Survey of Plant-Parasitic Nematodes in Pulse Crop Fields of the Canadian Prairies

by

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ABSTRACT

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The current distribution of economically important plant parasitic nematodes is relatively unknown in the Canadian Prairies for pulse crops. The majority of previous surveys were done several decades ago and may now be suspect as a result of recent molecular identification methods; as has been the case with the quarantine pest *Ditylenchus dipsaci* (Kühn) Filipjev. The nematode species *Ditylenchus dipsaci* can constrain export markets for economically important crops, such as peas. However, a more recent study has revealed that previous identifications of *D. dipsaci* in yellow pea (*Pisum sativum* L.) exports have actually been the non-quarantine species *Ditylenchus weischeri* Chizhov, Borisov & Subbotin. To further our understanding of these issues, a survey was conducted in three major pulse crops growing regions of Canada (Alberta, Saskatchewan and Manitoba) to determine the occurrence of plant-parasitic nematodes associated with pea, chickpea (*Cicer arietinum* L.) and lentil (*Lens culinaris* L.). A total of 465 plant and soil samples of pea, chickpeas, lentils and creeping thistle (*Cirsium arven*se L.), plants from 93 fields were analysed. Recovered nematodes were identified to genus by morphological features. Molecular analysis by species-specific PCR, PCR-RFLP and sequencing of the ITS (ITS1 + 5.8S + ITS2) of the rRNA gene were also used to identify recovered nematodes to species. Twenty genera of plant-parasitic nematodes were recovered from the soil and (or) the plants of pea, chickpea, lentil and creeping thistle, including *Anguina*, *Aphelenchoides*, *Ditylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Merlinius*, *Paraphelenchus*, *Paratylenchus*, *Pratylenchus*, *Subanguina*, *Paratrichodorus*, *Tylenchorhynchus* and *Xiphinema*.

Several fields had high density of plant-parasitic nematodes belonging to the genera *Ditylenchus, Pratylenchus, Paratylenchus, Helicotylenchus* and *Tylenchorhynchus,* which could be potentially problematic for the crops studied or for crops that are grown in rotation. Molecular analysis results indicate the recovery of *D. weischeri*, *D. dipsaci*, *Pratylenchus neglectus* (Rensch) Filipjev & Schuurmans-Stekhoven, *Xiphinema rivesi* Dalmasso and *Paratylenchus nanus* Cobb. *Ditylenchus weischeri,* a parasite of thistles and not crops, was recovered from 22 fields across Alberta, Saskatchewan and Manitoba. *D. dipsaci* was recovered from pods of one yellow pea field in Manitoba. These results confirm the high prevalence of *D. weischeri* on creeping thistle in pulse fields and the near absence of *D. dipsaci*.

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I dedicate this thesis to my son, Thatcher Leopold.

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LIST OF ABBREVIATIONS

AB	Alberta
AFLP	Amplified Fragment Length Polymorphism
bp	base pairs
ºC	degrees Celsius
cm	Centimeter
dH2O	distilled water
DNA	Deoxyribonucleic Acid
e.g.	Exempli Gratia
ed.	Editor(s)
F	Fahrenheit
Fig.	Figure
g	Gravity
g	Gram
hsp90	Heat shock protein
hr	Hour(s)
i.d.	internal diameter
i.e.	Id Est
ITS	Internal Transcribed Spacer
J2	Second-stage nematode
J3	Third-stage nematode
J4	Fourth-stage nematode
kg	Kilogram

MB	Manitoba
min	Minute(s)
mL	Milliliter
mm	Millimetre
µm	Micrometer
PCR	Polymerase Chain Reaction
RAPD	Random Amplification of Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
R.O.	reverse osmosis
rDNA	Ribosomal DNA
rRNA	Ribosomal RNA
sec	Second(s)
SK	Saskatchewan
% w/v	Percent weight/volume
5.8S	gene Non-coding component of the large ribosomal subunit
18S	gene Small nuclear ribosomal subunit
28S	gene Large nuclear ribosomal subunit

1. GENERAL INTRODUCTION

1.1 Nematode Biology

Nematodes are a group of biologically diverse roundworms belonging to the phylum Nematoda or Nemata (De Ley and Blaxter 2002). Most are microscopic, measuring less than 1 mm long. They are the most abundant animals on Earth and have successfully adapted to almost every habitat (Decraemer and Hunt 2006). Most species live freely in soil, maintaining the health of the ecosystem by participating in a wide range of important processes, including decomposing organic matter, cycling nutrients, and biologically controlling insects and other microorganisms (Ingham et al. 1985; Bongers and Bongers 1998; Gugino et al. 2009). Some species, however, can be parasitic to animals (Gugino et al. 2009) and plants.

Plant-parasitic nematodes use an anatomical apparatus called stylet (or odontostylet in some groups) to puncture plant tissues and absorb nutrients (Nicol 2002). Most plant-parasitic nematodes feed on roots (Holterman et al. 2017); however some are able to feed on stems, leaves, and flowers (Nicol 2002). Plant-parasitic nematodes have evolved key traits that give them the ability to act as parasites on nearly all plant species (Bianco and Maizels 1989; Blaxter et al. 1998).

1.1.1 Survival Strategies

The success of nematodes can also be attributed to the incredible survival strategies acquired throughout their evolution. When environmental conditions are hostile, some nematode species can enter a dormancy state, where their metabolic process works at a minimal level (Perry at al. 2013). They remain in this state until conditions are favorable for their development once again (Perry at al. 2013). There are two types of dormancy: quiescence and diapause (Perry at al. 2013). Quiescence is a spontaneous process and can be triggered by unpredictable environmental

changes such as drought, low or high temperatures, or lack of oxygen (Perry at al. 2013). Quiescence can be stage-specific (obligate quiescence) when it happens as part of the nematode life cycle, e.g. before juveniles hatch from their eggs, or it can be non-stage-specific (facultative quiescence) (Perry at al. 2013). Diapause is a programmed development arrest and precedes environmental changes (Hand et al. 2016). Contrary to quiescence, when the nematode enters in a diapause state, it will only return when endogenous physiological prerequisites are met (Perry et al. 2013; Hand et al. 2016). In other words, even if environmental conditions are favorable for its development, the nematode will not leave diapause stage until intrinsic required conditions are satisfied (Hand et al. 2016).

Nematode survival strategies can allow them to survive for incredibly long periods of time. For instance, Shatilovich et al. (2018) recently showed the ability of two nematode species to survive 30,000-40,000 years in cryobiosis in permafrost deposit samples taken from the Arctic.

1.1.2 Feeding Strategies

Plant parasitic nematodes have three principal feeding-strategies: they can be ectoparasites, endoparasites, or semi-endoparasites (Decraemer and Hunt 2006). Ectoparasites have a long stylet that can penetrate plant tissue without the nematode entering the plant (Hussey and Williamson 1998). Most ectoparasitic nematodes are migratory, i.e., they can move freely in the soil and plant and can infect more than one plant throughout their life cycles (Hussey and Williamson 1998). A few ectoparasites lose their motility after feeding starts and are called semi-endoparasites (Lambert and Bekal 2002; Decraemer and Hunt 2006). Semi-endoparasites partially penetrate the host; in other words, their anterior is inside the roots while their posterior is in the soil matrix (Decraemer and Hunt 2006). After establishing a feeding site, they become swollen and lose their ability to move (Lambert and Bekal 2002; Decraemer and Hunt 2006). Endoparasites usually have smaller

stylets, and they use it to penetrate plants and move between the plant tissues until they find a suitable place to feed (Ferris and Ferris 1998). Migratory-endoparasites maintain their mobility throughout their life cycle; typically they feed and reproduce inside the host, but they can also migrate to another host (Lambert and Bekal 2002). Sedentary-endoparasites comprise a highly specialized group of plant-parasitic nematodes; they form fixed feeding sites and lose their motility after feeding starts (Hussey and Williamson 1998). Physical changes take place as females go through somatic muscle atrophy and assume a body-sac-like shape (Lambert and Bekal 2002; Decraemer and Hunt 2006). Some nematodes have more than one feeding strategy. For example, species of *Helicotylenchus* and *Hoplolaimus* can be semi-endoparasitic or migratory ecto-endoparasitic, according to the host plant (Perry and Moens 2011).

1.1.3 Life Cycle

Typically, a plant-parasitic nematode goes through six development stages during its life cycle (Decraemer and Hunt 2013). The earlier development stages are called juvenile stages. Juvenile stages start with the egg (first-stage juvenile), and then they go through four molts (four juvenile stages separated by a molt) before they reach maturity (adult stage) (Decraemer and Hunt 2013). In Longidoridae and Trichodoridae the first stage happens inside the egg (releasing the first-stage juvenile), while in Tylenchomorpha, two stages happen inside the egg, and one involves a molt (releasing second-stage juvenile) (Decraemer and Hunt 2013). Therefore, most plant-parasitic nematodes hatch from the egg as infective second-stage juveniles. In this stage, the nematode will live in soil without feeding until finding a host (Escobar et al. 2015). The life cycle can take only days or over a year, depending upon the species and environmental conditions (Ferris and Ferris 1998).

1.1.4 Plant Damage

Plant parasitic nematodes can cause damage in all plant parts, such as flowers, pods, stems, leaves, and roots (Lambert and Bekal 2002). Nematode feeding strategies determine the amount of tissue destruction suffered by the plant. For example, migratory ectoparasites cause less physical damage to the roots when compared to migratory endoparasites (Ferris and Ferris 1998). Migratory endoparasites are believed to cause the most direct damage, as they destroy plant tissues while traveling and feeding through the plant (Ferris and Ferris 1998). Sedentary endoparasites have complex feeding relationships with host plants (Hussey and Williamson 2013). They modify the plant cells around the vascular tissues, forming permanent feeding sites to supply the nematode with an abundant flow of food (Hussey and Williamson 2013). The physical damage they cause is limited to the cells around the feeding site; some species will induce galls in the roots (Hussey and Williamson 2013).

Nematodes can be classified according to where they cause damage to the plant as: aboveground nematodes or belowground/root nematodes (Ferris and Ferris 1998). Nematodes that feed on aboveground plant parts can further be classified into three groups: (i) species infesting aboveground plant parts, such as ovaries, e.g., *Anguina tritici* (Steinbuch) Chitwood; (ii) foliar nematodes, e.g. *Aphelenchoides bessey* Christie; and (iii) stem nematodes, e.g., *Ditylenchus dipsaci* (Schomaker and Been 2013). Some symptoms caused by aboveground nematodes are reduced vigor, gall formation in flower primordium, twisting/distortion of leaves and stem, leaf discolouration, and necrosis (Perry and Moens 2011).

Nematodes can reduce the capacity of the roots to absorb and translocate water and nutrients through feeding or by damaging the roots' anatomy (Jaques and Jarvis 1994). Symptoms caused by root nematodes may be expressed in above and below ground parts of the plants; however, in most cases they are not specific and can be misdiagnosed as other issues, such as

nutrient deficiency (Jaques and Jarvis 1994). For instance, damage caused by *Pratylenchus* can be misdiagnosed as nutrient deficiency because the nematode causes lesions to the roots, limiting nutrients uptake by the plant (Compton 2015). Additionally, the lesions caused by the nematode can be a point of entrance for potential pathogens, leading to misdiagnosis of root diseases, thus making diagnosis even more difficult (Abawi and Chen 1998). Some of the symptoms caused by root-feeding nematodes include stunting, wilting, discolouration of foliage, formation of root galls or knots, root lesions, reduced root biomass, stubby roots, curly tips, and root rot (Schomaker and Been 2013).

Visible symptoms can sometimes be used as predictors of yield reductions (Schomaker and Been 2013). Reduced yields can be determined if the plant parts that are attacked are the commercialized products; otherwise, symptoms and yield reduction caused by nematodes can have a more complex relationship (Schomaker and Been 2013). However, in general, symptoms are unspecific, and damage to plants from plant-parasitic nematodes typically goes unnoticed at first and is often misdiagnosed and attributed to other issues (Gokte-narkhedkar 2006; Tenuta 2014). Therefore, as plant-parasitic nematode populations build, growers are often unaware of the damage to come in subsequent years (Tenuta 2014).

1.2 Nematode Identification

Nematode diversity is estimated to exceed one million species (Blaxter et al. 2005). Only approximately 26,000 to 40,000 nematode species have been described so far, which represents roughly 5-10% of the nematode species estimated on the planet (Blaxter et al. 2005; Creer et al. 2010). This identification gap can be blamed on the difficulty in differentiating nematode species,

which can be done following classical morphology or using molecular approaches (Porazinska et al. 2012).

Precise nematode species identification is imperative to address quarantine regulations (Powers 2004), predict crop loss, and make nematode control management efficient (Eisenback,1998). As methods to control nematodes have become more species-specific or even race specific, such as plant resistance, crop rotation, and selective chemical control, correct nematode identification has become increasingly important (Eisenback, 1998). Morphological, biochemical, and molecular approaches have been used to study nematode taxonomy (Seesao et al. 2016). In this study, only morphological and molecular approaches will be discussed, as these were the techniques chosen to identify the nematodes in the survey.

1.2.1 Classical Morphology Identification

Nematodes are considered particularly difficult to identify due to their minute body size, inter-genus morphological similarities, a limited number of distinguishable taxonomic characteristics, overlapping body measurements (Powers 2004), and the existence of sibling species (genetically different but morphologically identical) (Decraemer and Hunt 2006; Ahmed et al. 2015). Description of new species and routine nematode species identification using classical morphology is a time-consuming task that can be accomplished only by highly experienced nematologists (Powers et al. 2011). Identification to genus and species is achieved using guidance from taxonomic keys (Mai et al. 1996; Mekete et al. 2012).

Despite the incredible biological variability that nematodes have in terms of life cycles, host preferences, habitats, etc., they have a preservative morphology (Decraemer and Hunt 2006). Approximately 99% of all nematodes described so far have a basic body plan consisting of a thin, long, tube-like form tapered towards the cephalic region and tail (Decraemer and Hunt 2006).

Nevertheless, analyses of nematode morphological features under light microscopy is, in most cases, sufficient to identify nematodes to genus (Eisenback 1998). Nematode identification to species, however, requires a much more detailed examination.

All plant-parasitic nematodes possess a common morphological feature, the stylet, even though parasitism has arisen independently multiple times in the phylum (Bird et al. 2014). The stylet is one of the most important features in plant-parasitic nematode identification (Yeates et al. 1993). Nonetheless, it is not an exclusive structure of plant-parasitic groups, as many predators and fungal feeders have their own lineage-specific stylet (Holterman et al. 2017). The stylet morphology differs considerably between clades (Bird et al. 2014), facilitating nematode identification at higher taxa. However different, they have the same basic function, which is to withdraw nutrients from plant cells (Bird et al. 2014).

In addition to the stylet, some other basic features within the digestive system used to identify nematodes are the number of esophagus parts, the presence or absence of a medium bulb and its valve position, the lip region shape, and the presence or absence of overlapping intestines. Within the secretory–excretory system, the number and position of ovaries and the position of sexual organs and anus are some of the basic morphological features used to identify nematodes. Body size, mature female body shape (swollen or slender), the presence of offset head region, tail shape, and size are also relevant in nematode identification. Morphological identification is accomplished with the help of diagnostic features, such as apparatus formed by the cuticle, namely, caudal alae, vulva flaps, longitudinal lines, and perineal patterns in swollen females. Even the shape of the body after death (curved, spiral, straight, etc.) is used in nematode morphological identification.

Sexual dimorphism is uncommon, but when present it can be helpful for morphological identification. For example, in the *Heterodera* genus, females have a characteristic swollen body in the adult stage, which resembles a lemon, while the male is filiform throughout its life cycle (Eisenback 1998).

Sometimes, the structures required to identify species can only be seen using electron microscopy (De Ley et al. 2005). This is time-consuming, as morphological measurements are often required. Great experience is needed to distinguish and perform morphological measurements in nematode species (De Ley et al. 2005), hence the increasing applications of molecular tools in nematode species identification.

1.2.2 Molecular Identification

Molecular taxonomic analysis enhances classical descriptive taxonomy and sometimes even surpasses it (Decraemer and Hunt 2006). This is certainly true for diagnosis of cryptic or sister species, where molecular analyses have been crucial, as classical identification lacks the necessary accuracy that can be reached by molecular analysis (De Ley et al. 2005; Decraemer and Hunt 2006). This is exemplified in the case of *Ditylenchus* Filipjev (Anguinidae, Tylenchida) species, where recently, *D. weischeri*, which is a sibling species of *D. dipsaci*, was described as a separate species, based mostly on molecular analyses but also morphological and behavioral characters (Chizhov, Vladimir N.; Borisov, Boris A.; Subbotin 2010). *Ditylenchus dipsaci* and *D. weischeri,* as sibling species, naturally have very similar morphology, yet they can be distinguished by a few morphological features, such as shorter tails, shorter spicules, larger c index, larger vulva-anus distance to tail-length ratio, longer vulva-anus distance, and longer posterior sac in adults of *D. weischeri* (Tenuta et al. 2014) (Figure 1.1). However, morphometric measurements are time-consuming and require adult nematodes, while molecular techniques can be applied even when

there is a need for fast identification, as in quarantine pest screening, when only juvenile stages are available for identification, or when the material to be identified is limited (Ahmed et al. 2016).

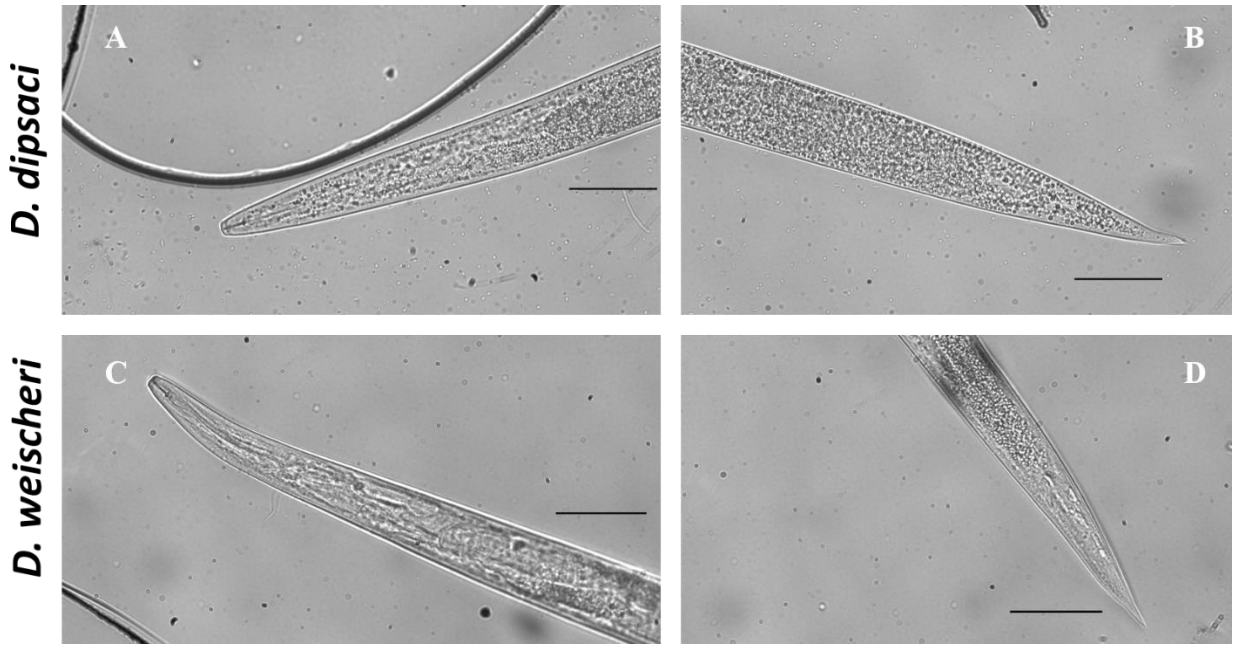

Figure 1.1 Light photomicrographs of *D. dipsaci* and *D. weischeri* showing similar morphological characteristics. (A) anterior end and (B) tail region of *D. dipsaci* recovered from garlic from Manitoba (Photo Credit Jehn Francisco Gamurot) and (C) anterior end and (D) tail region of *D. weischeri* recovered from Canadian thistles from Manitoba. Scale bar = 35 µm.

There is a wide variety of routine, rapid, and robust DNA-based molecular diagnostics developed for application in nematology (Oliveira and Monteiro 2011). Most of these diagnostics are based on polymerase chain reaction (PCR) techniques and DNA sequences variations (Ahmed et al. 2016). Additional techniques rely on random DNA amplification sequences, such as randomly amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and sequence characterized amplified DNA regions (SCAR) (Ahmed et al. 2016; Seesao et al. 2017).

PCR-based methods can be used for the identification of known species and also have the potential to diagnose new species. Some drawbacks are difficult standardization and lack of availability of automated processes (Pedram et al. 2015; Roos and Grant 1993; Blok and Powers 2009; Seesao et al. 2016).

The PCR-RFLP method uses enzymes to digest PCR products, producing different size fragments that can be used to distinguish species, even siblings species. Some disadvantages of this method are that it can only be used to identify known species, it is time-consuming, and it can not be applied in mixed samples or samples with multiple individual nematodes (Seesao et al. 2016). Subbotin et al. (2005) applied this method as an additional tool to distinguish *D. weischeri* from *D. dipsaci*. However, because of its disadvantages, Madani et al. (2015) developed conventional and real-time PCR primers based on the Hsp90 gene sequence as a more practical alternative to routinely identify *D. weischeri* and *D. dipsaci* species.

DNA sequencing has been routinely applied in nematode identifications in the last few years (De Ley et al. 2005; Pereira et al. 2010; Mahran et al. 2010). Genome sequencing projects for nematodes have been carried out and will certainly add to the scientific body of knowledge, allowing the design of new molecular markers for nematode identification studies (Seesao et al. 2016).

The success and reliability of the PCR-based methodology is dependent on the selection of a suitable DNA region (Seesao et al. 2006). The internal transcribed spacer regions (ITS1 and ITS2) of ribosomal RNA are commonly used for molecular characterisation of nematodes (Subbotin et al. 2005; Ahmed et al. 2016). ITS regions are variable, allowing distinction between taxa, but yet enough conserved, and therefore can be used in phylogenetic analyses to identify common ancestors (Ahmed et al. 2016). Similarly, the D2-D3 expansion segment of the nuclear

28S rDNA subunit can be used to characterize nematode species, and as it is a rapidly evolving region, even close species can be distinguished (De luca et al. 2004; De Ley et al. 2005; Hajieghrari et al. 2007). Many other DNA regions or marker or genes have been selected for molecular characterization of nematodes, including the 18S rRNA gene, mitochondrial cytochrome oxidase 2 (COX2), and mitochondrial cytochrome oxidase 1 (COXI) (Seesao 2016).

Ultimately, both molecular and morphological approaches have significant and separate explanatory power, but each can be subject to noise and data corruption (Coomans 2002). Nevertheless, the use of PCR-based molecular diagnostics has demonstrated improved sensitivity, accuracy, and confidence when combined with conventional morphological descriptive methodology (Oliveira et al. 2011).

1.3 Stem Nematodes (*Ditylenchus* spp.)

The *Ditylenchus* group is taxonomically complex. They have a conserved morphology; therefore, identifying species from this genus can be challenging, even for taxonomy experts, which hampers effective management (Douda 2005). The genus has more than 80 species described to date (Tenuta at al. 2014) nevertheless, only a few are parasites of higher plants; the majority of the species feed on fungi. The most agriculturally important species within this genus are *D. dipsaci*, *D. destructor* Thorne, *D. gigas* Ovlas, Troccoli, Palomares-Rius, De Luca, Liebanas, Landa, Subbotin & Castillo, *D. augustus* (Butler) Filipjev, and *D. arachis* Zhang, Liu, Janssen, Zhang, Xiao, Li, Couvreur & Bert (Vovlas et al. 2011; Duncan and Moens 2013; Singh et al. 2013b; Tenuta et al. 2014).

1.3.1 *Ditylenchus dipsaci*

Ditylenchus dipsaci is among the most devastating pests within the *Ditylenchus* genus, and it is ranked as one of the most economically damaging plant-parasitic nematodes worldwide, although it is more problematic in temperate regions, where cool and moist conditions favor its reproduction (Subbotin et al. 2005; Jones et al. 2013; Singh et al. 2013b). *Ditylenchus dipsaci* has a quarantine status in many countries due to its high virulence and extensive host range, which includes many economically important crops (Hockland et al. 2013).

Pulse crops such as peas, chickpeas, faba (fava) bean (*Vicia faba* L.), and lentils are among the plants that *Ditylenchus dipsaci* can parasitize or is associated with (Singh et al. 2013a; Pokharel et al. 2015). Other important crops include onions (*Allium cepa* L.), garlic (*Allium sativum* L.), alfalfa, oats (*Avena sativa* L.), potatoes (*Solanum tuberosum* L.), and wheat (*Triticum aestivum* L.) (Douda Ondrej 2005; Hajihassani et al. 2016; Hajihassani and Tenuta 2017). It is estimated that this nematode can parasitize more than 500 plant species (Schmidt-Rhaesa 2014).

Populations of this nematode that differ in host preferences are often called host races and are named after the main crop that they parasitize, e.g., oat race (Starr et al. 2013). Over 30 physiological races have been described from *Ditylenchus dipsaci*. However, several studies have indicated that *Ditylenchus* "host races" are species complex, and molecular analyses conducted by Subbotin (2005) confirmed this. Based on molecular analyses, namely RAPD-PCR, AFLP, PCR-RFLP, and sequencing of the ITS rRNA region, *Ditylenchus* species belong to two clades, i.e., they came from two ancestors. One group is denominated *D. dipsaci sensu stricto* and has diploid chromosome numbers; the other is a complex of the *Ditylenchus* species and has polyploid chromosome numbers (Subbotin et al. 2005). At least seven species can be drawn from the *Ditylenchus* species complex (Subbotin et al. 2005). Two species have already been singled out, namely *D. weischeri* (Chizhov et al. 2010) and *D. gigas* (Vovlas et al. 2011). *D. gigas* is a parasite

of broad beans (Vovlas et al. 2011) while *D. weischeri* is a parasite of the creeping thistle (Hajihassani et al. 2016). Other species that are parasites of wild and ornamental plants are yet to be described (Subbotin et al. 2005). Attempts to distinguish races from *D. dipsaci sensu stricto* have not been successful (Subbotin et al. 2005).

Ditylenchus dipsaci are commonly referred to as stem and bulb nematodes, as they feed on mostly on aboveground plant parts such as stems, leaves, and flowers but also bulbs, tubers, and rhizome tissues (Subbotin et al. 2005; Duncan and Moens 2013).

The stem and bulb nematode cycle can be completed relatively quickly, between three to four weeks depending on environmental conditions such as temperature and moisture (Yuksel 1960). This nematode reproduces sexually within plant tissues during the growing season but not during cold weather (Wallace 1958; Griffith et al. 1997). *Ditylenchus dipsaci* goes through two molts inside the egg and hatches as a second-stage juvenile (Duncan and Moens 2013). All stages are infective, but only the juvenile fourth stage is the survival stage (Duncan and Moens 2013). In this stage, and in highly infested tissues, the nematodes agglomerate and form a mass called "eelworm wool." This mass of nematodes can survive in soil for many years in the absence of the host plant and in low moisture and temperature conditions (Pokharel et al. 2015). When moisture or warm conditions are present, their survival rates drop significantly (Duncan and Moens 2013). Due to its short cycle and the production of many eggs (200 to 500 eggs per female) this species can increase in number quickly (Yuksel 1960).

Symptoms caused by *D. dipsaci* infestation on peas, chickpeas, lentils and faba beans vary with environmental factors, nematode density, and plant cultivars. Stem tissues swell and go from brown-reddish to black (Caubel et al. 1998). Leaves and internodes have necrosis symptoms and can be misdiagnosed as a disease caused by fungus (Caubel et al. 1998). Symptoms can also be

seen in young pods, which turn brown-reddish in colour, and seeds, which become darker, smaller, and distorted. (Caubel et al. 1998; EPPO 2017).

1.4 Pulse Crops

Pulses are annual leguminous crops that are commercially harvested only for their seeds (Roy et al. 2010). Therefore, the major pulse crops include kidney beans, lima beans, dry peas, chickpeas, lentils, lupins, and various other varieties of dry beans (Roy et al. 2010). Dry peas and lentils are grown widely across Canadian Prairies, are rich in protein, and are important sources of both food and feed around the world (MacWilliam et al. 2014). Pulse crops also provide many benefits to crops in rotation, potentially improving crop quality and yield (MacWilliam et al. 2014). Pulse crop benefits include increased nitrogen uptake (which reduces dependence on nitrogen fertilizers), increased seed protein, improved efficiency of water use, reduced disease incidence, decreased weed and pest problems and carbon footprint reduction (Williams et al. 2014).

Canada is one of the world's largest producers of pulse crops, along with India, China, Myanmar, and Brazil (“Pulse Industry” 2015). The three most important pulse crops in terms of worldwide production are beans, chickpeas, and peas, according to statistics from the United Nations Food and Agriculture Organization (Bekkering 2015). In Canada, the main pulse crops are dry beans, dry peas, chickpeas, and lentils (Pulse Canada 2018).

Peas are the main pulse crop grown in Canada, and they accounted for 6% of the total cropped area in 2011. Additionally, Canada is the world’s largest producer and exporter of peas, with more than 85% of its production being exported to 130 countries (Pulse Canada 2018). Within Canada, significant portions of Alberta, Saskatchewan, and Manitoba are used for growing pulse crops (Patron 2015). Saskatchewan is a major province for pulse crop production, accounting for

approximately 79.3% of the total pulse-producing area in Canada, followed by Alberta, accounting for 16.2%, and Manitoba, with 2.3% of the total pulse crop of Canada (Statistics Canada 2015).

1.5 Nematode Pests of Pulse Crops

Little is known about the impact of plant-parasitic nematodes on pulse crop yield in the Canadian Prairies. In other pulse regions, nematodes are known to cause significant crop damage. In chickpeas and common beans, for example, nematodes are responsible for 13.7% and 10.9% of average yield loss worldwide (Askary 2017).

The most predominant plant-parasitic nematode parasitizing pulse crops worldwide are *Meloidogyne* spp., *Pratylenchus* spp., *Rotylenchus* spp., *Heterodera* spp., *Tylenchorhynchus* spp., and *Helicotylenchus* spp. (Askary 2017). More specifically, *D. dipsaci, Pratylenchus neglectus* (Rensch, 1924) Filipjev & S. Stekhoven, *Pratylenchus thornei* Sher and Allen, and *Paratylenchus hamatus* Thorne and Allen have been reported to cause damage in peas (Hafez et al. 2010), while *D. dipsaci, P. lentis, P. neglectus, P. thornei,* and *P. hamatus* have been reported to be associated with lentils, and *D. dipsaci, P. neglectus, P. thornei, Heterodera ciceri* Volvas, Grecor & Di Vito, *Meloidogyne artiellia* Franklin have been associated with chickpeas (Singh et al. 2013).

1.6 Distribution of Plant-Parasitic Nematodes in Western Canada

Previous interest in the population dynamics and distribution of nematodes has been primarily focused on the provinces of British Columbia, Ontario, New Brunswick, Quebec, Nova Scotia, and Prince Edward Island (Kimpinski and Thompson 1990; Potter and McKeown 2003). The few studies that described species present in the Prairie provinces have been concentrated on the Central and Southern area of Alberta (Webster 1972; Hawn 1973).

Early examples of research into plant-parasitic nematodes in the Prairies include a *Paratylenchus projectus* Jenkins survey study conducted in Alberta during 1970 and 1971 by Webster and Hawn 1973. In their study, *P. projectus,* which is a parasite of plants from the Fabaceae family, was found at high densities in Central Alberta (Webster and Hawn 1973). Another study, conducted in 1971 by Sewell, examined samples from diverse sources, such as scientists, farmers, florists, and materials confiscated at airports and ports. It was found that *Heterodera punctata* Thorne and *Aphelenchus avenae* Bastian were present in samples of native grasses in Saskatchewan and alfalfa in Alberta (Sewell 1977). In Southern Alberta in 1973, Hawn found a number of plant-parasitic nematodes, including *Paratylenchus* sp.*, Tylenchus* sp.*, Tylenchorhynchus acutus, Aphelenchoides* sp.*, Aphelenchus* sp., and notably *Ditylenchus dipsaci,* in irrigated soils where peas, green beans, alfalfa, potatoes, corn, carrots (*Daucus carota* subsp. *sativus*), and sugar beets were grown (Hawn 1973). *Ditylenchus dipsaci*, was found in 93% of the 28 pea fields analysed in their study (Hawn 1973). *Ditylenchus dipsaci* was also reported in Regina, Saskatchewan, causing stem swellings in Canadian thistles (*Cirsium arvense*) (Watson and Shorthouse 1979). Ebsary et al. (1984) re-examined specimens in the Canadian National Collection of Nematodes and described *Xiphinema occiduum* Ebsary, Potter & Allen in grass, sod, and wheat in Saskatchewan; in barley, sod, alfalfa, and wheat in Alberta; and in apple and strawberry in Manitoba. In Alberta, minor damage in vegetable crops has been associated with *Paratrichodorus spp.,* such as *P. allii* (Jensen) Siddiqi and *P. pachydermus* (Seinhorst) Siddiqi and *Trichodorus* spp. (Vrain and Ebsary 1994).

A number of *Pratylenchus* species have been described in the Prairies (Holzgang and Pearse 2006; Merrifield 2007; Yu 2008; Mahran et al. 2010). *Pratylenchus neglectus* was described for the first time in Brandon, Manitoba, from a corn field in 1971 (Mahran et al. 2010).

It has also been reported in potato in Alberta (Merrifield 2007; Forge et al. 2015) and strawberry in Saskatchewan (Holzgang and Pearse 2006). Other species were described by Yu (2008) based on preserved specimens from the Canadian National Collection of Nematodes. He identified *P. fallax* Seinhorst and *P. penetrans* (Cobb) Filipjev in Saskatchewan; *P. neglectus*, *P. penetrans*, and *P. pratensis* (de Man) Filipev in Manitoba; and *P. crenatus* Loof, *P. hexincisus* Taylor & Jenkins, *P. neglectus*, and *P. penetrans* in Alberta (Yu 2008).

In short, the importance of plant-parasitic nematodes in the Canadian Prairies is largely unknown, especially for pulse crops. Only a few studies have been performed, most of them several decades ago, and many of them are now suspect as result of newer molecular findings (and consequently new taxonomic classifications), the discovery of new nematode species, and changes in nematode distribution and population densities (Potter and McKeown 2003). This was the case with *D. dipsaci* previously reported in thistles in Saskatchewan (Watson and Shorthouse 1979). Newer findings by Tenuta at al. (2014) have indicated that *D. dipsaci*, previously found in Canadian thistles in Saskatchewan, was actually *D. weischeri*, a parasite of thistles and not major crops.

The fact remains that little is known about plant-parasitic nematode distribution in pulse crops fields in the Canadian Prairies. This research aims to decrease this knowledge gap and address questions regarding the distribution and possible importance of plant-parasitic nematodes of pulse crops in commercial fields in Canadian Prairies.

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2 SURVEY OF PLANT-PARASITIC NEMATODES IN PULSE CROP FIELDS OF THE CANADIAN PRAIRIES

2.1 Abstract

The quarantine pest nematode *Ditylenchus dipsaci* can hamper securing export markets for some crops. In Canada, it has been reported that previous identification of *D. dipsaci* in yellow pea export shipments was likely the non-quarantine species *D. weischeri*, a parasite of creeping thistle, and not crops. To further clarify if the quarantine pest *D. dipsaci* is found in pulse plants and to address the gap in understanding the distribution of plant-parasitic nematodes in the Canadian Prairies, a field survey was conducted on commercial yellow pea, lentil and chickpea fields in Alberta, Saskatchewan and Manitoba. Samples of pulse and creeping thistle (a.k.a. Canada thistle, *Cirsium arvense*) plants (flowers or pods, stem and leaves) and soil were collected from 93 fields. Nematodes were extracted from the plant materials using a modified Whitehead tray method and from the soil using the Cobb sieving sugar/floatation method. The first 100 nematodes observed for each sample were identified to genus by morphological features and frequency of occurrence in fields, mean population densities in soil and plant samples and prominence values (i.e. nematode frequency and density combined into one value) were calculated. Molecular analysis by species-specific PCR, PCR-RFLP and/or sequencing of the ITS (ITS 1 + 5.8S + ITS2) of the rRNA gene were used to identify selected nematodes to species. Twenty genera of plant-parasitic nematodes were recovered from the soil and (or) the plants of pea, chickpea, lentil and creeping thistle, including *Anguina*, *Aphelenchoides*, *Ditylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Merlinius*, *Paraphelenchus*, *Paratylenchus*, *Pratylenchus*, *Subanguina*, *Paratrichodorus*, *Tylenchorhynchus* and *Xiphinema*. Plant-parasitic nematodes were, in general,

less prominent in plant samples than in soil samples. In soil samples, *Paratylenchus* was the most prominent genus (241 prominence value) recovered from lentil soil, followed by *Tylenchorhynchus* (78) and *Pratylenchus* (74), both recovered from thistle soil in pea fields. In the plants above ground samples, *Ditylenchus* was the predominant genus (38) recovered from chickpea and *Aphelenchoides* was the second most predominant genus (2) recovered from pea samples.

Most of the fields analysed had low plant-parasitic nematode population density. Nevertheless, several fields had high levels of *Ditylenchus, Pratylenchus, Paratylenchus, Helicotylenchus* and *Tylenchorhynchus*, which could be potentially problematic for the crops studied or for crops that are grown in rotation. Molecular analysis results indicated the recovery of *Ditylenchus weischeri, D. dipsaci, Pratylenchus neglectus, Xiphinema rivesi* and *Paratylenchus nanus. Ditylenchus weischeri* was recovered from 22 fields (1 to 300 nematodes/g plant sample) across Alberta, Saskatchewan and Manitoba. *Ditylenchus weischeri* is not, however, considered an agricultural pest. *Ditylenchus dipsaci* was recovered at low density from pods of one yellow pea field (1.6 nematodes/g pods) but not from the soil. The positive field was resampled in the following year for the soil with failure to obtain *D. dipsaci. Pratylenchus neglectus* was identified in six pea and chickpea fields. Four of these fields had density levels (104 to 176 nematodes/100g dry soil) which could be potentially damaging to crops. *Pratylenchus neglectus'* host preference and possible crop damage remains to be determined. *Paratylenchus nanus* was identified in soil samples from one pea field in Saskatchewan, and it was also recovered at a density (813.65 nematodes/100g soil) above threshold levels established for economic damage in peas. This survey indicates that several fields in the Canadian Prairies have plant-parasitic nematode species at high density population levels and can potentially cause crop damage. Regarding *Ditylenchus*, which was our main goal,

this research could successfully confirm the high prevalence of *D. weischeri* on creeping thistle in pulse fields and the near absence of *D. dipsaci*.

2.2 Introduction

Canada is the world's leading exporter of pulses; more than 85% of its production is exported to 130 countries (Pulse Canada 2018). The main pulses grown in Canada are dry beans, dry peas, chickpeas and lentils (Pulse Canada 2018). The largest pulse growing areas are situated in the Canadian Prairies due to favorable agricultural conditions such as suitable climate and fertile soil (Statistics Canada 2015). Moreover, the Canadian Prairies have easier access to ports that lead to main importers such as India (for dry peas) and China and Turkey (for lentils and chickpeas) (Statistics Canada 2015). Commercial pulse fields are predominantly concentrated in Saskatchewan, which accounts for approximately 79.3% of the pulse producing area. Alberta is the second, accounting for 16.2% of the total field crop area, followed by Manitoba (2.3%) (Statistics Canada 2015).

Presence of some plant-parasitic nematodes can negatively impact the access to international market, as is the case with the quarantine pest *Ditylenchus dipsaci,* which has been particularly problematic for yellow pea exports from Canada to India. *Ditylenchus dipsaci* is an important crop pest of quarantine status in many countries, due to its wide host range and ability to cause extensive economic losses in many economically important crops (Schmidt-Rhaesa 2014). *Ditylenchus dipsaci* was reported by Watson and Shorthouse (1979) as the parasite infesting the Canada thistle (*Cirsium arvense*) in Saskatchewan. More recently, a new *Ditylenchus* species, *D. weischeri*, has been described parasitizing the thistle in Russia (Chizhov et al. 2010). Following

this new finding, Tenuta et al. (2014) conducted surveys and host suitability studies to determine the phytosanitary risks of pea grain exports containing *D. dipsaci* in the Prairie provinces. The results indicated that *D. weischeri*, and probably not *D. dipsaci*, was present in yellow pea grain from the harvest samples in 2009 and 2010 and in Canada thistle plants in Saskatchewan, Alberta and Manitoba (Tenuta et al. 2014). *Ditylenchus weischeri* is a parasite affecting the creeping thistle and is not an agricultural pest of crops grown in the Canadian Prairies (Hajihassani et al. 2016, 2017).

The current knowledge of nematode biodiversity, with an emphasis on phytopathogenic species, is predominantly based in the intensively cropped regions of British Columbia, Ontario, New Brunswick, Quebec, Nova Scotia and Prince Edward Island (Kimpinski and Thompson 1990; Potter and McKeown 2003). Very few surveys have been conducted in the past years in the Prairie provinces and had been focused in Alberta (Lethbridge, Olds and Taber area and the Peace River valley), while the rest of the cropped area was less researched (Potter and McKeown 2003). For instance, Hawn (1973) described plant-parasitic nematodes in the irrigated soils in Southern Alberta for alfalfa, pea, green bean, sugar beet, potato, field corn and carrot crops. He found a variety of plant-parasitic nematodes, including *Paratylenchus projectus, Tylenchorhynchus acutus* (Allen) Siddiqi, *Aphelenchoides* sp. and notably *Ditylenchus dipsaci*; likely to have actually been *D. weischeri*. Webster and Hawn (1973) conducted a survey to determine the distribution of the nematode *P. projectus*, a parasite of plants from the Fabaceae family, in the alfalfa fields of central and Northern Alberta. Sewell (1977) compiled the findings on nematode identification from samples throughout Canada, from varied sources such as scientists, farmers, florists and materials confiscated at airports and ports. In his paper, the only nematodes described in the Prairies were *Heterodera punctata* and *Aphelenchus avenae* present in the native grasses in Saskatchewan and

in alfalfa in Alberta, respectively (Sewell 1977). Minor damage in Alberta vegetable crops has been associated with *Paratrichodorus* spp. such as *P. allii*, *P. pachydermus* and *Paratrichodorus* spp. (Vrain and Ebsary 1994). Ebsary et al. (1984) re-examined specimens in the Canadian National Collection of Nematodes and observed *Xiphinema occiduum* in the Canadian Prairies in wheat, grass, barley, alfalfa, apple, strawberry and sod samples. Yu (2008) also identified species from the Canadian National Collection of Nematodes and described *Pratylenchus fallax* and *P. penetrans* in Saskatchewan; *P. neglectus, P. penetrans*, and *P. pratensis* in Manitoba; and *P. crenatus, P. hexincisus, P. neglectus,* and *P. penetrans* in Alberta. *Pratylenchus neglectus* was also found in potato fields in Manitoba and Alberta (Merrifield 2007) and in strawberry in Saskatchewan (Holzgang and Pearse 2006).

There are limited data regarding plant-parasitic nematodes associated with pulse crops in Canada; the surveys and research on nematode identification performed several decades ago may now be suspect as (Potter and McKeown, 2003) a result of recent molecular identification methods and changes in nematode distribution and population densities.

The impact of plant-parasitic nematodes on pulse crops yield in the Canadian Prairies is not known. In other pulse regions, nematodes can be significant pests of pea (*D. dipsaci, P. neglectus, P. thornei, P. hamatus*), lentil (*D. dipsaci, Pratylenchus lentis, P. neglectus, P. thornei, Paratylenchus hamatus*) (Hafez et al. 2010), chickpea (*D. dipsaci, P. neglectus, P. thornei, Heterodera ciceri, Meloidogyne artiellia*), and faba bean (*D. gigas, Heterodera goettingiana* Liebscher) (Singh et al. 2013). Furthermore, extensive damages have been reported due to some of these species. For instance, in Idaho, USA, two pea and one lentil fields infested with *P. neglectus, P. thornei* and *P. hamatus* had up to 90% crop losses (Riga et al. 2008). The fact remains

that the prevalence of plant-parasitic nematodes in pulse crops in Western Canada is relatively unknown.

2.3 Objectives

The main objective of this study was to help clarify if the quarantine pest *Ditylenchus dipsaci* is found in pulse crops on the Canadian Prairies. This information is of importance as the presence of this nematode can hamper security exports of yellow peas. To accomplish that, a field survey was conducted for plant-parasitic nematodes of the pulse crops, yellow pea, chickpea and lentil in three provinces Alberta, Saskatchewan and Manitoba, which are the major pulse producing regions in Canada.

More specifically, this thesis study aimed to:

(i) use molecular techniques to distinguish between *Ditylenchus dipsaci* and *D. weischeri* which can then confirm the presence of *Ditylenchus weischeri* in creeping thistle in fields and the lack of occurrence of *D. dipsaci* in crops and creeping thistle;

(ii) determine the frequency, population density and prominence of plant-parasitic nematode genera in yellow pea, chickpea, lentil and the perennial weed, creeping thistle, in soil and plants samples from commercial fields;

(iii) determine if the plant-parasitic nematode genera found in the pulse crops occur at population densities that may possibly damage crops and

(iv) determine the species identity of the prominent genera of plant-parasitic nematodes.

2.4 Materials and Methods

2.4.1 Soil and Plant Sampling

A total of 93 commercial fields planted to either yellow pea, lentil or chickpea were surveyed for the occurrence and identification of *Ditylenchus* and other plant-parasitic nematodes in Manitoba, Alberta and Saskatchewan during summers of 2014 and 2015 (Table 2.1, Figure 2.1). Fields were selected and sampled in collaboration with Dr. Michael Harding and his team and Dale Risula and Saskatchewan crop specialists Shannon Chant, Lyndon Hicks, John Ippolito, Kim Stonehouse, Cory Jacob, Kaeley Kindrachuk, Sherri Roberts, Cory Jacob, Shannon Friesen, Brian Olson and Danielle Stephens. The fields were visited at the mid-reproductive growth phase (R4 or R5) to maximize the likelihood of recovering foliar nematodes such as *Ditylenchus*. At this stage, the canopy is closed promoting humid conditions and the crop is still green promoting feeding on stems, leaves and pods for the nematode. Ten whole crop plants (above ground) were sampled from each field using a "W" pattern walk. One soil core (0–30 cm) was taken at the base of each plant sampled using a split-tube sampler (3.5 cm i.d.). The 10 cores for the plants for each field were pooled together to provide one sample and placed immediately into a chest cooler. Five Canadian thistle plants were randomly selected in each field and the whole above ground plant also sampled. Two cores (0–30 cm) were taken at the base of each of the five thistle plants, yielding a total of ten cores for a field that were then mixed together. Samples were refrigerated and shipped in chest coolers to the University of Manitoba Soil Ecology Laboratory.

Table 2.1. Number of fields sampled for lentil, chickpea and yellow pea by year and province.

Crop	2014		2015			Field Totals
	SK[a]	AB[b]	SK	AB	MB[c]	
Yellow Pea	25	23	6	10	7	71
Lentil	0	0	13	0	0	13
Chickpea	0	0	3	6	0	9
Totals	48		45			93

Provinces are SK[a]= Saskatchewan, AB[b]= Alberta, MB[c]= Manitoba

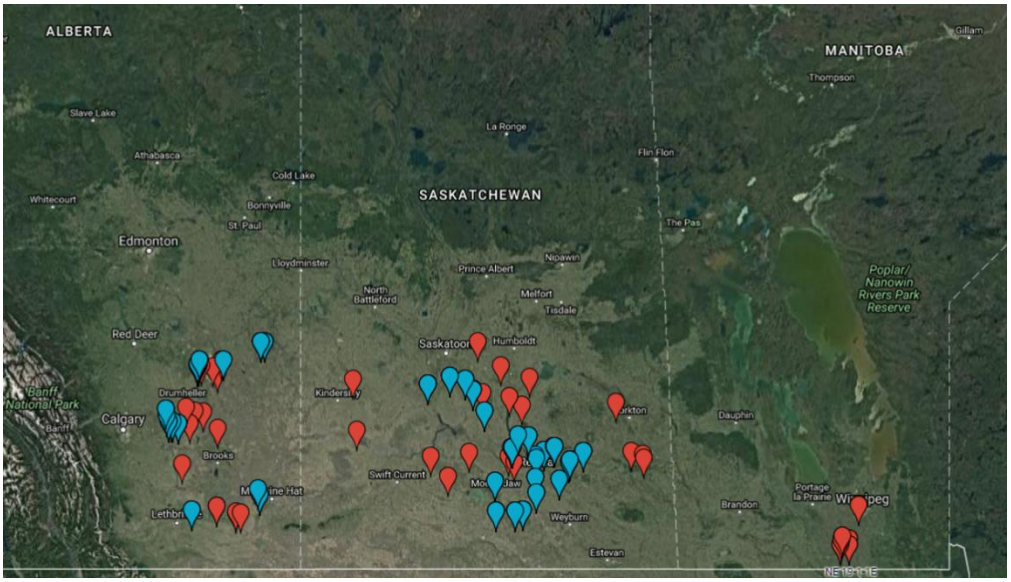

Figure 2.1 Locations of pulse fields sampled in 2014 and 2015 (n= 93).

2.4.2 Extractions and Counting

2.4.2.1 Nematode Extractions from Plant Material. Nematodes were extracted from plant materials using a modified Whitehead tray method (Whitehead and Hemming 1965) (Figure 2.2). Each plant sample was divided into 2 subsamples based on plant component. The first subsample comprised of stems and leaves, whereas the second comprised of flowers (for thistle) or pods and seeds (for pea, chickpea and lentil). The subsamples were chopped to a maximum length and

weight of 1 cm and 5 g for stem and leaves, 5 g for thistle flowers, and 10 g for seeds. The chopped components were placed on extracting pans. The pans had a base of a potting dish (18 cm in diameter) with a wire mesh (700µm screen size) and setup with one layer of filter paper (*Kimwipe*, Kimtech Science, Mississauga, ON, Canada) supported by three plastic rings (3mm thick). Reverse osmosis water was added to the dish to wet the paper and plant samples were placed on the paper. The units were covered and incubated in a controlled environment room at 21°C for six days (for stem and leaves samples) or four days (four pods and flowers samples) in the dark, water was added as necessary to keep plant material saturated. After incubation, the solution in the dishes was emptied onto a stack of sieves (top to bottom; 100-mesh (0.1397 mm openings) and 400-mesh (0.03302 mm opening)). The screens were rinsed several times with tap water, trapping the nematodes on the finest screen. The trapped plant material, including nematodes, were then washed into a 15mL conical centrifuge vial and immediately placed at 4°C until ready to be analyzed (within 24 hours).

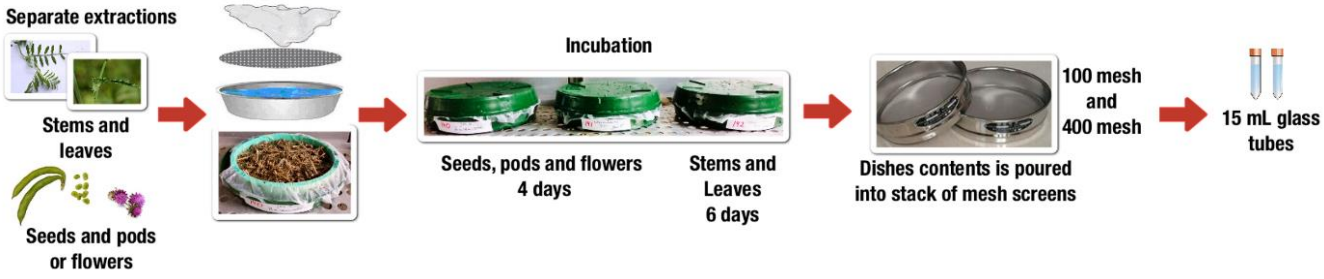

Figure 2.2 Illustration of nematode extraction from plant materials using a modified Whitehead tray method.

2.4.2.2 Nematode extractions from soil. Nematodes in the soil samples were extracted using the Cobb sieving sugar/flotation method (Ingham 1994) (Figure 2.3). Approximately 100g of fresh soil was placed in a 500mL plastic beaker and filled with R.O. water. A separate subsample of soil

(about 15g) was oven dried at 105°C for 24 hours for determination of dry weight of soil samples used for extractions. After mixing, the soil and water in the beaker were emptied onto a stack of 45-mesh (0.4699 mm opening) on top of a 400-mesh screen and rinsed several times. The trapped contents were backwashed into a 50mL plastic centrifuge tube filled with water and centrifuged for 5 min at 605.39 x gravity using a *Centra-CL2* centrifuge (Thermo IEC, Needham Heights, MA, USA). The clear supernatant was discarded, and pellet suspended in sucrose solution (454g L^{-1} ddH_2O) and centrifuged for 1 min 15 s at 202.92 x gravity. A 500-mesh (0.0254 mm opening) screen was used for the final recovery of nematodes suspended in the sucrose after centrifugation with the trapped material washed into a 15mL glass conical centrifuge vial and placed at 4°C. The total number of soil and plant samples types extracted for analysis is presented in Table 2.2.

Figure 2.3 Illustration of nematode extraction from soil using the Cobb sieving sugar/flotation method.

Table 2.2. Samples by type (flowers or pods and stems and leaves) analysed according to crop, province and year of sampling.

Province[a]	Crop	Above Ground Crop				Soil Under Crop		Above Ground Weed				Soil Under Weed		Totals
		2014		2015		2014	2015	2014		2015				
		Pods	[b]Other	Pods	Other			Flowers	Other	Flowers	Other	2014	2015	
SK	Yellow Pea	25	25	6	6	25	6	21	25	2	6	25	6	178
	Lentil	0	0	13	13	0	13	0	0	0	1	0	1	41
	Chickpea	0	0	2	3	0	3	0	0	0	0	0	0	8
AB	Yellow Pea	20	23	6	10	23	10	12	18	6	9	18	9	164
	Chickpea	0	0	6	6	0	6	0	0	5	6	0	3	32
MB	Yellow Pea	0	0	7	7	0	7	0	0	7	7	0	7	42
Sample Type Totals		45	48	40	45	48	45	33	43	20	29	43	26	465

[a] Provinces are SK = Saskatchewan, AB = Alberta, MB = Manitoba
[b] Stems and leaves

2.4.3 Morphological Characterization

The first 100 plant-parasitic nematodes were identified to the genus level by morphological characters using standard taxonomic keys (Mai and Mullin 1996; Mekete et al. 2012). A bright field microscope (BX-51, Olympus Canada, Inc., Richmond Hill, Canada) equipped with a digital imaging camera (Olympus Qcolor3) and Image-Pro Plus 6.2 (Media Cybernetics, Rockville, MD, USA) software was used to identify and obtain pictures of the specimens.

2.4.4 Molecular Identification

2.4.4.1 DNA Extraction. DNA extraction followed a slightly modified version of the protocol described by Tenuta et al. (2014), as detailed next. An individual nematode was hand-picked using a handling needle, transferred onto an embryo dish under a dissecting microscope and rinsed at least three times in sterile (autoclaved) ddH_2O, transferred to a 0.2 ml PCR reaction tube containing 10 µl sterile ddH_2O, 2 µl of Proteinase K (Roche, UK) and 12 µl of Direct PCR Lysis Reagent (Viagen Biotech, Los Angeles, CA, USA) and frozen at –80°C overnight. The tube was then placed in a Thermocycler (T100TM, Bio-Rad Laboratories Canada Ltd, Mississauga, ON, Canada) machine, heated for 60 min at 60°C and then for 10 min at 94°C. The DNA was stored at −20°C until ready to use for PCR.

2.4.4.2 Polymerase Chain Reaction (PCR). Nematode universal and species-specific PCR primer sets used for DNA analysis in this research are provided in Table 2.3. The PCR mixture consisted of 1 to 3 µl of DNA extraction solution, 2.5 µl of 10× PCR buffer reaction buffer, 1 µl of dNTPs mixture (dATP, dCTP, dGTP and dTTP), 0.2 µl DreamTaq DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 250 nM of each primer, and ddH_2O to a final volume of 25µl. The PCR amplification conditions for each primer set are given in Table 2.3. All the samples

were run with nematode universal D2A-D3B primers (De Ley et al. 1999), which amplified the D2 and D3 expansion region of the 28S rDNA gene and TW81-AB28 primers (Fanning et al. 1995), which amplified the Internal Transcribed Spacer region (ITS) of the 18s rRNA gene. This was done prior to analyses with species-specific primer sets to verify the presence of nematode DNA or to yield PCR products for DNA purification. The PCR amplification products were isolated by electrophoresis on 1.5% agarose gels with 0.5% TAE buffer, and visualized by staining with 1 µl of 10,000X concentrated of GelRed fluorescent dye (Biotium Inc, Hayward, CA, USA). Amplification products were visualized under UV elumination using a Gbox gel capture imaging system (SYNGENE, Synoptic, LTD. Cambridge, UK).

Table 2.3. Primers and thermocycling conditions used for molecular identification in this research.

Primer Name	Species	Primer Sequence 5'- 3'	Amplification Conditions	Reference
AB28_TW81	Universal ITS region	ATATGCTTAAGTTCAGCGGGT GTTTCCGTAGGTGAACCTGC	94°C 4 min; 35 cycles at 94°C 1 min, 55 °C 1.5 min; 72°C 2 min; and a final extension at 72°C 10 min	Fanning et al., 1995
D2A-D3B	Universal D2/D3 region	ACAAGTACCGTGAGGGAAAGTTG TCGGAAGGAACCAGCTACTA	94°C 2 min; 35 cycles at 94°C 1 min, 53 °C 30 s; 72°C 1 min; and a final extension at 72°C 4 min	De Ley et al., 1999
U831-Dipsaci_hsp90R	*D. dipsaci*	AAYAARACMAAGCCNTYTGGAC GWGTTAWATAACTTGGTCRGC	94°C 3 min; 33 cycles at 94°C 30 s, 50.5 °C 1 min; 72°C 1 min; and a final extension at 72°C 10 min	Madani et al., 2015
U831-Weischeri_hsp90R	*D. weischeri*	AAYAARACMAAGCCNTYTGGAC AGCACTAAAATTAAGYGTAAAGG	94°C 3 min; 33 cycles at 94°C 30 s, 55 °C 1 min; 72°C 1 min; and a final extension at 72°C 10 min	Madani et al., 2015
PNEG-D3B	*P. neglectus*	ATGAAAGTGAACATGTCCTC TCGGAAGGAACCAGCTACTA	95 °C 3 min; 35 cycles at 95 °C 1 min; 63 °C 1 min; 72 °C 1 min; and a final extension at 72 °C for 7min	AlBanna et al., 2004
PPEN-D3B	*P. penetrans*	TAAAGAATCCGCAAGGATAC TCGGAAGGAACCAGCTACTA	95 °C 3 min; 35 cycles at 95 °C 1 min; 62 °C 1 min; 72 °C 1 min; and a final extension at 72 °C for 7min	AlBanna et al., 2004
PSCR-D3B	*P. scribneri*	AAAGTGAACGTTTCCATTTC TCGGAAGGAACCAGCTACTA	95 °C 3 min; 35 cycles at 95 °C 1 min; 63 °C 1 min; 72 °C 1 min; and a final extension at 72 °C for 7min	AlBanna et al., 2004
PTHO-D3B	*P. thornei*	GAAAGTGAAGGTATCCCTCG TCGGAAGGAACCAGCTACTA	95 °C 3 min; 35 cycles at 95 °C 1 min; 68 °C 1 min; 72 °C 1 min; and a final extension at 72 °C for 7min	AlBanna et al., 2004
PVUL-D3B	*P. vulnus* Allen & Jensen	GAAAGTGAACGCATCCGCAA TCGGAAGGAACCAGCTACTA	95 °C 3 min; 35 cycles at 95 °C 1 min; 68 °C 1 min; 72 °C 1 min; and a final extension at 72 °C for 7min	AlBanna et al., 2004
AFragF1- AFragR1	*A. fragariae*	GCAAGTGCTATGCGATCTTCT GCCACATCGGGTCATTATTT	94°C 2 min; 40 cycles at 94°C 1 min; 53°C 40 sec; 72°C 1 min; and a final extension at 72°C for 10 min	McCuiston et al., 2007

2.4.4.3 Sequencing. The D2–D3 region of the 28S rDNA gene or the ITS region of the 18S rRNA gene were amplified using universal D2A-D3B primers (De Ley et al. 1999) and TW81-AB28 primers (Fanning et al. 1995) respectively. DNA fragments were purified either from agarose gels using QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany), or using QIAquick PCR Purification Kit (QIAGEN, Hilden, Germany), following the manufacturer's instruction. Amplification product concentration and quality was determined by spectrophotometry using a spectrophotometer (NanoDrop 2000, Wilmington, DE, USA) to insure reactions productions were good for sequence determinations. Purified PCR products were sequenced by Macrogen, Corp. (Rochville, MD, USA).

2.4.4.4 PCR - Restriction Fragment Length Polymorphism (PCR-RFLP). Amplified rDNA-ITS products of *Ditylenchus* spp. were subjected to restriction fragment analysis as an additional method of species identification. PCR-RFLP reactions were prepared using the same procedure as for Tenuta et al. (2014). The whole PCR product of successful gene amplification sample was isolated and cut from gels purified using the QIAquick Gel Extraction Kit, following the manufacturer's instructions. Then, the purified product was digested with five restriction enzymes (Bsh1236I, Hinf I, MspI, RsaI, and TaqI) in a buffer designated by the supplier (Fermentas, Thermo Scientific Inc., Waltham, MA, USA). Reactions were carried out following the manufacturer's recommendations. Digested DNA fragments were isolated on buffered (0.5%TAE) 1% agarose gel containing 0.5 ml of 10,000X concentrated GelRed fluorescent dye (Biotium, Hayward, CA, USA) and visualised on the GBox UV transilluminator.

2.4.5 Data Analysis

Population density of each nematode taxon in soil is reported on a dry weight basis (# $100g^{-1}$ dry soil) and for plant components on a fresh weight basis (# g^{-1}). Nematode frequency was calculated as a percentage of the number of samples containing a specific taxon divided by the number of samples analysed. Prominence, which is a single value for frequency and density combined, was calculated as (mean population density x √frequency/ 10) (De Waele and Jowaan 1988).

An ANOVA was performed on nematode density data using PROC GLM in SAS studio version 3.71 (SAS Institute Inc., Cary, NC). The data was modeled assuming a negative binomial distribution. Least-square means were compared using the Tukey-Kramer mean comparison procedure to examine whether the density of a taxon varied with soil type (crop soil * weed soil) or plant sample type (stem and leaves from crop * steam and leaves from weed * pods from crop * flowers from weed). Only positive samples were added into the analyzes, zero counts were disregarded.

2.5 Results

The present study was conducted to determine the plant-parasitic nematodes of economic importance present in the Canadian Prairies, focusing on the quarantine plant-parasitic nematode *D. dipsaci*. To accomplish this, nematodes were extracted from 93 commercial crop fields of peas, lentils and chickpeas (Table 2.1) in 2014 and 2015. Thistle plants were also analysed to confirm infestation by *D. weischeri* (Tenuta et al. 2014), contesting prior reports of infestation by *D. dipsaci* (Watson and Shorthouse 1979). Lentils and chickpeas were only sampled in 2015, and the lower

number of fields sampled reflect the lower number of area planted that year (Table 2.1). Therefore, most samples analysed were from yellow pea (82.5%), whereas 8.8% were from lentil and 8.6% from chickpea fields (Table 2.2).

Plant-parasitic nematodes were recovered from 60% of the samples analysed. Twenty percent of the samples had only free-living nematodes. Approximately 20% of the samples contained no nematodes and were all from above ground plant parts. Samples with zero counts for nematodes or that only contained free-living nematodes were not included in summary statistics of positive fields.

Twenty genera containing plant-parasitic nematodes were recovered from the soil and (or) plant parts of peas, chickpeas, lentils and thistle plants from the Canadian Prairies, namely *Anguina, Aphelenchoides, Aphelenchus, Coslenchus, Ditylenchus, Filenchus, Helicotylenchus, Hoplolaimus, Longidorus, Merlinius, Paratylenchus, Pratylenchus, Psilenchus, Subanguina, Paraphelenchus, Paratrichodorus, Tylenchorhynchus* and *Xiphinema* (Table 2.4, Figure 2.4). Nematodes belonging to the *Paraphelenchus, Tylenchus, Filenchus, Coslenchus* and *Psilenchus* genera, and possibly others belonging to the *Tylenchidae* family, were not accounted in this survey. Although some species of those taxon have been reported to be associated with damage in crops (Anwar et al. 2011), their pathogenicity to crops is unknown (Bafokuzara, 1996; Daramola and Afolami 2013) and the majority are algal and fungal feeders (Yeates et al. 1993). Additionally, they are not important plant-parasitic nematodes of pulse crops; therefore, they were not accounted in this survey.

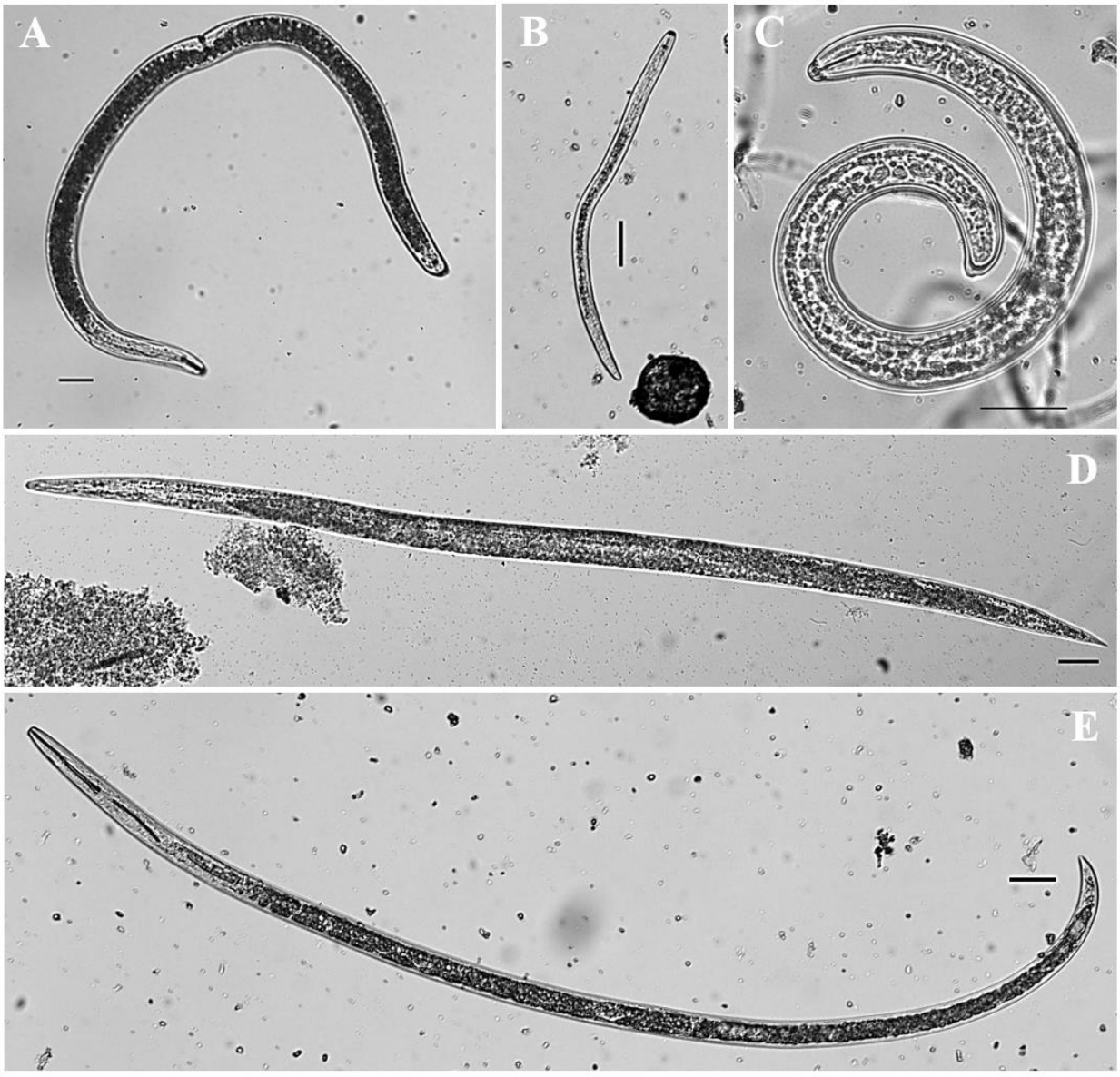

Figure 2.4 Light photomicrographs of nematodes extracted from soil and plant samples in this survey. (A) *Tylenchorhynchus* (B) *Pratylenchus* (C) *Helicotylenchus* (Photo Credit Terri Fairman, modified) (D) *Ditylenchus* (E) *Xiphinema*. Scale bars A, C = 20 µm; B, D = 35 µm and E = 70 µm.

Table 2.4. Number of fields and sample types (above ground plant parts and soil for crops and weeds) positive for plant-parasitic nematodes recovered from commercial fields sampled in 2014 and 2015.

Taxa	Positive fields	Positive sample types			
		Above Ground Crop	Above Ground Weed	Soil Under Crop	Soil Under Weed
Anguina	3	3	–	–	–
Aphelenchoides	81	40	16	43	33
Aphelenchidae	49	25	5	34	12
Ditylenchus	50	12	26	18	15
Helicotylenchus	25	–	–	15	12
Hoplolaimus	2	–	–	1	1
Longidorus	1	–	–	–	1
Merlinius	1	–	–	1	–
Paratylenchus	45	–	–	36	25
Pratylenchus	20	–	–	15	8
Subanguina	6	3	3	1	–
Paratrichodorus	1	1	–	–	–
Tylenchorhynchus	60	1	–	43	35
Xiphinema	6	–	–	3	3
Samples analysed	93	178	125	93	69

2.5.1 Prominence of Plant-Parasitic Nematodes in Pulse Crops Fields of the Canadian Prairies

Prominence values refer to how frequently and abundantly a nematode taxon is found in a field by combining the frequency and density into one value. Plant-parasitic nematode prominence differed among crops and sample types. In general, plant-parasitic nematodes were less prominent in plant samples than in soil samples.

2.5.1.1 Pea fields. *Ditylenchus* was the most prominent plant parasitic genus found in above ground samples from pea fields. It had a higher prominence in thistle samples (18.42) than in pea

samples (0.64). *Aphelenchoides* was the second most predominant genus with predominance values of 2.27 and 0.94 in above ground pea and thistle respectively. Other nematode genera had low prominence values (Table 2.5). *Anguina* and *Paratrichodorus* were found only in above ground crop samples and not in weed (Table 2.5). In soil samples, *Tylenchorhynchus* and *Paratylenchus* were the most predominant genera, with a 77.64 prominence value found in thistle soil and 70.81 in pea soil, respectively (Table 2.5). *Ditylenchus* was more prominent in soil samples from thistle (39.51) than soil samples from pea (9.98). *Pratylenchus* and *Paratylenchus* were more prominent in soil samples from pea than soil samples from weed.

2.5.1.2 Lentil fields. All plant-parasitic nematodes recovered from above ground lentil samples – namely *Anguina*, *Aphelenchoides, Aphelenchidae* and *Ditylenchus* – had very low prominence values, ranging from 0.04 to 0.23 (Table 2.6). In lentil soil samples, *Paratylenchus* was the most prominent genus (241.33). It was followed by *Aphelenchoides* (29.46), *Tylenchorhynchus* (15.81), *Ditylenchus* (9.98) and lastly, *Pratylenchus*, which was recovered at very low prominence value (1.88) (Table 2.6).

2.5.1.3 Chickpea fields. Only *Aphelenchoides* and *Ditylenchus* were found in above ground samples (Table 2.7). *Ditylenchus* had a relatively high prominence (37.56) in thistle samples from chickpea fields, and it was not present in chickpea samples (Table 2.7). In soil samples, *Pratylenchus* was the most prominent (73.88, in thistle soil). *Aphelenchoides* (19.90) and *Aphelenchidae* (2.84) were the only two other taxa recovered from thistle soil in chickpea fields. In chickpea soil samples, nematodes belonging to the taxon *Aphelenchoides* (26.47), *Paratylenchus* (23.25) and *Pratylenchus* (24.87) were also recovered at comparatively higher prominence.

Table 2.5. Number of positive samples, frequency and prominence of plant-parasitic nematodes recovered from commercial yellow pea fields in 2014 and 2015.

Taxa	Above Ground Crop			Above Ground Weed			Soil Under Crop			Soil Under Weed		
	N° positive samples	Frequency (%)	Prominence	N° positive samples	Frequency (%)	Prominence	N° positive samples	Frequency (%)	Prominence	N° positive samples	Frequency (%)	Prominence
Anguina	2	1	<1	–	–	–	–	–	–	–	–	–
Aphelenchoides	37	27	2	14	12	1	32	45	21	32	49	20
Aphelenchidae	17	13	1	5	4	<1	18	25	11	11	17	13
Ditylenchus	11	8	1	25	22	18	9	13	10	15	23	40
Helicotylenchus	–	–	–	–	–	–	14	20	51	12	18	47
Hoplolaimus	–	–	–	–	–	–	–	–	–	1	2	<1
Longidorus	–	–	–	–	–	–	–	–	–	1	2	1
Merlinius	–	–	–	–	–	–	1	1	1	–	–	–
Paratylenchus	–	–	–	–	–	–	26	37	71	25	38	41
Pratylenchus	–	–	–	–	–	–	12	17	48	6	9	26
Subanguina	3	2	1	3	3	<1	1	1	1	–	–	–
Paratrichodorus	1	1	1	–	–	–	–	–	–	–	–	–
Tylenchorhynchus	1	1	<1	–	–	–	33	46	51	35	54	78
Xiphinema	–	–	–	–	–	–	3	4	3	3	5	2
Samples analysed		135			113			71			65	

Table 2.6. Number of positive samples, frequency and prominence of plant-parasitic nematodes recovered from commercial lentil fields in 2014 and 2015.

Taxa	Above Ground Crop			Soil Under Crop		
	N° positive samples	Frequency (%)	Prominence	N° positive samples	Frequency (%)	Prominence
Anguina	1	4	<1	–	–	–
Aphelenchoides	3	12	<1	6	46	29
Aphelenchidae	7	27	<1	10	77	16
Ditylenchus	1	4	<1	5	38	10
Paratylenchus	–	–	–	6	46	241
Pratylenchus	–	–	–	1	8	2
Tylenchorhynchus	–	–	–	6	46	16
Samples analysed		26			13	

Table 2.7. Number of positive samples, frequency and prominence of plant-parasitic nematodes recovered from commercial chickpea fields in 2014 and 2015.

Taxa	Above Ground Crop			Above Ground Weed			Soil Under Crop			Soil Under Weed		
	N° positive samples	Frequency (%)	Prominence	N° positive samples	Frequency (%)	Prominence	N° positive samples	Frequency (%)	Prominence	N° positive samples	Frequency (%)	Prominence
Aphelenchoides	–	–	–	2	18	<1	5	56	26	1	33	20
Aphelenchidae	1	6	<1	–	–	–	6	67	14	1	33	3
Ditylenchus	–	–	–	1	9	38	4	44	5	–	–	–
Helicotylenchus	–	–	–	–	–	–	1	11	9	–	–	–
Hoplolaimus	–	–	–	–	–	–	1	11	1	–	–	–
Paratylenchus	–	–	–	–	–	–	4	44	23	–	–	–
Pratylenchus	–	–	–	–	–	–	2	22	8	2	67	74
Tylenchorhynchus	–	–	–	–	–	–	4	44	7	–	–	–
Samples analysed		17			11			9			3	

2.5.2 Nematode Abundance

Nematode abundance varied within crops, weed and sample types.

2.5.2.1 Pea. In above ground samples, except for *Ditylenchus*, all nematode genera found in above ground crops and above ground weed had mean densities lower than 8 nematodes/g of the sample. *Ditylenchus* had the highest density recorded in above ground samples (maximum density of 300 nematodes/g in thistle plants) (Table 2.8). *Ditylenchus* had significantly higher (p = 0.002) mean density in thistles flowers (mean of 55.17 nematodes/g) than in other above ground crop and thistle sample types.

In soil samples, the highest mean densities were recorded for *Paratylenchus* (131.44 nematodes/100g dry soil, in pea soil), *Helicotylenchus* (114.8 nematodes/ 100g dry soil in pea soil and 109.55 nematodes/100g dry soil in thistle soil), *Pratylenchus* (106.37 nematodes/100g dry soil in pea soil) and *Tylenchorhynchus* (105.57 nematodes/100g dry soil in thistle soil) (Table 2.8). Mean nematode populations did not significantly differ between pea soil and thistle soil for those above-mentioned genera. In contrast, *Ditylenchus* had significantly higher (p = 0.04) mean population in thistle soil samples (82.39 nematodes/100g dry soil) than in pea soil samples (27.69 nematodes/100g dry soil) (Table 2.8).

Table 2.8. Mean population densities for soil (nematodes per 100g dry soil mass) and plant sample type (nematodes per gram) positive for plant-parasitic nematode taxa from commercial yellow pea fields sampled in 2014 and 2015.

Taxa	Above Ground Crop						Above Ground Weed						Soil Under Crop			Soil Under Weed		
	Pods			Other[a]			Flowers			Other								
	Mean	SE[b]	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Anguina	<1	n/a[c]	n/a	1	n/a	n/a	–[d]	–	–	–	–	–	–	–	–	–	–	–
Aphelenchoides	<1 a	0.4	0.1-0.6	5 a	1	<1-25	<1 a	0.6	<1-1	<1	1	<1-10	32 A	5	4-159	28 A	4	1-97
Aphelenchidae	<1	n/a	n/a	2	1	<1-12	0	n/a	n/a	1	1	<1-3	22	6	3-79	31	10	2-91
Ditylenchus	1 b	1	0.4-2	4 b	2	1-14	55 a	26	<1-300	27 ab	11	<1-37	28 B	11	6-91	82 A	25	1-332
Helicotylenchus	–	–	–	–	–	–	–	–	–	–	–	–	115 A	37	4-506	110 A	38	7-328
Hoplolaimus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	2	n/a	n/a
Longidorus	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	7	n/a	n/a
Merlinius	–	–	–	–	–	–	–	–	–	–	–	–	10	n/a	n/a	–	–	–
Paratylenchus	–	–	–	–	–	–	–	–	–	–	–	–	131 A	32	3-1024	66 A	16	6-420
Pratylenchus	–	–	–	–	–	–	–	–	–	–	–	–	106 A	37	8-630	87 A	43	4-176
Subanguina	<1	n/a	n/a	6	5	<1-11	–	–	–	1	0.3	<1-1	8	n/a	n/a	–	–	–
Paratrichodorus	–	–	–	8	n/a	n/a	–	–	–	–	–	–	–	–	–	–	–	–
Tylenchorhynchus	–	–	–	1	n/a	n/a	–	–	–	–	–	–	74 A	15	4-659	106 A	22	3-980
Xiphinema	–	–	–	–	–	–	–	–	–	–	–	–	13 A	4	6-18	10 A	3	3-15

[a] other = stems and leaves
[b] SE = ± standard error
[c] n/a = value not provided because nematode genus was found in a single sample
[d] - = absence of nematode population of that taxon
Within above ground crop and weed, means followed by the same lowercase letter in row are not significantly different ($P < 0.05$).
Within soil crop and weed, means followed by the same capital letter in row are not significantly different ($P < 0.05$).

2.5.2.2 Lentil. All the four nematode taxa –*Anguina*, *Aphelenchoides*, *Aphelenchidae* and *Ditylenchus* – recovered from above ground lentil samples had low densities (equal or less than 0.71 nematodes/g) (Table 2.9). In lentil soil, *Paratylenchus* was recovered at high mean population density (355.23 nematodes/100g dry soil). *Ditylenchus* was recovered at a density of 16.01 nematodes/100g dry soil (range 4.27–40.33), and *Pratylenchus* was only recovered from one sample and had a density of 6.77 nematodes/100g dry soil.

Table 2.9. Mean population densities for soil (nematodes per 100g dry soil mass) and plant sample type (nematodes per gram) positive for plant-parasitic nematode taxa from commercial lentil fields sampled in 2014 and 2015.

Taxa	Above Ground Crop						Soil Under Crop		
	Pods			Other[a]					
	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Anguina	-[b]	-	-	<1	n/a	n/a	-	-	-
Aphelenchoides	<1	0.3	0.1-0.3	1	n/a	n/a	43	25	5-169
Aphelenchidae	<1	0.2	0.1-0.6	1	0.2	0.2-1	18	4	1-41
Ditylenchus	-	-	-	<1	n/a	n/a	16	7	4-40
Paratylenchus	-	-	-	-	-	-	355	125	7-901
Pratylenchus	-	-	-	-	-	-	7	n/a	n/a
Tylenchorhynchus	-	-	-	-	-	-	23	10	2-84

[a] other = stems and leaves
[b] – = absence of nematode population of that taxon

2.5.2.3 Chickpea. In above ground samples, only one taxon, *Aphelenchidae*, was recovered from chickpea samples (Table 2.10). *Aphelenchoides* and *Ditylenchus* were the only genera recovered from above ground thistle samples. *Ditylenchus* displayed relatively high density (124.57 in thistle steam and leaves), while *Aphelenchidae* and *Aphelenchoides* had low densities (ranging from 0.19 nematodes/g to 0.52 nematodes/g) (Table 2.10). In soil samples, *Pratylenchus* had the highest mean density (90.48 nematodes/100g dry soil) and it was recovered from thistle soil. In contrast, it was found at low density (16.76 nematodes/100g dry soil) in chickpea soil, although it did not differ statistically ($p = 0.08$). *Aphelenchoides* had the highest density (35.51 nematodes/100g dry soil) within chickpea soil samples, followed by *Paratylenchus* (34.88 nematodes/100g dry soil) and *Helicotylenchus* (26.85 nematodes/100g dry soil).

Table 2.10. Mean population densities for soil (nematodes per 100g dry soil mass) and plant sample type (nematodes per gram) positive for plant-parasitic nematode taxa from commercial chickpea fields sampled in 2014 and 2015.

Taxa	Above Ground Crop			Above Ground Weed						Soil Under Crop			Soil Under Weed		
	Other[a]			Flowers			Other								
	Mean	SE[b]	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range	Mean	SE	Range
Aphelenchoides	–[c]	-	-	1	n/a[d]	n/a	<1	n/a	n/a	36	19	2-108	34	n/a	n/a
Aphelenchidae	<1	n/a	n/a	-	-	-	-	-	-	18	3	7-25	5	n/a	n/a
Ditylenchus	-	-	-	-	-	-	125	n/a	n/a	8	3	4-16	-	-	-
Helicotylenchus	-	-	-	-	-	-	-	-	-	27	n/a	n/a	-	-	-
Hoplolaimus	-	-	-	-	-	-	-	-	-	4	n/a	n/a	-	-	-
Paratylenchus	-	-	-	-	-	-	-	-	-	35	18	3-72	-	-	-
Pratylenchus	-	-	-	-	-	-	-	-	-	16 A	7	7-27	90 A	37	53-127
Tylenchorhynchus	-	-	-	-	-	-	-	-	-	10	5	3-23	-	-	-

[a] other = stems and leaves
[b] SE = ± standard error
[c] - = absence of nematode population of that taxon
[d] n/a = value not provided because nematode genera was found in a single sample
Within soil crop and weed, means followed by the same capital letter in row are not significantly different ($P < 0.05$).

2.5.3 Species Identification of Main Plant-Parasitic Nematodes by Molecular Analyzes

2.5.3.1 *Ditylenchus* spp. Species identification of *Ditylenchus* was confirmed using species-specific PCR, PCR-RFLP and sequencing (positive results are summarized in Table 2.11).

Table 2.11. Molecular characterization of *Ditylenchus* species recovered from plant and soil samples from commercial pulse fields sampled in 2014 and 2015.

Field	Field Crop	Province	Sample #	Sample I.D.	Year	Description	Species-specific PCR	RFLP	Sequencing	Identity (%)	E value
1	Pea	SK	1	25F-1	2014	Thistle flowers	*D. weischeri*				
			2	25F-2	2014	Thistle flowers	*D. weischeri*				
			3	25F-4	2014	Thistle flowers	*D. weischeri*				
			4	25SL-5	2014	Thistle stems and leaves	*D. weischeri*				
2	Pea	SK	5	39F	2014	Thistle flowers	*D. weischeri*				
			6	39SL-1	2014	Thistle stems and leaves	*D. weischeri*				
			7	39SL-2	2014	Thistle stems and leaves	*D. weischeri*				
			8	39SL-3	2014	Thistle stems and leaves	*D. weischeri*				
3	Pea	SK	9	70-1	2014	Thistle soil	*D. weischeri*				
			10	70-2	2014	Thistle soil	*D. weischeri*				
			11	70-5	2014	Thistle soil	*D. weischeri*				
			12	70-6	2014	Thistle soil	*D. weischeri*				
4	Pea	SK	13	148SL-3	2014	Thistle stems and leaves	*D. weischeri*				
			14	148SL-4	2014	Thistle stems and leaves	*D. weischeri*				
5	Pea	AB	15	165-2	2014	Thistle soil	*D. weischeri*				
			16	165-3	2014	Thistle soil	*D. weischeri*				
			17	165-4	2014	Thistle soil	*D. weischeri*				
6	Pea	SK	18	186SL-1	2014	Thistle stems and leaves	*D. weischeri*				
			19	186SL-2	2014	Thistle stems and leaves	*D. weischeri*		*D. weischeri*	100	0.0
			20	186SL-3	2014	Thistle stems and leaves	*D. weischeri*				

Field	Field Crop	Province	Sample #	Sample I.D.	Year	Description	Species-specific PCR	RFLP	Sequencing	Identity (%)	E value
		SK	21	186SL-4	2014	Thistle stems and leaves	*D. weischeri*				
			22	186SL-5	2014	Thistle stems and leaves	*D. weischeri*				
7	Pea	SK	23	190SL-1	2014	Thistle stems and leaves	*D. weischeri*				
8	Pea	MB	24	7P -2	2015	Pea pods	*D. dipsaci*		*D. dipsaci*	99	2e-126
			25	7P-3	2015	Pea pods	*D. dipsaci*		*D. dipsaci*	98	2e-177
			26	7P-4	2015	Pea pods	*D. dipsaci*	*D. dipsaci*			
			27	7P-7	2015	Pea pods	*D. dipsaci*	*D. dipsaci*			
			28	7P-9	2015	Pea pods	*D. dipsaci*	*D. dipsaci*			
			29	7P-13	2015	Pea pods	*D. dipsaci*	*D. dipsaci*			
			30	7P-14	2015	Pea pods	*D. dipsaci*				
			31	8F-2	2015	Thistle flowers	*D. weischeri*		*D. weischeri*	99	0.0
9	Pea	MB	32	16F-1	2015	Thistle flowers	*D. weischeri*	*D. weischeri*			
			32	16F-2	2015	Thistle flowers	*D. weischeri*				
			33	16F-3	2015	Thistle flowers	*D. weischeri*				
			34	16F-4	2015	Thistle flowers	*D. weischeri*				
			35	16F-5	2015	Thistle flowers			*D. weischeri*	99	0.0
			36	16F-7	2015	Thistle flowers	*D. weischeri*				
			37	16F-10	2015	Thistle flowers	*D. weischeri*				
			38	16F-12	2015	Thistle flowers	*D. weischeri*				
10	Pea	MB	39	20F	2015	Thistle flowers			*D. weischeri*	98	0.0
			40	20SL	2015	Thistle stems and leaves			*D. weischeri*	98	0.0
11	Pea	MB	41	23P-1	2015	Pea pods	*D. weischeri*				
			42	24F-1	2015	Thistle flowers	*D. weischeri*	*D. weischeri*			
			43	24F-2	2015	Thistle flowers	*D. weischeri*				
			44	24F-3	2015	Thistle flowers	*D. weischeri*				

Field	Field Crop	Province	Sample #	Sample I.D.	Year	Description	Species-specific PCR	RFLP	Sequencing	Identity (%)	E value
		MB	45	24F-4	2015	Thistle flowers	*D. weischeri*				
			46	24F-9	2015	Thistle flowers	*D. weischeri*				
			47	24F-10	2015	Thistle flowers	*D. weischeri*				
			48	24F-11	2015	Thistle flowers	*D. weischeri*		*D. weischeri*	99	0.0
			49	24F-12	2015	Thistle flowers			*D. weischeri*	99	0.0
12	Pea	MB	50	28F-1	2015	Thistle flowers	*D. weischeri*		*D. weischeri*	99	7e-121
			51	28F-2	2015	Thistle flowers	*D. weischeri*				
			52	28F-3	2015	Thistle flowers	*D. weischeri*				
			53	28F-4	2015	Thistle flowers	*D. weischeri*				
			54	28F-5	2015	Thistle flowers	*D. weischeri*				
			55	28F-7	2015	Thistle flowers	*D. weischeri*				
			56	28F-8	2015	Thistle flowers	*D. weischeri*				
			57	28F-9	2015	Thistle flowers	*D. weischeri*				
			58	28F-10	2015	Thistle flowers	*D. weischeri*				
			59	28F-11	2015	Thistle flowers	*D. weischeri*				
			60	28F-12	2015	Thistle flowers	*D. weischeri*				
			61	28F-13	2015	Thistle flowers	*D. weischeri*				
			62	28F-14	2015	Thistle flowers	*D. weischeri*				
			63	28F-35	2015	Thistle flowers	*D. weischeri*				
			64	28F-40	2015	Thistle flowers		*D. weischeri*			
			65	28F-46	2015	Thistle flowers	*D. weischeri*				
13	Pea	AB	66	31P	2015	Pea pods	*D. weischeri*	*D. weischeri*			
			67	31SL-1	2015	Pea stems and leaves		*D. weischeri*			
14	Pea	AB	68	40SL-4	2015	Thistle stems and leaves	*D. weischeri*				
			69	40SL-5	2015	Thistle stems and leaves			*D. weischeri*	99	
			70	40SL-7	2015	Thistle stems and leaves			*D. weischeri*	99	

Field	Field Crop	Province	Sample #	Sample I.D.	Year	Description	Species-specific PCR	RFLP	Sequencing	Identity (%)	E value
			71	40SL-8	2015	Thistle stems and leaves			*D. weischeri*	99	
15	Pea	AB	72	41	2015	Pea soil	*D. weischeri*				
			73	42-2	2015	Thistle soil	*D. weischeri*				
16	Pea	AB	74	44SL	2015	Thistle stems and leaves			*D. weischeri*	99	0.0
17	Pea	AB	75	57	2015	Pea soil	*D. weischeri*				
			76	58-4	2015	Thistle soil	*D. weischeri*				
			77	58-5	2015	Thistle soil	*D. weischeri*				
			78	58-6	2015	Thistle soil	*D. weischeri*				
18	Pea	AB	80	60F-1	2015	Thistle flowers	*D. weischeri*				
			81	60F-2	2015	Thistle flowers	*D. weischeri*				
			82	60F-3	2015	Thistle flowers	*D. weischeri*		*D. weischeri*	96	9e-154
			83	60F-4	2015	Thistle flowers	*D. weischeri*		*D. weischeri*	97	0.0
			84	60F-5	2015	Thistle flowers	*D. weischeri*				
19	Chickpea	AB	85	76SL-10	2015	Thistle stems and leaves	*D. weischeri*				
			86	76SL-2	2015	Thistle stems and leaves	*D. weischeri*				
			87	76SL-3	2015	Thistle stems and leaves	*D. weischeri*				
			88	76SL-4	2015	Thistle stems and leaves	*D. weischeri*				
			89	76SL-5	2015	Thistle stems and leaves	*D. weischeri*				
			90	76SL-7	2015	Thistle stems and leaves	*D. weischeri*		*D. weischeri*	99	0.0
			91	76SL-8	2015	Thistle stems and leaves	*D. weischeri*				
			92	76SL-9	2015	Thistle stems and leaves	*D. weischeri*		*D. weischeri*	96	0.0

PCR with *D. dipsaci* and *D. weischeri* species-specific primers were performed in 91 DNA samples from 23 fields. DNA from 75 single individuals from 16 fields yield a single product with an approximate size of 200bp, consistent for *D. weischeri* identity (Table 2.11). DNA from seven individuals recovered from pea pods samples from one field located in Manitoba yield an approximate 200bp band size when tested with *D. dipsaci* primers. The same sample was extracted five times during the following months. The first two re-extractions yielded a total of nine individual nematodes also positive for *D. dipsaci*, according to species-specific PCR results and *Ditylenchus* spp. were not recovered for the last two re-extractions. This pea field, positive for *D. dipsaci* in pods and seeds from pea, was also positive for *D. weischeri* when samples from thistle flowers were tested. Representative results with species-specific primers are given in Figure 2.5.

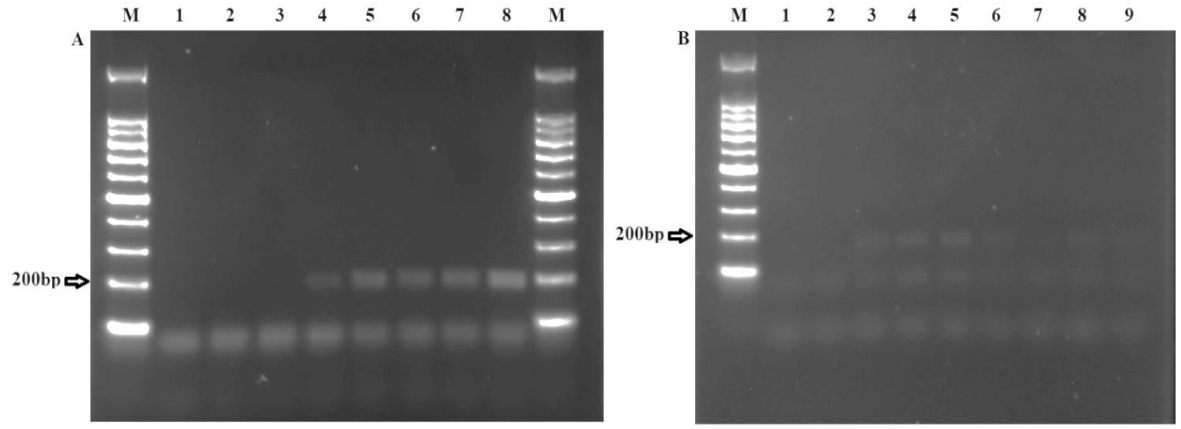

Figure 2.5 Representative gels with amplification products obtained in PCR with *Ditylenchus* species-specific primers. (A) *D. weischeri* species-specific primer. Lanes: 1 and 2, non-template control (water); 3, negative control, *D. dipsaci*, garlic, Ontario; 4 to 7, *D. weischeri*, thistle flowers, Manitoba; 8, positive control *D. weischeri*. (B) *D. dipsaci* species-specific primer. Lanes: 1, non-template control DNA (water); 2, negative control, *D. weischeri*; 3 to 8, *D. dipsaci* from pea pods, Manitoba; 9, positive control, *D. dipsaci*, garlic, Ontario; M: 100bp ladder (Qiagen).

Thirty DNA samples from six fields were also tested with *D. dipsaci* and *D. weischeri* primers but yielded no amplification.

Seventeen DNA samples from 11 fields could be assigned to species level through sequencing. They showed highest similarity with *D. weischeri* (16 samples) and *D. dipsaci* (1 sample) in blast search. Twelve of those samples that were sequenced were also previously tested with species-specific PCR, and the results were all consistent (Table 2.11). Eight *Ditylenchus* sequences, from eight fields, had low identity values and/or low query cover and could not be assigned to species (Appendix I.1). Either PCR or direct sequencing failed for seven DNA samples from seven fields.

PCR-RFLP. Nine DNA samples from five pea fields positive for *Ditylenchus* from pea pods, pea stem and leaves and thistle flowers were analysed for RFLP-whole ITS profiles. Seven DNA samples were tested through RFLP and specific-PCR, and the results were consistent (Table 2.11). Banding patterns obtained with Bsh123, HinfI, MspI, RsaI and TaqI restriction enzymes had an approximate size: unrestricted, (800), (280,220), (330,140), (480,310) and (350,240), respectively, for *D. weischeri*; and (510,310), (410,320), (350,150), (340,300,140) and (390, 210) for *D. dipsaci*, for the same restriction enzymes, respectively. Diagnostic patterns were similar to those published for *D. weischeri* from thistles from MB and SK samples and for *D. dipsaci* from garlic samples from Ontario (Tenuta at al. 2014). Restriction enzyme profile from two individual nematodes from pea pods and pea stem and leaves are illustrated in Figure 2.6.

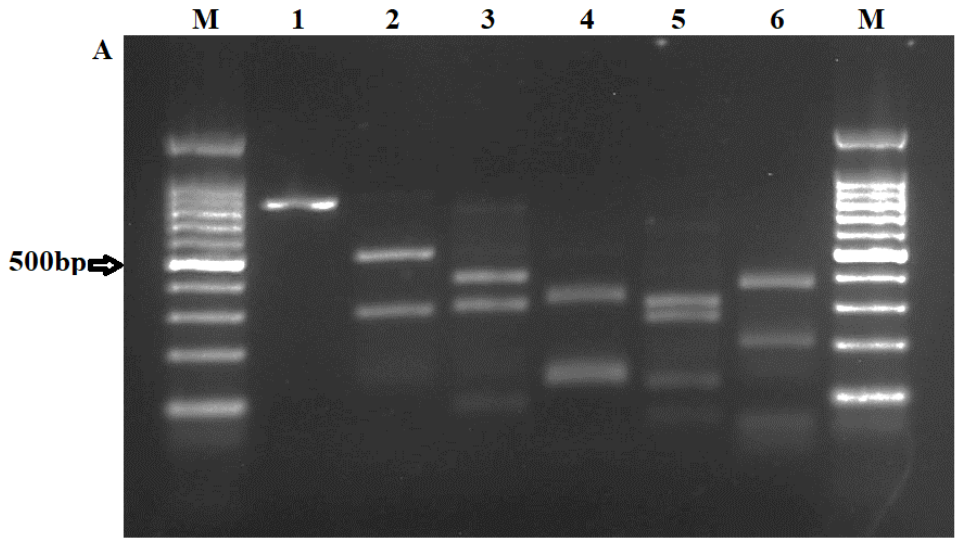

Figure 2.6 Representative ITS-PCR-RFLP profile for *Ditylenchus dipsaci*. Lane 1, unrestricted PCR product; Lanes 2 to 6: Bsh1236I, HinfI, MspI, RsaI, and TaqI. M: 100 bp ladder, (Promega, Madison, WI).

PCR-RFLP traits confirmed the results obtained through species-specific PCR and sequencing analyses (Table 2.11).

Ten fields had low *Ditylenchus* numbers and/or failed to yield *Ditylenchus* species during re-extractions and therefore were not analysed.

Thus far, the only species identified for *Ditylenchus* were *D. weischeri* and *D. dipsaci* from 19 out of 50 fields that were positive for this nematode. *Ditylenchus* species from eight fields could not be assigned to species through matching their sequencing into BLASTlast database (due to low matching identity score or query cover) (Appendix I.1). *Ditylenchus* individuals from six fields tested with *D. weischeri* and *D. dipsaci* primers were negative for both of those species. *Ditylenchus* individuals from seven fields could not be assigned to species because either PCR or

direct sequencing failed. Moreover, ten fields had low density numbers and/or could not be collected and therefore were not analysed.

2.5.3.2 *Pratylenchus* spp. PCR with universal and species-specific primers were performed in 59 individual *Pratylenchus* spp. from nine pea and chickpea fields from Alberta and Saskatchewan. PCR with *P. neglectus* species-specific primers, yield a single positive 290bp band for 16 out of 35 specimens tested. The samples positive for *P. neglectus* were from one pea, one chickpea and four thistle soil samples (samples belonged to five fields) (Table 2.12). The same DNA samples positive for *P. neglectus* and additional DNA samples prevenient from the same soil samples were also tested with *P. penetrans* and *P. thornei* species-specific primers. All the 25, 37 and 11 *Pratylenchus* DNA samples tested with *P. penetrans*, *P. thornei* and *P. scribineri* specific primers respectively either failed to produce a band or produced bands of the wrong size, indicating that those species were not present in the six fields tested (data not shown). We caution, however, since no positive controls were used in those reactions, it is not possible to conclude with certainty that those species were not present in those fields tested.

Three specimens were selected for sequencing of D2-D3 expansion segments of 28S rDNA gene. Sequence analysis results showed the highest similarity with *P. neglectus* for one specimen, while the two other sequences had low query covers and identity values and could not be assigned to species.

In total, *Pratylenchus* spp. from five fields out of six that were analysed were identified as *P. neglectus* (Table 2.12). The other 14 positive fields that were positive for *Pratylenchus* could not be analysed due to the small number of recovered nematodes during re-extractions.

Table 2.12. Molecular characterization of *Pratylenchus* species recovered from plant and soil samples from commercial pulse fields sampled in 2014 and 2015.

Field #	Province	Field Crop	Sample #	Sample I.D.	Year	Description	Species-specific PCR	Sequencing	Identity (%)	E value
1	Alberta	Pea	1	50-2	2015	Thistle soil	*P. neglectus*			
			2	50-4	2015	Thistle soil	*P. neglectus*			
			3	50-5	2015	Thistle soil	*P. neglectus*			
			4	50-6	2015	Thistle soil	*P. neglectus*			
			5	50-7	2015	Thistle soil	*P. neglectus*			
			6	50-8	2015	Thistle soil	*P. neglectus*			
			7	50-9	2015	Thistle soil	*P. neglectus*			
			8	50-10	2015	Thistle soil	*P. neglectus*			
2	Alberta	Chickpea	9	69-1	2015	Chickpea soil	*P. neglectus*			
3	Alberta	Chickpea	10	90-1	2015	Thistle soil	*P. neglectus*			
4	Saskatchewan	Pea	11	184-2	2014	Thistle soil		*P. neglectus*	99	0.0
			12	184-5	2014	Thistle soil	*P. neglectus*			
			13	185-1	2014	Pea soil	*P. neglectus*			
			14	185-4	2014	Pea soil	*P. neglectus*			
			15	185-5	2014	Pea soil	*P. neglectus*			
			16	185-6	2014	Pea soil	*P. neglectus*			
5	Saskatchewan	Pea	17	192-1	2014	Thistle soil	*P. neglectus*			

2.5.3.3 *Aphelenchoides*. *Aphelenchoides* nematodes suspected to be plant-parasitic, based on morphological features, such as lip and caudal region, were tested through species-specific PCR and sequenced. DNA samples from 22 *Aphelenchoides* nematodes belonging to two pea fields (four pea and thistle plant samples in Alberta) were tested with *A. besseyi*, *A. ritzemabosi* and *A. fragariae* (Ritzema - Bos) Christie and *A. subtenuis* Cobb species-specific primers. The results showed no specific amplification, indicating that the nematodes tested did not belong to those species.

Sequencing results of 12 specimens from five fields (lentil and pea plant samples from AB and SK) had low identification match and low query cover and therefore could not be assigned to species level. Six out of the 12 specimens sent for sequencing were also tested with species-specific PCR as mentioned above.

2.5.3.4 Other genera. Other species identified through sequencing were *Paratylenchus nanus* (one field, pea soil, AB), *Xiphinema rivesi* (one field, pea soil, AB) and *Aphelenchus avenae* (two fields, pea soil and pea stem and leaves, AB).

Moreover, blast searcher of the partial the D2–D3 region of the 28S rDNA gene and the ITS region of the 18S rRNA gene analysed did not confirm the species names or identities at genus level for one *Paratylenchus*, three *Tylenchorhynchus* and six non-identified genera.

2.6 Discussion

Nematodes belonging to twenty nematode taxa including *Anguina, Aphelenchoides, Aphelenchidae, Ditylenchus, Helicotylenchus, Hoplolaimus, Longidorus, Merlinius,*

Paratrichodorus, Paratylenchus, Pratylenchus, Subanguina, Tylenchorhynchus and *Xiphinema* were isolated from the soil and (or) the peas, chickpeas, lentils and thistle plants from the Canadian Prairies (Table 2.4).

2.6.1 *Ditylenchus* (Stem and Bulb Nematode)

Ditylenchus species were commonly found in this survey. Fifty of the 93 fields analyzed contained at least one *Ditylenchus* nematode. It was more commonly found in pea fields, and it was more prominent in weed than crop samples. In particular, pea soil samples had a much lower prominence (9.86) for *Ditylenchus* when compared to thistle soil samples (39.51). The same is true for above ground samples, where above ground pea samples had much lower prominence value (0.64) than above ground thistle samples (18.42). Population density varied from 1 to 300 nematodes/g, and significant higher mean densities were recovered from thistle flowers and soil samples when compared to above ground plant parts and soil from peas.

Two species among the *Ditylenchus* genus were identified based on molecular characterization, namely *D. weischeri* and *D. dipsaci*. In total, 19 fields were positive for *Ditylenchus* across Alberta, Saskatchewan and Manitoba (Table 2.11).

Ditylenchus weischeri was recently described as a new species as opposed to a race of *D. dipsaci* (Chizhov et al. 2010). Greenhouse host suitability and development and reproduction studies of *D. weischeri* show that it is not a parasite infesting commonly cultivated crops in the Canadian Prairies (Hajihassani et al. 2016, 2017). Although host study shows that *D. weischeri* can reproduce weakly in two pea varieties (Hajihassani et al. 2016), it needs an average temperature of 27ºC for its complete development and reproduction, and in the Canadian Prairies, this mean daily temperature is unusual and unsustained (Hajihassani et al. 2017). Additionally, *D.*

weischeri cannot be transmitted in harvest grain because it is not a seedborne parasite (Hajihassani et al. 2016).

Ditylenchus dipsaci is a damaging crop pest of quarantine status in many countries due to its wide host rage and ability to cause extensive economic losses in many important crops. It can parasitize about 500 plant species (Schmidt-Rhaesa 2014). It is a strong parasite of pea and garlic, and it can reproduce weakly in one chickpea and three bean varieties (Hajihassani et al. 2016). This nematode can survive freezing temperatures and drying conditions by entering a cryptobiotic state, allowing it to be viable for more than 25 years (Schmidt-Rhaesa 2014). During this cryptobiotic state, the nematode is heat and chemical resistant, and adding to the wide host range, control for this nematode is very difficult, justifying the high quarantine status this pest has around the world (Schmidt-Rhaesa 2014). *Ditylenchus dipsaci* is distributed worldwide, but it is particularly important in temperate regions (Schmidt-Rhaesa 2014). In Canada, *D. dipsaci* has been reported in British Columbia causing damage in alfalfa (Vrain and Lalik 1983). In Alberta, it has been found in association with pea, green bean, alfalfa, potato, corn, beet and carrot (Hawn 1973). In Ontario, it was reported parasitizing onion and garlic (Fushtey and Kelly 1975), and it has become a serious pest of garlic in this province (Yu at al. 2010). However, the accuracy of the findings from old surveys is now suspect because of the availability of molecular studies and new species that have been found or taxonomically rearranged. More recently, *D. dipsaci* have been reported to cause significant economic losses in two garlic fields in Southern Manitoba in 2015 (Hajihassani and Tenuta 2017). The grower had obtained the contaminated garlic seed pieces from Ontario, which is known for having *D. dipsaci*. In this survey, *D. dipsaci* was recovered at a low density (1.6 nematodes/g pods) from pods of one yellow pea field in Rhineland, Manitoba (but not from soil). Re-sampling of the same field and adjacent ones for soil the following year failed to

obtain *D. dipsaci*. Canola and soybean was in rotation that year and therefore only soil samples were collected. This is a concern since *D. dipsaci* has variable susceptibility responses to different crop varieties and could potentially infect pea fields grown around infested garlic fields (Hajihassani et al. 2016). Manitoba does not export field pea grain and therefore this finding does not interfere with Canadian yellow pea exports. Nonetheless, garlic producers should only grow and distribute clean plant material to avoid contamination with *D. dipsaci* on other fields and/or crops. A practical solution is using certified plant material or sending samples to a reliable nematode laboratory.

Ditylenchus dipsaci was described as a parasite of Canada thistle in Saskatchewan (Watson and Shorthouse 1979), but the results of Tenuta et al. (2014) indicate it was likely the closely related *D. weischeri*. The *Ditylenchus* species frequency found in pea in this survey is higher when compared to that found by Tenuta et al. (2014). They analysed 538 harvest grain pea samples in 2009 and 2010 in Saskatchewan, Alberta and Manitoba (Tenuta et al. 2014). In their study, weed debris were commonly found in the harvest pea samples provided by the growers. The weed debris found were then screened and analysed separately from pea grain samples. *Ditylenchus* was found at a higher frequency in this study – 14% of the 248 above ground pea samples compared to 2% of 538 pea harvest samples. They found 11 positive *Ditylenchus* samples in the weed debris samples while no *Ditylenchus* were present in the grain pea samples. In this survey, 25 above ground thistle samples and two samples from pea seeds and pods were positive for *Ditylenchus*. The *Ditylenchus* nematodes found in the two samples from pea seeds and pods had low density values (mean of 1 nematode/g plant) and were identified as *D. dipsaci* for one sample and *D. weischeri* for the other sample. As mentioned earlier, *D. dipsaci* has recently been reported in two garlic fields in Manitoba (Hajihassani and Tenuta 2017). *Ditylenchus dipsaci* was not found in the

samples analysed in the survey by Tenuta at al. (2014). It is important to note that Tenuta et al. (2014) analyzed yellow pea grain from harvest samples provided by farmers, while in this study, the samples were collected directly from fields.

In chickpea fields, *Ditylenchus* spp. was recovered from one stem and leaf weed sample at a density of 124.57 nematodes/g and from chickpea soil samples at a mean density 7.59 nematodes/g. *Ditylenchus* were not found in weed soils from chickpea fields which may be due to the low number of samples analyzed (only three). Two out of four chickpea soil samples positive for *Ditylenchus* were tested with *D. weischeri* and *D. dipsaci* species-specific primers and had no amplification. The other two samples were not identified due to the small number of nematodes recovered. PCR and sequencing results were positive for *D. weischeri* for nematodes recovered from the stem and leaf thistle sample recovered from the chickpea field.

In lentil fields, *Ditylenchus* was recovered from one lentil sample (stem and leaves) at very low density (0.2 nematodes/g) and from lentil soil (16.1 nematodes/100g soil). Weed samples were not analyzed for lentil fields. *Ditylenchus* recovered from two out of five lentil soils were tested with *D. weischeri* and *D. dipsaci* species-specific primers and displayed no amplification. Other lentil samples were not identified to species due to the small number of nematodes recovered for two samples, and PCR and DNA extraction fail for one sample.

Ditylenchus weischeri is not a parasite of lentils or chickpeas. *Ditylenchus dipsaci*, on the other hand, can reproduce weakly in chickpeas and have been reported infesting lentils (Greco and Di Vito 1994), although Hajihassani et al. (2016) demonstrated that *D. dipsaci* does not reproduce in the variety CDC Greenland tested. *Ditylenchus dipsaci* was not identified in the samples tested in chickpeas or lentil fields in this survey.

Eight *Ditylenchus* sequences had low identity values and/or low query cover and were not assigned to species. *Ditylenchus* genus has more than 80 described species, most of which are fungal-feeders; only a few are plant parasitic (Duncan and Moens 2013; EPPO 2017). *Ditylenchus* species from soil samples that could not be identified with species-specific primers or sequencing are likely fungal-feeder species that do not have their sequence in the BLAST database.

Nine fields had more than 100 nematodes/100g dry soil, and they were all from thistle samples (soil, flowers, stem and leaves) mostly from pea fields; however, one chickpea field had 124 nematodes/100g and it was from thistle's stem and leaves. *Ditylenchus* threshold values for economic damage vary greatly between nematode species, host crops, climate and soil types. A threshold of one *Ditylenchus* spp. nematode/100 mL soil has been reported for oats (Rivoal and Cook 1993). *Ditylenchus dipsaci* economic threshold of 10 nematodes/100 cm^3 soil has been reported for alfalfa in Kansas, USA (Todd and Jardine 1993). A threshold of two nematodes per gram of soil has been reported for onion (Bridge and Starr 2007) while a threshold of 100 nematodes/kg of soil has been reported for most crops, including onion, garlic and other alliums ("Bulb and Stem Nematode" 2009; Celetti and Potter 2010). However, *Ditylenchus* threshold can vary substantially; for *D. dipsaci*, even low population densities is a potential concern, as population for this nematode species can build up fast, causing great crop losses (Celetti et al. 2000).

The *D. dipsaci* positive pea field found in this survey in addition to the positive garlic fields recently reported, both in Manitoba, is a cause of concern. Preventive measures should be taken since this nematode is difficult to control once it has been established in high numbers.

2.6.2 *Pratylenchus* (Root-lesion Nematode)

Pratylenchus nematodes (root-lesion) were recovered from 20 out of 93 fields analysed in this survey and it was found more prominently in soil samples from pea. Root-lesion nematodes are migratory endoparasitic root feeding nematodes (Duncan and Moens 2013). They can infect a vast number of crops, such as legumes, cereals, potato, soybean, forage crops, maize, and many others; in fact, they may have the broadest host ranges among plant-parasitic nematodes (Duncan and Moens 2013). Many species in this genus cause substantial crop yield losses worldwide, therefore, from an economic point of view, *Pratylenchus* genus are among the most important group of plant-parasitic nematodes, next to *Heterodera* and *Meloidogyne* (Duncan and Moens 2013).

In this survey, nematodes recovered from crop and thistle soil samples from three pea and two chickpea fields in Alberta and Saskatchewan were positive for *P. neglectus*. It is possible that mixed populations were present in those fields since not all specimens were positive for *P. neglectus* according to PCR and sequencing results.

Pratylenchus neglectus and other *Pratylenchus* species have been identified in previous studies in Canada. Mahran et al. (2010) found *P. neglectus* in Manitoba when surveying commercial potato fields. Potter and Townshend (1973) encountered five species of *Pratylenchus*, namely, *P. neglectus*, *P. thornei*, *P. pratensis*, *P. crenatus*, and *P. penetrans*, when surveying corn, cereals, and forages in Ontario. Yu (2010) identified mounted *Pratylenchus* species from the Canadian National Collection of Nematodes. According to his research, *P. fallax* and *P. penetrans* were found in Saskatchewan; *P. neglectus, P. penetrans*, and *P. pratensis* in Manitoba; and of *P. neglectus, P. crenatus, P. hexincisus*, and *P. penetrans* in Alberta.

The economic importance of *P. neglectus* in the Canadian Prairies is not well understood. In Alberta, *Pratylenchus neglectus* has also been found in association with potato and wheat (Forge et al. 2015). In the USA, this nematode species is a major parasite of cereals (Smiley et al. 2005). In the Pacific Northwest region, *P. neglectus* has been reported damaging wheat crops, with yield losses generally in the range of 16 to 40%, although losses up to 71% have been reported when this nematode is associated with other plant-parasitic nematodes and soilborne infecting fungi (Smiley et al. 2005). In Idaho, USA, significant economic damage (up to 90% production loss) has been reported in dryland pea and lentil caused by *P. neglectus* in mixed population with *P. thornei* and *Paratylenchus hamatus* (Riga et al. 2008).

Host preferences studies have shown chickpea to be a moderate to good host for *P. neglectus* whereas pea is shown to be a poor host (Taylor et al. 2000; Smiley et al. 2014; May et al. 2016). A greenhouse host suitability study of thirty Pacific Northwest crops and cultivars shows that two lentils, three chickpeas, one oat and five canola cultivars were good hosts for *P. neglectus* among other crops (Smiley et al. 2014). However, all four pea cultivars tested, including two yellow pea, 'Universal' and 'Badminton', were poor to minor hosts for *P. neglectus* but were good hosts for *P. thornei* (Smiley et al. 2014). A rotational study conducted in Montana showed that *P. neglectus* density increased under winter wheat and canola making it good hosts whereas pea and lentil were not (May et al. 2016). They also observed that nematode populations were sustained from spring to fall under pea but declined through winter following this crop (May et al. 2016). In Australia, 81 cultivars from 12 field crop species were assessed in a host preference study and they had similar conclusions. They found that chickpea, wheat, and canola were good hosts for *P. neglectus*, field pea and triticale were poor hosts while barley, oat and durum wheat were moderate hosts (Taylor et al. 2000). There are also some contradictory host preferences for this nematode,

which can be partially explained by the existence of physiological races (Griffin 1991; Mahran et al. 2010). For example, populations of *P. neglectus* from Manitoba and Idaho differ from populations from Ontario concerning the host-parasite relationship to potato cultivar Russet Burbank (Hafez et al. 1999; Mahran et al. 2010).

Economic loss threshold has been reported for *Pratylenchus* for different crops and regions. For pulse crops, namely beans and cowpeas, a threshold of 50 and 100 nematodes/100 cm^3 of soil has been reported for *Pratylenchus* found in sand to sandy loam and clay loam to clay soils, respectively (Dickerson et al. 2000). Additionally, an action threshold level of 500 nematodes/100 cm^3 of soil has been proposed for wheat for the southwest region of the USA (Dickerson et al. 2000). However, experiments carried out in northeastern Oregon-USA concluded that initial *P. neglectus* populations of 200 nematodes per 100g of soil were sufficient to cause yield losses on wheat (Smiley et al. 2005). Also, for *P. neglectus*, even lower threshold levels may be enough to cause crop damage – for example, a threshold of 90 nematodes per 100g soil for this species has been reported for potato field in some regions of Bulgaria (Samaliev and Markova 2014). In general, a threshold of 100 nematodes/100mL soil for *Pratylenchus* spp. for most crops has also been determined (Rivoal and Cook 1993; Thompson et al. 2010; Fleming et al. 2016). In this survey, the greatest population densities of *Pratylenchus* spp. were found in soil samples from pea with mean density of 117.01 nematodes per 100g of soil (Table 2.8). Weed soil from pea fields had mean density of 87.07 nematodes per 100g of soil (Table 2.8). In chickpea fields, population densities were greater in weed soil samples than in crop soil, with mean of 90.48 nematodes per 100g soil compared to 6.63 nematodes per 100g soil found in only one soil crop sample (Table 2.9). Seven out of 20 fields positive for *Pratylenchus* had above 100 nematodes/100g of soil and could indicate potential problem for farmers. Half of the samples that had more than 100

nematodes/100g soil were from weed soil and the other half were from pea soil. Twelve fields had more than 50 nematodes/100g soil, which is the threshold proposed for some pulses for sand to sandy loam soils (Dickerson et al. 2000). *Pratylenchus neglectus* were found in higher population densities in thistles soil samples from pea fields. Samples that were positive for this nematode species had relatively high densities values ranging from 78.0 to 176.4 nematodes per 100g soil (four out of seven fields that had more than 100 nematodes/100g soil were positive for *P. neglectus,* data not shown). *Pratylenchus neglectus* was also found in pea soil sample at a density of 104.4 nematodes per 100g soil. Two samples from chickpea fields were positive for *P. neglectus*, one from crop soil had low density of 26.8 and one from thistle soil had 128.0 nematodes per gram soil. This suggests that most fields with *P. neglectus* were above the threshold. Finding high densities of *P. neglectus* in thistle soils was surprising since studies in the USA and Bulgaria have shown that thistle is a poor or non-host for this nematode species (Samaliev and Markova 2014; Smiley et al. 2014).

It is not possible to infer that *Pratylenchus* found in this survey has a parasitic relationship with the plants studied because infested fields could be directly correlated with cereal crops in rotations. Pea crops are typically grown in rotation with wheat which has been reported to be a good host of *P. neglectus* (May et al. 2016). Studies have shown that populations of *Pratylenchus* can increase dramatically when cereal is grown in high frequency in the rotations (Smiley et al. 2005). Additionally, in a host study for *P. neglectus* in Montana, the authors found that nematode populations were sustained but not increased during pea rotations (May et al. 2016). Therefore, nematode populations found in this survey could be surviving on peas, chickpeas and weeds but not increasing. Additionally, it is important to note that those samples may have mixed populations, which could influence host preferences.

More specifically, host suitability of pulse crops for *P. neglectus* in pulse crop cultivars grown in Western Canada is unknown. There is a need for field-based and controlled (greenhouse) studies on *P. neglectus* population density and crop performance. Whether these populations are feeding on or only surviving on peas and/or thistles or the crops in rotation needs to be ascertained through experiments under controlled and field conditions.

While *P. neglectus* is a concern to growers in other regions (May et al. 2016), its potential for yield constrains needs to be investigated in the Canadian Prairies as nematode losses will depend on, *inter alia*, presence of race types (Curtis et al. 2002), crop species, cultivar, growth stage, management factors such as cropping system, rotation, tillage practices and abiotic factors such as soil temperature, moisture and texture (Smiley et al. 2005). Therefore, further studies of this species is also necessary to evaluate its economic importance for growers in the Canadian Prairies.

2.6.3 *Paratylenchus* (Pin Nematode)

Almost half (48%) of the soil samples analysed from pulse crops fields were positive for *Paratylenchus* (Table 3). *Paratylenchus* was the most prominent genus recovered from lentil soil (241.33) and the second most prominent recovered from both pea (70.81) and chickpea (24.87) in this survey.

Paratylenchus is an ectoparasite of cosmopolitan distribution (Mai and Mullin 1996). It has been associated with an extensive list of plants, including pea, lentil, chickpea (Castillo et al. 2008), vegetables, grasses and fruit trees (Dropkin 1989; Jaques and Jarvis 1994). It is often associated with vegetable crops in eastern Canada, but it rarely causes economic damage (Jaques and Jarvis 1994).

Paratylenchus threshold limits have not been established for pulse crops. Nonetheless, a threshold range of 51 to 300 nematodes/100g of soil has been reported for grasses and cereals with this genus ("Nematode Management Action Plan" 2012; Niblack and Paul 2014, as cited in Fleming at al. 2016). Similar threshold values have also been proposed for corn, wherein *Paratylenchus* populations of 51 to 100 nematodes/cm^3 of soil pose a minor risk, 100 to 500 a moderate risk, 501 to 1000 pose a severe risk and nematodes above 1000 pose a very severe risk of crop damage (Compton 2015). In this survey, most lentil and pea fields had above threshold limits for *Paratylenchus*. Five pea fields had more than 300 nematodes/100g soil (four samples were from pea soil and one from thistle soil, ranging from 314.71 to 1023.51 nematodes/100g soil). At the lower end of the threshold, eight pea soil samples had more than 51 nematodes/100g soil (55.64 to 141.95), and eight thistle soil samples from pea fields had more than 51 nematodes per/100g soil (59.23 to 182.71), which can be interpreted as a minor risk of economic damage. In addition, *Paratylenchus* nematodes found in yellow pea fields had high mean densities in pea soil (131.44 nematodes/100g dry soil) when compared to thistle soil (66.0 nematodes/100g dry soil), but the result was not statistically significant. In lentil soil, *Paratylenchus* was recovered at a high mean population density (355.23 nematodes/100g dry soil); therefore, the majority of the fields had densities above the suggested threshold limits. Four lentil fields had more than 300 nematodes/100g soil (ranging from 322.65 to 900.90 nematodes/100g soil), and one field had more than 51 nematodes/100g soil (149.48 nematodes/100g soil). *Paratylenchus* was not recovered from thistles in lentil fields, but only one sample was analysed. In chickpea fields, while *Paratylenchus* was one of the most prominent nematodes, its mean density (34.88 nematodes/100g dry soil) was relatively low. Yet two chickpea soil samples had more than 51 nematodes/100g soil (60.44 to 71.62 nematodes/100g dry soil), which can be interpreted as a minor risk for economic damage

when a host crop is present as it is in the lower end of the threshold. *Paratylenchus* was not found on the three samples of weed soil that were analysed.

In this study, one *Paratylenchus* species, namely *P. nanus*, was identified through sequencing. The sequencing of the 28S RNA region of two specimens was analysed through BLAST, and they best matched a sequencing of *P. nanus* from North Dakota, locus MH237651. The specimens identified in this survey were from a soil sample from a pea field in Saskatchewan, where the population density for *Paratylenchus* was 813.65 nematodes/100g soil.

Paratylenchus nanus have been shown to be able to parasitize three pea cultivars in a reproduction study in which *Paratylenchus* was collected from infested pea fields from North Dakota (Plaisance et al. 2016). The study shows that *P. nanus* was successful at parasitizing the pea cultivars tested even at the lowest initial population level tested of 300 nematodes/100g soil although the reproduction rate was the highest at 600 nematodes/100g soil (Plaisance et al. 2016). The positive pea field for *P. nanus* found in this survey had a population density higher than 600 nematodes/100g soil, and it is possible that it is feeding on peas. *Paratylenchus nanus* was first described in North Dakota in the United States (Subbotin et al. 2014). In Canada, *P. nanus* has been collected in Saskatchewan from grasses (Raski 1975). This nematode species has a wide host rage, comprising grasses (Watson and Bell 2001) and fruit trees (Fisher 1967).

Previous surveys have also identified other *Paratylenchus* species. *Paratylenchus projectus* was spotted in Alberta in alfalfa, alsike and red clover (Webster and Hawn 1973). Damage in alfalfa was associated with a higher population density of this nematode in some areas (Webster and Hawn 1973). However, a study on forage legume yields in microplots suggests that *P. projectus* is not a major parasite of alfalfa as it is considered to be a poor host when compared

to trefoil and red and white clover, which are good hosts (Townshend and Potter 1981). *Paratylenchus projectus* has also been found in Ontario in pea (Senwel 1971), oats, barley, forage, wheat and corn (Potter and Townshend 1973). Other species of *Paratylenchus* described in Canada are *P. aciculus* Brown, *P. robustus* de Man, *P. aculentus* Brown, *P. neoprojectus* Wu & Hawn, *P. tenuicaudatus* Wu, *P. hamatus, P. tateae* Wu & Townshend, *P. brevihastus* Wu, *P. labiosus* Anderson & Kimpinski, and *P. variabilis* Raski. These species were described by Yu (2009) from preserved specimens at the Canadian National Collection of Nematodes, and most were associated with grasses (Yu 2009).

Data concerning the nematode damage caused by *Paratylenchus* is scarce in the Prairies provinces. Moreover, this nematode is often present in mixed populations with other genera, such as *Pratylenchus* and *Tylenchorhynchus*, making crop damage difficult to assess (Chitambar 2017). In other regions, however, extensive crop damage has been reported for pulse crops and other crops. Near Moscow, Idaho, United States, *Paratylenchus hamatus,* in mixed population with *Pratylenchus neglectus* and *P. thornei,* caused great yield reduction (up to 90%) in dry land peas and lentils (Riga at al. 2008). In Ontario, *Paratylenchus* has reduced yields of rhubarb at population densities of 500 nematodes/100g soil (Townshend and Potter 1973).

The high density in which *Paratylenchus* was recovered for most fields in this survey, especially lentil and pea fields, is a potential concern. It needs to be investigated whether it is parasitizing the crops in rotation and causing economic damage.

2.6.4 *Helicotylenchus* (Spiral Nematode)

Helicotylenchus are among the main plant parasitic nematodes that attack pulse crops, however as an ectoparasite it is considered to cause less harm when compared to endoparasites

(Askary 2017). *Helicotylenchus* are called spiral nematodes because they assume a "spiral" form when relaxed by heat. Damage caused by this nematode feeding in the roots is physical and it can serve as a port of entry for other soil pathogens such as fungus and bacteria (Yeates 1984). *Helicotylenchus* spp. have a extensive host range including chickpeas, common beans (Askary 2017), peas (Wouts and Knight 1993) and lentils (Marais and Swart 1996).

Out of the 25 fields that were positive for *Helicotylenchus*, seven had nematodes population densities above 100 nematodes/100g dry soil and they were all from either pea soil or thistle soil samples from pea fields. Three fields had population density ranging from 115.66 to 168.82 nematodes/100g of soil; three fields had population ranging from 222.88 to 299.26 nematodes/100g of soil and one field had high population density for both pea soil (506.03 nematodes/100g dry soil) and thistle soil (328.27 nematodes/100g dry soil) (data not shown). Relatively high densities of this nematode are necessary to cause damage to crops (Mekete and Reynolds 2011). Dickerson et al. (2000) reported threshold values according to soil type and crop for North Carolina. In beans and cowpeas, for example, a threshold of 200 nematodes per 100 cm^3 of soil for sandy to sandy loam and 300 nematodes per 100 cm^3 of soil for clay loam to clay soils were suggested (Dickerson et al. 2000). Moreover, thresholds of 300 to 400 nematodes per 100 cm^3 have been reported for *Helicotylenchus* nematodes in grasses and cereals (Mekete and Reynolds 2011; Niblack and Paul 2014). However, densities of 100 nematodes per 100 cm^3 were enough to cause damage to corn and soybean in Iowa,USA (Norton and Nyvall 1999). Action threshold limits for *Helicotylenchus* in peas are unknown. Taking beans and cowpeas threshold as a reference, only four fields had above threshold limits in this survey. Threshold limits for cereal are higher and only one field met the threshold limit proposed. These observations may indicate that *Helicotylenchus* could be a problem for cereals in rotation. However, caution is needed since

the threshold proposed for cereals was not studied in the Canadian Prairies and could vary greatly due differences in soil and climate.

Helicotylenchus economic importance in North America is not well understood. In North Central USA, *H. pseudorobustus* Steiner is the most prevalent *Helicotylenchus* species and it is considered a mild pathogen of corn (Norton, 1977; Norton et al., 1978). In Canada, *H. pseudorobustus* has been found in Manitoba and eastern provinces associated with grasses (Anderson 1974). *Helicotylenchus phalerus* Anderson and *H. spitsbergensis* Loof have been reported in Alberta (Anderson 1974) and *H. cornurus* Anderson has been reported in Saskatchewan associated with *Agrotis* sp. grasses. *Helicotylenchus digonicus* Perry is widespread in Canada and has been reported in Alberta, Saskatchewan and Manitoba, it has a wide host rage including corn, oats, flax, apple, pasture grasses, barley, alfafa (*Medicago sativa* L.) and cherry *Prunus* sp. (Anderson 1974). *Helicotylenchus dihystera* Cobb has been reported in Ontario (Townshend, 1984; CABI/EPPO, 2010). *Helicotylenchus microlobus* Perry which is a parasite of soybean (Taylor, 1960), is one of the most common *Helicotylenchus* species found in Minnesota and has recently found in North Dakota (Yan et al. 2017).

2.6.5 *Tylenchorhynchus* (Stunt Nematode)

Tylenchorhynchus was frequently found in fields sampled in this survey, 60 fields out of 93 fields analysed were positive for this genus. *Tylenchorhynchus* along with *Helicotylenchus* described above are the main ectoparasitic nematodes that parasitize pulse crops (Askary 2017). *Tylenchorhynchus* is a pest of chickpeas (Maqbool, 1987), common beans, corn, sorghum, wheat and other crops *(*Anderson and Potter 1991). In chickpeas, it can retard root system growth (Ali, 1995) and reduce rhizobium nodulation (Tiyagi and Alam 1990).

In a South Carolina USA guideline report, some thresholds for *Tylenchorhynchus* based on crop and soil type have been suggested (Dickerson et al. 2000). In that report beans had a 200 to 300 nematodes/100 cm^3 of soil threshold population, whereas wheat had 100 nematodes/100 cm^3 of soil for sand to clay soils for wheat and more than 500 nematodes/100 cm^3 of soil have been suggested for corn and soybean crops (Dickerson

et al. 2000). The threshold varied with soil type where upper limits were suggested for clay type soils while lower densities where suggested for sandy type soils. In this survey, mean densities of *Tylenchorhynchus* recovered from soil under crop (74 nematodes per 100g dry soil) in pea fields were lower than those found in the Montana wheat survey conducted by Johnson (2007). However, some fields had densities above the threshold limit proposed for wheat, beans, soybean and corn in other regions (Dickerson et al. 2000) and could indicate a potential concern for growers.

Economical importance of *Tylenchorhynchus* nematodes for pulse crops in Canada has not been determined. In Ontario, *T. claytoni* Steiner and *T. nudus* Allen have been identified (Potter and Townshend 1973). In a survey of wheat fields in Montana USA, *Tylenchorhynchus* were widely distributed and had high populations levels indicating that this nematode may be a concern for wheat producers in that region (Johnson, 2007).

2.6.6 *Aphelenchoides* (Bud and Leaf Nematode)

Aphelenchoides were one of the most frequently found nematode recovered in this survey; 81 out 93 fields analysed were positive for this genus. Although *Aphelenchoides* were frequently found in this survey, populations densities were relatively low for all crops and sample types.

Most *Aphelenchoides* spp. are mycophagous and are common in soil (Ruess 2000; Duncan and Moens 2013). Only a few *Aphelenchoides* species are important crop pathogens such as *A.*

bessey, *A. fragariae* and *A. ritzemabosi* (Duncan and Moens 2013). *Aphelenchoides* spp. suspected to be the main plant parasitic nematodes for this group based on morphological features were analysed using molecular identification methods. Sequencing results obtained did not have a high match with the sequences in the BLAST database. This indicate that major plant parasitic nematodes are not present in the fields tested. Moreover, species found in soil are likely fungal feeders and have probably not been described yet.

In Canada, there are only a few reports of this nematode. It has been associated with ornamental plants in British Columbia (Morrall 2000). In Minnesota, USA they are parasites of some crops and ornamental plants (Chen et al. 2012).

2.6.7 *Aphelenchidae*

Nematodes belonging to the *Aphelenchidae* taxa were recovered from 49 fields in this survey and populations density were relatively low for all crops and sample types. Nematodes from the *Aphelenchidae* family are mostly frugivorous and unbiquous in occurrence (Barker and Darling, 1965).

In this study only two specimens from the *Aphelenchidae* taxa have been sequenced, one from pea soil and the other from pea stem and leaves samples from two fields in Alberta. The results best-matched sequencings of *Aphelenchus avenae*. *Aphelenchus avenae* is primarily a fungal feeder however it can be a parasite of higher plants, although reports of pathogenicity of this nematode have been few (Barker and Darling, 1965; Kumari 2012). It has been associated with many plants worldwide, including faba bean (Azimi 2018) grasses (Skantar et al. 2011) and wheat (Walker 1984). It has also been reported in samples of native grasses in Saskatchewan and alfalfa in Alberta (Sewell 1977).

Although it is very likely that *Aphelenchus avenae* was present in many of the fields sampled in this survey, it may not be a concern to growers since severe economic damage caused by this nematode have not yet been reported (Coyne et al. 2014).

2.6.8 *Xiphinema* (Dagger Nematode)

Dagger nematodes occurred infrequently in yellow pea fields in crops (4% of 71 samples) and weed soils (5% of 65 samples) in this survey (Table 2.5), which corresponds to 6 positive fields. This genus has several economically important nematodes that cause additional damage to root tissues; many species are responsible for transmitting important plant nepoviruses (Decraemer and Geraert 2013) and, therefore, is in the list of Pests Regulated by Canada published by the Canadian Food Inspection Agency (CFIA 2018). *Xiphinema* spp. are frequently found in North America and have wide host distribution, including in some vegetables, fruit trees, wheat, corn, soybean, grasses and peas (Potter and Townshend 1973; Robbins 1993; Pinkerton et al. 1999). In this study, sequencings of the D2-D3 region of 28S rRNA gene from two individuals showed the highest similarity with *X. rivesi*; they were recovered from pea soil samples from Alberta. *Xiphinema rivesi* has been considered the most widespread species among the *Xiphinema americanum* group in North America (Robbins 1993) and is a vector of nepoviruses, namely *Cherry rasp leaf virus*, *Tobacco ringspot virus* and *Tomato ringspot virus* (Brown 1994). Because of its activity as a vector, this nematode has been given an A2 quarantine status by the European and Mediterranean Plant Protection Organisation (EPPO 2017). *Xiphinema rivesi* was earlier described in Eastern Canada found associated with alfalfa, oats, grapes and other crops. Surveys conducted in neighbour states in USA have also found *X. rivesi*. In Idaho, it has been associated with apple trees and potatoes (Hafez et al. 2010). In Washington, *X. rivesi* was found in a survey of cherry orchards. Those plants were contaminated with a cherry rasp leaf disease, and this

nematode is linked to the rapid spread of the *Cherry rasp leaf virus* responsible for causing this disease (Akinbade et al. 2014). As *Xiphinema rivesi* is not a parasite of pulse crops the presence of this nematode in pea fields in this survey could be attributed to crops in rotation. Other dagger nematodes species, however, have been associated with peas, namely *X. americanum* (Riggs and Niblack 1993) and *X. bakeri* (McElroy 1972). More recently, in a survey conducted in Minnesota, *X. americanum* was the most common nematode found around roots of peas and other crops (Taylor et al. 1958). *Xiphinema americanum* have been previously described in the provinces of Saskatchewan, Ontario, Nova Scotia and Quebec. Other species of *Xiphinema* that were reported in Canada include *X. occiduum* in Saskatchewan (associated with wheat and grass), Manitoba (associated with apple) and Alberta (associated with barley, alfalfa and wheat); *X. thornei* in British Columbia (Ebsary et al. 1984) and *X. chambersi* Thorne in Ontario (Ebsary et al. 1984; Akinbade et al. 2014).

Xiphinema was not prominent in the fields surveyed in this study. However, this is consistent with a survey carried out in the northwest region of Minnesota (Chen et al. 2012). Additionally, the low frequency and abundance of *Xiphinema* found in this survey suggests that these nematodes are not a concern for growers in the regions surveyed.

2.6.9 *Longidorus, Paratrichodorus, Hoplolaimus, Merlinius*

Other important groups of nematodes isolated from soil samples in this study included *Hoplolaimus* spp., *Longidorus* spp., *Merlinius* spp., and *Paratrichodorus* spp.; however, the population density and frequency of these nematodes were low with prominence values no greater than 1.37. Species identification of the nematodes belonging to this genus was not possible due to the limited number of nematodes recovered.

2.6.9.1 *Longidorus* (Needle Nematode). *Longidorus* are relatively long nematodes and have a characteristic and easily identifiable odontostylet with a single guide ring (Decraemer and Geraert 2013). Eight *Longidorus* species, out of about 160 described in the literature so far, are vectors of nepoviruses (Taylor and Brown 1997; Gutiérrez-Gutiérrez et al. 2016). Species that carry nepoviruses can cause substantial crop damage even at very low population density, such as one or two nematodes/100 cm^3 soil (Niblack 2003). *Longidorus* species have been observed in Canadian crop fields from previous studies. *Longidorus elongatus*, for instance, has been reported in British Columbia and Ontario (EPPO 2017). This nematode is a vector of the *Raspberry ringspot virus* and *Tomato black ring virus* and has been associated with pea in North America (Norton et al. 1984) and with chickpea in India (Castillo et al. 2008). Other *Longidorus* species vectors of nepoviruses found in North America are *L. breviannulatus* Norton & Hoffmann and *L. diadecturus* Eveleigh &Allen. The former is associated with damage on creeping bent grass golf greens in Quebec (Simard et al. 2009) and is also found in Ontario (Allen 1986). Additionally, *L. breviannulatus* is a damaging parasite of corn in Iowa and Illinois (Malek 1980; Macguidwin 1989). The latter is a vector of peach rosette mosaic virus and has been found in Ontario (Eveleigh and Allen 1982).

2.6.9.2 *Paratrichodorus* (Stubby-root Nematode). *Paratrichodorus* nematodes, which were also found at low frequency in this survey, can also carry nepoviruses (Decraemer and Geraert 2013). Even at low population levels, they can cause substantial damage by transmitting Pea Early Browning virus and Tobacco Rattle virus, which cause the disease of pea and the Corky ringspot disease (Jensen and Allen 1964) of potato, respectively. Pea Early-Browning virus has not been reported in Canada (Acheraghian 2016); however, Tobacco Rattle virus is present in Saskatchewan, Alberta, British Columbia and the neighbouring USA states, such as North Dakota

and Minnesota (EPPO 2017). *Paratrichodorus* species are commonly found in blueberry-growing regions of North America, and one species, *P. renifer* Siddiqi is potentially damaging to blueberry plants (Forge et al. 2012). *Paratrichodorus minor* Colbran has been found in Canada and is widely distributed in the USA. This nematode has been associated with over a hundred crops, including yellow peas, wheat, soybean and potatoes (Schneider and Ferris 1987). *Paratrichodorus allius* Jensen was associated with crop losses in potatoes grown in the Pacific Northwest and in the Western USA due to the transmission of Tobacco Rattle virus (Gieck et al. 2007; Mojtahedi and Santo 1999).

2.6.9.3 *Hoplolaimus* (Lance Nematode). Lance nematodes are large migratory endoparasitic nematodes and have a characteristic tulip-shaped stylet knob (Decraemer and Geraert 2013). This genus has plant-parasitic species that feed on the roots of a large variety of crops including crops in rotation in the fields sampled in this study, such as cereals, soybeans and wheat (Dropkin 1989). *Hoplolaimus* species have been observed in crop fields in North America from previous literature, for example, *H. galeatus* Cobb, *H. coronatus*, among others (Taylor et al. 1958). *Hoplolaimus galeatus* has a wide host range that includes grasses, cotton and trees (Decraemer and Geraert 2013). *Hoplolaimus coronatus* Cobb was commonly found in flax fields in an earlier survey in Minnesota (Taylor et al. 1958). The low prominence values found for *Hoplolaimus* in the current study agree with the results of a survey conducted in organic fields in Minnesota (Chen et al. 2012).

2.6.9.4 *Merlinius* (Stunt Nematode). *Merlinius* were found in pea soil samples in very low numbers in this survey. Many species of *Merlinius* have been described in Canada (Anderson and Ebsary 1982); more specifically, *M. brevidens* Allen has been found in Ontario (Sewell 1977). *Merlinius brevidens* has been associated with yellow pea (Pinkerton et al. 1999), chickpea (Castillo

et al. 2008), wheat and other crops. However, economic damage has mostly been reported in wheat and barley crops (Langdon et al. 1961; Smiley et al. 2006).

Although some of those genera have nematode species that have great economic importance among major agricultural crops (Nicol et al. 2011), the low frequency and density of *Longidorus*, *Paratrichodorus*, *Hoplolaimus* and *Merlinius* found in this survey suggests that they are not a concern at this point among pulse growers in Western Canada.

2.7 Conclusion

The primary goal of this research was to determine the distribution of *Ditylenchus dipsaci* in pea crops in the Canadian Prairies, if present. The secondary goal was to determine other important plant-parasitic nematodes that may be present in commercial pulse crop fields in Saskatchewan, Alberta and Manitoba.

This research could successfully confirm that *D. weischeri* is prevalent in the pea fields in the Prairies provinces while *D. dipsaci* is nearly absent. *Ditylenchus weischeri* was frequently found in thistle samples. *Ditylenchus weischeri* is a parasite of thistle and not of commonly grown crops in the Canadian Prairies (Hajihassani et al. 2016; 2017). *Ditylenchus dipsaci* was only found in one pea field in Manitoba; it was recovered at a low density. *Ditylenchus dipsaci* had been reported to cause significant economic losses in two garlic fields in Southern Manitoba in 2015. The positive *D. dipsaci* pea field found in this survey in addition to the recently reported positive garlic fields, both in Manitoba, raise the potential for concern for local growers. Preventive measures should be taken since this nematode is difficult to control once it has been established in high numbers. Findings regarding *Ditylenchus* were consistent with Tenuta et al. (2014)

preliminary study, *D. dipsaci* is not a concern for security exports for yellow pea as it is nearly absent and the only field positive for this nematode is located in Manitoba, which does not export yellow peas.

Plant-parasitic nematodes belonging to twenty genera were recovered from soil and (or) plants of peas, chickpeas, lentils and thistle plants, including *Anguina*, *Aphelenchoides*, *Ditylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Longidorus*, *Merlinius*, *Paraphelenchus*, *Paratylenchus*, *Pratylenchus*, *Subanguina*, *Paratrichodorus*, *Tylenchorhynchus* and *Xiphinema*, were isolated from the soil and (or) plants of peas, chickpeas, lentils and thistle plants from the Canadian Prairies.

A few nematode groups of parasites of pulse crops namely, *Pratylenchus, Paratylenchus, Helicotylenchus* and *Tylenchorhynchus* were found at densities above the threshold levels in some fields and could be a concern for growers.

Pratylenchus neglectus was present in most samples that were above threshold limits for *Pratylenchus*. *Pratylenchus neglectus* is a very serious pest of wheat in the Pacific Northwest region in the USA and chickpea, canola and lentil have been shown to be good hosts for this nematode in other regions. However, in this survey, *P. neglectus* was more predominant in thistle soil samples. High populations of this nematode could be a problem for host crops commonly grown in rotation with pulses such as wheat and canola. Host preferences studies for *P. neglectus* in cultivar crops in the Prairie are necessary to determine the parasitism relationship with this nematode in crops grown in the Canadian Prairies.

Other species recovered from pulse crops in this survey were *Xiphinema revesi* and *Paratylenchus nanus.* Further analysis is needed to identify more nematodes recovered from the

high-density fields to help determine if the nematodes present in those fields are economically important plant-parasitic nematodes.

The data presented in this survey adds to the understanding of which plant-parasitic nematodes are a problem or a potential problem for pulse crops in the Canadian Prairies.

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3 GENERAL DISCUSSION

3.1 Important Findings

The results from this survey confirmed that *Ditylenchus dipsaci* is not widespread in commercial pulse crops fields in the Canadian Prairies. Only one sample was positive for *D. dipsaci* and it was from pea pods from a field located in Manitoba. The positive field was re-sampled the following year and no *D. dipsaci* was recovered, however canola was in rotation and only soil samples were analysed. Manitoba is not the major producer of peas in Canada and does not export pea grains. Nevertheless, caution should be taken to not allow this nematode to disperse to other areas.

Moreover, this survey indicates that a number of fields in the Canadian Prairies potentially have plant-parasitic nematode species at high density population levels, which can potentially cause crop damage. *Pratylenchus* spp. were found at high population densities for some fields. Some species of this nematode can be very damaging to pulse crops (Duncan and Moens 2013). *Pratylenchus neglectus* was identified in most of the high-density fields. This nematode species is known to cause great economical damage to wheat in the Pacific North West region in the USA (Smiley et al. 2005). Wheat and canola, common good hosts for *P. neglectus* (Smiley et al. 2005), are important crops grown in the Canadian Prairies. However, it is not clear if this nematode is parasitizing pulse crops in the Canadian Prairies and needs further research to be ascertained. Other nematodes recovered above threshold levels in this survey were *Tylenchorhynchus*, *Helicotylenchus* and *Paratylenchus*. Those nematode taxa are commonly found in soils around the world, and often are found at high densities. Some species from those taxa are important plant parasitic nematodes of pulse crops. One species identified was *Paratylenchus nanus* and it was

recovered at above threshold levels in pea field. This nematode can parasitize peas, however economical damage reports are not common. More analyses are necessary to identify those nematode to species before conclusions can be drawn.

Another species identified in this survey was *Xiphinema rivesi*, recovered from pea soil samples from Alberta. *Xiphinema rivesi* has been considered the most widespread species from the *Xiphinema americanum* group in North America and is a vector of nepoviruses. *Xiphinema rivesi* is not a parasite of pulse crops; the presence of this nematode in pea fields in this survey could be attributed to crops in rotation. However, *X. rivesi* was found in this survey at a very low frequency and density and it might not be a concern.

3.2 Implications

The results in this survey are of great importance for the Canadian pea industry as it confirms previous findings that have indicated that *Ditylenchus dipsaci* is not predominant in the major exporting regions (Saskatchewan and Alberta) of pulse crops in Canada. Canada is the world's major exporter of peas and Saskatchewan and Alberta provinces alone account for 96% of the exports (Statistics Canada (STC) and Agriculture and Agri-Food Canada 2018). Biosecurity measures have been increased in recent years to control dissemination of seed borne plant parasitic nematodes across countries. *Ditylenchus dipsaci* is a quarentine pest and it can hamper Canadian exports barriers to many countries. India, for example, has restricted regulations regarding *D. dipsaci* in peas importations. If a grain shipment is contaminated with seed borne nematodes, it has to be fumigated to eliminate the nematode. This treatment is costly for pea exporters. Tenuta at al. (2014) has recently shown that *Ditylenchus* present in pea seed samples from Canadian

Prairie growers were *D. weischeri* (a parasite of thistles) and not likely *D. dipsaci*. *Ditylenchus weischeri* has recently been described as a new *Ditylenchus* species parasitizing thistles in Russia (Chizhov et al. 2010) and is not a parasite of main crops grown in the Prairies (Hajihassani et al. 2016, 2017). Following Tenuta's publication, *Ditylenchus* samples from grain shipments that were previously thought to be infected with *D. dipsaci* were re-examined using updated molecular methods and the results were negative for *D. dipsaci*. Tenuta's finding have reduced non-necessary costs with fumigants and have opened exportation barriers. The present study confirms those findings. Even though *Ditylenchus* spp. was frequently found in pea fields, molecular identifications showed that *D. weischeri* was the predominant species. *Ditylenchus dipsaci* was found only in one pea field in Manitoba and it was not found in Alberta and Saskatchewan.

3.3 Problems/ Challenges/ Improvements

3.3.1 Nematode Identification

The biggest challenge in the execution of this survey was the nematode identification. Even for the identification at a higher lever such a genus, nematode identification can be a difficult, laborious task. For example, some genera from the *Tylenchidae* family are very similar morphologically and more time is required to assign them to genus. They are also very commonly found in soils worldwide (Daramola and Afolami 2013). In fact, in this survey, they were the most predominant taxa recovered from soil in the fields analysed. The economical importance of nematodes belonging to this group have not been established for crops commonly grown in the Prairies as the majority are fungal feeders (Yeates et al. 1993). For those reasons, half way through the analyses of samples, a management decision was made where those nematodes were assigned

to the family level instead of genus level, which made the task more feasible. Results from *Tylenchidae* analyses were not reported in this survey because the pathogenicity of these nematodes on pulse crops is unknown (Bafokuzara, 1996; Daramola and Afolami 2013).

3.3.2 Molecular analyses

Another challenge encountered during the execution of this survey was to obtain high quality sequence results. Tentative identifications for some samples through sequencing have failed. Analyses of the sequences chromatogram results lead to the conclusion that DNA purification was ineffective for those samples. The DNA concentration was also lower than recommended for sequencing which could be due to the PCR reactions low yield or loss of DNA during the purification process.

As PCR reactions are designed mostly for diagnostic purposes and not for producing high amounts of DNA, PCR conditions had to be adjusted. A few PCR parameters, such as PCR cycling conditions and magnesium chloride concentrations, were adjusted which resulted in more yield and less non-specific amplifications. Next, the purification process had to be improved. Two purification methods were applied to purify DNA: direct PCR purification and gel extraction. Their rate of success was higher when purification from gel extraction was performed instead of direct PCR purification, even though the yields were lower. One explanation for the higher success obtained using gel extraction is that DNA from gel extraction is already free of primer dimers and other non-specific amplifications, which contributes to a successful sequencing.

Another resource used in this study to improve the sequencing rate of success when sequence length and/or quality was not satisfactory, was to request a reverse/both direction sequencing service. Reverse directions sequencing was also applied successfully by other studies when desired

results were not achieved in the first sequencing analyses (Bhadury et al. 2005; Kitagami et al. 2017). We have also tried cloning to amplify difficult DNA. Cloning has shown a high rate of success even for DNA samples that had PCR products with low, faint bands. The cloning products yield a lot more PCR products with high DNA content which was subsequently successfully sequenced. However, the cloning process is laborious, and it requires a few days to be completed. It also adds to the overall analyses cost.

Similar problems with sequencing data were faced in other studies. Vogt et al. (2014) and Pereira et al. (2010) for example, sequencing the D2-D3 region of 28S rDNA had a success rate of about 60%. Higher success rates (>80%) have been reported for nematode amplification using the same regions (De Ley et al. 2005). Sequencing experiments that have a rate of success of approximately 80% or higher are considered successful.

Fast DNA degradation was also a challenge encountered in this study. The use of commercial kits to clean up genomic DNA is a solution to avoid fast DNA degradation and improve DNA quality. After the DNA is cleaned up it can be stored for longer, maybe years if an elution buffer is used to preserve it. Purified PCR products can also be stored for longer periods of time but keeping genomic DNA is more interesting because of the possibility to use it with different set of primers. Storage of genomic DNA for long periods of time is particularly useful for future research when positive/negative controls are needed for new PCR reactions.

The quality of DNA samples is also affected by storage conditions such as temperature, UV radiation and moisture (Alpers et al. 2003) and caution should be administered. Moreover, when the samples go through multiple freeze-thaw cycles, DNA degradation will be more likely to occur. In this study degradation due to multiple thawing-freezing were evident and that is

probably due to the presence of nucleases in the sample which contributes directly to DNA degradation (Kawane et al. 2014). Purification of the DNA samples will diminish some of those reactions helping to preserve the sample. An alternative is to aliquot the DNA and store the samples that will be used in a matter of days or weeks in -4 instead of -20 ºC or lower temperatures. In that way, the freeze-thaw cycles are avoided.

Further improvements in molecular analyses can also be achieved by attention to details. For instance, specific agarose gel concentration should be applied according to the expected band size of the DNA fragment analyzed to obtain better resolution of the DNA band (Qiagen 2001). For example, when analysing species-specific primers that amplified fragments less than 300 bp, 1.5 % w/v of agarose should be used and for amplification with universal primers were expected band size for the nematodes applied in the study were above 600 bp, 0.7 % w/v should be used (Qiagen 2001).

3.4 Future Research

Future surveys will be necessary to monitor changes in *Ditylenchus dipsaci* population dynamics. In this survey only one field in Manitoba was positive for *D. dipsaci*. In the following year, the same field was re-sampled, and the samples analysed were not positive for *D. dipsaci*. However, canola was in rotation and only soil samples were analysed. The *Ditylenchus dipsaci* positive field and adjacent ones should continued to be closely monitored for the presence of this nematode as its population can grow fast in the presence of a suitable host. Crop rotation is a control method option for this nematode and a recent study has shown that canola is a suitable choice as *D. dipsaci* did not survived in the canola variety tested (Hajihassani et al. 2016). Some spring wheat varieties tested were not good hosts for *D. dipsaci* either (Hajihassani et al. 2016).

The first report of *D. dipsaci* in Manitoba was published recently. There were garlic samples infested with *D. dipsaci* from two garlic fields (Hajihassani and Tenuta 2017). The contamination of the fields happened as a consequence of growing infested garlic pieces from Ontario (Hajihassani and Tenuta 2017). This is a concern because as mentioned before, this nematode population can build up fast in the presence of a good host. Growers should be educated on the importance of only importing plant materials free from pests and diseases. This is important specially for *D. dipsaci*, which because of its wide host range, is very difficult to eliminate and can cause great economic damage (Sturhan and Brzeski 1991).

Ongoing research is currently trying to resolve remaining uncertainties found in the present thesis study, such as the possible presence of other root lesion species, the preferred host(s) of *P. neglectus* and its yield impact in Prairie crops. Other ongoing research conduct by Tom Forges aim to determine possible plant parasitic nematodes in faba-beans fields in Alberta. This will help to complete plant parasitic nematode data on the most important pulse crops grown in Canada.

Additionally, fields that had high density levels for *Ditylenchus*, *Helicotylenchus*, *Paratylenchus* and *Tylenchorynchus* nematodes warrant further analyses for species identification. This will help determine if the nematodes present in those fields are economically important plant-parasitic nematodes.

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Appendix I

Appendix I.1. *Ditylenchus* sequencing results with low identity values of specimens recovered from plant and soil samples from commercial pulse fields sampled in 2014 and 2015.

Field #	Province	Field Crop	Sample I.D.	Year	Description	Sequencing	Identity (%)	E value
1	AB	Pea	31SL-3A	2015	Pea stems and leaves	*Ditylenchus sp.*	89	
2	AB	Pea	40SL-6	2015	Thistle stems and leaves	*Ditylenchus sp.*	85	
3	AB	Pea	59SL-2	2015	Pea stem and leaves	*Ditylenchus weischeri*	94	2.00e-139
4	AB	Chickpea	76SL-11	2015	Thistle stems and leaves	*Ditylenchus weischeri*	82	1e-138
5	SK	Lentil	137P-1	2015	Lentil pods	*Ditylenchus phyllobius*	88	
			137P-2	2015	Lentil pods	*Deladenus sp.*	83	8e-35
6	SK	Lentil	139P-1	2015	Lentil pods	*Deladenus proximus*	83	7e-35
7	AB	Pea	96-4	2014	Pea soil	*Ditylenchus sp.*	87	
8	SK	Pea	188-1	2014	Thistle soil	*Ditylenchus sp.*	77	