification of nematodes (Palomares-Rius et al. 2017). *Pratylenchus* species have since been identified using PCR-based tools such as RFLP, RAPDs (Orui 1996, Pourjame et al. 1999, Waeyenberge et al. 2000). Some methods can also be used in combination; for example, RAPDs and SCAR-PCR were used for the identification of different stages of *P. thornei* (Carrasco-Ballesteros et al. 2007).

Polymerase chain reaction (PCR) techniques allow the determination of genetic variation of nematodes at the DNA level. It involves the chemical synthesis of multiple copies of the DNA molecule that is being targeted in the assay (Nega 2014). This technique uses a very small amount of nematode DNA and enzymes and each new amplified DNA molecule serves as a template for further replication (Giorgi et al. 1994). DNA can be extracted from individual nematodes and can be detected using PCR with universal or species-specific primers (Yan et al. 2008). PCR assays with species-specific primers are among the commonly used methods and gel electrophoresis is used to observe and distinguish amplified products (Uehara et al. 1999, Al-Banna et al. 2004). Al-Banna et al. (2004) managed to design some species-specific primers for distinguishing *Pratylenchus* species. Results produced by PCR give either a yes or no outcome and do not warrant any further analysis of amplicons because the species-specific primers only amplify a single band of the species present (Al-Banna et al. 2004). DNA extracts of *P. neglectus* and *P. thornei* collected from soil were identified using this method (Yan et al. 2008). *P. loosi* and *P. coffeae* have been

distinguished from each other using PCR with species-specific primers (Uehara et al. 1998). Al-Banna et al. (2004) distinguished six *Pratylenchus* species by amplifying the D3 region of the rDNA using species-specific primers. The species identified were *P. brachyurus, P. neglectus, P. scribneri, P. penetrans, P. thornei and P. vulnus*. With species-specific primers, it was possible to distinguish between *P. vulnus* and *P. penetrans* which would be difficult to do with morphological identification (Al-Banna et al. 2004). Moreover, *P. neglectus* could be distinguished from these two species based on the primers designed in this study. Quantitative PCR (qPCR) is used for detection, quantification, and characterization of nucleic acids. Unlike qPCR, conventional PCR does not quantify nematode species (Mokrini et al. 2013).

Molecular tools are powerful in discriminating plant-parasitic nematodes between and within nematode genera. However, they do not replace classical methods of identification but rather supplement them to obtain accurate results (Inserra et al. 2001, Oliveira et al. 2011). Limitations associated with molecular techniques include cross-contamination of samples, false-positive results, false-negative reaction, and difficulty in optimizing and validating the tools and methods, DNA extraction protocols and conditions (Nega 2014). There is no ideal marker because all the molecular tools have some limitations; hence, they can be used in combination where applicable.

1.7. Management of *Pratylenchus* species

Once *Pratylenchus* species are introduced into the soil of a field, they are difficult to eradicate (Castillo and Vovlas 2007). Management tactics used should aim at reducing initial population densities, restricting nematode multiplication and keeping nematode populations below the economic threshold. Species must be identified accurately and estimations for root and soil numbers should be made.

1.7.1. Cultural control

One of the most effective ways to manage *Pratylenchus* species is to exclude them from a field or region before they become established (Castillo and Vovlas 2007). This can be achieved by using uninfested, certified planting material and minimizing the spread of infested soils via equipment and boots. Practicing sanitation can help exclude *Pratylenchus* that may be introduced in uninfested seedbeds, nurseries, and fields. Growers and farm workers must use clean farm equipment, planting materials and soil free from *Pratylenchus* species. Quarantine measures must

be put in place to also prevent the spread of plant-parasitic nematodes (Schrader and Unger 2003). Root lesion nematodes are generally difficult to control using crop rotations because they have a broad host range (Jones et al. 2016). Resistant and non-host crops can be incorporated into crop rotations to help lower nematode population densities. Marigold (*Tagetes* spp.) and some mustard (*Brassica* spp.) are poor hosts for the RLNs and can, therefore, be used for the suppression of nematode populations (Evans et al. 1993). When non-host crops are grown, populations of nematodes decline due to starvation as well as weak reproductive capacities (University of Georgia Cooperative Extension 2009). Excellent nematode control can occur with crop rotations involving non-host crops for 2 to 4 years (Barker 1997). The effectiveness of rotations varies with the nematode species being targeted and its ability to survive prolonged periods without a host (Jones et al. 2016). Growing moderately susceptible crops can result in lower nematode multiplication and population densities than would occur with susceptible ones.

Several compounds isolated from antagonistic plants are nematicidal to *Pratylenchus* species and these include dithioacetylenes, glycosides, and glucosinolates (Ferraz and de Freitas 2004). *Brassica* species contain glucosinolates within their tissues. Applications of root and leaf tissues of *Brassica* species were effective against *P. neglectus* (Potter et al. 1998). Plant-parasitic nematodes can also be suppressed using neem products (*Azadirachta* spp.), that is, oil, cake, powdered neem and seed (Musabyimana and Saxena 1999). In Ontario, *P. penetrans* was controlled by incorporating French marigold (*Tagetes patula*) and African marigold (*Tagetes erecta*) in crop rotations with flue-cured tobacco (Reynolds et al. 2000). Some plants and herbs such as thyme, oregano, rosemary, cinnamon, fennel also contain compounds nematicidal to *Pratylenchus* species. Extracts from Soapbark tree (*Quillaja Saponaria)* had nematicidal effects on *P. neglectus* and *P. penetrans* at 100 ppm in Chile and these contain polyphenols, triterpenoid saponins, salts and sugars (San Martin and Magunacelaya 2005)

According to Castillo and Vovlas (2007), among the cultural methods, fallowing is one of the practices useful for the control of *Pratylenchus* species and it works best in hot and dry climates, such as in the tropics (Agrios 2005). Summer fallow exposes the nematodes to dryness, starvation, and heat. In the north-western part of the USA, *P. neglectus* populations were significantly decreased by a summer fallow and ultimately, the grain yield was high (Perry and Moens 2006). *Pratylenchus neglectus* populations were reduced by a combination of fallowing

for about 5 weeks and destruction of French bean roots. This combination was much more effective in reducing *P. neglectus* numbers than when fallow was implemented alone (Ornat et al. 1999). The limitation of this method is that some nematodes may survive through anhydrobiosis and others migrate to the subsoil. Once host plants are available, nematode populations increase to damaging levels (Thompson et al. 2008). Moreover, fallow may increase soil erosion and soil structure may be lost. Economically, the method may not be feasible because there will be no farm income from fields under fallow.

Organic products such as manure, compost, and oil cakes can be incorporated into the soil to reduce population levels of plant-parasitic nematodes. The addition of organic manures may promote nematophagus fungi and antagonistic bacteria which help to minimize nematode infestation (Oka and Yermiyahu 2002). Liquid hog manure (LHM) can effectively reduce *Pratylenchus* populations. Liquid hog manure contains toxic compounds such as ammonia, volatile fatty acids and nitrous acid, all of which can kill nematodes (Conn et al. 2005, Oka 2010). Mahran et al. (2008) reported that LHM was used to control *Pratylenchus* species by up to 80%. Liquid hog manure also controls *V. dahliae* by attacking the fungus microsclerotia (Conn et al. 2005); therefore, it can be effectively used to reduce the PED disease caused by the interaction of the fungus and root-lesion nematodes. In the United States, a commercial product (Clandosan) containing chitin and urea was developed and registered for use in nematode control. However, the product did not show an adequate suppressive capacity against nematodes. Soil amendments containing chitin can release ammonia as they degrade, and the ammonia has a nematicidal effect (Duncan 1991).

1.7.2. Plant resistance to *Pratylenchus* spp.

The use of resistant cultivars to control plant-parasitic nematodes has several advantages over other methods; it is cost-effective, environmentally sound and requires little to no technology (Trudgill 1991, Melakeberhan 1998). The resistance of a plant to nematodes is defined by its ability to suppress nematode development and reproduction. Resistance is controlled by genes that cause the host to inhibit nematode multiplication (Trudgill 1991). Resistant plants do not prevent nematode invasion, but they restrict reproduction and increase in population. Resistance can either be complete or partial. Complete resistance is whereby a crop or cultivar does not allow nematode multiplication and this commonly occurs against *Meloidogyne* and *Heterodera* species (Nelson et

al. 1989). Partial resistance allows nematodes to multiply to an intermediate level (Cook and Evans 1987). Resistance to *Pratylenchus* species and other migratory parasites is generally partial. Partial resistance has been reported for *P. penetrans* in alfalfa, *P. vulnus* in *Prunus* spp., and *Rotylenchus reniformis* in soybean (Robbins et al. 1994, Thies et al. 1994, Alcañiz et al. 1996). Tolerant plants can withstand nematode infection without implications on growth or productivity (Roberts 2002). According to Smiley and Nicol (2009), resistance and tolerance to a nematode species are said to be genetically independent.

A good resistant crop or cultivar is that which is tolerant to withstand nematode attack and produce good yields even in fields infested with high nematode populations (Trudgill 1991). A crop can be resistant to one species (or race) referred to as narrow resistance or to more than one nematode species referred to as broad resistance (Sikora et al. 2005). It is common that a crop resistant to one nematode species may not be resistant to another related species in the same genus which means that a crop resistant to *P. neglectus* for example, may not be resistant to *P. thornei* and vice versa (Farsi et al. 1995). If a crop or cultivar offers dual resistance, then there is no need to identify the nematodes present to the species level if the nematodes belong to the same genera. Intolerant cultivars allow the high build-up of nematodes, thereby causing much damage to the host plants. Tolerant but not resistant cultivars cause an increase in nematode population densities to damaging levels (Trudgill 1991). Resistant plant germplasm for *Pratylenchus* species is still less available. However, there are some crops in which resistant germplasm has been identified and these include cereals, potato, sweet potato, banana, forage crops, strawberry, and woody plants (De Waele and Elsen 2002, Perry and Moens 2006).

In Australia, much work to determine host resistance and susceptibility has been conducted on wheat because it is attacked by at least eight *Pratylenchus* species (*P. thornei*, *P. neglectus*, *P. crenatus*, *P. penetrans*, *P. teres*, *P. scribneri*, *P. zeae*, *P. brachyurus*) and it is one of the major crops grown in this region (Jones and Fosu-Nyarko 2014). Most wheat yield losses reported in Australia are associated with *P. neglectus* and *P. thornei* and quantitative traits loci (QTLs) for resistance to these species were identified in hexaploid wheat lines (Zwart et al. 2005, Smiley and Nicol 2009, Yu et al. 2012). However, plants resistant to *P. neglectus* may not be resistant to *P. thornei* and vice versa. In Australia, some wheat cultivars were resistant to *P. thornei* but not resistant to *P. neglectus* (Perry and Moens 2006). There are a few wheat lines that resist

both *P. neglectus* and *P. thornei* offering dual-resistance and these crops can be grown without having to conduct identification of the nematode species (Smiley and Nicol 2009). Other plant hosts resistant to *P. neglectus* are pea, faba bean, lentil, Narbonne bean and safflower (Smiley et al. 2014). In barley germplasm, five quantitative trait loci (QTL) for resistance to *P. neglectus* were identified (Sharma et al. 2011). Additionally, a gene for resistance (*Rlnn1*) to *P. neglectus* has been identified on chromosome 7A and another gene was also found on chromosome 4D (Williams et al. 2002, Zwart et al. 2005). Some crops may be resistant to more than one nematode group or species, e.g., some cultivars of sweet potato are resistant to *P. coffeae* and *Meloidogyne incognita* (Perry and Moens 2006). Faba beans are resistant to *P. neglectus* as well as *P. thornei* and can, therefore, be used to break the nematode cycle in infested crops (Taylor et al. 2000, Grains Research and Development Corporation 2017). Resistance is a good management strategy for the control of nematode populations. Unlike resistance, tolerance does not offer long term management of nematodes (Smiley and Nicol 2009).

1.7.3. Pesticide Control

Chemical control of *Pratylenchus* species is mostly considered an option when the other tactics, cultural, physical and biological, have failed to reduce nematode population densities (Castillo and Vovlas 2007). Several nematicides have now been banned because of health concerns, ozone layer depletion, and environmental pollution issues. Moreover, chemicals are costly, and they can adversely affect soil fertility on long-term use (Castillo and Vovlas 2007). It is considered more feasible and profitable to use resistance than chemical control, provided resistant crops or cultivars are available (Perry and Moens 2006). Due to the high costs of chemicals, they are mainly used in high-value crops such as banana and coffee (Perry and Moens 2006). No chemical or biological nematicides are available for nematode control in wheat. (Smiley et al. 2014). Fumigants volatilize in soil and they are usually applied in the liquid form killing eggs, juveniles, and adults. Non-fumigants are not effective against the egg stage, but they interfere with the host location of the nematodes. Examples of nematicides and fumigants developed for nematode control include; fosthiazate, oxamyl, 1.3 dichloropropene (1.3D), ethomyl, sprotetramat, metam-sodium, aldicarb, fenamiphos, and carbofuran (Davis and MacGuidwin 2000). Although nematicides are unable to eradicate the nematodes, they can reasonably reduce the populations (Agrios 2005).

1.7.4. Integration of management strategies

In most cases, a single control method may not be entirely effective against a nematode species hence the need to use various control strategies in combination (Kerry 2000). Canada has adopted the use of integrated pest management (IPM) tactics to control plant-parasitic nematodes since the 1990s in order to move away from the use of nematicides (Bélair et al. 2018). The practices used in IPM include; crop rotation, sanitation, use of resistant cultivars, antagonistic plants, use of organic amendments, and green manure cover crops (Hildalgo-Diaz and Kerry 2008, Belair et al. 2018). For effective control of *Pratylenchus* species, control tactics that reduce nematode populations must be integrated. *Pratylenchus* species can be controlled by a combination of fallowing, organic amendments and including resistant or non-host crops in rotations (Thompson et al. 2000, Mahran et al. 2010). Soil solarization may be used in integration with chemical, biological and cultural control strategies (Katan and DeVay 1991). A combination of fallow and tillage was reduced population densities of *P. neglectus* (Ornat 1999).

The presence of *Pratylenchus neglectus* in the Canadian Prairie Provinces warrants further investigation on the hosts preferred by this nematode among the major crops grown locally. *P. neglectus* was found in chickpea and yellow pea fields that were sampled in Manitoba during a survey recently conducted between 2014 and 2016 by Gouvea-Pereira (2018). As previously mentioned, *Pratylenchus* species do not cause apparent symptoms; hence, their damage may go unnoticed. Most pulse and non-pulse crops being grown in the Canadian Prairie Provinces are potential hosts for the *Pratylenchus* spp. There is no knowledge yet about what crops *P. neglectus* is parasitizing and this urgently needs to be determined. As part of management, determining the host status of crops and their effects on nematode population densities is a crucial step especially for emerging plant-parasitic nematodes. Such information is important for growers to know which crops to grow to prevent the nematode

1.8. Hypotheses

The hypotheses for this thesis were that *Pratylenchus neglectus* is the species present in the prairies and as shown elsewhere, canola, wheat and pulse crops are hosts for the nematode. I hypothesized that on suitable host crops, populations of *Pratylenchus* spp. will increase within the

8-wk crop growth cycles. Lastly, I postulated that there would be no impact on crop growth because the starting nematode densities were very low.

1.9. Thesis Objectives:

The objectives of this thesis were:

(i) to determine the host suitability of the primary pulse and non-pulse crops grown in the Canadian Prairies to the species of *Pratylenchus* spp. present,

(ii) to identify the species of the *Pratylenchus* spp. present in Canadian Prairie soils and those recovered from roots of suitable host crops,

(iii) to determine how quickly the populations of *Pratylenchus* spp. will increase with successive planting in the growth-chamber of the major pulse and non-pulse crops available in the Canadian Prairies, and

(iv) to assess the effects of the abundance of *Pratylenchus* spp. on the performance of the crops following several successive growth cycles of the crops.

1.10. Literature cited

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2. SCREENING FOR THE CROP HOST PREFERENCE OF *PRATYLENCHUS* SPP. FOUND IN SOILS OF THE CANADIAN PRAIRIES

2.1. Abstract

Pratylenchus spp. have recently been reported in some pulse fields in the Canadian Prairie Provinces. This research study aimed at determining the species identity of *Pratylenchus* spp. found in the Canadian Prairie soils and their host preferences. Additionally, the study assessed the effects of host crops on the abundance of *Pratylenchus* spp. following successive crop growth cycles in the laboratory. Before the study, nematodes were identified using morphological features followed by molecular assays, that is, polymerase chain reaction (PCR) with species-specific primers and DNA sequencing of the D3 region of the 28S rDNA gene. Seven crops, including; canola, chickpea, lentil, pinto bean, spring wheat, soybean, and yellow pea were screened as hosts for *Pratylenchus* spp. undergrowth chamber conditions. Commonly grown varieties available in the Prairies were evaluated in this study. A control treatment consisting of pots without crops was included. Nematode inoculum and soils were collected from a field near Brooks in Alberta. The host screening experiment was conducted in three replicated trials and host suitability was assessed using the nematode reproductive factor (R_f = final density/ initial soil density) after an 8-wk crop growth period. In another experiment, the effect of crop hosts on the build-up of the *Pratylenchus* spp. densities was assessed across three successive growth cycles. The *Pratylenchus* spp. recovered in the soil was identified as *P. neglectus* using PCR assays and comparisons of DNA sequences to the GenBank database. Additionally, based on morphology, the nematode features matched with those of *P. neglectus*. There were no males observed among the *Pratylenchus* spp. examined. The absence of males is a character for *P. neglectus* because it reproduces by parthenogenesis. The hosts for *Pratylenchus* spp. ($R_f > 1$) determined in this study were canola, chickpea, pinto bean, soybean, and spring wheat. Lentil was a poor host ($R_f < 1$) for the *Pratylenchus* spp. Yellow pea was considered a non-host for the nematode and most of its roots were not parasitized by the nematode at all. In the second experiment, the densities of *Pratylenchus* spp. increased in pots planted to canola, chickpea, pinto bean, soybean, and spring wheat. *Pratylenchus* spp. reproduced best on soybean and a mean abundance of >1000 nematodes kg^{-1} soil was observed in the final cycle. Low nematode populations were observed in lentil pots in all the cycles. The populations of *Pratylenchus* spp. decreased drastically in pots planted to yellow pea, indicating that the crop hindered survival and reproduction of the nematodes across the three

cycles. In the control, populations of *Pratylenchus* declined across cycles due to the absence of hosts to invade. This study showed that there were significant differences in the host status of the crops and how they influenced the final population densities of *Pratylenchus* spp. in the soil. The results report canola, chickpea, pinto bean, soybean, and spring wheat as new hosts of *Pratylenchus* spp. found in the Canadian Prairie soils. These findings articulate that growing crops that are suitable as hosts of *P. neglectus* such as soybean can significantly increase nematode abundance in the soil.

2.2. Introduction

The root lesion nematodes of the genus *Pratylenchus* Filipjev (1936) are important plant parasites devastating many crops globally, causing losses of up to 85% (Nicol et al. 2011). About 75 species have been described in this genus and the most economically important species include *P. neglectus, P. thornei, P. penetrans, P. vulnus, P. zeae P. goodeyi, P. scribneri, P. loosi, P. brachyurus* and *P. pratensis* (Blair and Stirling 2007, Araya et al. 2016). *Pratylenchus* species are recognized for their wide distribution in almost all environments as well as their wide host range. *P. penetrans,* for example, is known to parasitize more than 350 plant species (Duncan and Moens 2006, Castillo and Vovlas 2007). *Pratylenchus neglectus* is distributed in temperate and subtropical regions worldwide and has been reported in Canada, China, United States, Europe, Australia, Japan, South Africa and northwestern India (Potter and Townshend 1973, Olthof et al. 1982, Taylor et al. 2000, Mahran et al. 2010, Qiu et al. 2016).

Pratylenchus species target feeding to the root cortex of host plants and inflict damage through intracellular feeding on the root tissues (Castillo and Vovlas 2007). Hosts of *Pratylenchus* species include; cereals, canola (*Brassica napus* L.), potato (*Solanum tuberosum*), legumes, cotton (*Gossypium hirsutum* L.), coffee (*Coffea arabica* L.), sugarcane (*Saccharum officinarum* L.), sugar beet (*Beta vulgaris* L.), forage crops and fruit trees (Blair and Stirling 2007, Castillo and Vovlas 2007). Although some *Pratylenchus* species share the same hosts, for example, *P. neglectus* and *P. thornei,* they generally all have different host preferences (Taylor et al. 2000). *Pratylenchus neglectus* mainly parasitizes pasture legumes and grains (Cook and Yeates 1993). In Australia and the Pacific Northwest of the USA, *P. neglectus* severely parasitizes wheat (*Triticum aestivum* L.) and causes yield losses of up to 16-23% and 8-36%, respectively (Vanstone et al. 1998, Taylor et

al. 1999). In Canada, root-lesion nematodes are the most common plant-parasitic nematodes in Canadian agroecosystems (Bélair et al. 2018). The distribution and population development of *Pratylenchus* species in any region are governed by the availability of suitable hosts (Duncan and Moens 2006). The crops commonly grown in the prairies include; grains, pulses, oilseeds, and pasture crops (Bélair et al. 2018). There has been a shift in prairie crop production with wheat-fallow rotations being modified to exclude fallow and include crops such as pulses and oilseeds, all of which can host *P. neglectus* (Bélair et al. 2018).

Pratylenchus neglectus is present in all prairie provinces, including Alberta, British Columbia, Manitoba, and Ontario (Yu 2008, Bélair et al. 2018). The distribution of *P. neglectus* has been intensively studied in Ontario tobacco, cereal and forage growing areas. In Southern Ontario, *P. neglectus* was found to be the *Pratylenchus* species dominating the cereal and forage crops (Potter and Townshend 1973). In Saskatchewan, *P. neglectus* was found on strawberry (*Fragaria* x *ananassa* Duchesne). Mahran et al. (2010) studied the prevalence of *Pratylenchus* species in Manitoba and *P. neglectus* was found in 39% of the 31 potato fields sampled. In the same study, Mahran et al. (2010) investigated the suitability of the potato cultivar, Russet Burbank as a host for *P. neglectus* and the nematode did not reproduce on it. In Alberta, high populations of *P. neglectus* caused significant losses and increased the severity of potato early dying disease in a potato crop preceded by wheat (Forge et al. 2015). This study suggested that potato was a good host for *P. neglectus*. There is a possibility that the *Pratylenchus* spp. has physiological races or pathotypes, which may differ in host preference (Griffin 1991).

Pratylenchus neglectus was observed in 20 of 93 commercial pulse crop fields examined during a survey for plant-parasitic nematodes across the Prairie provinces of Canada between 2014 and 2016 (Gouvea-Pereira 2018). *Pratylenchus neglectus* was found in densities above 100 nematodes per 100 g of soil which is the economic threshold for this nematode in many crops (Fleming et al. 2016). *Pratylenchus neglectus*, being an emerging plant parasite in the Canadian Prairie Provinces, little is known about its host preferences among the crops being produced. Information about the host preference of the nematode is important for growers in these farming regions to know which crops to include in their cropping systems to prevent a build-up of damaging *Pratylenchus* populations. Additionally, such knowledge is useful for planning and implementing effective nematode management strategies. In Manitoba, potato is mostly rotated with wheat and

canola, both of which constitute the most extensive acreage in Western Canada. Wheat, canola, and pulses are reported as good hosts for *P. neglectus* (Taylor et al. 2000, Fatemy et al. 2006), but these crops have not been examined for their host abilities here in Canada. It has been postulated that *Pratylenchus* species in the Prairies are parasitizing pulse and non-pulse crops in rotation with potato and this needs to be determined.

The nematode population densities in the soil at the end of the season depends on the crop species grown. Plant-parasitic nematodes tend to multiply readily in the presence of a suitable host crop. Some crops promote nematode multiplication while others are suppressive, and this is summed up by three terms; susceptible, tolerant and resistant. A plant is said to be resistant when it can restrict nematode multiplication (Trudgill 1998). Resistant crops play a significant role in reducing nematode population densities in the soil and they can, therefore, be included in crop rotations to keep nematode numbers below damage thresholds (Taylor et al. 2000). In resistant non-hosts, the nematode can penetrate the plant, but no reproduction takes place. In a poor host, nematodes survive with minimum reproduction provided the crop is present. The magnitude of damage and yield loss that occur intolerant crops is directly related to the nematode population densities in the soil (Vanstone et al. 1998, Taylor et al. 1999). Yield loss can be minimized by employing strategies that reduce nematode populations.

Population densities of *P. neglectus* in the soil can be reduced by growing resistant crops or by ensuring that susceptible crops are not grown subsequently in short succession (Brown 1987, Vanstone et al. 1998). *Pratylenchus* species, including *P. neglectus* can be suppressed by crops such as safflower, field pea, flax, and triticale (Taylor et al. 2000, Smiley et al. 2005). Good hosts allow the nematode to multiply readily, causing a significant increase in nematode populations (MacGuidwin et al. 1992, Forge et al. 2012). Additionally, *Pratylenchus* population densities are subject to increase with monocropping of suitable host crops as well as when host species precede each other in a growing season. Apart from the host plant, the success of nematode reproduction depends on several other factors, including nematode population size and soil temperature, which in turn affect the length of the life cycle, which is slower in weak and resistant hosts than in good hosts (Trudgill 1995).

Host screening is important for the identification of resistant crops and cultivars for use in the effective management of plant-parasitic nematodes (Sogut et al. 2013). Host suitability of crops

to plant-parasitic nematodes can be determined using different procedures that encompass the population densities of the nematode. The reproductive factor (R_f) also known as the multiplication rate (MR), a ratio of final nematode densities to initial densities (Pf/ Pi) is the most common method used to determine nematode reproduction capacity and to assess the host suitability of a crop (Oostenbrink 1966). The R_f is simply an indicator that reveals whether a crop is resistant or susceptible to a nematode species. The R_f has been used in several studies to assess host suitability of crops or cultivars (Griffin 1991, Fernandez et al. 1994, Taylor et al. 2000, Hajihassani et al. 2016). A crop with $R_f > 1$ is considered a suitable host (Taylor et al. 2000). There are many variations in the use of the R_f among studies, and it seems that most researchers develop their own categories for host status (Canto-Saenz 1983, Zhang and Schmitt 1994, Forge et al. 2012). Another approach to assess host status is to compare the final nematode densities of the test crop with that of a reference host (standard) (Vanstone and Russ 2001, Inomoto and Asmus 2010).

Pratylenchus species are difficult to control using crop rotation because these nematodes have a very wide host range (Williams et al. 2002). If farmers in the Canadian Prairies are to prevent a build-up of *Pratylenchus* species in the soil, they need to know the effect of different field crops being grown on nematode population densities. Management tactics which farmers in the Prairies can employ include; use of cover crops (e.g., Marigold), application of organic amendments (e.g., liquid hog manure) and they can ensure crops have adequate nutrition to enhance tolerance. Nematicides can be very useful but they are costly, and their use is now being restricted because of health and environmental concerns. Moreover, for many crops, there is no chemical control available (Smiley 2005). Population levels of *Pratylenchus* species in the soil should be assessed as this will provide information useful for predicting potential damage to crops as well as for the development of control measures (Mahran et al. 2010).

To minimize crop damage levels and yield losses, it is important to accurately identify the nematode species prevalent in a field or region and the plant hosts associated with these nematodes. Identification of nematode species present in a field provides useful information for the development of effective management practices. The traditional method of identifying *Pratylenchus* species is based on examining the morphology of adult females. Generally, females in this genus possess more diagnostic features than males (Loof 1991, Chen et al. 2004). Additionally, males are rare in some species, such as *P. neglectus* which reproduce

parthenogenetically. Morphological identification of *Pratylenchus* species is difficult and requires training, nematology expertise, and use of advanced microscopes (Fortuner 1989, Oliveira et al. 2011). However, molecular tools such as polymerase chain reaction (PCR) and DNA sequencing have been developed for nematode identification and these are now being used to supplement the traditional methods.

Currently, there is a need to determine the capability of *Pratylenchus* spp. to parasitize and reproduce on some of the major pulse and non-pulse crops grown in Canadian Prairies. Therefore, the objectives of this study were to (i) to identify the *Pratylenchus* spp. present in the test soil used in the studies here, (ii) screen pulse and non-pulse crops commonly grown in the Prairies for host suitability to *Pratylenchus* spp. under growth chamber conditions in the test soil, and (iii) to determine the effect of selected crops to ramp up densities of the *Pratylenchus* spp. when grown in subsequent cycles in the test soil.

2.3. Materials and methods

2.3.1. Soil Collection

Soil naturally infested with *Pratylenchus* spp. was collected from some fields near the town of Brooks, Alberta, to determine the population densities of *Pratylenchus* spp. and to identify the field with the highest densities of *Pratylenchus.* In a survey for plant-parasitic nematodes conducted by Gouvea-Pereira and Tenuta in 2016, the density of *Pratylenchus* spp. was highest in two commercial fields in cereal-pulse-oilseed rotation in south-central Alberta near the town of Brooks. Soils were again collected in the fall of 2017 from these fields in collaboration with Dr. Michael Harding (Government of Alberta, Agriculture and Forestry, Brooks, AB). Twenty soil samples, ten from each field, were collected randomly using a core from a depth of 0-30 cm. The soils collected from the two fields were put in labelled plastic bags and were brought to the laboratory of Dr. Michael Harding for analyses. The nematode population densities in those fields were examined so that the one with the highest could be used in the growth chamber studies here. The field that had the mean highest nematode numbers between the two fields was determined and selected for collection to be brought back to the Soil Ecology Laboratory at the University of Manitoba.

The soil was collected at two different depths, 0-15 and 16-30 cm at randomly selected spots using a shovel. *Pratylenchus* spp. mostly occur within a depth of 0-30 cm. The soil collected was put in 20 labelled 25L plastic pails. The pails of soil were driven to the University of Manitoba and stored in the walk-in cooler at 4^{o}C until use. The dominant soil series for the samples collected was a dark brown Chernozemic characterized by a dark-brown to black A horizon. The pH range was around 6-7.8 and SOM was 3-5% (Alberta Soil Information Centre 2016). The soils were silty clay loams. The gravimetric moisture content (GMC) of the soils was measured by oven drying the soil samples at 105^{o}C for 48 hr.

Figure 2.1 Collection of naturally infested soils from a field near the town of Brooks, Alberta: Lanny Gardiner (left) and Priscillar Wenyika (right).

2.3.2. Nematode extraction from the soil

To determine the nematode densities, nematodes were extracted from the soil using modified Cobb's sieving and decanting, followed by the sugar flotation method from 100 g samples (Ingham 1994). Soil samples collected from each field were examined individually. The soils (100 g) were put in 500 ml plastic beakers and the beakers were filled with water. Using another plastic beaker, the soil solutions were mixed back-and-forth for about 10 times. The soil

solutions were passed over the U.S.A. Standard Test 45 and 400 mesh sieves followed by a centrifugation step at 1.9 x 1,000 r.p.m for 5 min. The supernatant was discarded and replaced with a sucrose solution. The soils were mixed with the sucrose solution using a metal spatula. The soil-sucrose solutions were centrifuged at 1.1 x 1,000 r.p.m for 1 min and 15 sec. In the final step, nematodes suspended in the sucrose solution were collected in a U.S.A Standard Test 500 mesh sieve. The nematodes collected were examined and counted under a dissecting microscope in gridded Petri dishes. Due to difficulty in counting nematodes at the edges of the Petri dishes, I counted nematodes within a square marked in the middle of the dish. The square was demarcated in such a way that it represented exactly half the area of the dish and this was done by counting the number of grid squares. The number obtained after counting nematodes within the square was multiplied by two to obtain the total dish count. The presence and abundance of *Pratylenchus* spp. were determined by identifying the nematodes morphologically during counts.

2.3.3. Morphological identification of *Pratylenchus* spp. in the soil

Pratylenchus spp. present in soils collected from the field selected to be used in the host screening study was extracted and used for identification. Recovered nematodes were collected in 15 ml conical vials, which were then centrifuged at 2,170 r.p.m for 5 min to ensure all the nematodes collected at the bottom. After centrifuging, the supernatant was removed by pipetting, leaving the nematodes in 0.5 ml water in the vial. Drops of a nematode suspension from each vial were placed onto a microscope slide, covered with a coverslip and sealed with nail polish. Nematodes were heat-killed so digital images could be better taken (Golden 1990). Nematode identification was made using an inverted microscope (Motic AE21, Microscope World, Carlsbad, CA). *Pratylenchus* species were identified to genus by examining morphological characters on adult females using an identification key in Mai et al. (1996). The characters used in identification included; the head shape, short stylet with basal knobs, esophagus overlapping the intestine ventrally, vulva position to total body length, and bluntly conoid tail. Images of *Pratylenchus* individuals were obtained using a compound microscope (Olympus BX51, Olympus Canada, Inc., Richmond Hill, ON) equipped with a digital imaging camera (QColor3, Olympus, Tokyo, Japan) and an Image-Pro Plus 6.2 software (Media Cybernetics, USA). There were other PPNs from the genera of *Helicotylenchus*, *Paratylenchus*, and *Tylenchorhynchus* that were observed during the

identification of *Pratylenchus* spp. under the microscope, but their numbers were not counted. Most of the nematodes observed were non-plant parasitic and none of these nematodes were identified to determine the species.

2.3.4. Molecular DNA identification of *Pratylenchus* spp. in the test soil

Nematode DNA was extracted from single individuals of *Pratylenchus* recovered from the test soil. The nematodes were hand-picked using a thin, plastic brush strand, washed three times in autoclaved, double-distilled water (ddH_2O), and each placed into a 0.2 ml PCR reaction tube containing 12 µl of autoclaved, double-distilled water (ddH_2O), 2 µl of 100 mg Proteinase K (Roche, UK), and 12 µl of Direct PCR Lysis Reagent (tail buffer) (Viagen Biotech, Los Angeles, CA, USA). The tubes were lightly vortexed and stored overnight in a freezer at -80ºC. The next day the tubes were incubated at 65ºC for 90 min, 95ºC for 10 min and an infinite hold of 20ºC in a thermocycler (Eppendorf CF2060, Master-cycler Pro-S, Hamburg, Germany). Each reaction tube was then stored at -20ºC before amplification by conventional PCR.

Conventional PCR with universal and species-specific primer sets was used to identify the nematode species. PCR was performed on DNA of 30 individuals of *Pratylenchus* spp. The primer sets and the amplification conditions used in the PCR assays are listed in Table 2.1. The AB28-TW81 primer set targeted the internal transcribed spacer (ITS) region of the ribosomal RNA while the D2A-D3B targeted the D2 and D3 expansion regions of the 28S rDNA (Fanning et al. 1995, De Ley et al 1999). The species-specific primer sets PNEG-D3B, PPEN-D3B and PTHO-D3B to identify *P. neglectus*, *P. penetrans* and *P. thornei*, respectively (Al-Banna et al. 2004). The PCR reaction mixture was made up of 19.05 µl sterile water, 2.5 µl buffer, 1.0 µl dNTPs, 0.25 µl Dream Taq polymerase enzyme and 0.6 µl for each of the two primers. The thawed nematode DNA (1 µl) was then added to the PCR reaction tubes and cycled using an Eppendorf CF2060 thermocycler. The PCR amplification conditions are provided in Table 2.1. A 1.7% agarose gel (Invitrogen, Carlsbad, CA, USA) stained with 1 µl of 10,000X concentrated GelRed fluorescent nucleic acid gel stain (Biotium, Fremont, CA, USA) was used to separate and visualize the amplified products. The gel was run at 95 V for 90 min in a gel box (Fisher Biotech Electrophoresis FB-SB-710). Amplified bands were compared to a 100 bp DNA ladder (ProMega) to obtain fragment sizes. The amplified products were observed under UV light using a Syngene G-BOX:

F3 (Frederick, Maryland, USA) imager with the GeneSys software (v.1.3.1.0). Images of gels were generated using a Synoptics 3.8MP camera, set at an exposure of 360 ms, light setting at TLUM (mid-wave) and filter setting at UV032.

DNA sequencing of the D3 region of *Pratylenchus* spp. individuals from the test soil was also performed to identify the species. PCR products from reactions that produced single bands (positive) when amplified with universal primers for *Pratylenchus* spp. and species-specific primers for *P. neglectus* were purified using either a QIAquick PCR Purification Kit (250) (QIAGEN, Hilden, Germany) or a QIAquick Gel Extraction. DNA samples of *Pratylenchus* that were negative for *P. neglectus* and species-specific primers for *P. penetrans* and *P. thornei* were also purified and sequenced to determine the species identity. A Nanodrop 2000 spectrophotometer v.1.0 (Thermo Fisher Scientific, Wilmington, DE, USA) was used to quantify DNA (ng μl^{-1}) and to determine purity (A260/280 ratio) in the purified PCR products. Macrogen Inc. USA did direct sequencing of the D3B expansion region of 28S rDNA (in both directions). The primers used for sequencing were the same ones used for PCR assays. Nematode sequences were compared to the GenBank database using the basic local alignment search tool (BLAST) provided by the National Centre for Biotechnology Information (NCBI) to find the best match to *Pratylenchus* species. The expected value (E-value), and percent identity match were used to determine how significant the species identification matches were.

Table 2.1 Universal and species-specific primer sets, and amplification conditions used in polymerase chain reaction (PCR) assays

Primer set	Region/ Species	Primer Sequence	Amplification conditions	PCR product size (bp)	Reference
AB28_TW81	ITS region (universal)	ATATGCTTAAGTTCAGCGGGT GTTTCCGTAGGTGAACCTGC	94ºC 4 min; 35 cycles at 94ºC 1 min, 55ºC 1.5 min, 72ºC 2 min, and a final extension 72ºC 10 min	900	Fanning et al. 1995
D2A_D3B	D2/ D3 region (universal)	ACAAGTACCGTGAGGGAAAGTTG TCGGAAGGAACCAGCTACTA	94ºC 2 min; 35 cycles at 94ºC 1 min, 53ºC 30 sec min, 72ºC 1 min, and a final extension 72ºC 4 min	800	De Ley et al. 1999
PNEG_D3B	*P. neglectus*	ATGAAAGTGAACATGTCCTC TCGGAAGGAACCAGCTACTA	95ºC 3 min; 35 cycles at 95ºC 1 min, 63ºC 1 min, 72ºC 1 min, and a final extension 72 ºC 7 min	290	Al Banna et al. 2004
PPEN_D3B	*P. penetrans*	TAAAGAATCCGCAAGGATAC TCGGAAGGAACCAGCTACTA	95ºC 3 min; 35 cycles at 95ºC 1 min, 62ºC 1 min, 72ºC 1 min, and a final extension 72ºC 10 min	278	Al Banna et al. 2004
PTHO_D3B	*P. thornei*	GAAAGTGAAGGTATCCCTCG TCGGAAGGAACCAGCTACTA	95ºC 3 min; 35 cycles at 94ºC 1 min, 68ºC 1 min, 72ºC 1 min, and a final extension 72ºC 7 min	288	Al Banna et al. 2004

2.3.5. *Pratylenchus* spp. detection in soils for the Host Screening study

The collections of the test soil were thoroughly mixed by repeatedly turning over the soil using a shovel. Sixteen subsamples of approximately 100 g were obtained and used to determine the starting densities of *Pratylenchus* spp. in the test soil. Nematodes were extracted from these subsamples again using the modified Cobb's sieving and decanting, followed by the sugar flotation method. Nematodes recovered from the soil were counted under the microscope (Olympus SZ61, Tokyo, Japan), as previously described. I counted the total nematodes in a sample and then the number of *Pratylenchus* in that sample.

2.3.6. Growth Chamber Studies

An experiment with three replicated trials and one 8-week growth cycle of the crops, referred here as the Host Screening Study, was conducted under growth chamber conditions (Bio-Chambers Inc. TPRB-149) to determine the capability of *Pratylenchus* spp. to infect and reproduce on the major field crops grown in the Canadian Prairies. Each trial had seven pulse and non-pulse crops and control pots (pots without crops), all of which had eight replicates. The growth pots of the first trial were retained for two repeated growth cycles of each crop, referred here as the Population Increase Study, to determine how quickly populations of *Pratylenchus* increase with repeated growth cycles of the crops. The crops screened were; canola (*Brassica napus* L.) var. L252, chickpea (*Cicer arietinum* L.) var. CDC Leader, lentil (*Lens culinaris* Medik) var. CDC Maxim, pinto bean (*Phaseolus vulgaris* L.) var. Windbreaker, soybean (*Glycine max* L.) var. Y4, yellow pea (*Pisum sativum* L.) var. CDC Meadow, and Canada Western Red Spring Wheat (*Triticum aestivum* L.) var. AAC Brandon. The varieties used were the most popular for each of the crops grown on the Canadian Prairies. A control treatment consisting of pots without crops was also included in all trials and growth cycles. The experiment was laid in a completely randomized design (CRD) fashion in the growth chamber. Each pot was considered an experimental unit. Crops were raised under growth chamber conditions at 23± 2^{o}C and humidity 65% for 8 weeks from emergence. 16-cm diameter polyethylene plastic pots were filled with 2 kg of the test soil. Seeds of chickpea, lentil, soybean, yellow pea, and pinto bean were germinated on paper towels and then three sprouted seeds of each were transplanted into pots. Three canola and spring wheat seeds were planted directly into pots. After emergence, seedlings were thinned to one

plant per pot. The pots were watered every other day. Weeds that emerged were eliminated by hand.

2.3.7. Nematode extraction from roots and soils

The density of *Pratylenchus* spp. in roots and soil was determined for each pot. A period of two weeks was used to allow the death of roots and migration of some of the *Pratylenchus* to the soil. Roots were recovered from each pot by hand, separating them from the soil. The soil from the pot was then placed into a polyethylene bag and mixed. Plant roots collected from each pot were washed in tap water, weighed and cut into small segments of between 1-2 cm. Nematodes in roots were extracted using the Whitehead-tray method (Whitehead and Hemming 1965) in which 15 g roots were incubated in trays for at least three days. Any crop that had a total root weight of more than 15 g, the roots were split, and the extra root material was extracted in another tray for effective nematode extraction. The extraction trays were plastic 18-cm diameter pot saucers, with a sheet of laboratory tissue (Kimtech wipes) spread on a nylon mesh screen (700 µm screen size) supported by 3 mm-thick plastic rings. Roots were placed on the laboratory tissue and tap water added to the tray until they were all submerged. Each day the water was checked to ensure roots remain submerged. Roots were repeatedly incubated for three days until there were no *Pratylenchus* species observed in the root samples extracted. Cobb's sieving and decanting method followed by sugar floatation was used for soil extractions. Recovered nematodes were counted as described previously and densities expressed as total *Pratylenchus* recovered from roots of each pot. The density of *Pratylenchus* spp. in soil was determined as previously described and expressed as the number of nematodes per kg of soil.

The nematodes collected from both roots and soil were put in glass vials, which were centrifuged at 2,170 rpm for 5 min (International Equipment Co, USA) prior to counting. The supernatant was pipetted leaving out about 4 ml of the nematode solution. All nematodes extracted from roots were counted in gridded Petri dishes under a dissecting microscope. The total number of nematodes in a sample, and the total number of *Pratylenchus* species in the same sample were counted. Nematodes observed in half of the dish were counted and multiplied by two to get the total dish count.

2.3.8. Reproductive potential for *Pratylenchus* spp.

The reproductive factor (R_f) of *Pratylenchus* spp. was determined by dividing the final recovered densities (soil + roots) by the initial nematode densities in the soil. The R_f values were categorized as follows; R_f greater than 1 ($R_f > 1$) = host, R_f less than 1 ($R_f < 1$) =poor host and R_f less than 0.1 ($R_f < 0.1$) = non-host. (Taylor et al. 2000). The R_f was calculated for all the seven crops as well as for the control for the three replicate trials.

2.3.9. Statistical analysis

A mixed-model analysis of variance (ANOVA) was used to analyse the data generated from the host screening study. Assumptions of normality were tested before any analysis could be conducted. The $\log_{10}$ transformation method was used to normalize data and missing values were corrected for. Missing values were from a few pots from one of the trials that did not emerge. In this study, each crop was considered a treatment and each pot was a replicate. The control was not included in statistical analysis, but it was included in graphical comparisons of nematode densities. All the tests were conducted at $\alpha = 0.05$. Mean comparisons for R_f values and all measure butments of growth parameters generated from the host screening study were conducted using the Tukey-Kramer test and the pdmix 800 macro (Saxton 1998). The least-squares means (LSmeans) *t*-tests were used to test if R_f values were significantly different from 1 where necessary. A simple linear regression (SLR) model used to determine the relationship between *Pratylenchus* abundance and different crop species grown in repeated cycles. Statistical analyses conducted in this study were all done using SAS (v9.4; SAS Institute, Cary, NC).

2.4. Results

2.4.1. *Pratylenchus* spp. of the test soil

The density of *Pratylenchus* in the soils ranged from 0 to 260 nematodes per kg^{-1} of soil and 0-180 nematodes per kg^{-1} of soil in the first and second fields, respectively. Based on these population densities, Field 1 was selected to be used as the test soil and collected to bring back to our laboratory for use in the Host Screening Study. The population density of the test soil brought to our laboratory ranged from 40 to 220 nematodes per kg^{-1} of soil. The mean population density of the test soil was 120 nematodes per kg^{-1} soil.

2.4.1.1. Morphology of *Pratylenchus* spp.

The morphological characters of *Pratylenchus* spp. observed under the light microscope included flat lips, robust stylet with well-developed knobs, and an esophagus overlapping the intestines ventrally. The nematodes observed had a round, prominent median bulb, a skinny, bluntly rounded to conoid tail, and a vulva positioned at the posterior end. When resting, the nematodes laid straight or ventrally curved. No males were found among the nematodes examined in this study. Images were taken of specimens of *Pratylenchus* spp. mounted on slides using a compound microscope are shown in Fig 2.2.

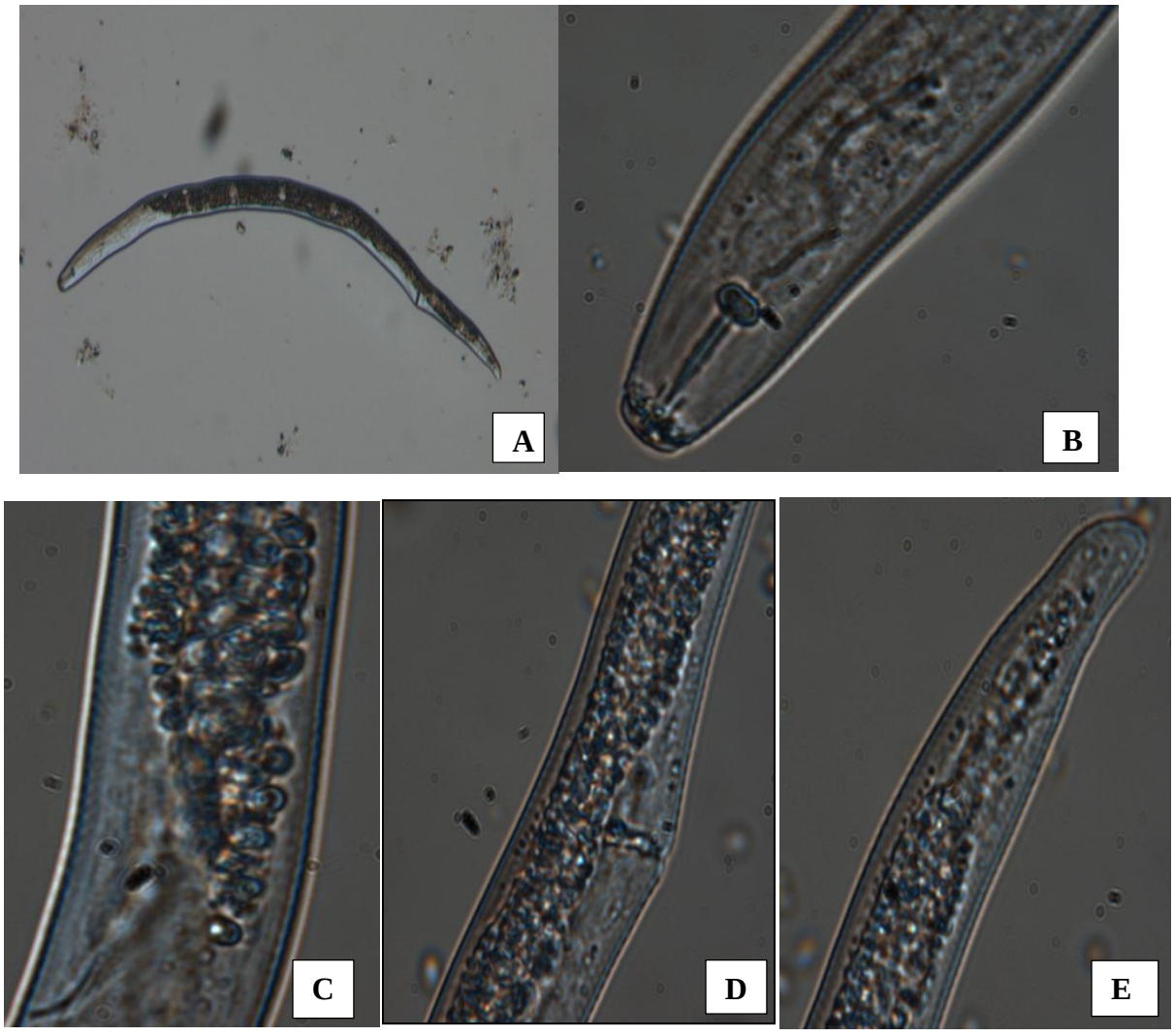

Figure 2.2 Light microscope images of *Pratylenchus* spp. specimens extracted from soils, A) image of whole nematode, B) head of *Pratylenchus*, C) esophagus overlapping intestines, D) vulva at the posterior end of the nematode, and E) tail (rounded).

2.4.1.2 Molecular identification

Polymerase chain reaction (PCR) with universal and species-specific primers was performed on 30 individual *Pratylenchus* species recovered from the test soil. The species-specific primer set (PNEG-D3B) was used to amplify the D3 expansion region of the large subunit of the 28S rDNA of the *Pratylenchus* species. Deoxyribonucleic acid (DNA) samples amplified with universal primers D2A-D3B and TW81-AB28 produced single bands of 800 and 900 bp, respectively (Table 2.2). The expected band size for *Pratylenchus* spp. positive for *P. neglectus* was 290 bp. Twenty individual *Pratylenchus* nematodes were identified as *P. neglectus* using PCR with species-specific primers (PNEG-D3B) (Table 2.2) and the other 10 were also identified as *P. neglectus* using DNA sequencing (Table 2.2).

Table 2.2 Molecular characterization of *Pratylenchus* species recovered from the test soil prior to the start of the host screening study using PCR with universal and species-specific primer sets

Nematode individual	Universal primer sets	Species-specific primer sets			
	D2A-D3B (800 bp)	**TW81-AB28 (900 bp)**	**PNEG-D3B (290 bp)**	**PPEN-D3B (278 bp)**	**PTHO-D3B (288 bp)**
20P	/	+	+	/	/
P1	/	+	+	-	-
P3	/	+	+	-	-
P7	/	+	+	-	-
P8	/	+	+	-	-
P11	/	+	+	-	-
P19	/	+	-	/	/
P23	/	+	-	-	-
P344-2	/	+	+	-	-
P344-3	/	+	+	-	-
P2	+	/	-	/	/
P5	+	/	-	/	/
P330	+	/	-	/	/
PN-02	+	/	+	/	/
PN-03	+	/	+	/	/
PN-04	+	/	+	/	/
PN-06	+	/	+	/	/
PN-07	+	/	+	/	/
PN-09	+	/	-	/	/
PN-13	+	/	-	/	/
PR-1	+	/	-	/	/
PR-2	+	/	+	/	/
PR-6	+	/	+	/	/
PR-01	+	/	+	/	/
PR-02	+	/	-	/	/
PR-03	+	/	+	/	/
PR-04	+	/	-	/	/
PR-06	+	/	+	/	/
PR-07	+	/	+	/	/
PR-08	+	/	+	/	/

+: Nematode species positive for the primer set, -: negative for the primer set, /: not checked

Single bands of 290 bp only specific for *P. neglectus* were observed on the amplified product using gel electrophoresis (Fig 2.3). The other species-primer sets tested were for *P. penetrans* and *P. thornei* and none of these were amplified positively on the DNA samples. A few samples that produced bands with universal primers but negative for all species-specific primers for *P. neglectus*, *P. penetrans* and *P. thornei* were sequenced to find best matching *Pratylenchus* spp. and the results are presented in Table 2.3.

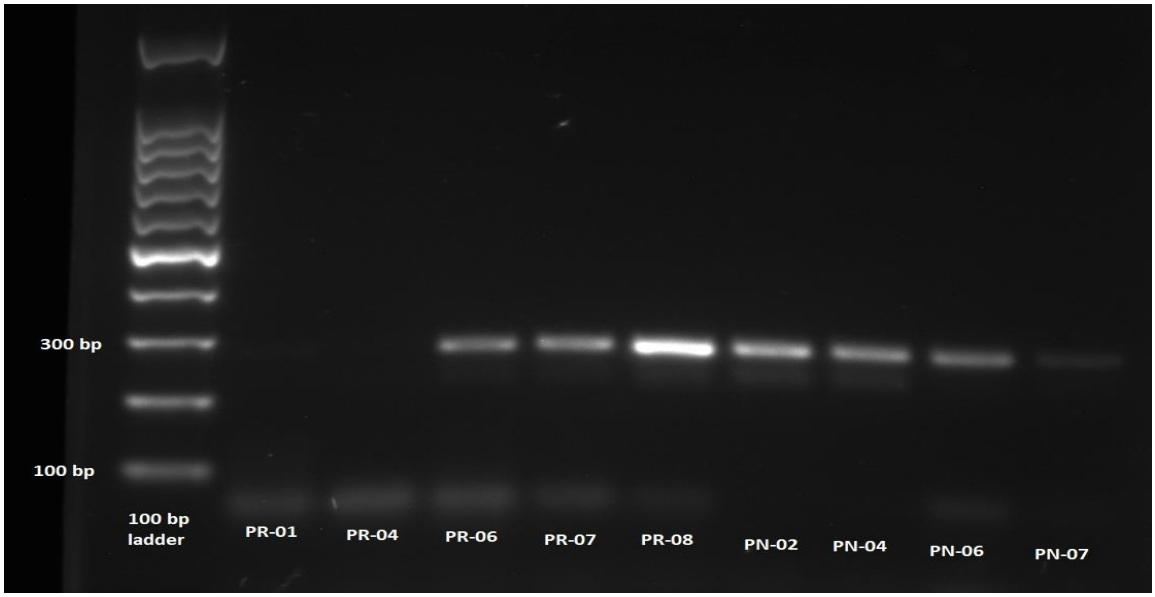

Figure 2.3 Agarose gel electrophoresis pattern of PCR products of genomic DNA of *Pratylenchus* individuals amplified with species-specific primers (PNEG-D3B) (Al-Banna et al. 2004). A product of 290 bp indicates an individual positive for being *P. neglectus*. Lanes: 100 bp DNA ladder, PR-01, PR-04, PR-06, PR-07, PR-08, PN-02, PN-04, PN-06, and PN-07 are *Pratylenchus* individuals recovered from the test soil prior to conducting the host screening study.

A total of 10 *Pratylenchus* individuals including the samples that tested both positive and negative for *P. neglectus* were sequenced. The DNA sequences obtained from the nematode samples analysed were compared in the GenBank using BLAST to check the species identity match. The sequences provided the best match to *P. neglectus* with identity matches of 98-100% and E-values of between $1e^{-94}$ to $4e^{-108}$. The E-value describes how many times one would expect to find a match of the sequence size by chance in the database. (Table 2.3). Four DNA samples (P5, P23, PN-13, and PR-02) that did not produce bands with *P. neglectus* primers as well as with primers for *P. penetrans* and *P. thornei* when sequenced, they had best identity matches with *P. neglectus* (Table 2.3). These DNA samples had identity matches of 98-100%. The PCR and the sequencing results indicated the absence of other *Pratylenchus* spp. apart from *P. neglectus* in DNA sample collections analysed in this experiment. The sequencing results agreed with those obtained from and PCR assays with species-specific primers.

Table 2.3 Molecular identification of *Pratylenchus* species recovered from the soil prior to the host screening study using DNA sequencing of the D3 region based on identity match to the NCBI Blast database.

Nematode individual	Match	Accession	Identity (%)	E-value	No. of accessions for *P. neglectus* (98-100%)
P1	*P. neglectus*	JX261951	100	$4e^{-108}$	21 (100) 5 (99) 1 (98)
P3	*P. neglectus*	EU130854	99	$2e^{-95}$	24 (99) 1 (98)
P5	*P. neglectus*	JX261951	100	$3e^{-94}$	34 (100) 4 (99) 1 (98)
P11	*P. neglectus*	JX261951	100	$1e^{-102}$	23 (100) 3 (99) 2 (98)
P23	*P. neglectus*	EU130854	100	$3e^{-95}$	23 (100) 2 (99) 2 (98)
PN-02	*P. neglectus*	JX261946	98	$1e^{-101}$	22 (98)
PN-07	*P. neglectus*	JX261947	99	$3e^{-87}$	36 (99) 14 (98)
PN-13	*P. neglectus*	EU130854	99	$1e^{-94}$	24 (99) 2 (98)
PR-01	*P. neglectus*	JQ303333	100	$3e^{-95}$	40 (100) 10 (99) 6 (98)
PR-02	*P. neglectus*	KU198962	99	$2e^{-101}$	44 (99) 4 (98)

Values in parenthesis are the identity percentages that correspond to the number of accessions with best matches for *P. neglectus.*

2.4.2. Host screening study for *Pratylenchus* spp. under growth chamber conditions

Seven pulse and non-pulse crops, including canola, chickpea, lentil, pinto bean, soybean, spring wheat, and yellow pea were successfully grown and screened as hosts for *Pratylenchus* spp. under growth chamber conditions. A single variety for each of the selected crops was evaluated.

Figure 2.4 Images of crop plants in the host screening study near the end of growth cycle 2. A: pots of all crops on a growth-chamber bench, B: soybean (left row) and chickpea (right row), C: pinto bean, D: canola, E: wheat, F: yellow pea (left) and lentil (right), G: chickpea, H: soybean

2.4.2.1.1. Crop host screening of the *Pratylenchus* spp.

The analysis of variance (ANOVA) for the reproductive factor (R_f) of *Pratylenchus* spp. for three replicated trials are presented in Table 2.4. There was a significant crop x trial interaction for the reproductive factor of *Pratylenchus* spp. (R_f) (Table 2.4). The significant interaction between crop and trial arose because nematode multiplication occurred, resulting in an increase in populations in some of the crops (e.g., chickpea and soybean) while populations declined in other crops (e.g., lentil, yellow pea). There was a significant effect of the crop on the reproduction of *Pratylenchus* spp. on the various crops examined in the Host Screening study. Similarly, a significant effect of the growth cycle was observed. The results showed that the multiplication of *Pratylenchus* spp. varied with the crop grown.

Table 2.4 Analysis of variance for the reproductive factor (R_f) of *Pratylenchus* spp. across three replicate trials of selected pulse and non-pulse crops in the host screening study grown in pots.

Source of variation	**df**	**MS**	***F***	***P***
Crop	6	10.03	23.67	< .0001
Trial	2	4.67	11.02	< .0001
Crop*Trial	12	3.04	7.16	< .0001

The reproductive factor values of *Pratylenchus* spp. on the selected crops grown in three replicate trials are presented in Fig 2.5. The results across the three trials indicated that the nematode reproduced ($R_f > 1.00$, $P < 0.05$) on soybean (SE = 0.09), canola (SE = 0.143) and chickpea (SE = 0.12). The mean R_f value for pinto bean across the trials ($R_f = 0.98$) was not statistically different from 1 based on *t*-tests and was, therefore, considered a host for the nematode. The R_f values of *Pratylenchus* for lentil (SE = 0.09), spring wheat (SE = 0.103), yellow pea (SE = 0.09) and the control (SE = 0.06) were less than 1 (Fig 2.5). The results indicated that lentil and yellow pea had poor hosting abilities for the *Pratylenchus* spp. in the control, the results showed that the nematode failed to reproduce in the absence of host plants.

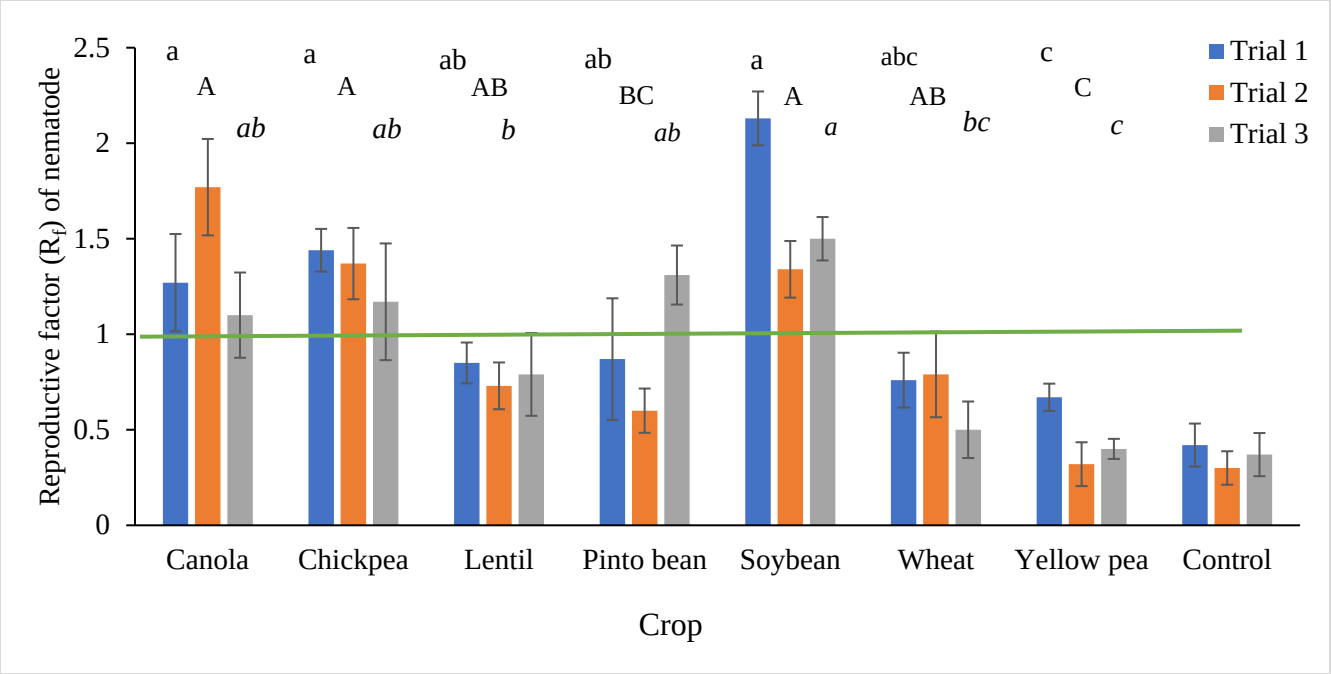

Figure 2.5 Mean reproductive factor (R_f) values of *Pratylenchus* on selected pulse and non-pulse crops across three replicate trials of selected crops in the host screening study grown in pots. Mean R_f values for the control (pots without crops) are also provided. Means comparison for R_f of the trials are denoted by letters in rows. For a trial, bars in a row with the same letters or numbers are not significantly different from each other based on Tukey's honest significance difference test ($P < 0.05$).

2.4.2.2. Crop host effect on the build-up of the population density of the *Pratylenchus* over several crop growth cycles

The analysis of variance (ANOVA) results for *Pratylenchus* spp. abundance recovered at the end of the three crop growth cycles is presented in Table 2.4. The ANOVA showed a significant interaction of the crop and growth cycle in relation to *Pratylenchus* abundance following the three growth cycles. The interaction resulted because the nematode abundance was greater in subsequent cycles than in the first cycle for crops such as soybean and chickpea while there was a decline in abundance on crops such as lentil and yellow pea. The crop effect on the abundance of *Pratylenchus* spp. was significant. The cycle also had a significant effect on the final nematode abundance of *Pratylenchus* spp. in the soil. The results indicated that both the crop type and the number of cycles in which the crop was grown significantly influenced the build-up rate of *Pratylenchus* spp.

Table 2.5 Analysis of variance for nematode total (soil plus root) abundance of *Pratylenchus* recovered at the end of each of the three growth cycles of selected pulse and non-pulse crops.

Source of variation	**df**	**MS**	***F***	***P***
Crop	6	50.7	51.14	< .0001
Cycle	2	87.2	6.32	0.0027
Crop* Cycle	12	63.5	7.13	< .0001

The relative effect of each crop on the abundance of *Pratylenchus* spp. across three cycles is shown in Figures 2.6 to 2.8. Nematode abundance was significantly influenced by crop and cycle. In the first cycle, nematode abundance varied with the type of crop grown as shown by the mean populations per kg of soil; soybean (μ = 198), pinto bean (μ = 152), canola (μ = 150), chickpea (μ = 120), lentil (μ = 93), spring wheat (μ = 90) and yellow pea (μ = 80). However, in Cycle 1, nematode abundance among all crops was not statistically different. The abundance of nematodes recovered at the end of the second and third cycles varied among the selected pulse and non-pulse crops. Nematode populations increased in canola, chickpea, pinto bean, soybean, and spring wheat from one cycle to the next. The results indicated that the total abundance of *Pratylenchus* spp. recovered from roots and soil increased with subsequent cycles of suitable hosts. For this group of crops, nematode abundance was lower in the first cycle compared to the second and third cycles, especially for soybean. Soybean had the highest final nematode abundance across all three cycles. *Pratylenchus* densities decreased in pots with lentil, yellow pea and the control (pots without crops). Lentil, yellow pea and control had the lowest nematode populations across all three cycles. The results showed that lentil and yellow did not allow *Pratylenchus* spp. to survive and reproduce.

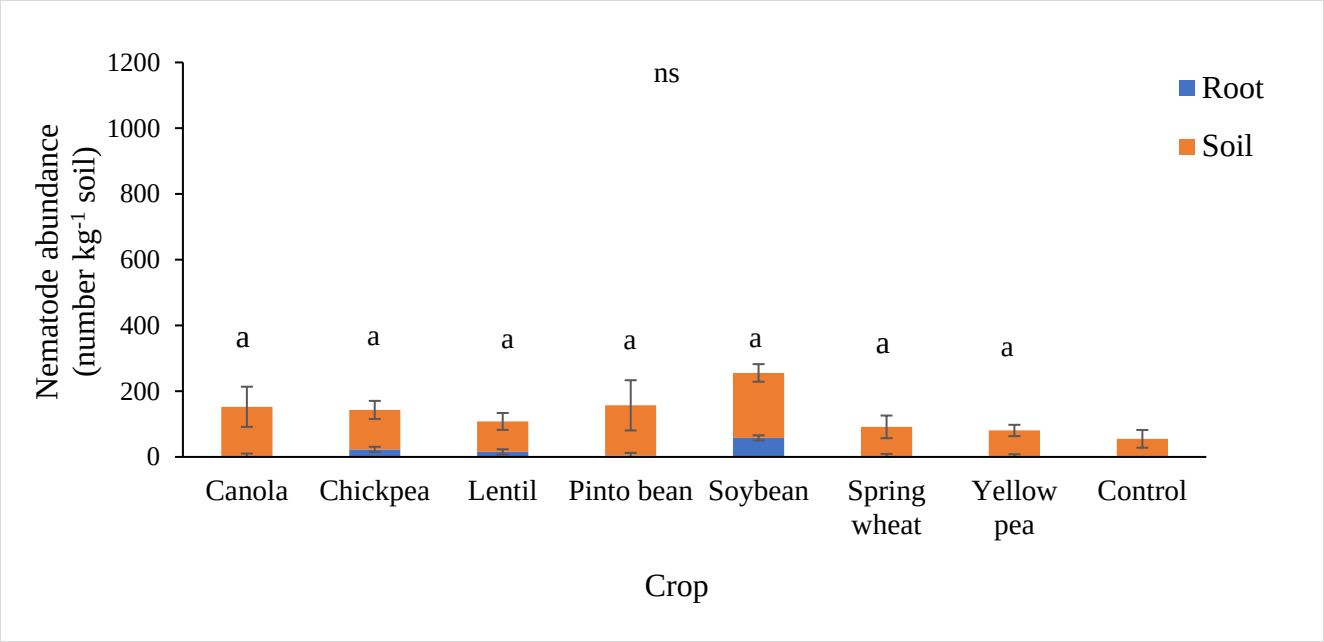

Figure 2.6 Mean abundance of *Pratylenchus* spp. in root and soil following the completion of the first growth cycle of selected pulse and non-pulse crops in pots. Mean nematode abundance in the soil for the control (pots without crops) is also provided. Bars for total abundance (root plus soil) with the same letters are not different from each other, according to Tukey's test ($P < 0.05$). ns= not significant

The changes in nematode abundance following the second growth cycles of selected crops are presented in Fig. 2.7. The densities of *Pratylenchus* spp. increased on chickpea, pinto bean, soybean, and spring wheat in Cycle 2. Soybean had the highest densities of *Pratylenchus* spp. at the end of the second growth cycle. More nematodes were recovered in canola soil in the first cycle than in the second growth cycle. However, more nematodes were recovered from canola, chickpea, soybean, pinto bean and spring wheat in the third growth cycle. Densities of *Pratylenchus* spp. recovered from lentil, yellow pea and the control were lower in the second growth cycle than in the first cycle.

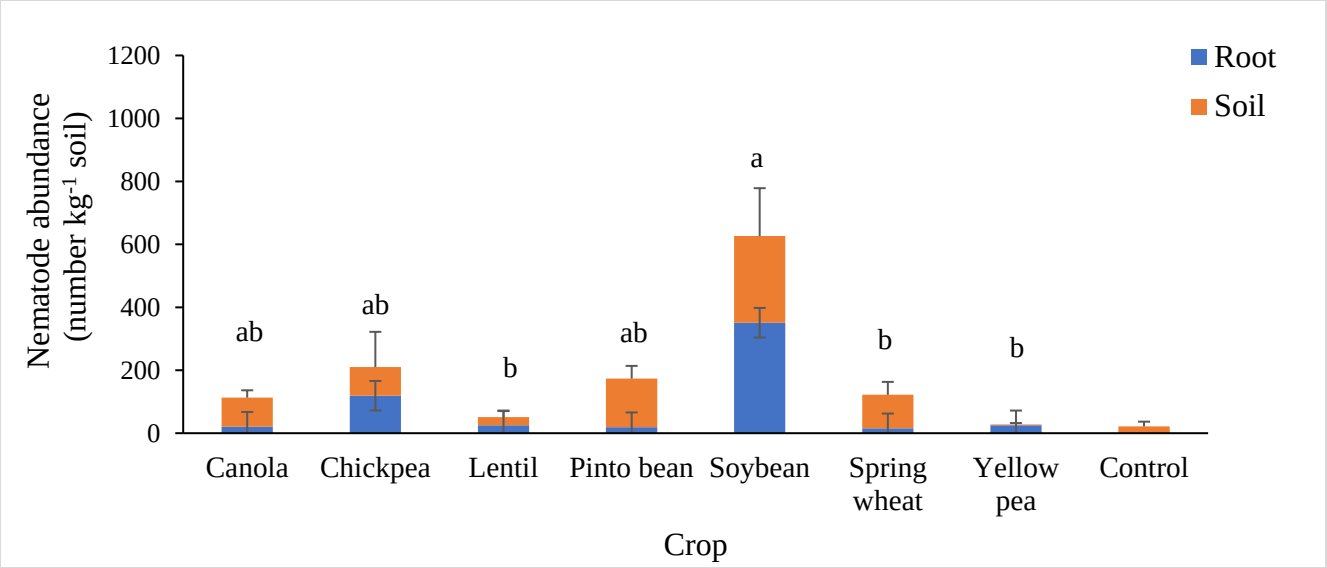

Figure 2.7 Mean abundance of *Pratylenchus* spp. in root and soil following the completion of the second growth cycle of selected pulse and non-pulse crops in pots. Mean nematode abundance in the soil for the control (pots without crops) is also provided. Bars for total abundance (root plus soil) with the same letters are not significantly different according to Tukey's test ($P < 0.05$).

Final populations of *Pratylenchus* spp. recovered from roots and soils on soybean were about 1,000 per kg^{-1} of soil in the third cycle (Figure 2.8). Canola, chickpea, pinto bean, and spring wheat had final mean populations between 160 and 250 nematodes per kg^{-1} of soil in the third cycle. In the third and final cycle, very low final *Pratylenchus* populations of < 30 nematodes per kg^{-1} of soil were recorded for lentil, yellow pea, and the control. These results indicated that the total abundance of *Pratylenchus* spp. was influenced by the crops grown and the number of successive cycles that each crop was grown in that soil.

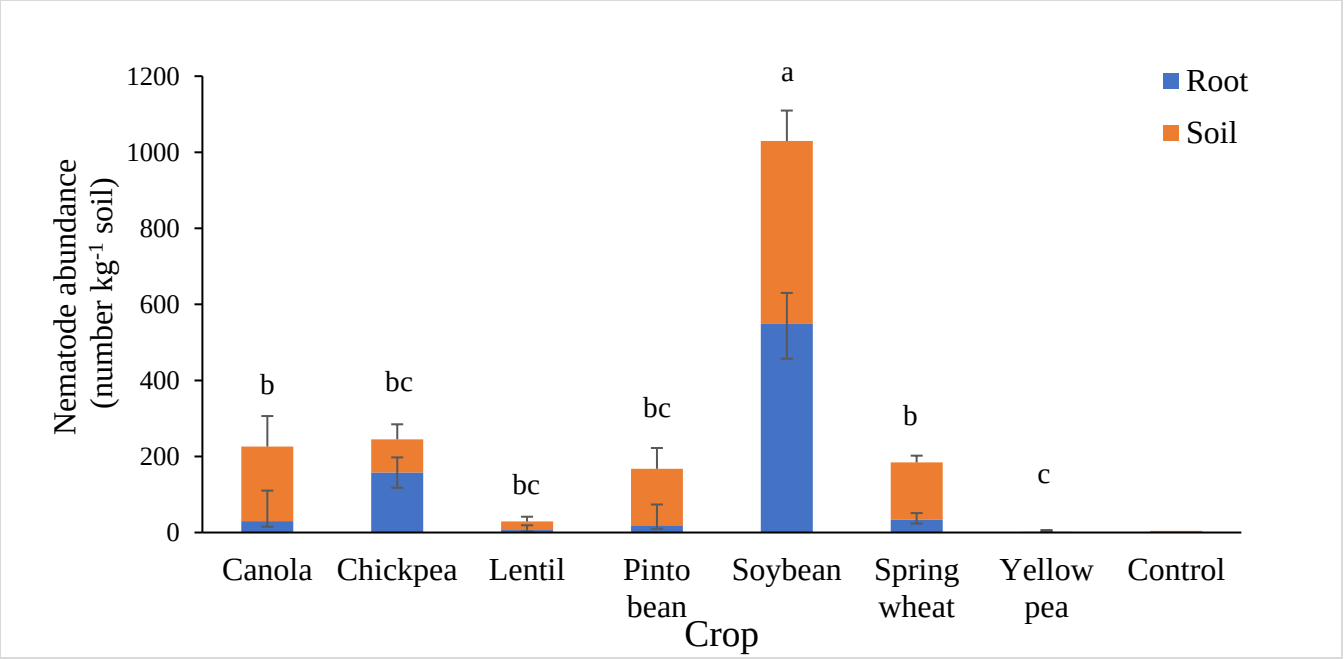

Figure 2.8 Mean abundance of *Pratylenchus* spp. in root and soil following the completion of the third growth cycle of selected pulse and non-pulse crops in pots. Mean nematode abundance in the soil for the control (pots without crops) is also provided. Bars for total abundance (root plus soil) with the same letters are not significantly different according to Tukey's test ($P < 0.05$).

The starting density and the build-up of *Pratylenchus* spp. across three subsequent cycles is shown in Fig. 2.9. It was observed that the final densities of *Pratylenchus* spp. differed among crops and with cycle. The rate of increase in populations of *Pratylenchus* spp. was linear with the crop growth cycle and each cycle was 8-wk long (Table 2.5). The build-up rate of the nematode was highest on soybean with subsequent growth cycles of the crop. *Pratylenchus* spp. in chickpea and pinto bean increased slightly in the second and third growth cycles. More *Pratylenchus* were recovered from canola in the third cycle than in the first and the second growth cycles. There was no build-up of the nematode observed on lentil, yellow pea, and control. These results indicated that the crops examined had different hosting abilities to *Pratylenchus* spp. and multiplication rate can significantly be influenced by the number of growth cycles.

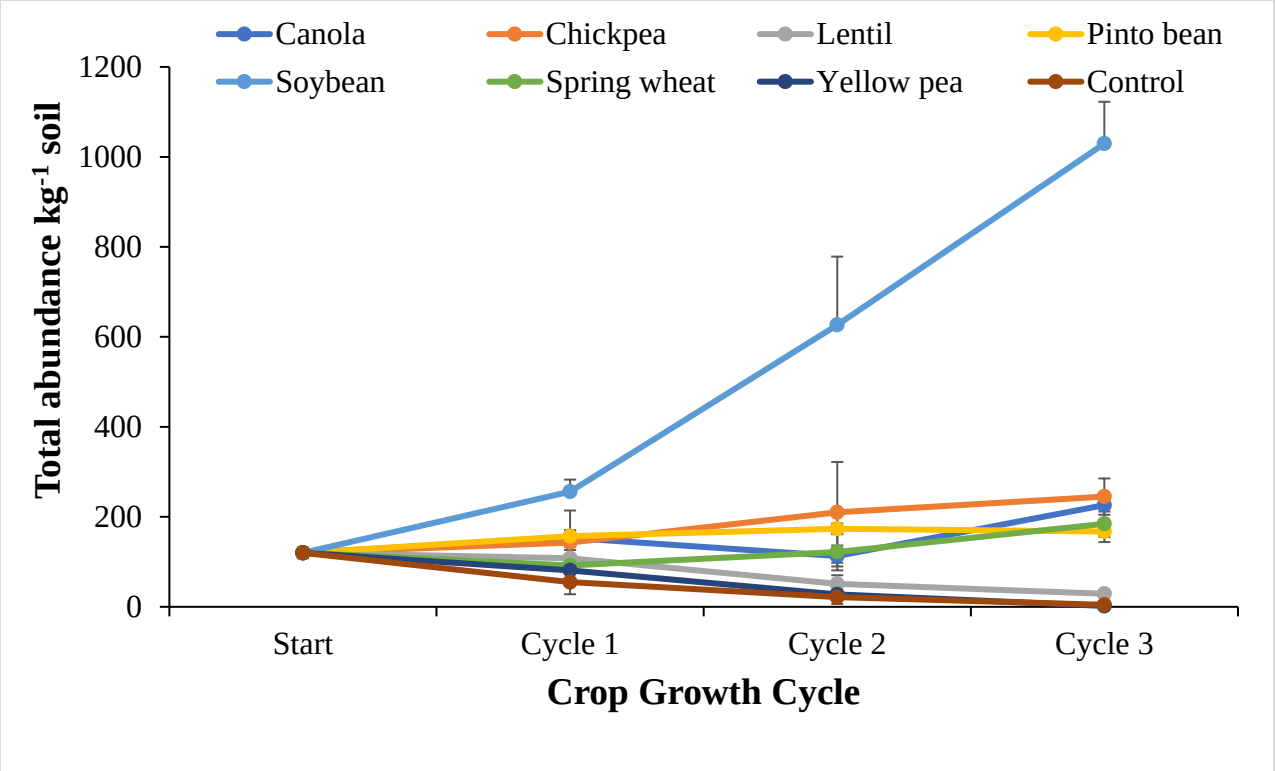

Figure 2.9 Build up rate of *Pratylenchus* spp. total abundances with subsequent crop growth cycles (Cycles 1-3)

Regression analysis of *Pratylenchus* abundance in three repeated growth cycles of different crops is provided in Table 2.5. The results from the regression analysis showed that the abundance of *Pratylenchus* spp. increased with repeated growth cycles of canola, chickpea, pinto bean, spring wheat, and soybean. Soybean had a significantly higher build-up rate of *Pratylenchus* spp. when grown repeatedly in different cycles. The abundance of *Pratylenchus* spp. decreased with repeated growth cycles of lentil and yellow pea.

Table 2.6 Regression of *Pratylenchus* abundance on different crops following three repeated growth cycles

Crop	*Y*	Slope SE	r^2	*P*
Canola	48.1x + 227	27.43	0.09	0.0897
Chickpea	53.2x + 265	26.39	0.16	0.0057
Lentil	-80.4x + 262	7.79	0.78	< .0001
Pinto bean	9.5x + 313	30.59	0.003	0.7576
Soybean	332.2x + 278	49.36	0.60	< .0001
Spring wheat	43.38x + 193	14.32	0.23	0.0050
Yellow pea	-87.7x + 233	6.44	0.86	< .0001

2.5. Discussion

2.5.1 Identity of the *Pratylenchus* in the test soil

Pratylenchus species were identified morphologically under the microscope. Morphological identification of *Pratylenchus* species was based on adult females as they possess more distinctive characters than males. In this study, nematodes were identified only to the genus using morphological features common for *Pratylenchus* spp. and these include black lips, short, robust stylet, esophagus overlapping the intestines ventrally, vulva positioned near the posterior end and bluntly rounded tails. There were no males found among the nematode species observed. This is an expected feature for *P. neglectus,* which reproduces parthenogenetically. Males are usually rare or absent among such species as *P. neglectus* (Castillo and Vovlas 2007). Other species such as *P. penetrans* reproduce amphimictically and males are common among such species (Castillo and Vovlas 2007). PCR assays and DNA sequencing were used for further species determination to supplement the morphological identification to give meaningful results.

For species determination, PCR with universal and species-specific primers followed by DNA sequencing of the D3 region of the rDNA was used to ensure accurate and reliable identification of the *Pratylenchus* species. The universal primer set pairs, TW81-AB28 and D2A-D3B used in this study to identify *Pratylenchus* spp. to the genus, target different regions on the DNA molecule, that is, the ITS-rDNA and D2-D3 expansion region, respectively (Subbotin et al.

2000, Amiri et al. 2002). The ITS and the D3 regions are the sites most commonly used as taxonomic markers for distinguishing between nematode species. The primers as mentioned earlier have been successfully used in identifying *Pratylenchus* and other plant-parasitic nematodes (Tanha-Maafi et al. 2003, Subbotin et al. 2008, Oliveira et al. 2017). The *Pratylenchus* D3 region is highly conserved and specific, even to the species level (Al-Banna et al. 1997). Additionally, the D3 region does not show any variation among nematodes belonging to the same species making it a useful marker for distinguishing *Pratylenchus* species (Al-Banna et al. 1997, 2004).

Single bands of approximately 290 bp in length were observed for all the individuals that were positive for *P. neglectus* amplified using the PNEG-D3B primer sets. The amplicon sizes obtained in this study matched with the expected band size for *P. neglectus* as reported by Al-Banna et al. (2004). The majority of the samples that were tested with the PNEG-D3B primer set were positive for *P. neglectus.* Any species that were negative for the *P. neglectus* primers were sent for sequencing to reveal the species identity. The DNA samples that tested positive with amplification using the universal (D2A-D3B) and species-specific (PNEG-D3B) primers, as well as those that were negative for the *P. neglectus* primer sets, were also sequenced. All the DNA samples that were sequenced had best identity matches with *P. neglectus.* PCR assays with other species-specific primers for *P. penetrans* (PPEN-D3B) and *P. thornei* (PTHO-D3B) did not yield any bands indicating the absence of these species in samples used in the current study. Additionally, sequencing results did not show any matches with *P. penetrans* and *P. thornei.* It was expected that *P. neglectus* would be the species present in the soil samples collected from the Canadian Prairies.

These results also agree with those reported by Gouvea-Pereira and Tenuta (2018), in which they identified *P. neglectus* as the prevalent species in the prairies using PCR and DNA sequencing. However, in their study, a few of their sequenced DNA samples had low reliability matching to *P. neglectus* suggesting that there could be other *Pratylenchus* species present (personal communication). This was not the case in this study as no other species were matching to the DNA samples used either used in PCR assays or sequencing. The presence of *P. neglectus* in the prairies has also been reported by Mahran et al. (2010) in potato fields in Manitoba. Moreover, based on previous reports, we did not expect to find any *P. penetrans* and *P. thornei* species. Using other species-specific primers other than the PNEG-D3B pair was useful to

determine if there were any other species present apart from *P. neglectus*. Moreover, for samples that tested negative for *P. neglectus*, it was necessary to test other species-specific primers, including those for *P. penetrans* and *P. thornei*. *Pratylenchus* species often occur in mixtures hence the need to test for the presence of different species. For DNA samples that tested negative for *P. neglectus*, this could suggest the presence of other species and that necessitates the need to amplify the samples using other species-specific primers and to perform DNA sequencing.

The species-specific primers used in this study targeted the D3 region of the 28S rDNA. Several studies have reported successful identification of *Pratylenchus* species using molecular methods and as such future studies can always incorporate these techniques (Al-Banna et al. 1997, Waeyenberge et al. 2000, Carrasco-Ballesteros et al. 2007, Troccoli et al. 2008, Yan et al. 2012). The results obtained in this study showed that the ITS region could reliably be used for the detection of *Pratylenchus* species. *Pratylenchus* species exhibit much variation in their sequences and size of the ITS region of the rDNA (Uehara et al. 1999, De Luca et al. 2011). Therefore, the ITS region offers better discrimination compared to the D2-D3 region and its versatility as a taxonomic marker makes it useful in the identification of any nematode species or population (Vrain and McNamara 1994, Powers et al. 1997, De Luca et al. 2011). Unlike the D3 region, the ITS region is said to have evolved faster and has undergone a lot of substitutional changes (Irdani 2008, De Luca et al. 2011). The ITS region is specific and has lower intraspecific polymorphism; hence it can be used to distinguish between populations of different species as well as within the same species (Powers et al. 1997, Subbotin et al. 2000b, Blouin 2002). Universal primers detect the presence of a nematode and species-specific ones then identify the nematode to the species level. Species-specific primers easily distinguish between species within the same genus or those that are closely related (Al-Banna et al. 1997). Additionally, when performing tests with species-specific primers, it is expected that amplification will only occur in those samples containing the target species. Results from the PCR with species-specific primers warrant no further tests on the amplified product; amplicons are produced at the presence of a nematode species and vice versa.

Species in the genus *Pratylenchus* mostly occur in mixtures with their counterparts, but in some cases, only one species may be present in a field (Smiley 2010). Fatemy et al. (2006) and Riga et al. (2008) reported the occurrence of *P. neglectus* in mixed populations with its closely

related species, *P. thornei.* Based on practice in this study, morphological identification alone may not be enough to reveal the identity of nematodes to species level, especially when using light microscopy because one cannot view the detailed morphological characters of the nematodes. The first step to effective nematode management is the identification of species present. Correct identification of nematode species and determination of host preferences of the species in a field or area is important for planning on and erecting management strategies. This also helps in deciding which crops to grow in order to prevent the build-up of damaging levels of the nematodes.

Sequencing results did not show the presence of any other species apart from *P. neglectus*. The sequencing results validated the findings from morphological and PCR assays that the nematode species present was *P. neglectus*. DNA sequencing is useful in ensuring that any arising false positives are eliminated from the results (Volossiouk et al. 2003). From these findings, we concluded that *P. neglectus* is present in the prairie provinces of Canada based on the lack of males which is a feature for *P. neglectus*, the PCR assays positive for *P. neglectus* specific-primers and DNA sequences matching to *P. neglectus.* It is, therefore, advisable for growers to know the nematode species present in their fields and the plant hosts associated with the species so that they can strategically plan on which crops to grow.

2.5.2 Host preference of the *Pratylenchus* in the test soil

This study reveals relevant information about the host preferences of *P. neglectus* on the major crops grown in the Canadian Prairies. Prior to this study, the *Pratylenchus* spp. was identified using morphological and molecular methods. The species present in the soils were identified as *P. neglectus* and there was a need to determine the hosts preferred by this nematode. In the current study, the R_f for *Pratylenchus* spp. was assessed first across three replicate trials in the first experiment and then across three cycles in another experiment. Based on the R_f values recorded within the three trials, the hosts for *P. neglectus* (R_f >1) were canola, chickpea, pinto bean, and soybean. Spring wheat had $R_f < 1$ in all trials but had a R_f of greater than 1 in the repeated growth cycles. Lentil and yellow pea were either less or not susceptible to the nematode. The control (pots without crops) also had lower final nematode populations in all the trials. Although the initial population densities of *Pratylenchus* were low, we expected that nematode reproduction would occur on suitable host crops, thereby increasing the final nematode population densities.

The species of crops influenced the reproduction of *Pratylenchus* spp. In several countries, *P. neglectus* has been reported on wheat, canola, chickpea (Mojtahedi et al. 1992, Smiley et al. 2014, Yan et al. 2016). Additionally, some reports stated that *P. neglectus* can readily multiply on soybean, chickpea and wheat (Mountain 1954, Guevara-Benitez et al.1970, Taylor et al. 2000).

P. neglectus reproduced best on soybean (R_f >1) indicating that the crop is a good host for the nematode. The nematode highly infected soybean roots compared to the other crops examined. This is the first report of *P. neglectus* on soybean in the Canadian prairies. Root-lesion nematodes are among the important pests of soybean (Yan et al. 2017). Soybean is susceptible to several *Pratylenchus* species including *P. neglectus,* and can be severely damaged by these nematodes (Mountain 1954, Majić et al. 2008, Khan 2012, Lima et al. 2017, Franchini et al. 2018). Much damage to soybean by *Pratylenchus* spp. occurs in less fertile soils and where monoculture has been practiced for many years (Illinois Agricultural Extension 1999). In South Africa, among other species, *P. neglectus* is also associated with soybean (McDonald et al. 2001, Fourie et al. 2015). Contrary to these reports, Owen et al. (2013) stated that soybean is resistant or a poor host for *P. neglectus*. In some reports, *Pratylenchus* species were considered as minor pests of soybean (Kinloch 1998, Warner 2006). Generally, in soybean fields, RLNs do not occur together with soybean cyst nematode (*Heterodera glycines* Ichinohe), but when they do, their populations are always lower. However, numbers of RLNs tend to increase when the soybean cyst is absent (Warner 2006).

Damage thresholds for root-lesion nematodes in soybean range from 70 to 1,400 nematodes per 100 cm^3 soil depending on species (Castillo and Vovlas 2007). In the USA, damage thresholds of *Pratylenchus* spp. in soybean range from 500 -1,000 g^{-1} dry root. Soybean is mostly reported as a host of other *Pratylenchus* species, including *P. agilis, P. alleni, P. brachyrus, P. scribneri,* and *P. thornei,* (Golden and Rebois 1978, Majić et al. 2008, Lima et al. 2017) rather than *P. neglectus*. In Canada, soybean is mainly produced in Ontario followed by Manitoba and Quebec and its production is increasing in Western Canada due to growing export markets of the crop (McMillan 2017). *Pratylenchus neglectus* is distributed in Manitoba and Ontario (Yu 2008) and I anticipate that these findings will provide useful knowledge to Canadian soybean producers. These findings, therefore, bring new insights to Canadian growers that soybean is a good host for *P. neglectus* and

can allow significant multiplication of the nematode. In soybean fields where high populations of *P. neglectus* occur, management strategies must be implemented to prevent crop damage and yield losses.

Second to soybean, in host suitability to *Pratylenchus* spp. was chickpea. High nematode numbers were also observed in the roots indicating that chickpea is a good host for the nematode. Smiley et al. (2014) also reported that chickpea is a good host of *P. neglectus.* The association of *P. neglectus* and chickpea has been reported in Australia, North Africa, Spain and the USA (Thompson et al. 2008, Smiley et al. 2014). In a study by Taylor et al. (2000), chickpea was highly susceptible to *P. neglectus* and it produced high final nematode densities. Many varieties of chickpea are known to increase reproduction and, ultimately, population densities of *P. neglectus* (Smiley 2015). However, chickpea was considered a poor host of a *P. neglectus* population from Italy (Di Vito et al. 2002).

Canola was also a good host for *P. neglectus,* and our findings agree with those reported by Taylor et al. (2000) in which canola was among the most susceptible crops to the nematode. We expected that canola would be a host for *P. neglectus* based on other studies previously done in Australia and the Pacific Northwest (Taylor et al. 2000, Smiley et al. 2005). Canola was a good host for this lesion nematode in a rotational study in Montana and nematode populations persisted under this crop from fall to spring seasons (May et al. 2016). Mahran et al. (2010) postulated that the dominant occurrence of *P. neglectus* in Manitoban fields in their survey for *Pratylenchus* species was because cereals and canola were used as rotation crops. This led to the hypothesis that *P. neglectus* is potentially parasitizing wheat and canola in the Canadian prairie provinces. The recent findings are significant because in Manitoba, for example, canola is one of the rotation crops for potato, earlier studies in Manitoba showed that potato was not a good host for *P. neglectus* (Mahran et al. 2010). *P. neglectus* could be a problem in the prairies considering canola is widely grown in these regions. Although canola and other *Brassica* spp. have good hosting ability for *P. neglectus*, they have neutral and bio-fumigation effects on these nematodes (Potter et al. 1998, May et al. 2016). Potter et al. (1999) reported that most canola cultivars are susceptible to *P. neglectus,* but a few others are nematicidal. *Brassica* spp. contain glucosinolates responsible for the production of isothiocyanates upon damage of plant tissues and these compounds have

nematicidal effects on *Pratylenchus* spp. (Potter et al. 1999). This suppressive effect of canola was observed when a rotation, including the crop, reduced populations of *P. neglectus* (Parker 1994).

Low populations of *P. neglectus* were observed on wheat in the three trials and these were attributed to very low initial soil population densities used in the trials. However, when wheat was grown repeatedly in two growth cycles, the populations of *Pratylenchus* spp. increased across the cycles suggesting that the crop was only susceptible at higher nematode infestations. In susceptible wheat cultivars, it is common to find very high population densities of several thousands of *Pratylenchus* spp. per g of infected root tissue (Smiley 2015). In crop rotation research conducted in Montana, *P. neglectus* reproduced best on wheat followed by canola yielding very high populations and this affirms that both crops are good hosts for this nematode (May et al. 2016). In Ontario, *P. neglectus* has previously been identified on wheat and other cereal and forage crops (Potter and Townshend 1973). Among other *Pratylenchus* species, *P. neglectus* occurred more frequently in these crops and this is probably because of these crops' good hosting abilities. Forge et al. (2015) reported that *P. neglectus* was associated with wheat in rotation studies conducted in Alberta. Susceptibility of wheat to *P. neglectus* has previously been reported in Australia and North America (Mojtahedi et al. 1992, Taylor et al. 2000).

In the wheat-growing areas of Australia, where RLNs are a threat to crop production, *P. neglectus* is one of the most damaging nematodes in those regions (Vanstone et al. 1998). The results from our experiment indicate the potential of *P. neglectus* to parasitize wheat and this should be alarming to growers if the nematode occurs in high populations. Damage to wheat has been associated with populations above 500 nematodes per 100 cm^3 of sandy to sandy loam and clay loam to clay soils and such populations require action for control (Dickerson et al. 2000). In Montana, populations of *P. neglectus* increased in wheat and canola, and these crops allowed the nematode to persist and reproduce effectively (May et al. 2016). Furthermore, very high populations of *P. neglectus* were observed when wheat preceded canola. Wheat and canola are considered good hosts for this lesion nematode and growing these crops in soils infested with *Pratylenchus* spp. would lead to an increase in populations (Smiley et al. 2005a, Wu et al. 2013). This suggests that growing wheat and canola successively or the season after season may result in a build-up of very high *Pratylenchus* spp. populations. Continuous annual production of wheat

causes higher populations of *Pratylenchus* spp. than wheat-fallow rotations (Smiley et al. 2004). Wheat production in the Canadian Prairies constitutes a large acreage (Bélair et al. 2018) and it is best to scout for *Pratylenchus* spp. in wheat fields to prevent the establishment and population growth of these nematodes. Wheat may cause high populations of *P. neglectus* which will impact the next crop. Although wheat is a good host for *P. neglectus*, a resistance gene (*Rlnn1*) has been identified in some wheat cultivars. Two wheat cultivars, Excalibur and Krichauff were reported to exhibit strong resistance to *Pratylenchus* species (Williams et al. 2002).

In this study, *Pratylenchus* spp. also reproduced on pinto bean with moderate populations. Bean cultivars are good hosts for *P. neglectus* (Castillo and Vovlas 2007, Sikora et al. 2018). Di Vito et al. (2002) reported that French bean was heavily infested with *P. neglectus* and their findings suggested that the crop was a good host. A field survey conducted in Turkey showed that dry bean root samples were highly infected by *P. neglectus* (Sağlam and Sözen 2018). In Ontario, bean has been reported to be a good host for *P. neglectus* and it was recommended for use in mass culturing of the nematode because it is easy to culture and has a good hosting ability (Olthof 1979). Although dry bean acreage is small, the crop is also rotated with cereals in western Canada, both of which are hosts for *P. neglectus* (Forge et al. 2015).

Contrary to my findings, Söğüt et al. (2014) reported that *P. neglectus* failed to infect and reproduce on 15 bean cultivars tested in their study. This suggests that bean cultivars differ in host suitability to *P. neglectus.* In dry beans, most crop damage by root-lesion nematodes and rapid nematode reproduction occurs in sandy soils. The *Pratylenchus* populations recovered from pinto bean in this study were below the economic threshold. Economic thresholds of *Pratylenchus* spp. in dry bean were estimated at >250 nematodes per gram of root. In sand to sandy loam and clay loam to clay soils, thresholds of *Pratylenchus* species for beans were estimated at ranges of 50 to 100 nematodes per 100 cm^3 of soil (Dickerson et al. 2000). Growers must, therefore, plan rotational cycles that do not promote the build-up of *P. neglectus* populations in the soil. For host crops such as chickpea and wheat, growers should select tolerant cultivars (if available) and grow them in rotation with resistant crops to maintain nematode populations below the damage thresholds (Grains Research and Development Corporation 2009).

Lentil was considered a poor host for the nematode. In agreement with our findings, Vanstone (2007) listed lentil among crops that are resistant to *P. neglectus*. In Montana, lentil was identified as a good rotational crop for wheat to reduce *P. neglectus* populations. *Pratylenchus neglectus* populations in lentil plots declined to about 27% of the original level (May et al. 2016). In contrast, lentil was considered a good host for *P. neglectus* in the Mediterranean region (Di Vito et al. 2002). Lentil was a host for *P. neglectus* in the Pacific Northwest (Riga et al. 2008). Yellow pea was a non-host for *P. neglectus* in the current study. Most of the yellow pea roots were not parasitized by the nematode at all. Smiley et al. (2014) reported that some cultivars of yellow pea were poor hosts for *P. neglectus*. The nematode did not reproduce on yellow pea and the final population densities in comparison with the initial densities were lower in soils planted to this crop.

In a study by Taylor et al. (2000), pea produced very low final densities of *P. neglectus*. Field pea reduced the population densities, and this resulted in yield increase intolerant wheat crops grown subsequently (Taylor et al. 2000). Similarly, in Montana, population densities of *P. neglectus* declined with the planting of lentil and pea in field plots (May et al. 2016). In Montana, a low population of *P. neglectus* was detected when wheat was rotated with field pea (May et al. 2016). The reduction of final nematode densities is an indication that a crop or cultivar is resistant or a non-host. The reported results of this study contradict the findings by Riga et al. (2008) who reported that *P. neglectus* populations increased in all pots with dryland peas (cvs. Columbian and Small sieve) and lentils (cvs. Red Chief and Pardina). Additionally, lentil and field pea were susceptible hosts for *P. neglectus* in Australia (Smiley 2010, Smiley et al. 2014). Vanstone (1999) stated that field pea is resistant to *P. neglectus* and also *P. thornei* which makes it a good break crop where these nematodes occur individually or in mixed populations. However, this is not true for all field pea cultivars hence the need to assess host statuses of different cultivars available locally.

Crop plants of the same species may differ in their hosting abilities, and hosting ability of crops is said to be species and cultivar-specific (Taylor et al. 2000, Smiley and Nicol 2009). It is, therefore, necessary to screen different cultivars of crops to determine their host status before making conclusions. In Australia, there are several contradicting reports on host suitability of lentil and pea, with some researchers considering them resistant to *P. neglectus* (Taylor et al. 2000,

Smiley et al. 2014, May et al. 2016) and others denoting the crops susceptible (Smiley 2010, Riga et al. 2008). Smiley and Nicol (2009) reiterated that when assessing the hosting abilities of crops, local cultivars should be used. The variations in host preferences may also be due to the existence of physiological races. Mahran et al. (2010) reiterated that *P. neglectus* may exist in different physiological races, thereby exhibiting differences in host preferences and parasitism. Griffin (1991) identified physiological types in virulence of *P. neglectus* on alfalfa. In the Netherlands, physiological races of *P. neglectus* were also reported to occur (Loof 1960). *Pratylenchus* species surviving in different geographic regions may prefer different host crops and the hosting ability of both legumes and cereals is considered species and cultivar-specific (Thompson et al. 2000, Carver 2009).

In the control (pots without crops), there was a decline in the numbers of *Pratylenchus* at the end of the 8wk-growth period of each trial. This clearly showed that nematode survival and reproduction is rendered unsuccessful at the absence of plant hosts. *Pratylenchus* species exhibit obligate parasitism and in the absence of a suitable host, they decline sharply in population densities (Castillo and Vovlas 2007). Nematode populations decline with exposure to extended periods of fallow and further decline occurs with the absence of host and/or fallow periods of up to 6 months (Smiley 2010, Thompson et al. 2017). Fallowing helps in controlling population densities of *P. neglectus* as it causes nematode death by starvation (Tyler 1933, Saynor 1972). However, during the absence of host crops, nematodes can survive in dry roots or root residues and in the soil (Smiley 2010, Thompson et al. 2017); thus, fields must be left free of weeds, crop residues and volunteer crops (Ornat et al. 1999). *Pratylenchus* spp. can also survive by anhydrobiosis or in quiescence during periods where there are no hosts to invade (Mani 1999, McSorley 2003). In the absence of suitable hosts, plant-parasitic nematodes can survive up to a year in the soil. The major threats to nematode survival rates during the absence of hosts are moisture and temperature (Roberts et al. 1981, Goodell and Ferris 1989). In the Canadian Prairies, fallowing is used for maintaining water reserves in drier parts and for weed control in wet regions (Agriculture and Agri-Food Canada 2016). However, fallowing is less practiced in the Prairies, and it cannot be recommended for use as a method to monitor *Pratylenchus* populations due to its contribution to environmental degradation.

An increase in population densities of *Pratylenchus* spp. in the soil is greatly accelerated by growing susceptible crops (Taylor et al. 2000); hence, this should be avoided. Populations of *Pratylenchus* species in soils are greatly influenced by cropping history. Suitable hosts of *Pratylenchus* species should be rotated with poor or non-host crops because multiple generations of the nematode will be maintained as long as suitable hosts are available (Kandel et al. 2013). Non-host crops are either immune, allowing no nematode penetration or resistant, allowing nematode penetration, little parasitism, but no reproduction occurs (Hunt et al. 2018). Smiley et al. (2014) observed lower population densities of *Pratylenchus* spp. in resistant wheat cultivars than in susceptible ones. A wheat crop is sown after another wheat crop had higher root infestations than wheat grown after faba beans (Vanstone 1999). Numbers of *Pratylenchus* in wheat following field peas can be up to 20 times lower than in wheat after wheat (Vanstone 1999). This suggests that resistant pea cultivars play a significant role in crop rotations to reduce populations of *Pratylenchus* species in infested fields.

The use of the R_f by assessing initial and final densities to determine host status was meaningful because it revealed whether the nematodes reproduced or not and to what extent the reproduction occurred. With the R_f, it became easy to categorize the suitable and poor host crops of *P. neglectus.* The R_f is considered a good indicator of the nematode-host relationship (Bélair and Benoit 1996, McKenry and Anwar 2006). Taylor et al. (2000) and Hollaway et al. (2000) also demonstrated the applicability of using the R_f to assess host suitability of crops to *Pratylenchus* species. Although not used in this study, another way to determine the host suitability of plants would be to use final nematode population densities (P_f) (Fernandez et al. 1994). This approach was also used by Taylor et al. (2000) in which they compared final nematode densities from the soil and roots of a test crop with those obtained from a known reference host and non-host for the nematode identified in previous studies. The R_f and the P_f both can reveal the suitability of a plant to host a nematode species simplistically.

2.5.3 Population density build-up of the *Pratylenchus* with sequential growth cycles of the selected crops

Total nematode abundance for *Pratylenchus* spp. in the soil was assessed over three subsequent growth cycles. To determine the final nematode abundance or population densities of

P. neglectus, the final nematode numbers in both roots and soils were counted because *Pratylenchus* species exhibit both ectoparasitic and endoparasitic feeding. Some of the nematodes may be inside roots and while others occur in the soil or rhizosphere (Zunke 1990, Smiley 2010). Counting nematodes in soil plus roots helps to give a reliable quantification of the populations likely to be present in a sample or field (Taylor et al. 2000, Castillo and Vovlas 2007). For each crop and the control, the reproductive factor (R_f) of *Pratylenchus* spp. was also assessed to determine how total nematode abundance was affected by host crops over the cycles. Nematode abundance was affected by crop as well as the number of cycles in which the crops were grown.

The initial populations in Cycle 1 were very low, but it was expected that the nematode populations would increase on suitable hosts with repeated cycles of the crops. The results showed that the final population densities of *P. neglectus* increased in pots planted to canola, chickpea, pinto bean, soybean, and spring wheat. In lentil and yellow pea, there was a decline in *Pratylenchus* densities. In the first cycle, there was no significant difference in nematode abundance among all the test crops examined. However, in the second and third crop growth cycles, the host crops affected the population densities of *Pratylenchus* spp. differently. Generally, there were more *Pratylenchus* spp. recovered from the roots of host plants than from the soil for some of the crops. This was especially true for chickpea and soybean in Cycles 2 and 3. Fatemy et al. (2006) also reported that greater numbers of *Pratylenchus* spp. occurred in roots than in the soil. *Pratylenchus* species are commonly found in high populations inside plant roots (Mai et al. 1996). Inside the roots of suitable hosts, nematode populations can multiply to up to 1,000-2,000 nematodes g^{-1} of root (Davis and MacGuidwin 2000).

Soybean had the highest total abundance of *Pratylenchus* spp. across all three growth cycles. Regression analyses showed that the population densities of the nematode further increased with repeated growth cycles of the soybean crop. The nematode abundance recorded for *Pratylenchus* spp. in soybean was significantly different from that of other crops in the second and third cycles indicating that the crop was the most preferred host for *P. neglectus.* In the third cycle, soybean had a mean total abundance > 1,000 nematodes kg^{-1} soil which is above the damage threshold level for *Pratylenchus* spp. including *P. neglectus* (Fleming et al. 2016). The results showed that *P. neglectus* has the potential to increase to damaging levels when suitable hosts are grown. The final mean population of *P. neglectus* on soybean was > 100 nematodes per g^{-1} fresh

root. This can be a damaging population in some crops, for example, in cereals such populations can cause about 10% yield loss if they occur at the grain filling stage. These results indicated that *P. neglectus* has the potential to increase to damaging levels on soybean even when it occurs in small populations. The final populations on soybean were about four times higher than the initial soil populations started with. *Pratylenchus neglectus* reproduced effectively on soybean multiplying the initial densities by over a 1000-fold at an optimum temperature of 30^{o}C (Acosta and Malek 1979). On susceptible crops, *Pratylenchus* spp. multiply readily and their numbers can increase greatly to damaging levels (Thompson et al. 2008). As was observed for soybean and a few other crops, population densities of plant-parasitic nematodes can significantly increase during the growing cycle or season of the host crops (Smiley 2015). Moreover, with repeated growth cycles of a preferred host, nematode population densities will further increase.

Chickpea had the second highest *Pratylenchus* abundance after soybean. The populations of *Pratylenchus* spp. in pots planted to chickpea increased from Cycle 1 to Cycle 3. However, the nematode abundance in chickpea pots was below the economic threshold for *Pratylenchus* species. In the second and third cycles, populations of *Pratylenchus* spp. also increased in pots planted to canola, pinto bean and spring wheat. However, the final total populations observed in these crops were all below the damage threshold levels for the nematode. The damage levels of *Pratylenchus* spp. differ with species, soil type, and host crop but they can range from 50 to 1, 800 nematodes per 100 g^{-1} or 1,000 to 36, 000 per 2,000 g^{-1} soil (Potter and Olthof 1993). The damage threshold level for most *Pratylenchus* spp. including *P. neglectus* is 1,000 nematodes kg^{-1} soil (Fleming et al. 2016). Taylor et al. (2000) reported the effects of 81 field crops on the population densities of *P. neglectus* in Australia and high final nematode densities were found on chickpea and wheat. Initial populations of *Pratylenchus* spp. double with a single growing cycle of susceptible crops such as chickpea and wheat (Eastwood and Smith 1995). It is, therefore, likely that growing these crops in fields infested with *P. neglectus* may result in increased population densities of the nematode in the soil.

In pots planted to lentil and yellow pea, there was a significant decline in nematode populations from Cycle 1 to Cycles 2 and 3. The decline of nematode numbers in lentil and yellow pea indicates poor hosting abilities of these crops to *P. neglectus*. Similar to our findings is a report

provided by Vanstone (2007), in which lentil was resistant to *P. neglectus*. In Montana, populations of *P. neglectus* decreased when lentil was grown (May et al. 2016). Of all the crops screened, yellow pea had the lowest final densities of *Pratylenchus*. There were no *Pratylenchus* spp. observed on almost all the roots of yellow pea and the soils too had very low counts of the nematode. These results suggest that growing yellow pea may help reduce soil populations of *Pratylenchus* spp.

The population densities of *Pratylenchus* spp. declined dramatically from cycle to cycle in pots that had no crops indicating that the nematodes needed a host for survival. Similarly, Smiley et al. (2014) reported the decline of *Pratylenchus* spp. at the absence of crop hosts in 12-wk and 16-wk growing cycles. In the absence of a plant host or suitable host crop, nematodes fail to survive and reproduce, resulting in a decline of nematode numbers. Death of nematodes occurs due to starvation and loss of reproductive capabilities (Agrios 2005, Kandel et al. 2013). Lower populations of *Pratylenchus* spp. were reported after summer fallow than there were after a wheat crop (Nombela et al. 1998). Farmers should not practice fallowing to manage populations of *Pratylenchus* species due to land degradation issues. However, the decline of nematode populations in the absence of plant hosts is not always guaranteed unless there are no root residues, volunteer crops or weeds that can host the nematodes. Root-lesion nematodes can survive through anhydrobiosis in the absence of host crops and this is common during fallow periods (Storey et al. 1982, Tobar et al. 1996). *P. neglectus* is said to be very adaptive to survival in dead roots and soil during periods where plant hosts are unavailable (Smiley 2015).

2.6 Conclusions

In conclusion, this study revealed that *Pratylenchus* spp. present in the Canadian Prairie soils is *P. neglectus* based on morphological observation and molecular diagnosis. The hosts for *P. neglectus* were canola, chickpea, pinto bean, soybean, and spring wheat, and populations effectively multiplied on these crops over subsequent growth cycles. To the best of our knowledge, this is the first report of *P. neglectus* infecting these pulse and non-pulse crops in the Canadian prairies. Soybean was the most preferred host for *P. neglectus,* and significantly high nematode

build-up was observed with repeated growth cycles of the crop. Soybean is being reported here as a new host for *P. neglectus* in Canada with the potential to increase to populations above the economic threshold levels. Despite the low starting densities of *Pratylenchus* spp. used in this study, the nematodes reproduced effectively, and population densities increased in the preferred host crops. This suggests that even with low initial densities, *Pratylenchus* species can infect and reproduce provided a suitable host is present. Based on our findings, we suggest that subsequently growing or monocropping crops such as chickpea and soybean in fields infested with *Pratylenchus* spp. should be avoided as this can cause a significant increase in nematode population densities to damaging levels. Since yellow pea was a non-host to *P. neglectus,* the crop can be used in rotations as a break-crop to reduce population densities of the nematode. As a recommendation, future studies can focus on culturing and mass rearing of the nematode and investigate the varietal responses for canola, chickpea, pinto bean, soybean, and spring wheat since these crops exhibited good hosting abilities for the nematode. Since this study was performed under growth chamber conditions, it would be valuable to know how nematode populations will change under field conditions.

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3. EFFECT ON CROP PERFORMANCE, ENDOMIGRATORY NATURE AND SPECIES IDENTITY OF *PRATYLENCHUS* RECOVERED FROM ROOTS OF CROP PLANTS IN THE CANADIAN PRAIRIES

3.1. Abstract

Pratylenchus spp. are present in the Canadian Prairie soils and more knowledge is required to understand better the parasitism relationship of these nematodes to the crops grown locally and their influence on the growth of host plants. The current study aimed at assessing the effect of increasing *Pratylenchus* spp. density on the performance of selected field crops commonly grown in the region in the Canadian Prairies under growth chamber conditions. Also, the study evaluated the endomigratory nature and effects of the feeding of *Pratylenchu*s spp. on roots of host crops. Another important aspect of this study was to determine the identity of *Pratylenchus* spp. recovered from the roots of host crops using morphological and molecular identification techniques. A pot experiment, with three repeated growth cycles, was conducted to assess if high densities of *Pratylenchus* spp. affected crop performance of canola, chickpea, lentil, pinto bean, soybean, spring wheat, and yellow pea. Crops were grown in soils naturally infested with *Pratylenchus* spp. for 8 weeks. Plant height was measured at 2-wk intervals from 2 to 8 weeks after emergence. Above-ground and root biomasses were measured at the end of each crop growth cycle. Total plant biomass was evaluated as the sum of the above-ground and root biomasses. Nematodes recovered from roots were identified morphologically by measuring morphometric characters of adult females, and results were confirmed by using polymerase chain reaction (PCR) with species-specific primers followed by DNA sequencing of the 28S rDNA. Statistical analyses for the plant growth parameters (height, above-ground and root biomass) were done using the simple linear regression (SLR) model and Pearson's correlation analyses. Roots of crops were examined under the microscope to detect any *Pratylenchus* spp. inside tissues. Plant roots were also checked for lesions at the end of each crop growth cycle. Increasing densities of *Pratylenchus* spp. reduced plant height of canola, lentil, pinto bean, spring wheat and yellow pea, with subsequent growth cycles. Plant height of chickpea and soybean was not affected by high densities of nematodes, although these crops were good hosts for the nematode. There was evidence of a reduction in above-ground and root biomasses of canola, lentil, pinto bean, spring wheat and yellow pea at greater *Pratylenchus* densities. Populations of *Pratylenchus* spp. did not significantly

impact the above-ground and root biomasses of chickpea and soybean. Regression analyses clearly showed that the crops examined were affected differently in terms of performance at greater densities of *Pratylenchus* spp. *Pratylenchus* spp. can significantly reduce the growth of host crops when they occur in high numbers. It is, therefore, important for farmers with nematode-infested fields to manage nematode populations to a minimum to prevent crop damage and yield losses. Acid fuchsin-stained eggs and juveniles of *Pratylenchus* spp. were observed inside the roots of chickpea and soybean, indicating the endomigratory behavior of these nematodes. Small, brownish lesions were observed on the roots of chickpea. The roots of other crops examined did not show any lesions. *Pratylenchus* spp. recovered from roots of host crops including canola, chickpea, lentil, soybean, and wheat were identified as *P. neglectus* using morphometric measurements of characters such as stylet length, body length (L), vulva position, *a*, *b*, and *c*-ratios. There were no males observed among the *Pratylenchus* individuals and this agrees with the parthenogenic nature of *P. neglectus*. Additionally, the molecular characterization of *Pratylenchus* spp. using PCR with species-specific primers and DNA sequencing of the D3 region of the rDNA confirmed that the nematode species was *P. neglectus*. More work is needed to determine the effect of *Pratylenchus* spp. on the performance of other crop cultivars, as well as under field conditions. Future studies may also aim to utilize greater initial densities of *Pratylenchus* spp. to assess the potential for crop damage and yield constraints on various crops grown in Canadian prairies.

3.2. Introduction

The root-lesion nematode (RLN), *Pratylenchus neglectus* (Rench) Filipjev Schuurmans Stekhoven is an obligate, migratory endoparasite parasitizing a wide range of crops. It is distributed in many temperate and tropical regions of the world (Taylor et al. 2000). *Pratylenchus neglectus* attacks many crops, including wheat, chickpea, canola, alfalfa, potato, and oat cultivars (Taylor et al. 2000, Castillo and Vovlas 2007). *Pratylenchus neglectus* is known to reduce yields in cereals and pulse crops (Di Vito et al. 1992, Vanstone et al. 1998, Taylor et al. 1999). In many regions of Australia, *P. neglectus* mainly infects wheat and causes yield losses of up to 27% (Vanstone et al. 1998, Taylor et al. 1999).

During feeding, *Pratylenchus* species puncture roots using their stylets and they release enzymes that degrade plant cell walls, thereby making penetration easy (Castillo and Vovlas 2007).

Pratylenchus species can complete the whole life cycle feeding and migrate inside the roots, but they can also feed as ectoparasites (Zunke 1990, Castillo and Vovlas 2007). The migration of these nematodes inside roots induces much damage to host plants. Plants parasitized by *Pratylenchus* species develop brownish, necrotic lesions on roots and ultimately, their growth is reduced. Infection by root-lesion nematodes also results in loss of marketable quality in crops such as carrots, peanuts, and potato (Bernard and Laughlin 1976). High population densities of *Pratylenchus* species on plants cause chlorosis, stunted growth, fewer lateral roots, reduced leaf number and size (Taylor et al. 1999). Plant growth parameters are good indicators of the effects of plant-parasitic nematodes on crops and reduction in these parameters indicates plant physiology impairment due to pathogenesis. Symptoms caused by *Pratylenchus* species are not as obvious as those caused by the cyst and root-knot nematodes, and they can, therefore, be mistaken for nutrient deficiency or moisture stress.

Damaging thresholds of *Pratylenchus* species range from 50 -1,800 per 100 g^{-1} soil (Potter and Olthof 1993). In Oregon and Washington, the damage threshold of *P. neglectus* was estimated at 2, 500 nematodes per kg^{-1} dry soil, and 2,000 nematodes per kg^{-1} for *P. penetrans* (Smiley et al. 2005a). Population densities of > 300 nematodes per g^{-1} of fresh root weight have been reported to cause yield losses (Bridge and Starr 2007). Damage thresholds for *Pratylenchus* species vary with nematode species and host plant available. Environmental factors also influence the amount of crop damage and yield loss that will occur in infected plants (Taylor et al. 1999). More economic damage by *Pratylenchus* species occurs with dry conditions of inadequate moisture and in nutrient-deficient soils (Vanstone et al. 2008). *Pratylenchus* species cause significant crop losses, which can be a huge threat to food security. The magnitude of yield losses caused by *Pratylenchus* species depends on the prevalent nematode species (Olthof, 1986). Moreover, in intolerant crops, the amount of yield loss attributed to these species is influenced by the nematode population densities present in the soil (Taylor et al. 1999). Reducing population densities of RLNs in the soil can, therefore, minimize damage and yield losses caused by these nematodes (Brown 1987).

It is difficult to assess the damage caused by *Pratylenchus* species using symptoms as they are not discrete, and they resemble symptoms caused by nutrient deficiencies, moisture stress and other pathogenic microorganisms (e.g., bacteria, fungi). Additionally, symptoms caused by

Pratylenchus species may be aggravated by secondary infection by fungal or bacterial pathogens and unfavourable environmental conditions (Taheri 1996). Several methods have been developed for use in assessing crop yield losses to *Pratylenchus* species and use of nematicides has been part of this progress (Doyle et al. 1987, McDonald et al. 1987, Badra and Adesiyani 1990, Thompson 1990). Williams et al. (2002) measured yield loss caused by *P. neglectus* using the nematicide, aldicarb in which they investigated the relationship between the population density or multiplication of *P. neglectus* and grain yield. Grain yield was compared between nematicide treated and non-treated trials. Yield loss has also been assessed by analyzing the correlation between yield and nematode population densities (initial or final) (Stynes and Veitch 1983, Prot and Savary 1993, Vanstone et al. 1998). Yield loss and nematode multiplication rates are both affected by moisture, temperature, fungal interaction, and nutrient availability (Barker and Noe 1987, Taheri et al. 1994).

Damage caused by *Pratylenchus* species is also associated with their interactions with other pathogens, including fungi and bacteria which often increase the intensity of symptoms (Powell 1971, Mai et al. 1977, Smiley 2010). *Pratylenchus* species often cause more damage when in combination with other pathogenic microorganisms in synergistic interactions. Through feeding, migration, and lesion formation, *Pratylenchus* species create entry channels for fungi and bacteria, which induce secondary infection on susceptible plants (Perry and Evert 1983). *Pratylenchus* species commonly form disease complexes with fungal pathogens of the genus *Fusarium* and *Verticillium*. They are also reported to interact with root-rot pathogens such as *Pythium, Phytophthora* and *Rhizoctonia* (Back et al. 2002a). The most common interaction of *Pratylenchus* species is with the wilt fungus, *Verticillium dahliae* Kleb 1913 to cause the potato early dying (PED) syndrome. The nematode interacts synergistically with *V. dahliae* in this disease complex and the occurrence of these two parasites increases disease severity on infected plants (Martin et al. 1982, Riedel et al. 1985).

Pratylenchus species often occur in mixed populations and it is, therefore, necessary to ensure careful and accurate identification of these nematodes (Taylor et al. 2000, Devran and Söğüt 2009). Morphological identification is useful in identifying *Pratylenchus* species, but it has certain limitations that render efficiency and reliability of the process. Measurement of morphometric characters is an important aspect of morphological identification, but where the overlap of

characters occurs, identification may be inaccurate. This is especially true for closely related and morphologically similar species such as *P. coffeae* and *P. pseudocoffeae* Zimmerman (1898). Scanning electron microscopy can be used to replace light microscopy because it provides reliable and detailed nematode morphological information, which can lead to accurate identification (Inserra et al. 2005). Variations in nematode morphology may be caused by exposure to different environmental conditions and alterations during specimen preparation, therefore, suggesting that the use of morphometrics alone for *Pratylenchus* identification may not be reliable (Al-Banna et al. 2004). Identification with morphometric characters may be supplemented by using SEM and molecular techniques to ensure accurate and reliable conclusions are drawn.

Pratylenchus neglectus is considered the most predominant species in Manitoba (Bélair et al. 2018). The nematode has been reported in potato fields in Manitoba, but the nematode did not reproduce on potato (cv. Russet Burbank) (Mahran et al. 2010). *Pratylenchus neglectus* was recently identified by Gouvea-Pereira (2018) during a survey for plant-parasitic nematodes in pulse fields conducted in the prairies between 2014 and 2016. The nematode was found in 20 of the 93 fields sampled and these findings prompted us to further investigate on the hosts preferred by *P. neglectus* as well as the impact caused by these nematodes on the growth of host crops. There is a need to assess the host-nematode relationship between *Pratylenchus* spp. and the crops being grown in the Canadian prairies.

3.3 Hypotheses

The hypotheses for this research chapter were that increasing population densities of *Pratylenchus* spp. in the soil would have an impact on crop growth and that the nematodes would cause necrotic lesions on roots of infected crops following repeated growth cycles. I postulated that the *Pratylenchus* species to be recovered from the roots of host crops is *P. neglectus* based on previous work on species identification.

3.4 Thesis objectives

The objectives of this study were:

1) To determine if an increase in density of *Pratylenchus* spp. with successive crop growth cycles would affect the performance of selected pulse and non-pulse crops grown under growth chamber conditions
2) To assess the effect endomigratory nature of *Pratylenchus* spp. on roots of selected crops
3) To determine the species identity of *Pratylenchus* recovered from roots of host crops

3.5. Materials and methods

3.5.1. Nematode inoculum

The soil used in the current study was previously used in one of the cycles in the host screening study. The soil was originally collected from a naturally infested field in Brooks, Alberta. The field from which this soil was collected has previously been reported to be infested with *Pratylenchus* spp. through a survey conducted between 2014 to 2016 (Gouvea-Pereira 2018). The final nematode densities in the soil from the first crop growth cycle were used as the initial densities for the subsequent crop growth cycles. The initial nematode densities in soil were determined for each pot using subsamples of 100 g. Each pot was filled with about 1.8 kg of nematode-infested soil and one plant was grown per pot.

3.5.2. Effect of *Pratylenchus* spp. densities on crop performance

Using the previously described pot experiment in Chapter 2, conducted in three repeated crop growth cycles, the effect of increasing densities of *Pratylenchus* spp. on the performance of seven selected field crops was assessed. The crops examined were canola, chickpea, lentil, pinto bean, soybean, spring wheat, and yellow pea. All crops were grown in soils naturally-infested with *Pratylenchus* spp. Plant height was measured at 2, 4, 6 and 8 weeks of growth. Crops were fertilized at 2 and 4 weeks after emergence using Miracle-Gro (20:20:20), a water-soluble fertilizer. About 1 teaspoon of fertilizer was dissolved in 4 l water. Harvested fresh above-ground

biomass and root biomass were measured at the end of each growth cycle. Total plant biomass was the sum of the above-ground and root biomasses per single crop.

3.5.3. Acid fuchsin staining of nematodes

Roots of chickpea and soybean were used to visualize stained *Pratylenchus* spp. inside root tissues using a modified sodium-hypochlorite acid fuchsin stain method by Byrd et al. (1983). The roots were washed using tap water to remove all the soil and debris. After washing, the roots were soaked for 4 min in an 8.25% chlorine bleach (NaOCl) solution to clear the tissues. Roots were agitated occasionally during soaking, and they were rinsed using tap water for about 45 sec. To wash away any residual NaOCl, roots were soaked in tap water for 15 min. Nematodes were stained by boiling roots in 50 ml tap water containing 1 ml of acid fuchsin for 1 min in the microwave. After cooling, the root tissues were de-stained by boiling in acidified glycerin, prepared by adding a few drops of hydrochloric acid (5N HCl) in 30 ml of glycerin. The nematodes were then examined under a compound microscope (BX51, Olympus Canada, Inc., Richmond Hill, Canada) at X40 to X100 and images were photographed using a digital camera (QColor3, Tokyo, Japan) installed with an Image-Pro Plus 6.2 software (Media Cybernetics, USA).

3.5.4. Morphological identification

Nematode samples were centrifuged at 2,170 r.p.m for 5 min and the supernatant was removed by pipette, leaving a volume of 0.5 ml in the vial. To visualize *Pratylenchus* spp. recovered from roots, drops of the nematode solution were pipetted onto microscope slides, covered with coverslips and sealed with nail polish. Glycerol was added to the slide mounts and nematodes were gently heat-killed so that the measurements could be quickly taken (Golden 1990). Nematode identification was made using an inverted microscope (Motic AE21, Microscope World, Carlsbad, CA). Nematodes were identified to genus morphologically using adult females based on the head, stylet, esophagus, and vulva position to total body length using an identification key by (Mai et al. 1996). The following morphometric characters; body length (L), maximum body width, vulva position, tail length, and stylet length were measured.

Additionally, *a* (body length/ greatest body width); *b* (body length/head to the joint of the esophagus) and *c* (body length/tail length) ratios were calculated according to De Man (Xie 2000).

The morphometric measurements were taken based on the De Man indices (L, V, s, a, b, c) (De Man 1876, 1880) and 30 nematodes were measured. All measurements were done at X40 magnification but the total body length (L) was done at X10 using an eyepiece micrometer. A compound microscope (Olympus BX51, Olympus Canada, Inc., Richmond Hill, Canada) equipped with a digital imaging camera (QColor3, Olympus, Tokyo, Japan) and an Image-Pro Plus 6.2 software (Media Cybernetics, USA) was used for taking the nematode images and morphometric measurements.

3.5.5. DNA extraction and Polymerase chain reaction (PCR)

DNA was extracted from a total of 40 single nematodes, collected from plant roots of host crops including canola, chickpea, lentil, soybean, and spring wheat. As previously described, nematodes were pipetted into a small Petri dish, washed three times using sterile dH_2O before being hand-picked into PCR tubes containing 12 µl ddH_2O. Tail buffer (12 µl) and 100 mg proteinase K (2 µl) were added to the tubes containing the nematode solution. After a slight vortex and centrifuge, the nematode solutions were frozen at -80ºC overnight. The next day, the tubes were heated at 65ºC for 90 min and 95ºC for 10 min. Afterward, the nematode DNA samples were labeled by date and given a code and then stored at -20ºC before use. Nematode DNA was amplified using universal and species-specific primers. The forward primer D3A (5'-GAC CCG TCT TGA AAC ACG GA-3') paired with the reverse primer D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') were used for amplification of the D3 region of the 28S rDNA as according to Al-Banna et al. (1997). PCR with species-specific was performed with the forward primers for *P. neglectus* (PNEG), *P. thornei* (PTHO), *P. penetrans* (PPEN) paired with the universal reverse primer, D3B. PCR assays were conducted based on the protocol provided by (Al-Banna et al. 2004). The reaction mixture for the species-specific primers was as follows:15.4 µl dH_2O, 2.5 µl buffer, 1 µl dNTPs (250 µM), 1 µl $MgCl_2$ (2.5 mM), 2 µl for each primer (0.8 mM), 0.1 µl Dream Taq polymerase (Thermo Fisher Scientific, Waltham, MA, USA). A volume of 1 µl of nematode DNA was used in each reaction mixture. The amplification conditions were; denaturation at 95ºC for 3 min, 95ºC for 1 min, 63ºC for 1 min, 72ºC for 1 min and additional step at 72ºC for 7 min for 35 cycles. The PCR products were analysed using electrophoresis on a 1.7% agarose gel (Invitrogen, Carlsbad, CA) stained with GelRed dye (Biotium, Fremont, CA). Wells were loaded with DNA samples stained with blue-orange dye (ProMega, Madison, USA) and 100 bp DNA

ladder (ProMega, Madison, USA) was used to compare sizes of amplicons. DNA of *Ditylenchus dispaci* was used as a negative control. The gel was run at 85 V for 1 hr in TAE buffer and amplified products were viewed under UV light using G-Box: F3 (Syngene, Frederick, MD). The gel was observed using the GeneSys software (v.1.3.1.0) equipped with a Synoptics 3.8MP camera, set at an exposure of 360 ms, light setting at TLUM (mid-wave) and filter setting at UV032.

3.5.6. DNA Sequencing

Sequencing of the D3 region was done by Macrogen, Corp (Rockville, MD, USA) on 40 DNA extracts of single nematodes recovered from roots. Before sequencing, DNA samples were amplified using both universal and species-specific primers previously mentioned. To confirm the positive results from PCR assays with species-specific primers, PCR products were sequenced. Any samples that were negative for the species-specific primers were also sequenced to determine the species identity. Before sequencing, PCR products were purified using purification kits; the Invitrogen (Thermo Fisher Scientific) and QIAquick Gel Purification kit (QIAgen, Germany). A Nanodrop 2000 spectrophotometer v.1.0 (Thermo Fisher Scientific, Wilmington, DE) was used to quantify DNA (ng μl^{-1}) and to determine purity (A260/280 ratio) of the PCR products. The sequencing results were compared to other sequences recorded in the GenBank database using the basic local alignment search tool (BLAST) to find the matching *Pratylenchus* species.

3.5.7. Statistical analysis

All data generated from this study were statistically analysed using SAS v9.4 (SAS Institute, Cary, NC). Data collected included plant height, above-ground, root biomass and nematode population densities in roots and soils. A mixed-model (PROC MIXED) analysis of variance (ANOVA) was used to analyse the data generated from the study. Assumptions of normality were tested, and data were transformed by $x^1 = \log_{10}(x+0.1)$ where necessary. All the tests were conducted at $\alpha = 0.05$. The Tukey-Kramer test and the pdmix 800 macro (Saxton 1998) were used for mean comparisons. Linear regression analysis was used to determine the relationship between densities of *Pratylenchus* and plant growth parameters (plant height, above-ground, and root biomass). Final plant height, above-ground biomass, root biomass and total biomass recorded

at the end of each crop growth cycle were regressed with *Pratylenchus* densities (number per kg^{-1} soil). Pearson's correlation was also used to analyse data from plant growth parameters. However, results from the correlation were not reported and discussed but were provided in the Appendix (Section 5.2).

3.6. Results

3.6.1. Effect of *Pratylenchus* densities on Crop Performance

Pearson's correlation and regression analyses provided similar information about the effect of *Pratylenchus* densities on plant growth, but only the regression analyses are reported since they provide quantitative information about the magnitude of the effect.

3.6.1.1. Effect of *Pratylenchus* spp. on total plant biomass with repeated growth cycles

An analysis of variance (ANOVA) table for total plant biomass of selected crops grown in three successive growth cycles is presented in Table 3.1. There was a significant interaction between crop and growth cycle. The ANOVA showed significant effects for both crop and cycle to total plant biomass.

Table 3.1 Analysis of variance for total plant biomass at the end of each of the three growth cycles of selected pulse and non-pulse crops.

Source of variation	df	MS	*F*	*P*
Crop	6	1261896	89.87	< .0001
Cycle	2	438333	31.22	< .0001
Crop*Cycle	12	261366	18.61	< .0001

The mean total plant biomass recorded for each crop across three different growth cycles is presented in Fig 3.1. This figure did not show any reduction in total biomass of crops from one growth cycle to the next. However, further analyses were performed to determine the relationship between high *Pratylenchus* densities and above-ground and root biomass.

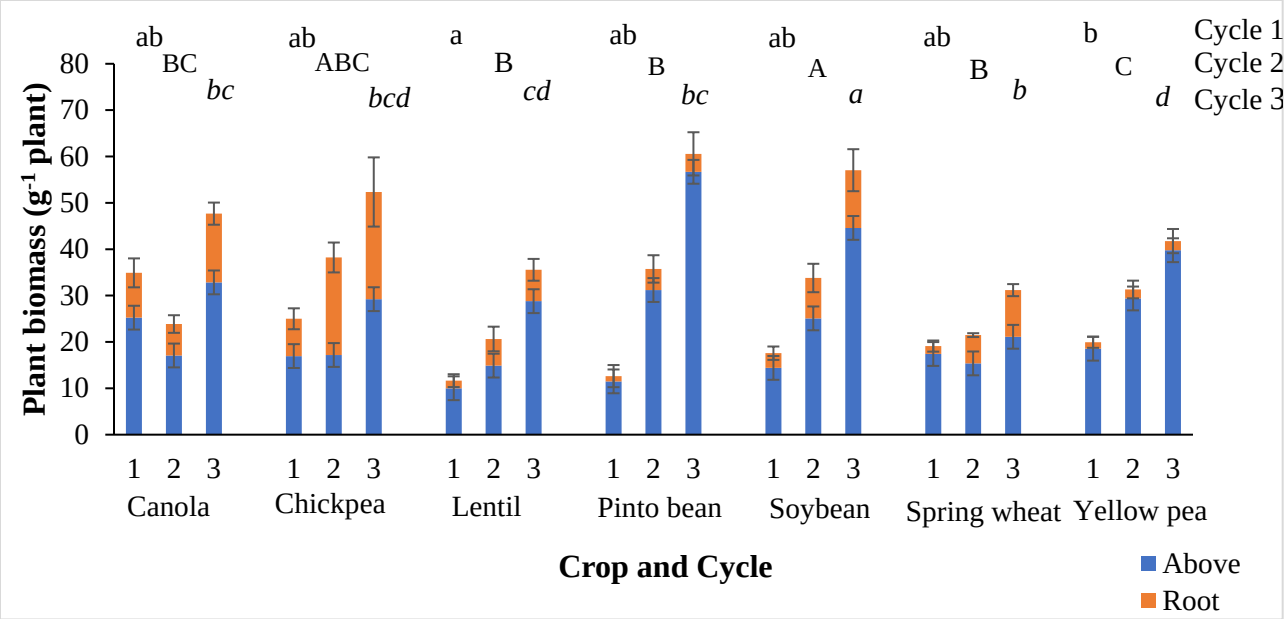


Figure 3.1 Mean above-ground and root biomass for the selected pulse and non-pulse crops at the end of each of the three growth cycles. Multiple comparisons for mean total (above-ground and root) plant biomass generated using the Tukey test and means with the same letter or number are not significantly different from each other. Comparisons of crops within a cycle are denoted by letters and numbers: lower case letters for Cycle 1, upper case letters for Cycle 2 and italicized letters for Cycle 3.

3.6.1.2 Effect of *Pratylenchus* spp. on above-ground biomass with repeated growth cycles

An ANOVA table for the above-ground plant biomass of selected crops following three successive growth cycles is presented in Table 3.2. There was a significant interaction between crop and growth cycle. The ANOVA showed significant effects for both crop and cycle for the three crop growth cycles of selected crops.

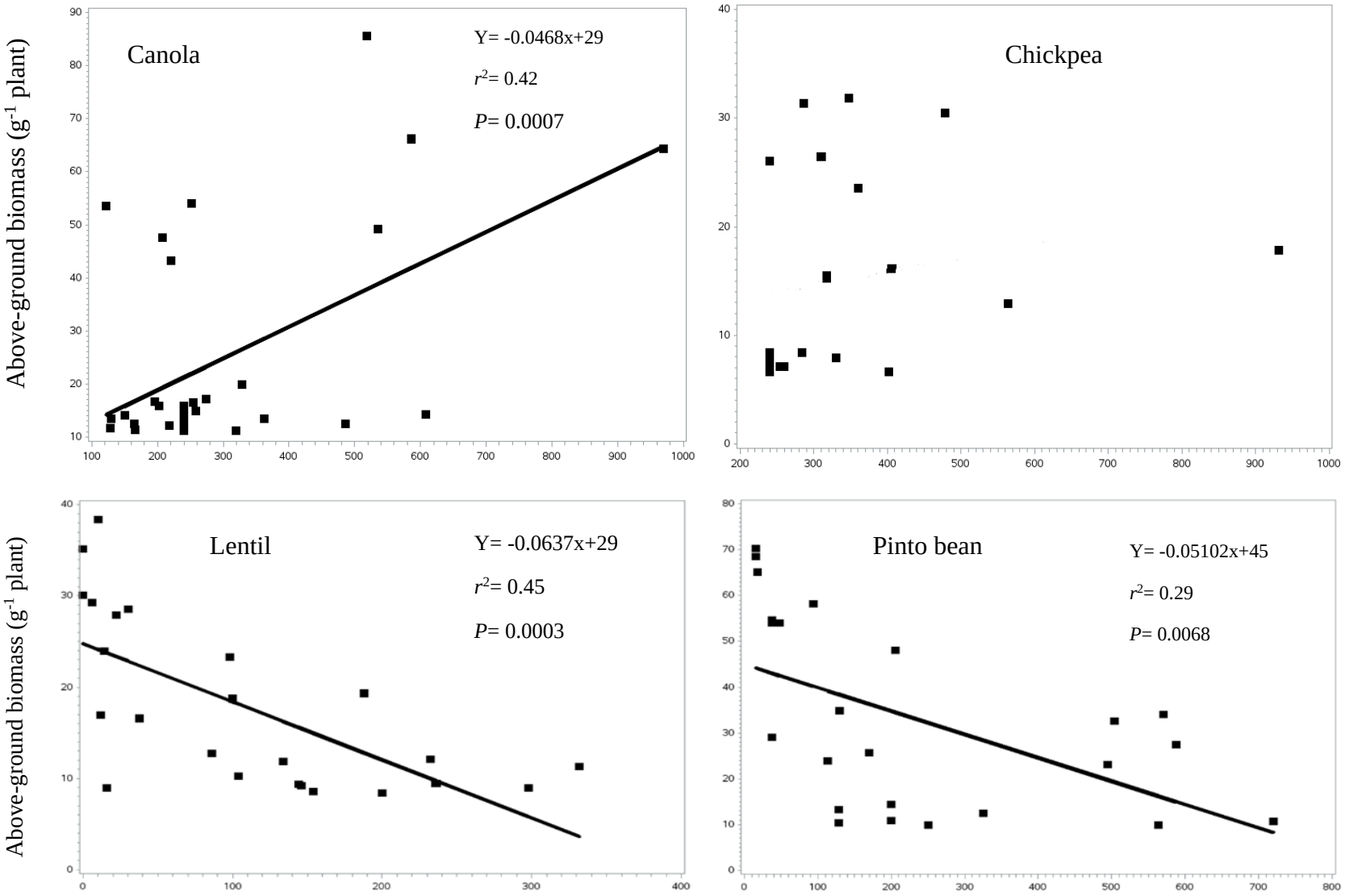
Table 3.2 Analysis of variance for above-ground plant biomass at the end of each of the three growth cycles of selected pulse and non-pulse crops.

Source of variation	df	MS	*F*	*P*
Crop	6	1267.18	34.55	< .0001
Cycle	2	5949.93	162.22	< .0001
Crop*Cycle	12	314.06	8.56	< .0001

Regression analyses indicated that increasing *Pratylenchus* density reduced the above-ground biomass for canola, lentil, pinto bean and yellow pea following three growth cycles and results are presented in Table 3.3. Chickpea, soybean, and spring wheat were exceptional; their biomasses did not decrease with increasing *Pratylenchus* densities. Above-ground biomass of soybean increased with increasing densities of *Pratylenchus* spp. following the three growth cycles.

Table 3.3 Regression analyses for the above-ground plant biomass of selected pulse and non-pulse crops following three repeated growth cycles

Crop	***Y***	**Intercept SE**	**Slope SE**	r^2	***P***
Canola	-0.0468x + 28	3.44	0.00119	0.42	0.0007
Chickpea	0.0015x + 17	5.11	0.0152	0.0007	0.9244
Lentil	-0.0637x + 25	2.24	0.0149	0.45	0.0003
Pinto bean	-0.0051x + 29	7.32	0.01708	0.29	0.0068
Soybean	0.0155x + 13	6.57	0.0063	0.22	0.0223
Spring wheat	-0.0187x + 5	2.65	0.0115	0.11	0.1194
Yellow pea	-0.0673x + 34	2.51	0.0174	0.40	0.0008


Canola
Y= -0.0468x+29
r2= 0.42
P= 0.0007
Chickpea
Lentil
Y= -0.0637x+29
r2= 0.45
P= 0.0003
Pinto bean
Y= -0.05102x+45
r2= 0.29
P= 0.0068
Above-ground biomass (g-1 plant)
Above-ground biomass (g-1 plant)
Pratylenchus density (number kg-1 soil)
Pratylenchus density (number kg-1 soil)

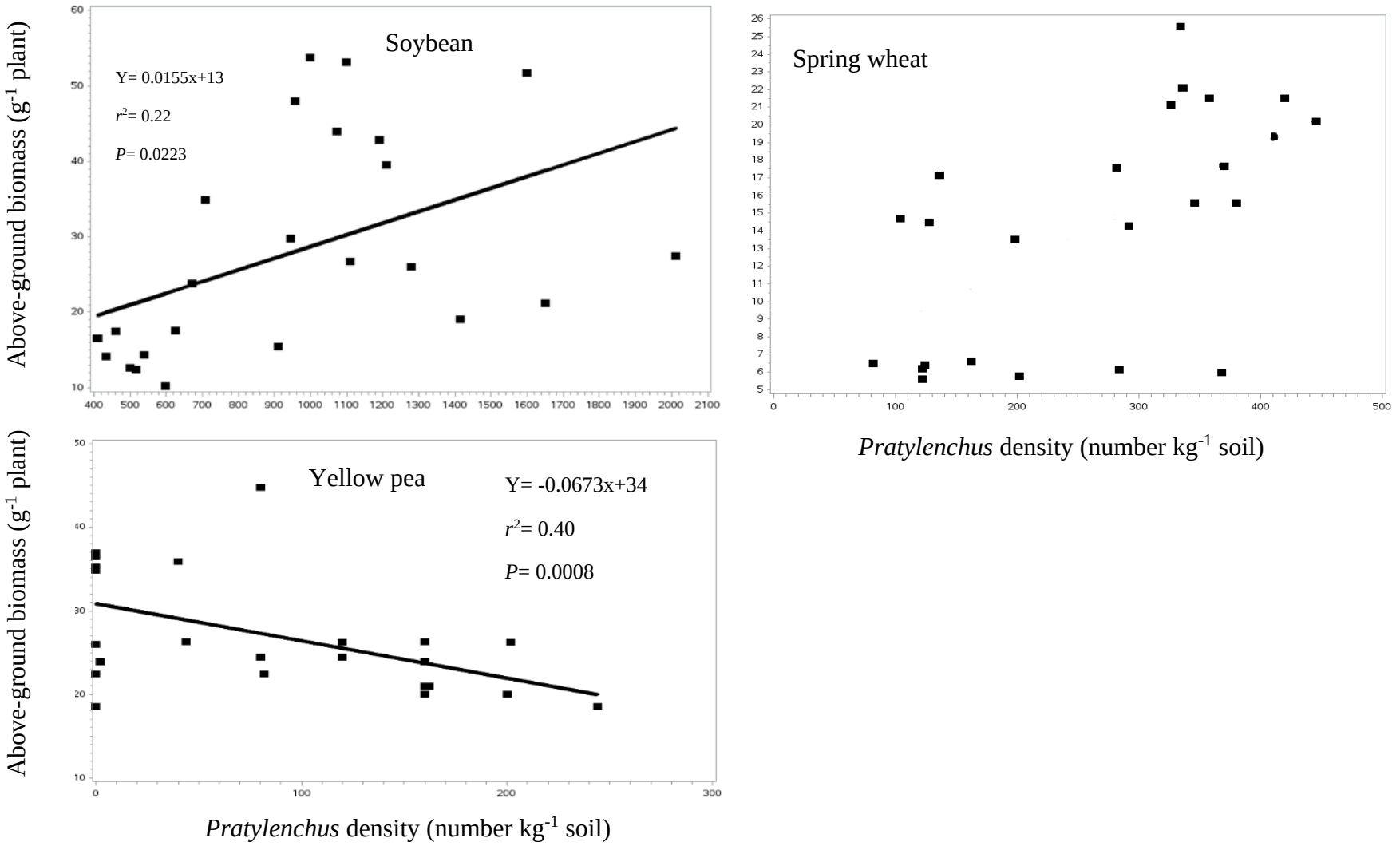


Figure 3.2 Regression analyses of *Pratylenchus* abundance of above-ground biomass of selected pulse and non-pulse crops following three growth cycles

3.6.1.3 Effect of increase in *Pratylenchus* spp. densities on root biomass with repeated growth cycles

An ANOVA for the root biomass at the end of the three crop growth cycles is presented in Table 3.4. There was a significant crop x cycle interaction for root biomass. The crop and cycle effects on root biomass were also significant.

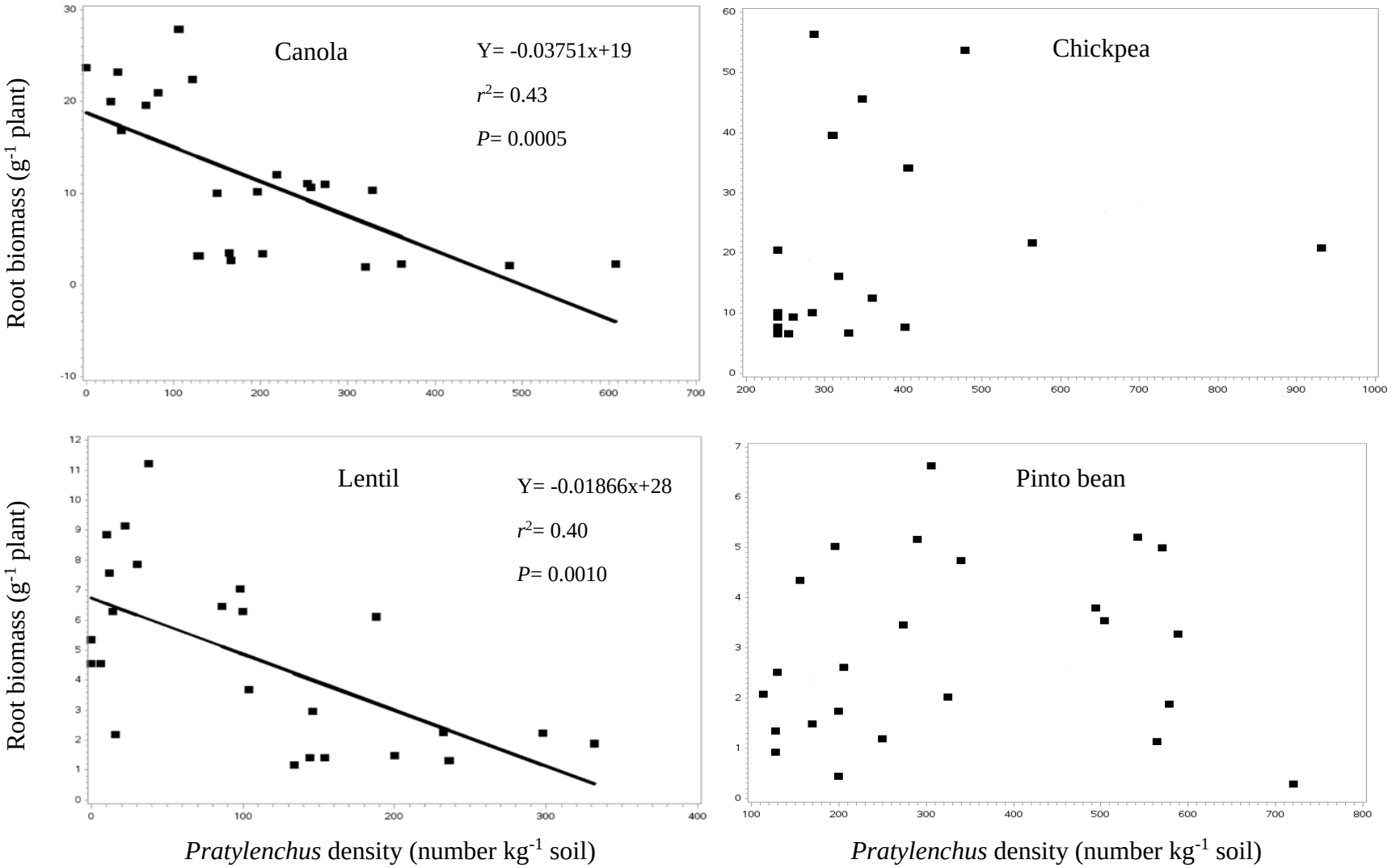
Table 3.4 Analysis of variance for root biomass at the end of each of three growth cycles of selected pulse and non-pulse crops.

Source of variation	df	MS	*F*	*P*
Crop	6	615.21	80.31	< .0001
Cycle	2	1239.69	161.83	< .0001
Crop*Cycle	12	119.90	15.65	< .0001

Regression analyses for effect of an increase in *Pratylenchus* density on root biomass of canola, lentil pinto bean, and spring wheat indicated a reduction in root biomass of these crops with increasing *Pratylenchus* density (Table 3.5). No reduction in root biomass was observed on chickpea, pinto bean, soybean, and yellow pea with increasing *Pratylenchus* density. Root biomass of chickpea and soybean increased with increasing *Pratylenchus* densities across the cycles.

Table 3.5 Regression analyses for root biomass of selected pulse and non-pulse crops following three repeated growth cycles

Crop	*Y*	Intercept SE	Slope SE	r^2	*P*
Canola	-0.03751x + 19	2.75	0.0009	0.43	0.0005
Chickpea	0.0075x + 21	8.90	0.0275	0.006	0.7907
Lentil	-0.0187x + 7	0.74	0.0049	0.40	0.0010
Pinto bean	-0.0025x + 3	0.63	0.0016	0.10	0.1496
Soybean	0.0065x + 2	1.87	0.0018	0.38	0.0015
Spring wheat	-0.0133x + 1	1.57	0.0064	0.16	0.0516
Yellow pea	-0.00208x + 2	0.19	0.0021	0.04	0.3260


Canola
Y= -0.03751x+19
r2= 0.43
P= 0.0005
Chickpea
Lentil
Y= -0.01866x+28
r2= 0.40
P= 0.0010
Pinto bean
Root biomass (g-1 plant)
Root biomass (g-1 plant)
Pratylenchus density (number kg-1 soil)
Pratylenchus density (number kg-1 soil)

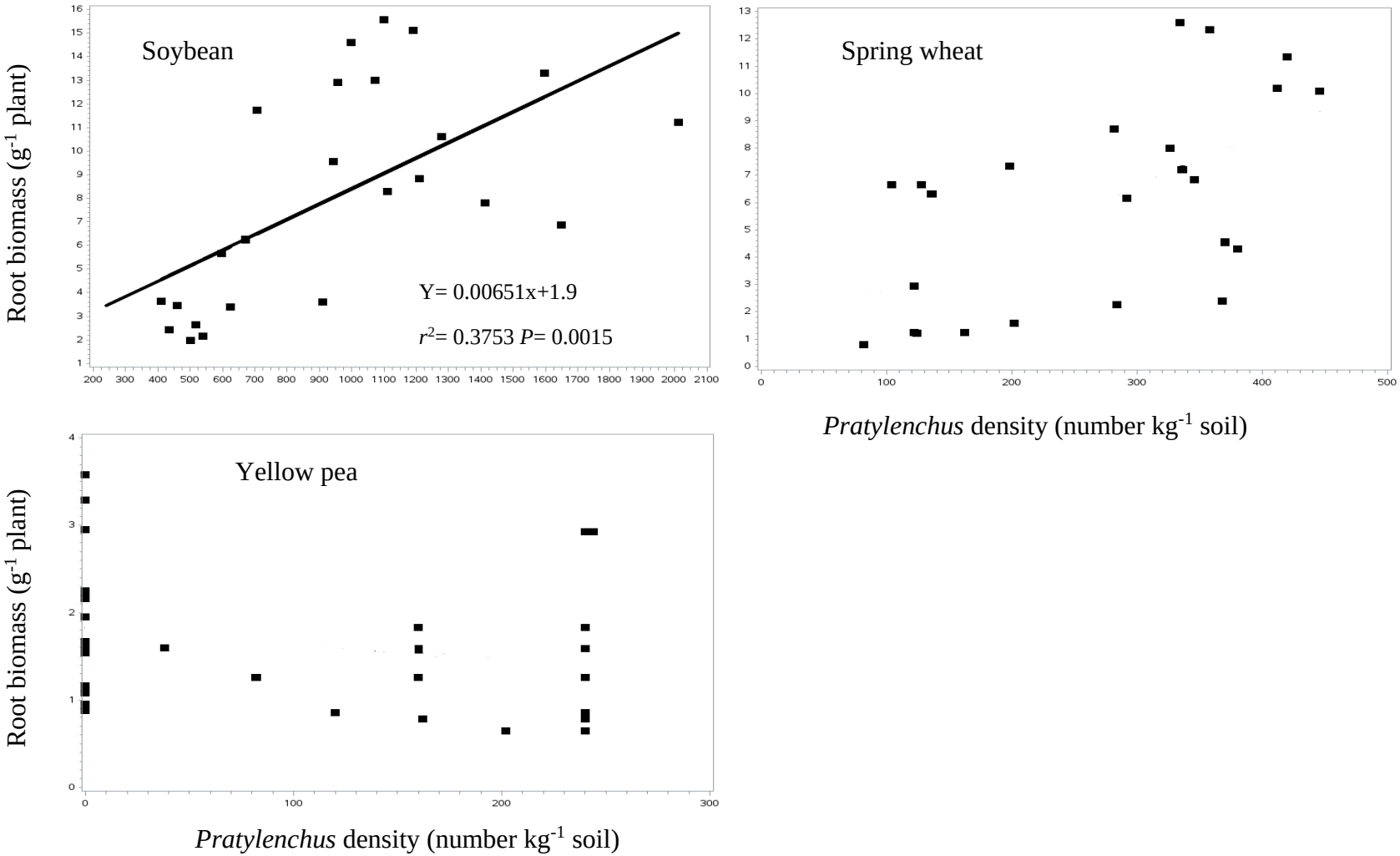


Figure 3.2 Regression of *Pratylenchus* density on root biomass of selected pulse and non-pulse crops following three repeated growth cycle

3.6.1.4 Effect of increase in *Pratylenchus* spp. densities on total biomass of different crops with repeated growth cycles

An analysis of variance table for total plant biomass at the end of three growth cycles is provided below (Table 3.6). There was a significant interaction between the crop and the cycle. The ANOVA showed significant effects for both crop and cycle.

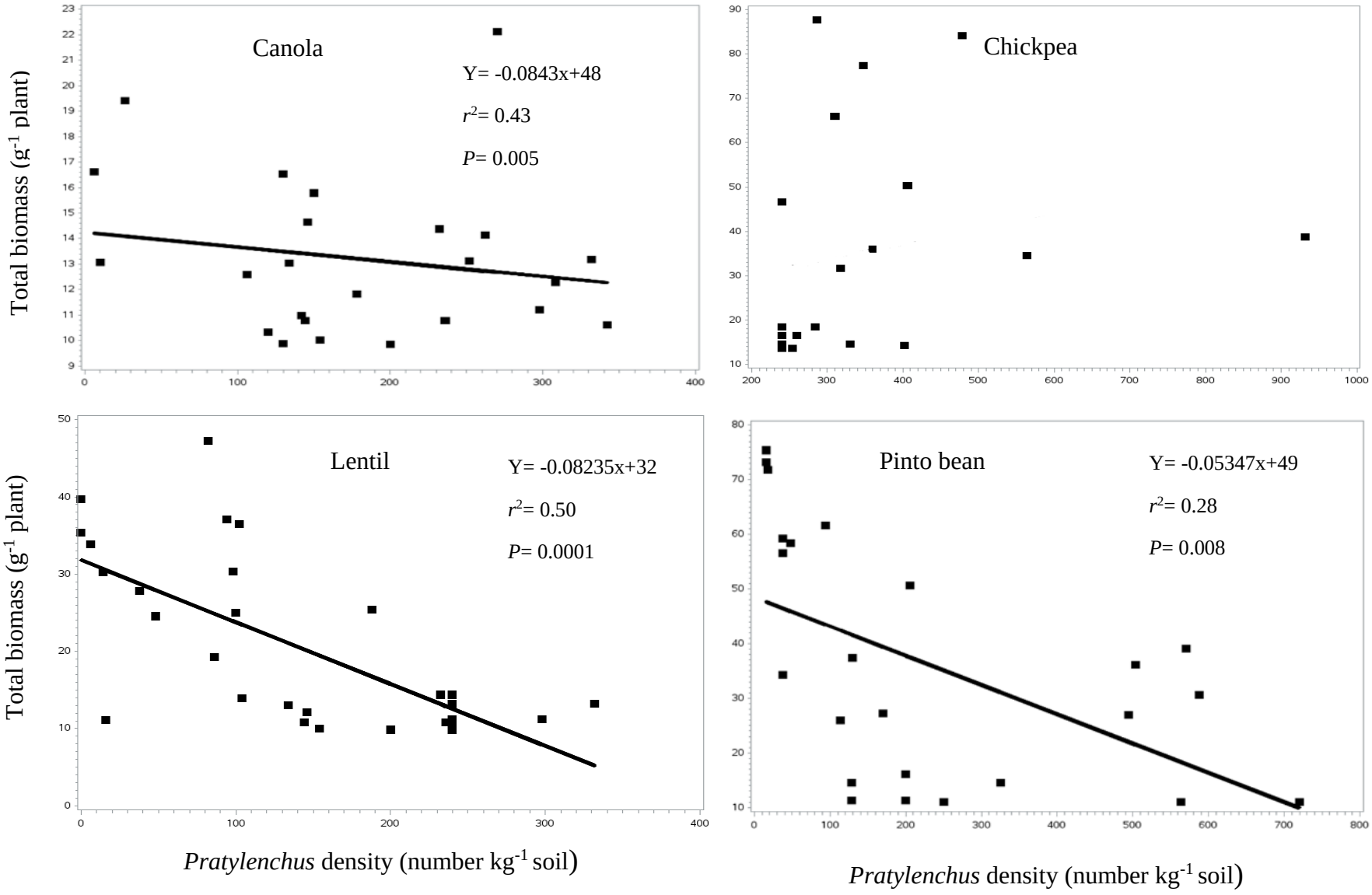
Table 3.6 Analysis of variance for total plant biomass at the end of each of three growth cycles of selected pulse and non-pulse crops.

Source of variation	df	MS	*F*	*P*
Crop	6	1261896	89.87	< .0001
Cycle	2	438333	31.22	< .0001
Crop*Cycle	12	261366	18.61	< .0001

Regression analyses on *Pratylenchus* density on total biomass indicated that total biomass of canola, lentil, pinto bean, and yellow pea were negatively affected by increasing densities of *Pratylenchus* spp. Total biomass of chickpea, soybean and spring wheat was not affected negatively by an increase in nematode density. There was a positive relationship between total biomass of soybean and the increasing densities of *Pratylenchus*. Regression analyses of *Pratylenchus* density on total biomass of selected pulse and non-pulse crops are presented below (Table 3.7).

Table 3.7 Regression analyses for total biomass of selected pulse and non-pulse crops following three repeated growth cycles

Crop	*Y*	Intercept SE	Slope SE	r^2	*P*
Canola	-0.0843x + 48	6.13	0.0207	0.43	0.0005
Chickpea	0.0089x + 39	13.6	0.0412	0.004	0.8321
Lentil	-0.0824x + 32	2.68	0.0174	0.50	0.0001
Pinto bean	-0.0535x + 32	7.84	0.0183	0.28	0.0080
Soybean	0.0220x + 15	8.30	0.0080	0.26	0.0113
Spring wheat	-0.0319x + 5	4.09	0.0176	0.13	0.083
Yellow pea	-0.0694x + 36	1.58	0.0185	0.40	0.0011


Canola
Y= -0.0843x+48
r2= 0.43
P= 0.005
Chickpea
Lentil
Y= -0.08235x+32
r2= 0.50
P= 0.0001
Pinto bean
Y= -0.05347x+49
r2= 0.28
P= 0.008
Total biomass (g-1 plant)
Total biomass (g-1 plant)
Pratylenchus density (number kg-1 soil)
Pratylenchus density (number kg-1 soil)

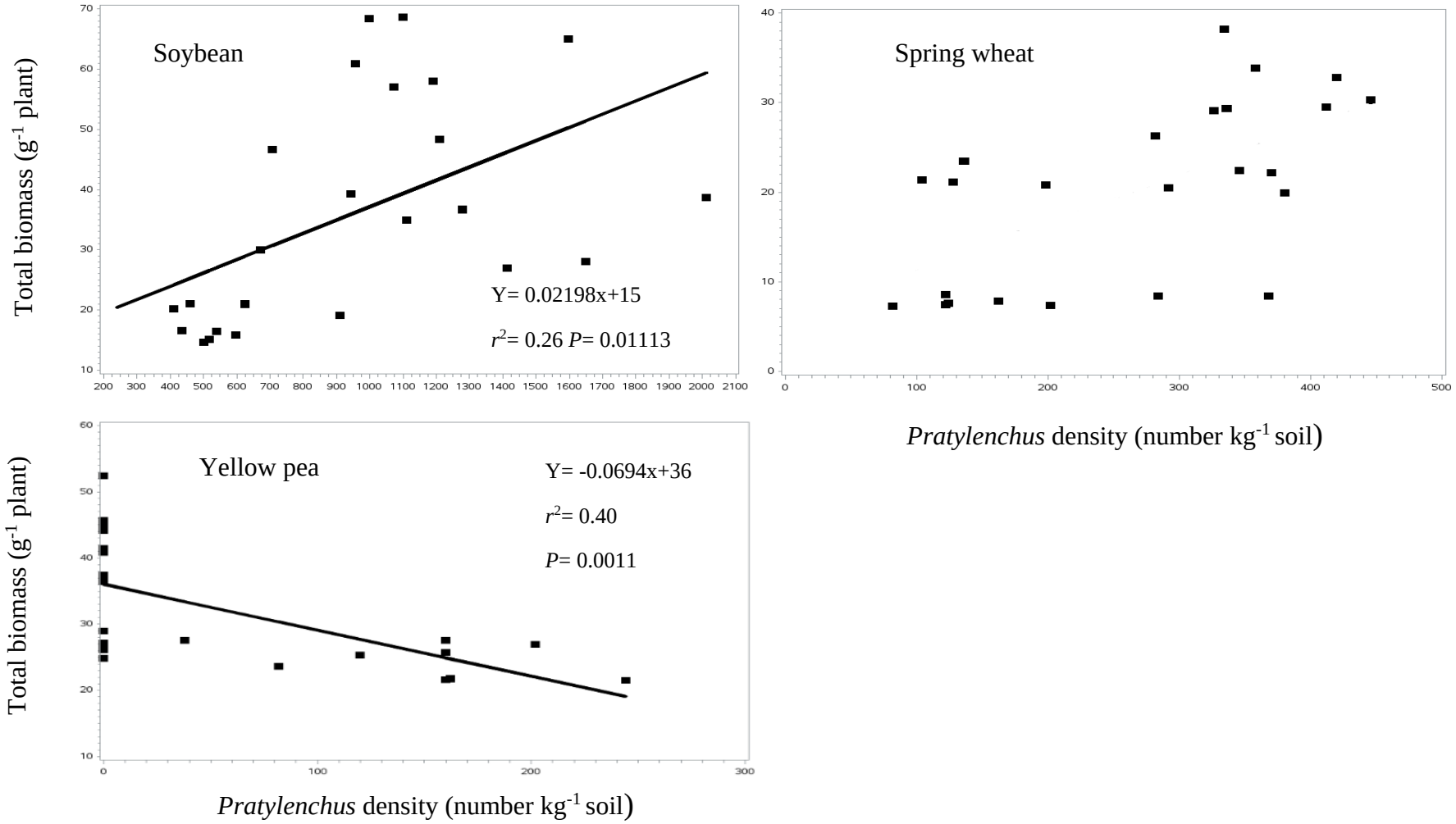


Figure 3.3 Regression of *Pratylenchus* density on selected total biomass of pulse and non-pulse crops following three repeated growth cycles.

3.6.1.5. Effect of increase in *Pratylenchus* spp. densities on crop height with repeated growth cycles

An ANOVA for plant height at the end of three crop growth cycles is presented in Table 3.8. There was a significant interaction between crop and cycle effects on crop height. Both crop and cycle effects were significant.

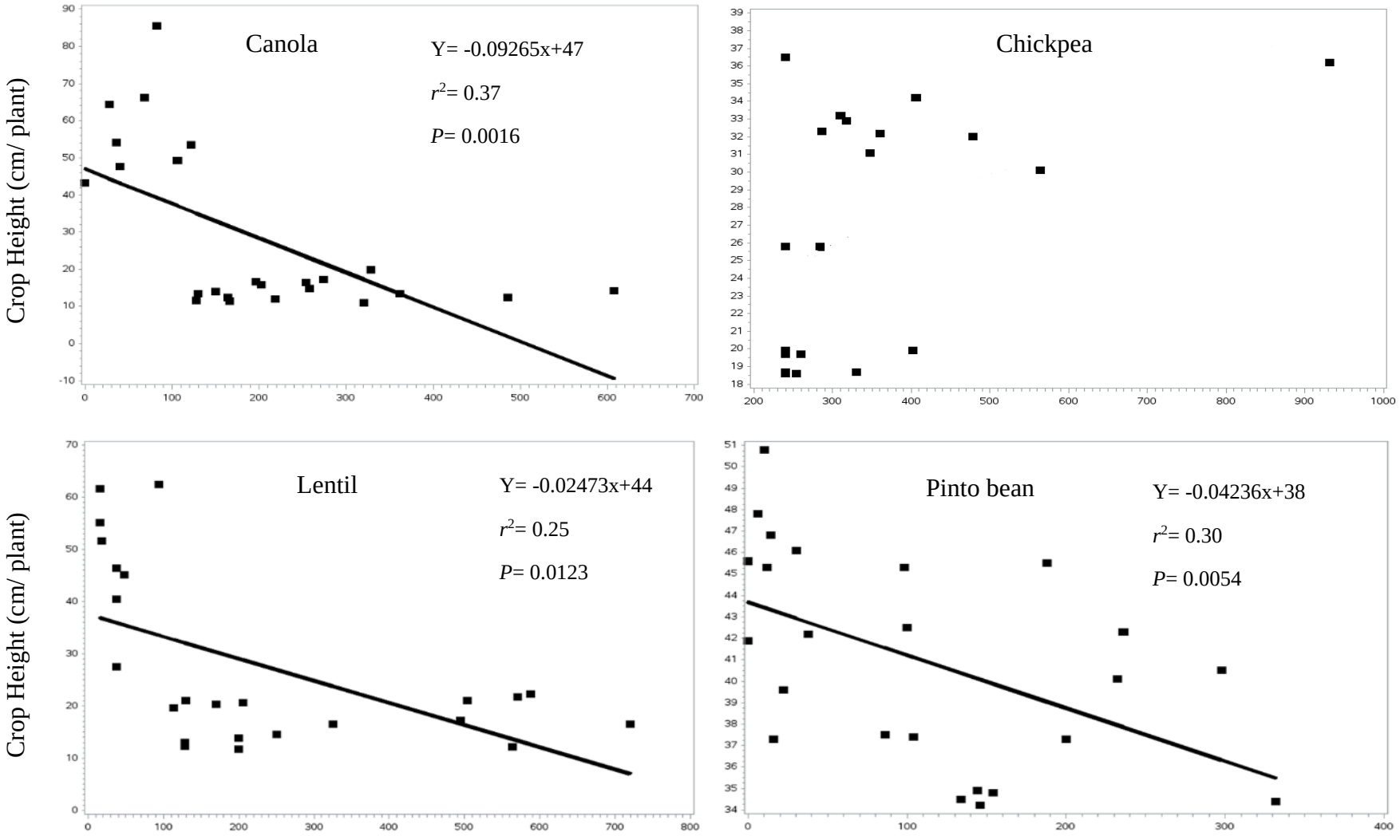
Table 3.8 Analysis of variance for final plant height at the end of each of the three growth cycles of selected pulse and non-pulse crops.

Source of variation	df	MS	*F*	*P*
Crop	6	7050.02	210.11	< .0001
Cycle	2	5143.09	153.28	< .0001
Crop*Cycle	12	558.20	16.64	< .0001

High densities of *Pratylenchus* spp. affected the plant height of crops differently. Regression analyses of the effect of *Pratylenchus* density on crop height are presented below. The results showed that crop height was reduced with increasing *Pratylenchus* densities in most crops such as canola, lentil, pinto bean, spring wheat, and yellow pea. Lentil showed a curvilinear relationship between *Pratylenchus* density and plant height (Fig 3.5). Increase in the density of *Pratylenchus* spp. did not cause a reduction in plant height of chickpea and soybean. There was a positive linear relationship between the height of soybean and *Pratylenchus* density ($P = 0.0285$) and the growth of soybean increased with increasing densities of *Pratylenchus* in the soil following repeated growth cycles.

Table 3.9 Regression analysis for the height of selected pulse and non-pulse crops following three repeated growth cycles

Crop	***Y***	**Intercept SE**	**Slope SE**	r^2	***P***
Canola	-0.0927x + 47	6.24	0.0258	0.37	0.0015
Chickpea	0.0131x + 20	3.33	0.0097	0.12	0.1985
Lentil	-0.0247x + 44	1.38	0.0091	0.25	0.0012
Pinto bean	-0.0424x + 38	5.70	0.0137	0.30	0.0054
Soybean	0.0089x + 18	3.96	0.0038	0.20	0.0285
Spring wheat	-0.0307x + 67	3.25	0.0113	0.25	0.0124
Yellow pea	-0.060x + 72	1.81	0.0196	0.29	0.0063


Canola
Y= -0.09265x+47
r2= 0.37
P= 0.0016
Chickpea
Lentil
Y= -0.02473x+44
r2= 0.25
P= 0.0123
Pinto bean
Y= -0.04236x+38
r2= 0.30
P= 0.0054
Crop Height (cm/ plant)
Crop Height (cm/ plant)
Pratylenchus density (number kg-1 soil)
Pratylenchus density (number kg-1 soil)

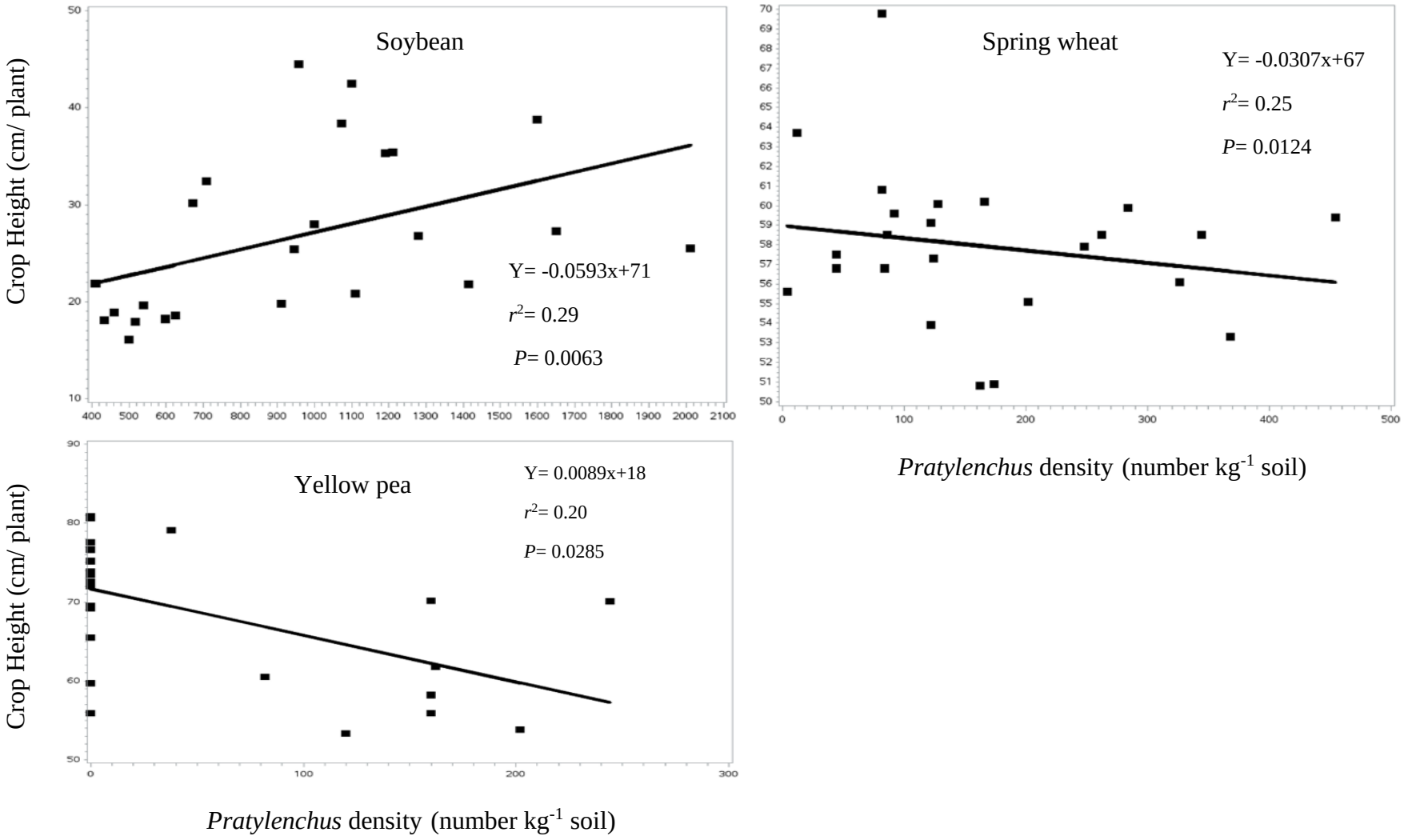


Figure 3.4 Regression of *Pratylenchus* density on plant height of selected pulse and non-pulse crops grown in three repeated growth cycles.

3.6.2 Observation of endomigratory feeding of *Pratylenchus* spp.

Acid fuchsin stained eggs and juveniles of *Pratylenchus* spp. were observed inside the roots of chickpea and soybean recovered at the end of the third cycle (Fig 3.6).

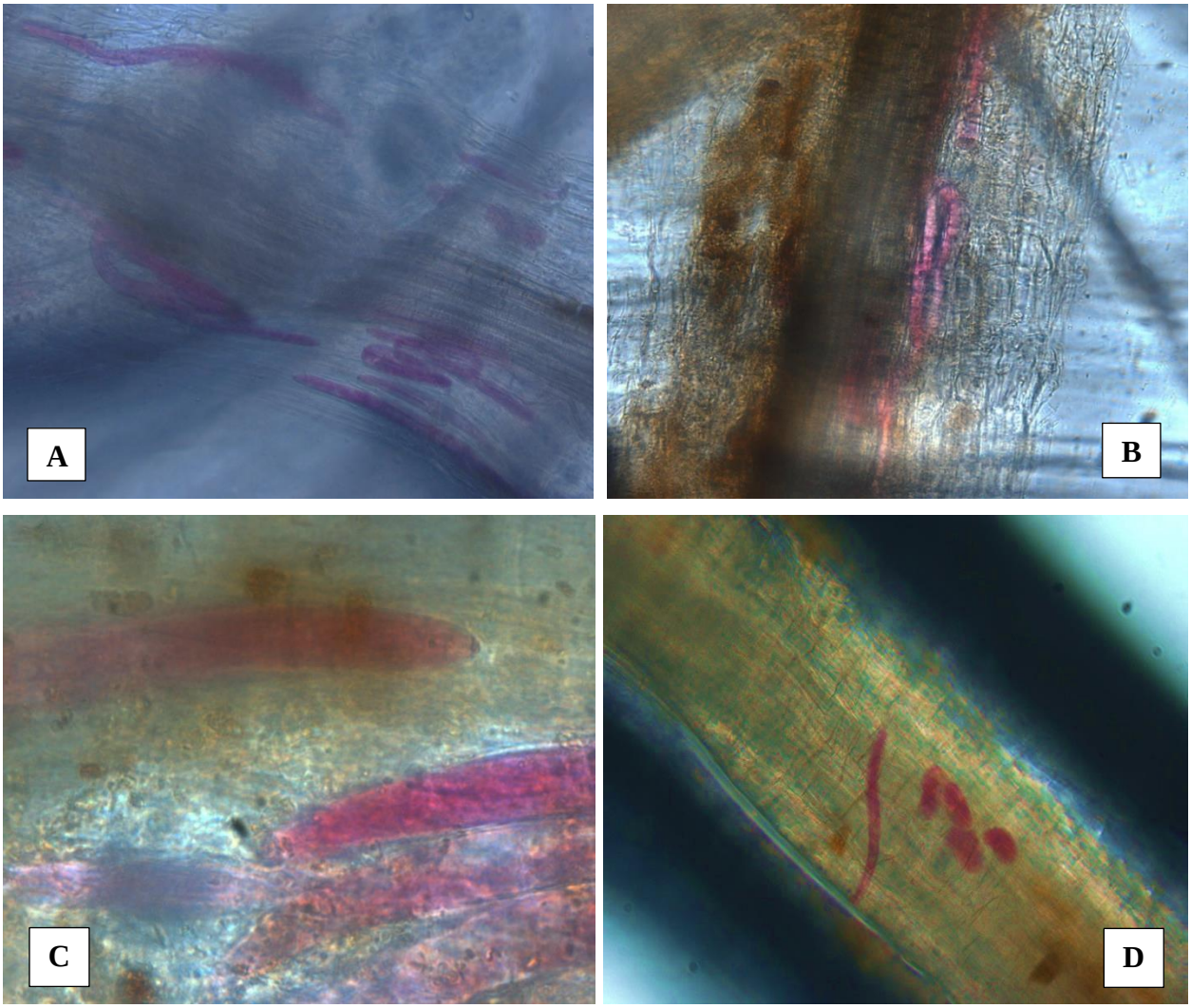


Figure 3.5 *Pratylenchus* (A through D) inside root tissues of chickpea and soybean cleared and stained with acid fuchsin as seen under the microscope (40X). Eggs (D) also observed in a root.

3.6.3 Effects of *Pratylenchus* feeding on roots of host crops

Roots of chickpea parasitized by *Pratylenchus* spp. had brown, necrotic lesions following the third cop growth cycle (Fig 3.7). The other crops examined, including canola, lentil, pinto bean, soybean, and spring wheat did not show any lesions on roots.

Figure 3.6 Brown, necrotic lesions (arrows) on chickpea roots following growth Cycle 3.

3.6.4 *Pratylenchus* species recovered from roots

3.6.4.1 Morphological Identification

Images of adult females of *Pratylenchus* spp. recovered from roots of chickpea, lentil, pinto bean, and soybean recovered at the end of Cycle 3 as observed under a light microscope are provided in Fig 3.8. No male *Pratylenchus* spp. nematodes were found.

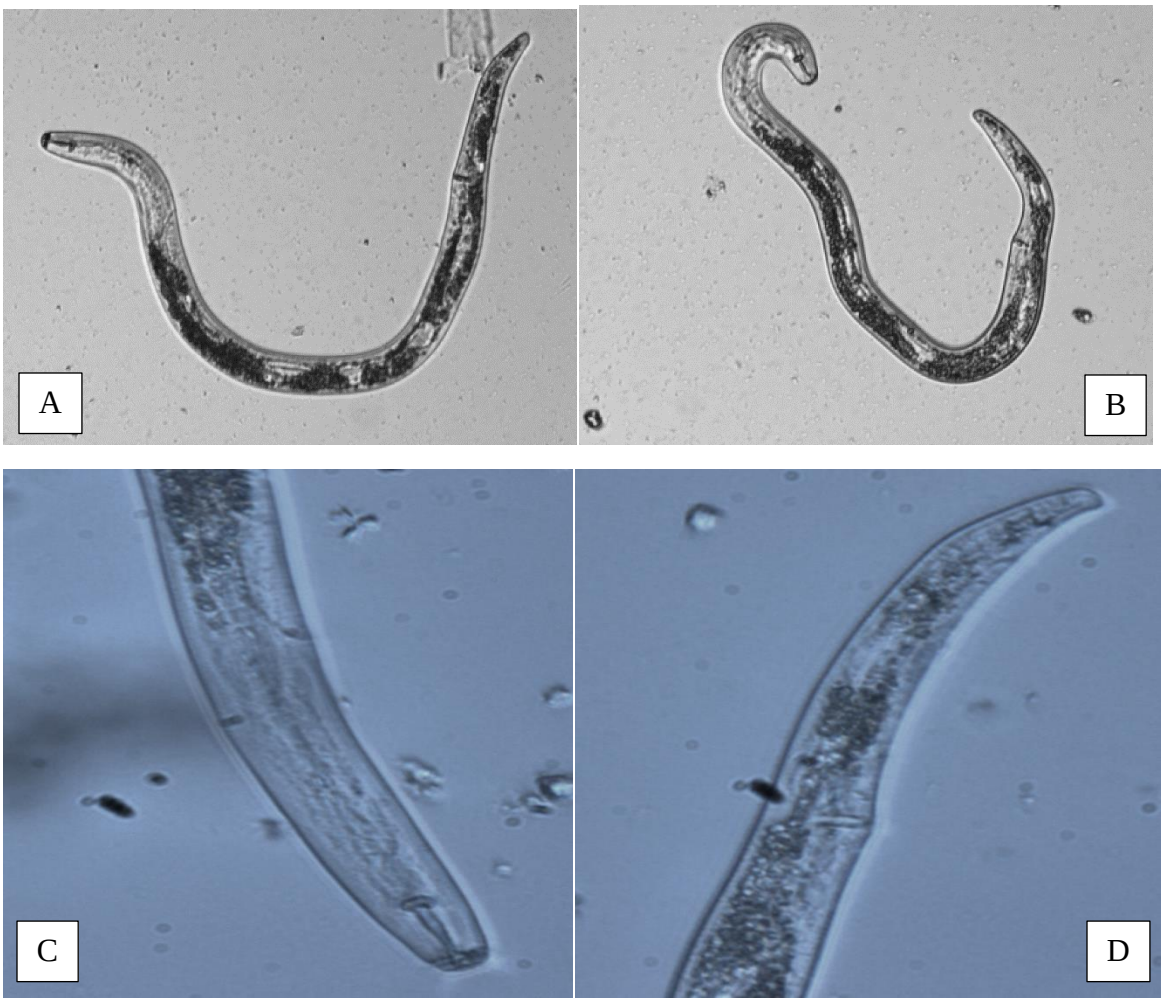


Figure 3.7 Light microscope images of *Pratylenchus* spp. specimens recovered from roots in the third growth cycle A) and B) are images of whole nematode, C) head of *Pratylenchus,* and D) vulva at the posterior end of the nematode (tail)

3.6.4.2 Morphometric measurements

The morphometrics of the adult females of *Pratylenchus* spp. recovered from roots examined following the end of Cycle 1 are presented in Table 3.10. Adult females (*n* = 30) were measured and the morphometric measurements taken on nematode individuals revealed that the species was *P. neglectus*. The measurements provided as a range (mean ± standard deviation) and the mean of each characteristic are also presented. The mean morphometric measurements were as follows: body length, 491.6 ± 51.4 (400-650) µm, stylet length, 17.1 ± 1.2 (14.6-19.5) µm, a, body width/ body length 21.0 ± 1.7 (18.3-25.0) µm, b, body length/ head to the joint of esophagus 5.1 ± 0.4 (4.4-5.9) µm, c, body length/ tail length 23.2 ± 1.4 (20.0-25.0) µm, vulva position 83.0 ±

1.4 (80.4-85.4), distance from the anterior end to the bulb, 96.4 ±11.6 (75.0-115.0) µm. The morphometric measurements taken for the 30 nematodes are provided in Appendix 1.

Table 3.10 Measurement of morphometric characters of adult females of *Pratylenchus* spp. recovered from roots of chickpea, lentil, and soybean at the end of Cycle 1 in comparison with previous reports by Handoo and Golden (1989) and Qui et al. 2016.

Morphometric character	This study	Handoo and Golden 1989	Qui et al. 2013
L	400-575	310-580	399.5-596.6
Vulva %	80.4-85.4	75.5-86.6	79.1-85.0
Stylet	14.6-19.5	15.0-19.0	16.0-19.6
a	18.3-25.0	16.5-32.2	19.2-23.1
b	4.4-5.9	4.9-7.8	4.8-6.9
c	20.0-25.0	13.8-26.8	20.7-25.4
Tail length	18.3-27.0	n/a	n/a
Distance from anterior end to bulb	75-115.0	n/a	n/a

n=30; Measurements are given in µm: mean ± standard deviation (range). *a*- body length/ greatest body width, *b*-body length/head to the joint of esophagus, *c*-body length/tail length, n/a-not available (provided). The ratios *a*, *b* and *c* were calculated according to de Man (Xie 2000).

3.6.4.3 Molecular characterization of *Pratylenchus* spp.

DNA of all individuals studied was successfully amplified using the D3A-D3B universal primer set yielding a band 345 bp in size (Table 3.6). Species-specific primer sets PNEG-D3B, PPEN-D3B and PTHO-D3B were used to detect the presence of *P. neglectus*, *P. penetrans* and *P. thornei* respectively (Al-Banna et al. 2004). DNA samples positive for *P. neglectus* species-specific primers (PNEG-D3B) yielded a single band of 290 bp in size. There were no bands observed using *P. penetrans* and *P. thornei* primer sets. There were no bands observed in the non-template control (NTC) without DNA and the negative control containing *Ditylenchus dispaci* species.

Table 3.11 Molecular characterization of *Pratylenchus* species recovered from roots of chickpea, lentil, and soybean at the end of Growth Cycle 3 using PCR with species-specific primer sets.

Nematode individual	Universal primer	Species-specific primer sets		
	D3A-D3B (345 bp)	PNEG-D3B (290 bp)	PPEN-D3B (278 bp)	PTHO-D3B (288 bp)
PN-CP1	+	+	-	-
PN-CP2	+	+	-	-
PN-CP5	+	+	-	-
PN-CP7	+	+	/	/
PN-CP9	+	+	/	/
PN-CP10	+	-	-	-
PN-L3	+	+	/	/
PN-S4	+	+	-	-
PN-S8	+	-	-	-
PN-S9	+	+	-	-
PN-S10	+	-	-	-
PN-S12	+	+	-	-
PR-13	+	+	/	/
PT-13	+	-	/	/
PT14	+	+	/	/
PT29	+	+	/	/
PT30	+	+	/	/
PT41	+	+	/	/
PT42	+	+	/	/
PT44	+	+	/	/
PT48	+	+	/	/
PT49	+	+	/	/
PT-51	+	+	/	/
PT-55	+	+	/	/

+: Nematode species positive for the primers used, -: negative for the mentioned species, /: not checked, values in parenthesis are expected band sizes for the primer sets used.

The amplified products of *Pratylenchus* spp. using universal primers is shown in Fig. 3.9. *Pratylenchus* spp. yielded a product size of 345 bp with the universal primers. *Pratylenchus spp.* individuals collected from roots of various crops, including chickpea, lentil and soybean produced a band of 290 bp in size, which was the expected band size for *P. neglectus* (Fig. 3.10). DNA of *Pratylenchus* spp. that were sequenced had best matches with *P. neglectus* with identity matches of 98-100% (Table 3.7). Both PCR and sequencing results showed that the *Pratylenchus* spp. recovered from the roots of host crops is *P. neglectus*.

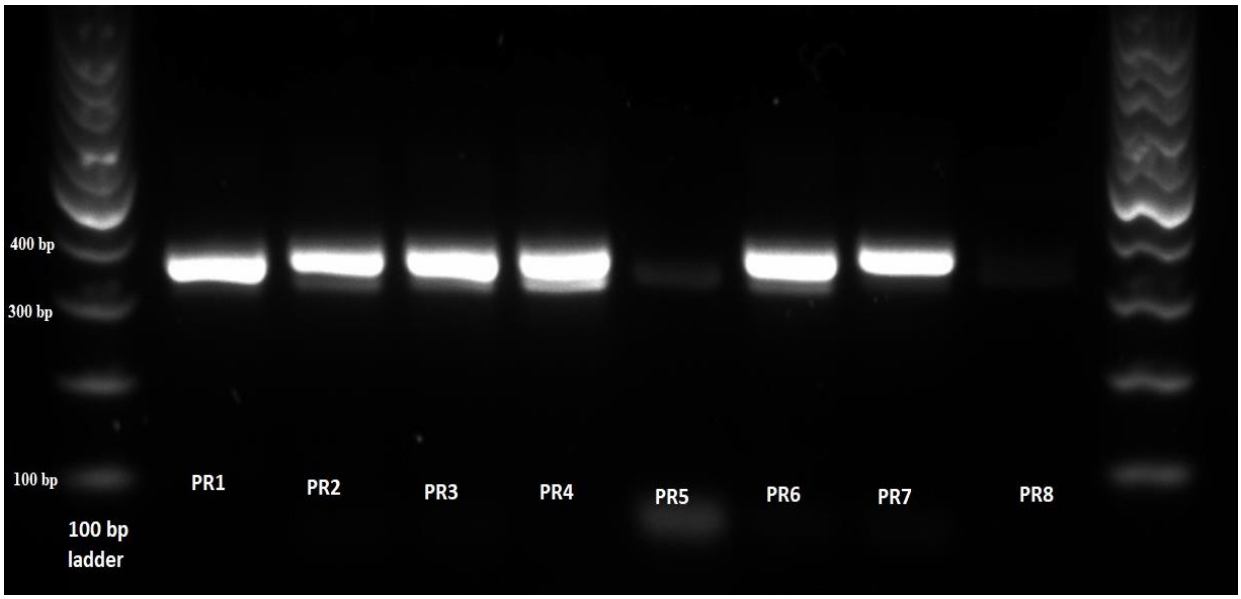


Figure 3.8 Agarose gel electrophoresis pattern of PCR products of genomic DNA of *Pratylenchus* individuals amplified with universal primers (D3A-D3B) (Al-Banna et al. 1997). A product of 345 bp indicates the presence of *Pratylenchus* spp. Lane 1 and 10 are 100 bp DNA ladders, lanes 2 to 9 (PR1, PR2, PR3, PR4, PR5, PR6, PR7, PR8) are *Pratylenchus* individuals recovered from roots of chickpea and soybean at the end of Growth Cycle 3.

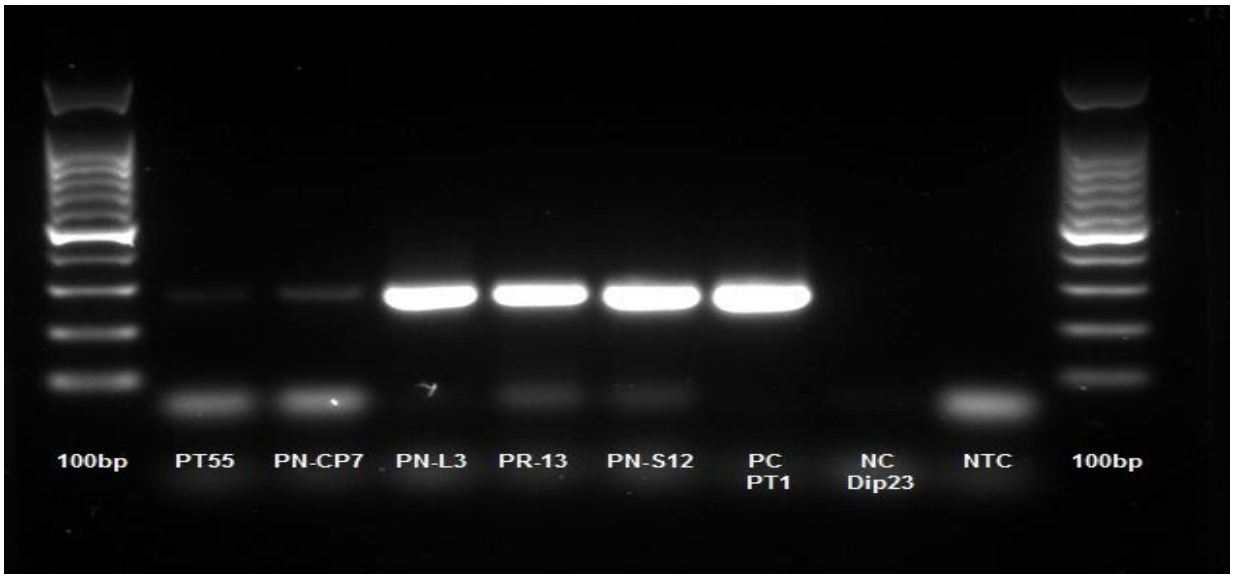


Figure 3.9 Agarose gel electrophoresis pattern of PCR products of *Pratylenchus* spp. amplified using species-specific primers (PNEG-D3B) (Al-Banna et al. 2004). Lanes: 100 bp DNA ladder, *Pratylenchus* individuals (PT55, PN-CP7, PN-L3, PR-13, PN-S12) recovered from roots of chickpea, lentil and soybean at the end of Growth Cycle 3, positive control for *P. neglectus* (PC-PT1), negative control with DNA of *Ditylenchus dispaci* (NC-Dip23), non-template control (NTC) with water and a 100 bp DNA ladder.

Table 3.12 Molecular identification of *Pratylenchus* species recovered from roots of canola, chickpea, lentil, pinto bean, soybean, and spring wheat at the end of Growth Cycle 3 using DNA sequencing of the D3 region based on identity match to the NCBI Blast database.

Nematode individual	Primer set	Size (345 bp)	Match	Accession	Identity (%)	E-value	No. of accessions for *P. neglectus*
PT1	D3A-D3B	+	*P. neglectus*	EU130854	100	$3e^{-110}$	27 (100) 5 (99) 2 (98)
PF-2	D3A-D3B	+	*P. neglectus*	EU130854	100	$2e^{-100}$	22 (100) 2 (99) 3 (98)
PF-3	D3A-D3B	+	*P. neglectus*	KM580548	100	$2e^{-85}$	28 (100) 2 (99) 3 (98)
PF-4	D3A-D3B	+	*P. neglectus*	KM580548	98	$7e^{-89}$	22 (98)
PF-5	D3A-D3B	+	*P. neglectus*	JX261951	100	$3e^{-95}$	24 (100) 2 (99) 2 (98)
PF-7	D3A-D3B	+	*P. neglectus*	KM580548	100	$3e^{-98}$	22 (100) 2 (99) 1 (98)
PF-8	D2A-D3B	+	*P. neglectus*	EU130854	100	$1e^{-98}$	22 (100) 5 (99) 1 (98)
PF-9	D2A-D3B	+	*P. neglectus*	EU130854	100	$1e^{-98}$	21 (100) 4 (99) 1 (98)
PF-10	D2A-D3B	+	*P. neglectus*	JX261951	100	$4e^{-98}$	23 (100) 3 (99) 2 (98)
PF-11	D2A-D3B	+	*P. neglectus*	JX261951	99	$1e^{-92}$	31 (99) 2 (98)
PF-12	D2A-D3B	+	*P. neglectus*	AJ545023	99	$3e^{-79}$	17 (99)
PN-Ca7	D3A-D3B	+	*P. neglectus*	KM580548	99	$4e^{-96}$	22 (99) 7 (98)
PN-CP1	D3A-D3B	+	*P. neglectus*	EU130855	100	$2e^{-109}$	30 (100) 5 (99)
PN-CP3	D3A-D3B	+	*P. neglectus*	EU130854	100	$5e^{-95}$	23 (100) 2 (99) 2 (98)

Nematode individual	Primer set	Size (345 bp)	Match	Accession	Identity (%)	E-value	No. of accessions for *P. neglectus*
PN-CP7	D3A-D3B	+	*P. neglectus*	EU130854	100	$5e^{-94}$	22 (100) 2 (99) 1 (98)
PN-CP10	D3A-D3B	+	*P. neglectus*	EU130854	100	$1e^{-97}$	22 (100) 4 (99)
PN-L1	D3A-D3B	+	*P. neglectus*	AJ545026	98	$2e^{-92}$	21 (98)
PN-L3	D3A-D3B	+	*P. neglectus*	KM585048	100	$7e^{-102}$	22 (100) 2 (99)
PN-S4	D3A-D3B	+	*P. neglectus*	EU130854	100	$3e^{-94}$	23 (100) 2 (99) 3 (98)
PN-S8	D3A-D3B	+	*P. neglectus*	AJ545023	100	$6e^{-100}$	22 (100) 2 (99) 3 (98)
PN-S10	D3A-D3B	+	*P. neglectus*	KM580548	100	$2e^{-96}$	23 (100) 2 (99) 1 (98)
PN-S12	D3A-D3B	+	*P. neglectus*	EU130854	100	$3e^{-95}$	26 (100) 2 (99) 2 (98)
PN-W2	D3A-D3B	+	*P. neglectus*	KM580548	99	$4e^{-99}$	21 (100) 2 (99)
PN-W4	D3A-D3B	+	*P. neglectus*	AJ545023	98	$7e^{-96}$	24 (98)
PR2	D3A-D3B	+	*P. neglectus*	JX261951	100	$4e^{-93}$	23 (100) 3 (99) 2 (98)
PR7	D3A-D3B	+	*P. neglectus*	EU130854	100	$4e^{-87}$	45 (100) 5 (99) 6 (98
PR8	D3A-D3B	+	*P. neglectus*	AJ545023	100	$2e^{-101}$	22 (100) 2 (99) 3 (98)
PR11	D3A-D3B	+	*P. neglectus*	EU130854	100	$2e^{-9}$	37 (100) 5 (99) 2 (98)
PR13	D3A-D3B	+	*P. neglectus*	AJ545025	100	$2e^{-95}$	23 (100) 2 (99) 3 (98)
PT7	D3A-D3B	+	*P. neglectus*	KM580548	100	$7e^{-97}$	22 (100) 2 (99) 1 (98)

Nematode individual	Primer set	Size (345 bp)	Match	Accession	Identity (%)	E-value	No. of accessions for *P. neglectus*
PT-13	D3A-D3B	+	*P. neglectus*	EU130854	99	$5e^{-95}$	25 (99) 3 (98)
PT14	D3A-D3B	+	*P. neglectus*	EU130854	100	$8e^{-94}$	21 (100) 2 (99) 3 (98)
PT21	D3A-D3B	+	*P. neglectus*	AJ545013	100	$6e^{-87}$	21 (100) 2 (99)
PT24	D3A-D3B	+	*P. neglectus*	KM580548	99	$1e^{-99}$	22 (100) 3 (99) 1 (98)
PT27	D3A-D3B	+	*P. neglectus*	EU130854	99.5	$1e^{-92}$	22 (99) 3 (98)
PT37	D3A-D3B	+	*P. neglectus*	AJ545024	100	$3e^{-97}$	22 (100) 2 (99) 1 (98)
PT41	D3A-D3B	+	*P. neglectus*	EU130855	100	$1e^{-109}$	30 (100) 16 (99) 2 (98)
PT54	D3A-D3B	+	*P. neglectus*	EU130854	100	$1e^{-95}$	43 (100) 2 (99)
PT55	D3A-D3B	+	*P. neglectus*	AJ545023	100	$3e^{-95}$	23 (100) 2 (99) 13 (98)

3.7 Discussion

3.7.1 Effect of *Pratylenchus* spp. on crop performance

In this study, the effect of increasing *Pratylenchus* density on the performance of canola, chickpea, lentil, pinto bean, soybean, spring wheat, and yellow pea was assessed in over three successive growth cycles of the same plant species in the same soil. Final plant height, above-ground biomass and root biomass of the selected crops were measured at the end of each cycle. Linear regression analysis was used to determine the relationship between *Pratylenchus* density and crop performance. Regression analyses showed that there were differences in the response of crops to increasing *Pratylenchus* density across the cycles.

Plant biomass of some of the crops including canola, lentil, pinto bean, spring wheat, and yellow pea was reduced by high *Pratylenchus* populations across the three repeated cycles. The results suggested that at higher densities of *Pratylenchus* spp., the performance of canola, lentil, pinto bean, spring wheat, and yellow pea was reduced. For canola, pinto bean and spring wheat, the results were not surprising because these crops were susceptible to *Pratylenchus*. Although soybean was the most preferred host to *Pratylenchus* spp., both its above-ground and root biomass were not reduced by greater densities of the nematode. Similarly, plant biomass of chickpea was not reduced by *Pratylenchus* spp. across the cycles. Regression analyses showed that plant biomass of soybean increased with increasing densities of *Pratylenchus*.

Plant height of chickpea and soybean was not reduced at greater *Pratylenchus* density despite the crops being good hosts for the nematode. The plant height of soybean increased with increasing densities of *Pratylenchus* in the soil. There was evidence of a reduction in plant height of canola, lentil, pinto bean, spring wheat and yellow pea at higher densities of *Pratylenchus* spp. Riga et al. (2008) reported that *P. neglectus* significantly reduced plant height and yield of dryland peas and lentils. Pulses are among the suitable hosts of *Pratylenchus* spp. and hence susceptible to a reduction in growth at high nematode densities. Correlation analyses agreed with regression analyses; however, they are not discussed here, and they are provided in the Appendix. Correlation analyses were not discussed because they are less powerful and redundant compared to regression analyses. The regression results showed that soybean could withstand high nematode densities and maintain their normal growth. Growing soybean can lead to an increase in densities of *Pratylenchus* spp. in the soil and these nematode populations may cause a reduction in the growth of other crops grown after soybean. The varieties of chickpea and soybean used in this study may have been tolerant of high nematode populations.

Since there were no crops grown in nematode-free soil (control) included in this study, there is a possibility that repeated cycles of the same crop may have affected growth. Differences in soil nutrition between cycles may have also caused differences in crop growth. Soils used in the last cycle may have retained more nutrients after the application of fertilizer than the ones used in the first cycle. The use of control crops would have helped in determining how plant growth was affected by the high densities of *Pratylenchus*. Crop species may significantly differ in how they

respond to high densities of plant-parasitic nematodes. Apart from plant-parasitic nematodes, plant biomass may also be affected by other factors such as fertility, moisture, and pH.

Pratylenchus spp. are known to cause a reduction in the growth of crops, plant biomass accumulation and the number of fibrous roots. Fatemy et al. (2006), reported that plants including canola were heavily infected by *Pratylenchus* spp. had stunted growth and also developed dark lesions on roots. Riga et al. (2008) reported that *P. neglectus* impacted the growth and yield of dryland peas (cvs. Columbian and Small sieve) and lentils (cvs. Red Chief and Pardina) in Idaho and plant height was reduced by between 50-70% compared to controls. The dryland pea samples they collected from fields infested with *Pratylenchus* spp. had means of 551 and 2,178 nematodes per gram of dry root. The lentil samples had means between 279 and 987 nematodes per gram of root. Their findings point out that growth and yield reduction occurred at high populations of *Pratylenchus* spp.

Griffin (1991) also reported the reduction of growth by *P. neglectus* in alfalfa under growth chamber conditions. In their study, at initial nematode populations of 500-5,000, plant survival was not affected by *P. neglectus*. Plants had 10-40% mortality, with initial populations of 5,000 and 10,000 nematodes. Some of the *P. neglectus* populations also reduced shoot dry weights at initial populations of 10,000. Root growth in inoculated plants was less than that of uninoculated ones. Root dry weight was reduced at initial populations (P_i) of 1,000 and above. Similar to the report by Riga et al. (2008), their study revealed that crop performance was mostly impacted at higher initial populations of *Pratylenchus*. In this study, the initial nematode populations across all the cycles were very low (120 nematodes kg^{-1} soil) as compared to those reported by Griffin (1991). *Pratylenchus neglectus* to suppressed shoot growth of potato in a greenhouse study after 3 weeks at P_i of 1,540 kg^{-1} soil. Shoot weight was reduced at 6 weeks by a P_i of 1,540 kg^{-1} soil. The number of marketable and total tubers was also suppressed at initial populations of 1,884 *P. neglectus* kg^{-1} soil. These reports show that *P. neglectus* can significantly impact crop growth, especially when the soil is highly infested with the nematode.

No visible symptoms were observed in the above-ground plant parts of the studied plant species. It is also unknown if the high densities of *Pratylenchus* recovered in soybean affected nodulation of the crop. The soybean crop was not inoculated. All the crops were fertilized at two and four weeks of growth. Symptoms of *Pratylenchus* spp. damage include; chlorosis, stunted

growth and brown lesions on roots (Perry and Moens 2013). Once nematodes parasitize plant roots, their normal function is interrupted, causing them to become less efficient in drawing water and nutrients. This ultimately implicates growth and plants become stunted and chlorotic. Riga et al. (2008) reported that *P. neglectus* caused stunted growth, chlorosis and wilting in dryland peas and lentils.

Reduction in plant-growth parameters such as plant biomass and yield is the most widely reported effect resulting from the host-parasite infection (López-Gómez and Verdejo-Lucas 2018). *Pratylenchus* species affect growth in susceptible plant hosts resulting in stunting and delay in maturity (Duncan and Moens 2013). However, with very low or moderate population densities of *Pratylenchus* species, reduction in crop growth may not be observed (Saeed et al. 1998). Much crop damage is expected to occur if susceptible crops are grown annually, resulting in a build-up of soil populations (Smiley 2015). Growers in the prairies can prevent crop damage and yield losses by incorporating non-host crops of *P. neglectus* in their rotation cycles.

3.7.2 Endomigratory feeding of *Pratylenchus* spp.

Nematodes were found both inside roots and, in the soil, and this is characteristic of *Pratylenchus* species. *Pratylenchus* spp. inhabit both the soil and roots during their life cycle. As previously mentioned, more *Pratylenchus* spp. were recovered from the roots of host plants for some of the crops than from the soil in the second and third cycles. This indicates that the nematodes were migrating from the soil to feed on inside roots. Fatemy et al. (2006) also reported a similar observation in which greater numbers of *Pratylenchus* spp. occurred in roots than in the soil. *Pratylenchus* species are endomigratory parasites and they spend most of their life cycle inside root tissues of host plants (Williams et al. 2002). Additionally, they can migrate from the roots to the soil and vice versa (Jones and Fosu-Nyarko 2014). The ability of these nematodes to migrate explains why some nematodes were found in roots and others in the soil. *Pratylenchus* spp. most commonly feed inside roots (endoparasitically), but juveniles occasionally feed outside roots (ectoparasitically) on root hairs. However, all the life stages of *Pratylenchus* spp. can be recovered from both roots and soil (Perry and Moens 2013).

Eggs and juveniles of *Pratylenchus* spp. were observed inside the roots of chickpea and soybean after staining with acid fuchsin. This observation indicated the endoparasitic nature of the *Pratylenchus* spp. As was observed on the images of stained root tissues taken, eggs may be laid inside the roots of host plants. *Pratylenchus* females may also deposit their eggs in the soil (Jones and Fosu-Nyarko 2014). Eggs of *Pratylenchus* spp. can be found in clusters because these nematodes exhibit gregarious behavior (Perry and Moens 2013). The juveniles observed inside the roots of chickpea and soybean occurred in small clusters. *Pratylenchus* spp. attract each other once root penetration is initiated, leading to the formation of clusters of nematodes inside host roots (Perry and Moens 2013).

3.7.3 Effects of *Pratylenchus* feeding on roots of host crops

In our study, we observed brown lesions on some of the chickpea roots, and these symptoms are characteristic of *Pratylenchus* species attack. *Pratylenchus* spp. form lesions on young roots, thereby inhibiting root development. Chickpea was susceptible to *P. neglectus* after hence the possibility of this crop to develop root lesions. Vanstone (2006) reported similar observations of orange-brown lesions on chickpea roots infected by *P. neglectus*. Similar observations on chickpea roots parasitized by *Pratylenchus* spp. were also made by McKay (2019), who reported the presence of brown lesions on roots of chickpea. Other microorganisms may have also aggravated lesions observed on chickpea in the soil, such as fungi and bacteria. Roots of most of the crops examined in this study did not show any symptoms of attack by the root-lesion nematode. I speculate that the absence of lesions on the roots of most crops was due to low initial and final populations of the nematodes in the soil. *Pratylenchus* species cause brown to reddish necrotic lesions on infected root tissues (Duncan and Moens 2013), and the intensity of these lesions depends on the level of nematode infestation (Kinloch 1998). With high populations, there is a possibility of increased root damage as nematodes compete for sites to parasitize. Yan et al. (2016) observed light brown lesions lateral roots of wheat at average populations of 20 to 24 nematodes per gram of root. *Pratylenchus neglectus* caused dark lesions on highly infested roots of canola (Fatemy et al. 2006). Root lesions created by *Pratylenchus* spp. create entry channels for microorganisms such as bacteria and fungi (Perry and Moens 2013).

Moreover, roots will rot because of secondary infection by these pathogens (Agrios 2005). Obvious symptoms may not be observed on plants if the *Pratylenchus* populations are low to

moderate (Davis and MacGuidwin 2000). If roots are undamaged, it is an indication that food resources are available for the nematode population and that there are new sites for the nematodes to infect (Ferris 1985).

3.7.4 Species identification of *Pratylenchus* recovered from roots of crop plants

Pratylenchus spp. recovered from roots of host crops were identified using morphological characters of adult females. However, molecular techniques were used to confirm the results of the morphological analyses. Characteristics of all the nematodes identified as *Pratylenchus* were a robust stylet, an esophagus overlapping the intestines ventrally and a slit-like vulva at the posterior end. *Pratylenchus* have few morphological characters that can be used to distinguish these species, and these include the number of lip annuli, presence or absence of spermatheca, number of lines in the lateral field, presence or absence of males and shape of the tail (Loof 1978, Handoo and Golden 1989). Some of these diagnostic features require the use of scanning electron microscopy (SEM) because the dissecting microscope may not show detailed characters. The identification of *Pratylenchus* species is generally difficult because of intra-specific variations as well as overlapping morphometric characters occurring among them. The morphometric characters measured in this study include; body length, stylet length, body width, distance from anterior end to bulb, vulva position, tail length and a, b, c ratios corresponding to De Man indices (L, s, a, b, c, V) (De Man 1876, De Man 1880). In our study, 30 individual nematodes were used for morphological identification and taking measurements of morphometric characters to cover a wide range of variation among the species. Since populations of *Pratylenchus* may occur mixtures of species, measurements should be taken on multiple nematode specimens (e.g., 20-30) before a nematode description is provided to prevent misidentification (Fortuner 1984, Perry and Moens 2006, Mahran et al. 2010). The number of nematode individuals used in our study matched this standard of using multiple specimens to target accurate identification. The morphometrics reported in this study lay in the same range of the original descriptions recorded for *P. neglectus* by Handoo and Golden (1989).

Body length measurements obtained in the current study were in the same range as those found by Qiu et al. (2016). The vulva position (80.4-85.4%) was within the range reported by

Handoo and Golden (1989). The stylet lengths reported by Qiu et al. (2016) and in this study were longer than those recorded by Wu et al. (2013). The a-value in this study was more than the range reported by Wu et al. (2013) and Qiu et al. (2016). However, the ranges for the a-ratio recorded were less than the descriptions by Handoo and Golden (1989). The b-values reported here were less than those reported for isolates from China (Qiu et al. 2016). The c-ratios of specimens used in this study were less than those recorded by Handoo and Golden (1989) but they were in the same ranges as those reported by Qiu et al. (2016). Differences in morphometric measurements may be due to environmental conditions or alterations to nematode morphology that occur during the preparation of specimens. Morphological identification, when used alone, may not be reliable due to the alterations to nematode morphology.

No males were observed among the nematodes identified and this is characteristic of *P. neglectus* as males are rare in this species (Handoo and Golden 1989). *P. neglectus* reproduces by parthenogenesis (Castillo and Vovlas 2007). Mahran et al. (2010) found only three males among the populations of *P. neglectus* that were present in potato fields in Manitoba. About half of the known *Pratylenchus* spp. reproduce by parthenogenesis. Among such species, males are absent or rare and the females lack sperm in the spermatheca (Perry and Moens 2013). However, males are common in other species such as *P. penetrans and P. vulnus* which exhibit amphimictic reproduction (Castillo and Vovlas 2007). The identification of *Pratylenchus* species is usually restricted to the morphology and morphometric characters of adult females. However, when present, males can also be used in identification and taking measurements of morphometric characters (Roman and Hirschmann 1969).

The *Pratylenchus* spp. recovered from roots of host crops was identified as *P. neglectus* based on PCR with species-specific primers and sequencing of the D3 region. Prior to tests with species-specific primers, nematode DNA samples were amplified using the D3A-D3B primer sets and all the samples tested positive yielding a product of 345 bp. According to Al-Banna et al. (1997), this was the expected band size for *Pratylenchus* species when amplified with these universal primers. Species-specific primers for *P. neglectus*, *P. penetrans* and *P. thornei* were used to detect the *Pratylenchus* spp. recovered from the roots of the various crops. The species-specific primers used in this study mainly target a specific region of the D3 expansion segment of the 26S rDNA (Al-Banna et al. 2004). The PCR assays yielded the expected product sizes (290 bp) for *P.*

neglectus as according to (Al-Banna et al. 2004). The tests conducted with these species-specific primers were consistent and most of the DNA samples were positive for primer sets specific for *P. neglectus*. Mahran et al. (2010) also identified *P. neglectus* from potato fields in Manitoba using species-specific primers. The negative control containing DNA of *Ditylenchus dispaci* did not yield any band indicating that the primers used only amplified the targeted D3 expansion region of the DNA molecule. There were no bands observed for the species-specific primers for *P. penetrans* and *P. thornei*, indicating the absence of these species in the *Pratylenchus* collections studied. If *Pratylenchus* spp. present other than *P. neglectus* were present, they would have been detected using species-specific primers. Species-specific primers enable amplification of only a single band for the species present (Al-Banna et al. 2004).

A few samples that were negative for PNEG-D3B primers specific for *P. neglectus* were sequenced, including other samples positive for these primers. Forty DNA samples that were sequenced showed higher similarity to *P. neglectus* in the GenBank than any other species. The identity matches of these samples to *P. neglectus* ranged from 98-100%. The sequences matched with eight accessions for *P. neglectus* all of which were from published journal articles; hence, they were reliable. There was no evidence for the presence of other *Pratylenchus* spp. among the nematodes recovered from the roots of host crops. The absence of males and morphometric results obtained in this study agree with those obtained from molecular assays that the *Pratylenchus* spp. recovered is *P. neglectus*. Molecular techniques are useful for confirming results obtained from morphological identification (Handoo et al. 2008). These methods supplement each other, thereby leading to reliable conclusions.

3.8 Conclusions

High population densities of *Pratylenchus* spp. can significantly reduce the performance of host crops. However, with low populations of nematodes, plant growth parameters (e.g., plant biomass and plant height) may not be implicated. Additionally, symptoms may not be visible when the population densities of the nematodes are not very high. *P. neglectus* was the species recovered from roots of host crops including canola, chickpea, pinto bean, soybean, and spring wheat. Results from morphometric measurements, PCR assays and sequencing, were consistent and pointed out to the *Pratylenchus* spp. identity being *P. neglectus*. The observation of lesions on roots of

chickpea indicates the potential of these nematodes to negatively impact suitable host crops if the nematodes occur in high population densities. There is also a possibility that these nematodes will affect crop performance with repeated growth cycles of suitable hosts. Growing suitable hosts such chickpea and soybean successively can result in a build-up of high *Pratylenchus* populations, which will, in turn, affect the current crops or the crops in rotation. It is, therefore, important for growers to plan rotation cycles wisely to prevent crop damage and yield losses to *Pratylenchus* spp. Growers can include lentil and yellow pea between cycles of suitable hosts to

3.9 Literature cited

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4 OVERALL SYNTHESIS

Pratylenchus neglectus was first reported in Manitoba and Ontario in 1955 and was later reported in Ontario and Alberta in 1982 and 2007 respectively (Baker 1956, Olthof et al. 1982). Yu (2008) reported that *P. neglectus* was among the 12 *Pratylenchus* species recorded in the Canadian National Collection of Nematodes (CNCN) database with the nematode occurring in Alberta, British Columbia, Manitoba, and Ontario. In 2010, *P. neglectus* was identified in potato fields in Manitoba by Mahran et al. (2010) during their investigation on *Pratylenchus* species prevalent in Manitoba. A few years later, Forge et al. (2015) reported the occurrence of *P. neglectus* in Alberta, and the nematode was associated with potato and wheat. More recently, *P. neglectus* has been identified in 20 of 93 pulse fields surveyed for plant-parasitic nematodes in the prairies between 2014 and 2016 (Gouvea-Pereira 2018). This survey revealed the occurrence and distribution of *Pratylenchus* species in some of the fields, but there was no knowledge as to what crops the nematodes are potentially parasitizing. It is well known that the presence of suitable hosts for these nematodes in farming regions influences their distribution as well as population growth (Duncan and Moens 2006).

To address the uncertainty of the host preferences for *P. neglectus*, the objective of this thesis was to assess the host status of the major field crops that are being grown in the Canadian Prairies under growth chamber conditions. The research also investigated the effects of selected crops on population densities of *P. neglectus* when grown in subsequent cycles. The thesis included morphological and molecular identification of the nematode species as well as conducting a host screening study on seven different pulse and non-pulse crops. The ability of *P. neglectus* to infect and reproduce on a crop was determined by the use of the nematode reproductive factor (R_f), which is a very common approach used for host suitability tests (Zhang and Schmitt 1994, Taylor et al. 2000, Hajihassani et al. 2016). *Pratylenchus neglectus* is an emerging plant-parasitic nematode in the Canadian Prairies, and as such there was a need to identify the hosts for this nematode considering that it was observed in high population densities sufficient to cause crop damage (Gouvea-Pereira 2018).

Naturally infested soils collected from a field previously reported having *Pratylenchus* species were used as nematode inoculum. Since naturally infested soils were used, it was imperative to identify the *Pratylenchus* species present before the experiment to ensure accurate

information is relayed. Moreover, accurate identification of root-lesion nematodes is useful for research and also serves as an important foundational step when developing management strategies (Carneiro et al. 2017). This study provides useful information on the hosts of *P. neglectus* most of which have not yet been reported here in Canada. Our results also reveal how likely populations of *P. neglectus* are to change with repeated growth cycles of hosts and non-host crops. Additionally, the study reports the effects of *Pratylenchus* populations on crop performance, the endomigratory nature of the nematodes as well as the species identification of nematodes recovered from roots of host crops.

4.1 Important findings

The *Pratylenchus* spp. present in the Canadian Prairies was identified as *P. neglectus* based on morphometric measurements, PCR with species-specific primers, and sequencing of the D3 region of the rDNA. *P. neglectus* was expected to be the species present based on previous reports by Mahran et al. (2010) and Gouvea-Pereira (2018) who also identified the nematode in Manitoba and other prairie provinces. It was hypothesized that canola and spring wheat would be good hosts for *P. neglectus* based on studies reported by other researchers (Taylor et al. 2000, Fatemy et al. 2006). In Manitoba, potato (cv. Russet Burbank) was shown not to be a host for *P. neglectus* and for that reason it was suspected that the nematode is parasitizing other crops in rotation with potato, that is, canola and wheat (Mahran et al. 2010). Contrasting results to Mahran et al. (2010), in Alberta, (Forge et al. 2015), found that potato was a good host for *P. neglectus*. Other crops being grown in the prairie provinces have not been assessed for suitability as hosts for *P. neglectus*.

The most important findings in this research were that most of the major field crops being grown in the prairies are suitable hosts for *P. neglectus*. Out of the seven crops screened, five of them are susceptible to the root-lesion nematode. The results from this study revealed that that canola, chickpea, pinto bean, soybean, and spring wheat are hosts for *P. neglectus*. Soybean was the most preferred host among the crops evaluated. *Pratylenchus neglectus* has been previously reported on these crops in other agricultural regions such as Australia, Pacific Northwest (Taylor et al. 2000, Smiley et al. 2005, Thompson et al. 2008, Vanstone et al. 2008, Wu et al. 2013). Overall, *P. neglectus* reproduced more on soybean followed by chickpea, canola, pinto bean, and spring wheat. To the best of my knowledge, this is the first report of *P. neglectus* infecting these

crops here in Canada. This study reports soybean as a new host for the nematode. Lentil exhibited poor host ability for the nematode. *Pratylenchus neglectus* least infected yellow pea. As hypothesized, canola and spring wheat were hosts for *P. neglectus*. However, wheat had lower final populations of *P. neglectus* in the three trials of the first cycle, but the nematode populations increased when the crop was grown in two other subsequent cycles. This suggests the possibility that the wheat cultivar studied may not be a very good host for *P. neglectus*, or that the crop was only susceptible to higher initial soil populations of the nematode. Based on the current findings, it should be noted that *P. neglectus* may not be a big threat to soybean production, although high densities of the nematode were recovered from the crop. However, high densities of *Pratylenchus* in the soil may affect the next crop grown after soybean.

It was not surprising that high populations of *P. neglectus* were observed on chickpea because the crop is a good host for the nematode (Taylor et al. 2000, Smiley et al. 2014). The most interesting and least anticipated findings were that of soybean being a good host for *P. neglectus*. There is limited literature reporting soybean as a host for *P. neglectus* but not for other species such as *P. brachyurus, P. scribneri* and *P. thornei.* However, these results bring an addition of soybean to the list of hosts susceptible to *P. neglectus* in Canada. The final populations of *P. neglectus* observed on the soybean crop are potentially detrimental as they were above the threshold levels. Growers producing soybean in fields infested with *P. neglectus* are likely to encounter high populations at the end of the crop season. Crop damage and yield losses may occur in the next susceptible crop grown after soybean if the *Pratylenchus* populations are not managed. Another possibility is that if other hosts for *P. neglectus* (e.g., canola, wheat) are preceded by soybean in rotations, they are prone to substantially high parasitism and yield reduction. I, therefore, suggest that growers should ensure these crops do not succeed each other in rotations because they may cause significant population build-up of this nematode.

With canola and spring wheat hosting *P. neglectus*, growers in the prairies need to consider the diagnosis of soil and root samples as an initiative plan towards the management of this nematode because these crops occupy large acreage. Where canola, soybean, and spring wheat are grown in rotation, farmers may want to include a non-host crop in between these crops to suppress *Pratylenchus* populations. Based on findings from this study and a report by Forge et al. (2015), bean growers in the prairies should also be on the lookout for *P. neglectus* to prevent any crop

damage because the crop is known to be a good host. *P. neglectus* was identified in chickpea and yellow pea fields in a survey by Gouvea-Pereira (2018), but in this study, the nematode only reproduced on chickpea and not yellow pea. This shows that it is important to assess the host status of crops because different crops affect final nematode populations differently as was observed in this study. Population densities of root-lesion nematodes readily increase with the presence of a suitable host.

Lentil was a poor host for *P. neglectus* in this study, but since there are conflicting reports on the host suitability of this crop there is need to examine other cultivars (Taylor et al. 2000, Castillo and Vovlas 2007, Riga et al. 2008). Moreover, *P. neglectus* failed to survive and reproduce on yellow pea leading to a decline in the final populations. These discoveries are important for growers producing pulse crops in the prairie provinces because lentils and peas constitute most of the pulse production and exports. Canada is among the leading producers and exporters of pulses in the world, striking an export value of 4.1 billion in 2016 (Agriculture and Agri-Food Canada 2017) and better these crops not be implicated by *Pratylenchus* species.

Another question that needed to be answered was whether *P. neglectus* populations had an impact of on growth of each of the crops and if there would be any symptoms on above and below-ground plant parts. We expected negative correlation between nematode populations and growth parameters of soybean as it had the highest *Pratylenchus* abundance. However, this was not the case and the crop had a low positive correlation suggesting that soybean growth was not affected by high *Pratylenchus* density. There were no symptoms observed on the above-ground parts of all the crops. Moreover, although soybean had the highest final nematode populations, no lesions were observed on the roots of this crop. If plant-parasitic nematodes occur at low to medium populations, they may not cause any symptoms or damage to the crop (Davis and MacGuidwin 2000). Farmers must know that symptoms caused by *Pratylenchus* species may not be obvious; hence can be mistaken for nutrient deficiency or moisture stress (Castillo and Vovlas 2007). Growers may need to get a nematology expert to analyze their samples. Characteristic symptoms of *Pratylenchus* species on infected crops which farmers can check for are brown, necrotic lesions on roots. Where yellowish/chlorotic patchy areas occur in a field, farmers can get root and soil samples for nematode analysis as a precautionary measure.

The findings of this study were that growth parameters, that is, plant height, above-ground and root biomass of canola, lentil, pinto bean, spring wheat and yellow pea, were reduced by high *Pratylenchus* density. There was a moderately negative correlation between nematode densities and plant biomass for these crops. With repeated crop growth cycle, impact on growth may be observed provided that the nematode soil numbers increase with each growing season and that the crops grown are suitable hosts. As expected, nematode populations increased during the 8-wk growth cycle on suitable hosts. This growth period was enough for the nematodes to complete at least 1-2 reproduction cycles since *Pratylenchus* species complete their life cycle in 3-8 weeks (Warner 2006, Castillo and Vovlas 2007).

It is key to know the host ranges of *Pratylenchus* species because such knowledge is useful for the development of management strategies (Castillo and Vovlas 2007). Findings from this study provide growers with fundamental knowledge which they can use in selecting crops to grow and in assessing the need for nematode diagnosis in their fields. Poor hosts or resistant cultivars can be used as break crops to reduce nematode populations in the soil. From these findings, it is suggested that growers can incorporate yellow pea in rotation with other crops to reduce population densities of *P. neglectus* in infested fields. At the absence of suitable plant hosts, populations of *P. neglectus* declined and this shows that fallow periods can be helpful to growers as nematode populations may be significantly reduced. In the prairies, fallowing is practised to preserve moisture for the next growing season and control weeds and as such can be used to target nematode populations. Fallowing and growing resistant crops or cultivars are considered the most effective control methods for managing root-lesion nematodes (Taylor et al. 2000, Kratochvil et al. 2004). However, fallowing may not be feasible to practice in the prairies due to its effect on land degradation. The effectiveness of non-host crops in control of *Pratylenchus* species has been equated to the use of nematicides (Taylor et al. 1999).

4.2 Interesting findings

It was interesting that although the experiment was started with very low initial densities of *P. neglectus* in the soil, the final populations significantly increased on suitable hosts. This suggests that populations of *Pratylenchus* species can readily multiply if suitable plant hosts are available. Soybean had the highest populations of *P. neglectus* but surprisingly there were no

lesions observed on the roots of the crop. However, it is unknown if the nematode affected nodule formation on soybean. In the very first cycle, the absence of lesions on most of the roots of crops examined was attributed to the low initial nematode densities in this cycle. In some cases, *Pratylenchus* species may not cause visible symptoms when they occur in low numbers. Soybean is often associated with other *Pratylenchus* spp. (e.g., *P. brachyurus*, *P. scribneri*) rather than *P. neglectus* (Majić et al. 2008, Lima et al. 2017) hence, these findings will add more insight into the host status of soybean to root-lesion nematodes. Thompson et al. (2008) listed soybean as highly resistant to *P. neglectus*, however, results from this study contradict their report, and this, therefore, ignites the need to assess the host status of other cultivars. Although soybean had the highest abundance of *Pratylenchus*, it was interesting to find out that the performance of this crop was not reduced, rather crop growth increased with increasing nematode populations. We expected soybean to have its height and plant biomass compromised as nematode densities increased. The *Pratylenchus* spp. did not prefer lentil and yellow pea as hosts and it was interesting that regression analyses showed that plant height, above-ground and root biomasses of these crops were reduced as nematode density increased.

This study also investigated how fast and to what extent would population densities of *P. neglectus* increase with successive crop growth cycles. Since the host status of the different crops had been assessed, it was probable that populations of *P. neglectus* would increase readily on suitable host crops previously identified in the host screening experiment. High nematode numbers were observed with repeated cycles of canola, chickpea, pinto bean, soybean and spring wheat which were all good hosts of the nematode. As was observed in this study, susceptible crops allow the nematode to express high reproductive fitness (Castillo and Vovlas 2007). Population growth of plant-parasitic nematodes is influenced by resistance or tolerance of host plants (Roberts 2002). Nematode multiplication was highest on soybean across all three growth cycles. Yellow pea has the potential to inhibit both survival and multiplication of *P. neglectus* hence can be used in infested fields. *P. neglectus* can become a big problem in the prairies if susceptible crops are continually grown and if there are no proper management strategies in place.

4.3 Challenges and improvements

The major challenge in this study was culturing the nematode inoculum for use in the host screening study and further analyses. Initially, the plan was to mass culture *P. neglectus* on carrot

discs to obtain inoculum. This method has been successfully used by other researchers in culturing *P. neglectus* as well as *P. thornei* by incubating inoculated carrot discs at temperatures between 20-25ºC (Taylor 2000, Vanstone and Russ 2001). Before this study, callused carrot discs were inoculated with about 100 single nematodes and were incubated at 25ºC, aiming at nematode multiplication. Unfortunately, the nematode cultures failed and there was no reproduction observed on the carrot discs and the nematodes declined in numbers instead. Verdejo-Lucas and Pinochet (1992) cultured *P. neglectus* on carrot discs, but the nematode had the lowest reproduction compared to other species. It was postulated that carrot is probably not a good host for *P. neglectus* or that the nematode occurs in different physiological races; hence, infectivity and reproductive capacity will be influenced by the species present (Griffin 1991). As an alternative, canola callus was used to culture the nematode as previously intended. However, this was not a successful endeavor. The canola plants grew very slowly; hence, it was time-consuming, and there was not enough plant material for massive nematode cultures. With the knowledge that wheat and canola are good hosts for *P. neglectus* as reported elsewhere (Taylor et al. 2000, Fatemy et al. 2006), another plan was to culture the nematode in pots planted to these crops. Plants were inoculated with pure cultures of *Pratylenchus* species. After assessing the final *Pratylenchus* densities recovered from roots plus soil at the end of the culturing period, it was observed that no nematode multiplication had occurred. Finally, naturally infested soils were collected from a field in Alberta in which *Pratylenchus* spp. were previously identified. It is, therefore, important that future studies aim at finding other ways of mass culturing *P. neglectus* such as using soybean and bean crops. As shown in this study, soybean was a good host for the nematode and had the potential to promote the build-up of higher *Pratylenchus* densities. Olthof (1979) recommended the use of bean to rear *P. neglectus* and it is speculated that this might work to solve the current problem. An improvement to these scientific studies would have been to include control crops grown in nematode-free soils in addition to crops grown in *Pratylenchus*-infested soils to assess and compare their differences in growth or performance at high nematode densities. There were a few DNA samples that showed double bands when using universal primers and it was suspected that little contamination occurred during DNA extraction. This was solved by careful maintenance of aseptic conditions and samples that showed streaking of double bands the appropriate fragment was excised from the agarose gel. Samples were then purified using QIAquick Gel Extraction Kit (QIAGEN, Germany). Another problem encountered during molecular tests using PCR was low DNA concentration in some of

the samples. The protocol was modified by using 40 µl buffer elution buffer instead of 80 µl to increase concentration. For purified PCR samples that had a DNA concentration of less than 20 ng μl^{-1}, the purified products were used as templates in a re-amplification procedure following the protocol for *P. neglectus* assay reported earlier but with 25 cycles instead of 35. The resultant PCR product was re-purified using the QIAquick PCR Purification Kit to yield a concentration higher than 20 ng μl^{-1}. The targeted 20 ng μl^{-1} was to meet the sequencing requirements by Macrogen (Maryland, USA).

4.4 Summary of conclusions

This being the first report of *P. neglectus* infecting major pulse and non-pulse crops grown in Canada, there is a need to investigate management practices that might be useful in fields infested with this root-lesion nematode. Implementation of nematode management tactics begins with accurate nematode identification and relating species to their suitable hosts. Scanning electron microscopy can be used to replace light microscopy as it is known to give reliable and detailed nematode morphological information (Inserra et al. 2005). This will be particularly useful in situations where *Pratylenchus* species occur in mixed populations. Growers can use rotation with poor/non-host crops (e.g., pea, safflower) to reduce population densities.

With repeated growth cycles of the same crops, populations of *P. neglectus* increased from cycle to cycle in suitable hosts whilst in poor/ non-host crops; there were drastic reductions in populations. These findings are important for growers, especially those with fields infested with *P. neglectus*. It is a call for growers to ensure that crops that are susceptible to *P. neglectus* are not planted subsequently to prevent a build-up of damaging populations. Survival and reproduction of *Pratylenchus* spp. are compromised in the absence of crop hosts, therefore, where possible farmers can maintain fields free of crop residues, volunteer crops, and weeds. Weeds susceptible to *P. neglectus* include; wild oat (*Avena sterilis* L.), common wild oat (*Avena fatua* L.), barnyard grass (*Echinochloa crusgalli* L.) and Palmer amaranth (*Amaranthus palmeri* S. Wats). Based on findings from this study and literature, it can be noted that nematode numbers in the soil will decline due to starvation and desiccation. There is a need for further study on the prevalence of *P. neglectus* in soybean growing areas and/or fields in Canada.

4.5 Future recommendations

Field experiments should validate the findings from the host screening study conducted under growth chamber conditions. Field experiments give a better understanding and reveal useful information on how different environmental conditions may affect nematode population densities. Additionally, conducting field studies is useful because the experiment can be done on a much larger scale giving a good representation of what is likely to happen to the crops and nematodes in the field. So far, a single cultivar has been tested for each of the crops and more information is still needed to know how different varieties respond to *P. neglectus* infestations. In general, the host preference for *P. neglectus* is quite controversial and host screening must be done on local crop cultivars (Smiley and Nicol 2009). The variations in host preferences are possible because *P. neglectus* occurs in various physiological races (Griffin 1991, Mahran et al. 2010). There is a need for assessing the responses of other varieties of chickpea, canola, pinto bean, soybean, and spring wheat to *P. neglectus* since these crops were found to be hosts for this nematode. This will drive us to the knowledge about the performance of the different crop cultivars grown in Canada, and growers can then select cultivars that suit their needs. More fields, in which the suitable host crops are being grown may need to be tested for the occurrence and distribution of *Pratylenchus* spp. Future studies can also investigate the effects of *P. neglectus* on nodulation and root growth of soybean. Weed species can also be assessed for host suitability to *P. neglectus* as some species may promote nematode survival and reproduction during the absence of crop hosts. Growers should avoid frequent growing of susceptible crops in fields infested with these nematodes.

Moreover, susceptible crops should not be grown successively to prevent an increase of *P. neglectus* to damaging levels. Much crop damage can occur when *Pratylenchus* species interact with other microbial pathogens such as fungi to form disease complexes (Castillo and Vovlas 2007). There are some reports of *P. neglectus* interacting synergistically with the fungus *Verticillium dahliae* causing the potato early dying (PED) syndrome (Hafez et al. 1999). Future studies can also investigate if the *P. neglectus* populations present in the prairies can interact with other microorganisms such as *Verticilllium* species in canola and other crops.

Future studies can also seek to resolve methods for mass culturing of *P. neglectus* inoculum. In summary, this study reveals that the *Pratylenchus* spp. prevailing in the Canadian Prairies is *P.*

neglectus, and information about its host preferences that has been lacking has also been revealed. We believe these findings will be especially useful to pulse and non-pulse growers in western Canada, and through this knowledge, they can effectively plan on their cropping systems. Farmers can grow yellow pea or include it in rotations if they want to reduce the population densities of *Pratylenchus* species. This work reveals not only new findings but also leaves unanswered questions for researchers and growers; hence future studies must aim at bringing answers that help to understand better threats, challenges, and losses associated with *P. neglectus*.

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5 Appendix

Appendix 5.1 Morphometric measurements of *Pratylenchus* species

Appendix Table 1 Morphometric measurements of *Pratylenchus* species. Lengths are in µm.

	Morphometric character								
No.	**Body length (L)**	**Distance to Vulva**	**Vulva %**	**Stylet**	**a**	**Distance from anterior**	**b**	**Tail length**	**c**
1	425	355	83.5	15	20	80	5.31	20	21
2	435	370	85.01	18	22	85	5.12	20	21.8
3	425	350	82.4	17.08	20	85	5	20	21.3
4	500	415	83	17.08	21.96	110	4.55	22	22.7
5	470	390	83	17.08	25	95	4.95	18.8	25
6	460	385	83.7	17	22	90	5.11	21	21.9
7	430	360	83.7	15	21	80	5.38	20	21.5
8	480	410	85.4	17.08	19.5	107	4.49	20	24
9	650	530	81.5	19.52	24.4	120	5.42	27	24.1
10	460	380	82.6	17.08	21	93	4.95	20	23
11	445	370	83.1	18	20	80	5.56	20	22.3
12	400	320	82.5	15	20	75	5.33	20	20
13	465	380	81.7	19	21	85	5.47	20	23.3
14	460	370	80.4	15.86	21	80	5.75	21	21.9
15	525	440	83.8	18.3	20.74	98	5.36	25	21
16	545	450	82.6	18.3	21.96	115	4.73	25	21.8
17	480	410	85.4	15	21	100	4.8	20	24
18	530	440	83	17.1	22	110	4.82	23	23
19	530	445	84	17.1	21	110	4.82	22	24.1
20	480	400	83.3	15.9	19.52	105	4.57	19.5	24.6
21	520	435	83.7	17.1	21.96	100	5.2	22	23.6
22	490	395	80.6	17.08	19.52	98	5	19.5	25.1
23	475	385	81.1	17.08	18.3	90	5.28	20	23.8
24	550	465	84.5	18.3	24.4	95.2	5.78	21.96	25
25	455	370	81.3	14.64	18.3	100	4.55	18.3	24.9
26	490	410	83.7	17.08	19.8	90	5.44	20	24.5
27	485	400	82.5	17.08	19.52	110	4.41	19.5	24.9
28	500	425	85	18.3	20.74	95	5.26	21	23.8
29	555	470	84.7	18.3	23.18	107	5.19	25	22.2
30	575	485	84.3	18.3	23.18	105	5.48	25	23

Appendix 5.2 Pearson's correlation for the effect of increasing *Pratylenchus* density on crop performance of selected pulse and non-pulse crops grown in the Canadian Prairies

Pearson's correlation for the effect of increasing *Pratylenchus* density on growth of selected pulse and non-pulse crops following three repeated growth cycles. The plant growth parameters assessed include; plant height, above-ground biomass, root biomass, and total plant biomass.

Appendix Table 2 Pearson's correlation for crop height of selected pulse and non-pulse crops following three repeated growth cycles

Crop	**Correlation (*r*)**	***P***
Canola	-0.60818	0.0016
Chickpea	0.35176	0.1985
Lentil	-0.50288	0.0123
Pinto bean	-0.54994	0.005
Soybean	0.44711	0.0285
Spring wheat	-0.50197	0.0124
Yellow pea	-0.54128	0.0063

Appendix Table 3 Pearson's correlation for above-ground plant biomass following three repeated growth cycles

Crop	**Correlation (*r*)**	***P***
Canola	-0.64428	0.0007
Chickpea	0.02682	0.9244
Lentil	-0.6731	0.0003
Pinto bean	-0.5372	0.0068
Soybean	0.4471	0.0285
Spring wheat	-0.3265	0.1194
Yellow pea	-0.6360	0.0008

Appendix Table 4 Pearson's correlation for root biomass of selected pulse and non-pulse crops following three repeated growth cycles

Crop	**Correlation (*r*)**	***P***
Canola	-0.65653	0.0005
Chickpea	0.07494	0.7907
Lentil	-0.62871	0.0010
Pinto bean	-0.30335	0.1496
Soybean	0.61262	0.0015
Spring wheat	-0.40186	0.0516
Yellow pea	-0.20942	0.3260

Appendix Table 5 Pearson's correlation for total biomass following three repeated growth cycles

Crop	**Correlation (*r*)**	***P***
Canola	-0.65606	0.0005
Chickpea	0.05987	0.8321
Lentil	-0.71006	0.0001
Pinto bean	-0.5284	0.0080
Soybean	0.50757	0.0113
Spring wheat	-0.36111	0.0830
Yellow pea	-0.62491	0.0011