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Occurrence of *Ditylenchus weischeri* and Not *D. dipsaci* in Field Pea Harvest Samples and *Cirsium arvense* in the Canadian Prairies

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Abstract: The stem nematode, a parasite of the herbaceous perennial weed, *Cirsium arvense* (L.) Scop. and identified as *Ditylenchus dipsaci* (Kühn) Filipjev, was reported in the Canadian prairies in 1979. Recently, *D. weischeri* Chizhov parasitizing *Cirsium arvense* was described in Russia, and it has been shown that this species is not an agricultural pest. In this study, we examined *Ditylenchus* species found in field pea (*Pisum sativum* L.) grain harvest samples in 2009 and 2010 and from *C. arvense* shoots in pea fields in the Saskatchewan, Alberta, and Manitoba provinces. Samples from 538 fields (mainly yellow pea) were provided by 151 growers throughout the main pea-growing area of the Canadian prairies. Of the samples collected, 2% were positive for *Ditylenchus*. The population density of the nematode ranged between 4 and 1,500 nematodes kg^{-1} pea harvest sample and related to presence of *C. arvense* seeds. Positive samples occurred in 2009 but not in 2010 and were from throughout the pea-growing area of the Canadian prairies and not related to cropping history. *C. arvense* collected from yellow pea fields in Saskatchewan and Manitoba, but not Alberta, were infested with *Ditylenchus*. Morphological and molecular (ITS-PCR-RFLP) traits indicated that this species belongs to *D. weischeri*. The results indicated the stem nematode found in yellow pea grain is *D. weischeri* which resided with *C. arvense* seeds and debris to pea samples. Unlike *D. dipsaci*, *D. weischeri* is not a nematode pest of economic importance; therefore, its presence in the pea harvest samples was not a concern.

Key words: Canadian prairies, *Cirsium arvense*, creeping thistle, detection, *Ditylenchus*, *D. weischeri*, pea.

The nematode genus *Ditylenchus* Filipjev contains more than 80 species, only few are parasites of higher plants. The stem and bulb nematode, *D. dipsaci* (Kühn) Filipjev, is a migratory endoparasite that feeds within the parenchymatous tissues of more than 500 plant species in the Fabaceae, Liliaceae, and Chenopodiaceae (Bridge and Star, 2007). *Ditylenchus dipsaci* occurs in most temperate areas of the world and is a parasite of many crops including onion (*Allium cepa* L.), garlic (*Allium sativum* L.), carrot (*Daucus carota* L.), pea (*Pisum sativum* L.), lentil (*Lens culinaris* Medikus), sunflower (*Helianthus annus* L.), potato (*Solanum tuberosum* L.), strawberry (*Fragaria ×ananassa* Duchesne), sugar beet (*Beta vulgaris* L.), and alfalfa (*Medicago sativa* L.). The current prevalence of *D. dipsaci* on crops in the prairie provinces of Canada (Alberta, Saskatchewan, and Manitoba) is unknown though previously reported for alfalfa in Alberta (Hawn, 1973). In general, the symptoms of *D. dipsaci* damage of crops include swollen and distorted stems and petioles, with brown to black lesions. For grain legumes, discoloration and distortion of pods and seeds is observed (Bridge and Star, 2007). Infested crops can become stunted with spongy and damaged plant tissue predisposing the plant to colonization by other plant pathogens (Hawn, 1963; Vrain, 1987). In addition to being the infective stage, the fourth-stage juvenile (J4) is the survival stage of *D. dipsaci* in plant tissue, seed, and soil. Dry seeds of the host plants carrying this pest are an important means of dissemination from one region to another (Hooper, 1971). The juveniles enter a state of cryptobiosis and can survive 3 to 5 yr and even decades of desiccation (Sturhan and Brzeski, 1991). The longevity and wide host range of *D. dipsaci* can make ridding of fields of the pest difficult. As a result, *D. dipsaci* is a quarantine nematode in many countries thus preventing the importation of infested soil, seed, and plants.

About 30 host races of *D. dipsaci* have been described based on host preferences (Hooper, 1971; Sturhan and Brezeski, 1991). Some races of *D. dipsaci* cannot interbreed whereas those that can may have different host preferences than the parent races (Webster, 1967). Thus, *D. dipsaci* is believed to be a complex containing several morphologically similar and phylogenetically related species, including *D. dipsaci sensu stricto, D. gigas, D. weischeri*, and several other still undescribed species (Subbotin et al., 2005; Chizhov et al., 2010; Vovlas et al., 2011). Only *D. dipsaci sensu stricto* and *D. gigas* are considered pests of crops with the latter being specific to broadbean (*Vicia faba* L.). Recently, Jeszke et al. (2014) showed *Ditylenchus* from *Allium cepa*, *Cichorium endivia*, and *Phlox paniculata* in Poland belonged to *D. dipsaci sensu strict* and that from *V. Faba* to be *D. gigas*.

Near the City of Regina in Saskatchewan, Canada, Watson and Shorthouse (1979) reported *D. dipsaci* infesting the herbaceous perennial weed, *Cirsium arvense* (L.) Scop., commonly referred to as creeping or Canada thistle. Chizhov et al. (2010) recently described a new species, *D. weischeri*, infesting *C. arvense*, in fields,

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road sides, and ditches near Moscow, Russia. The symptoms of infected shoots of stem swelling or galls were similar to that reported by Watson and Shorthouse (1979) for *D. dipsaci* on *C. arvense*. *Ditylenchus weischeri* is presently known as a specialized parasite of *Cirsium* species only. Differentiation of *D. weischeri* from *D. dipsaci sensu stricto* is based on differences in some morphological characters (shorter tails in adults, larger c index, shorter spicules, longer vulva-anus distance, larger vulva-anus distance to tail length ratio, and longer posterior sac) and whole ITS-rRNA and hsp90 gene sequences (Chizhov et al., 2010). Therefore, the occurrence of *D. dipsaci* on *C. arvense* in the prairie provinces of Canada requires reexamination. Because, *C. arvense* is a widely distributed persistent perennial weed of temperate climates around the world, correct differentiation of *D. weischeri* from *D. dipsaci sensu stricto* is important.

Canada is the world's largest producer and exporter of field pea. It is Canada's most important pulse crop and grown over a large geographic area, including significant portions of the provinces of Saskatchewan and Alberta, and some parts of Manitoba. The value of field pea exports averaged $890 million (Canadian dollar) per year (2010–2012) with Saskatchewan and Alberta accounting for 78% and 18%, respectively, of the exports (Agriculture and Agri-Food Canada, 2013). Export shipments of field pea are monitored by the Canadian Food Inspection Agency for *D. dipsaci* where the importing country has quarantine restriction for the pest. In light of the description of the new species, *D. weischeri*, a parasite of *C. arvense*, observations of *D. dipsaci* on *C. arvense* and in grain samples denied cross-border importation require reevaluation.

The objectives of this study were to determine the (i) occurrence and species identification of *Ditylenchus* species in field pea grain harvest samples from the Canadian prairie provinces, (ii) relation of the nematode in grain samples to geographical origin and management (e.g., crop rotation, weed seeds in samples), and (iii) *Ditylenchus* species from infested *C. arvense* shoots obtained during surveys in the prairie provinces.

Materials and Methods

Collection of field pea harvest samples and field history: Field pea growers in Alberta and Saskatchewan were contacted to provide pea harvest samples for the 2009 and 2010 growing seasons. In addition, pea grain samples were obtained from the Manitoba Field Pea Variety trials in 2009 and 2010. All participants agreeing to provide pea harvest samples were sent grain envelopes along with a questionnaire. The questionnaire asked for pea variety, cropping history, observation of any disease symptoms on the pea crop, and legal location of the field. Each envelope accommodated about 700 g of harvest grain from one field and was mailed to the University of Manitoba. Samples were designated by number in order with a total of 538 samples received.

Nematode extraction from harvest samples: Nematodes were extracted from the grain harvest samples using a sieving technique. About 300 g of sample was weighed and soaked in 4 liter of aerated tap water for 24 hr. Three test sieves of mesh size #24, #60, and #500 (W.S. Tyler Industrial Group, Mentor, OH) corresponding to 710-, 250-, and 25-μm diam. openings respectively, were stacked. Soaked samples were poured through the screen assembly. The assembly was agitated by hand while being rinsed three times, each with 2 liter of tap water. Then the #24 screen was washed with tap water to pass small particles onto the other screens and the screen removed from the assembly. The same was then done for the #60 screen. Finally, the #500 screen was washed. Nematodes and debris retained on the #500 screen was collected in 20 ml of tap water. *Ditylenchus* nematodes recovered were counted using gridded petri dishes and a stereo microscope. Densities of the nematode were expressed as numbers kg^{-1} harvest sample.

Analysis of weeds seeds in pea grain of harvest samples: During the analysis performed on grain harvest samples in 2009, *Ditylenchus* nematodes were observed emanating from weed seeds. Therefore, weed seeds of the remaining unextracted portion samples from the 2009 survey were separated from pea grain by dry screening onto #24 and #60 screens, and the weed seeds were hand-picked using a brush and forceps. The weed seeds were identified to species. Seed weights of each weed species are reported as g weed seeds kg^{-1} grain sample. Nematode extraction was then performed separately on the pea grain and the total weed seeds and debris from the dry-screened pea harvest grain samples. In 2010, nematode extraction was only done on recovered material from dry screening of total weed seeds plus debris and pea grain. Nematode extraction and analysis was conducted using the same wet-screening method as described previously. Densities of nematodes are reported as number kg^{-1} pea grain and number g^{-1} weed seeds plus debris.

Analysis of field Cirsium arvense shoots: Sampling of pea fields was conducted in July 2010 in Manitoba for the presence of *Ditylenchus* in *C. arvense* shoots in two pea fields (designated B and C) and an adjacent field to each planted with a different crop (Fields 81 and 84). In addition, two road side locations (designated RS1 and RS2) were sampled for *C. arvense*. Canola (*Brassica napus* L.) and spring wheat (*Triticum aestivum* L.) was growing in Fields 81 and 84, respectively, whereas the other two fields, Fields B and C, were planted with yellow pea. Pea harvest samples from Fields 81 and 84 had been positive in 2009 for *Ditylenchus* and *C. arvense* seeds. Fields B and C were adjacent to Fields 81 and 84, respectively. Road Sides 1 and 2 were located next to Fields 81 and 84, respectively.

A total of five *C. arvense* shoots were collected randomly from each field with shoots being at least 10 m from each other to ensure they were from different plants. Two shoots each from the road side locations were collected. Whole shoots were cut at the soil surface level and placed in a polythene bag and place in an ice chest, transported to the laboratory and stored at 4°C until processing. All *C. arvense* inflorescences were excised from the vegetative tissue (leaves and stems) and evaluated separately. After gently washing with tap water, florets in the inflorescences were teased apart and leaves plus stems were chopped to 1- to 10-cm pieces and all soaked separately in aerated tap water overnight. Extraction of the nematodes was done using the wet screening method described previously. Nematodes were expressed as g^{-1} dry weight of stems plus leaves or inflorescences.

Additionally, *Ditylenchus* in soil was determined from the locations of the collected *C. arvense* shoots. Five soil cores (2-cm by 20-cm deep) were collected with a soil probe beneath the *C. arvense* shoots and bulked. Five subsamples weighing 100 g each were drawn from each composite soil sample and nematodes extracted using a modified wet screening and Baermann funnel technique (Barker, 1985). Final nematode counts were expressed as numbers 100 g^{-1} dry weight of soil.

In August 2011, additional collections of *C. arvense* shoots in yellow pea fields for the presence of *Ditylenchus* was done in Manitoba, Saskatchewan, and Alberta. A total of 14, five, and four yellow pea fields were sampled in Manitoba (southwest region; designated CT11F1-F14), Saskatchewan (near the town of Scott; designated CT11F5-F19), and Alberta (Edmonton area; designated CT11F20-F23), respectively. Three shoots, cut at soil level, were collected from each field. The samples were extracted for nematodes in inflorescences and stems plus leaves as described above.

Morphological identification: From the pea harvest samples, mainly *Ditylenchus* juveniles were recovered except for one sample where five *Ditylenchus* males were recovered to perform morphometric analysis. Nematodes of all developmental stages were obtained from the *C. arvense* shoot. Thus, morphometric analysis by determination of De Man indices (De Man 1880, Chizhov et al., 2010) of males and females was done on 8 to 10 male and female specimens recovered from *C. arvense* shoots. The determinations were the following: body (L), stylet, tail, esophagus, spicule, gubernaculum, bursa, tail without bursa, and postvulval uterine sac length; maximum body and body at anus widths; distance anterior terminus to median bulb valve, hemizonid, excretory pore and vulva; and distance vulva to anus. Indices a (body length/greatest body diameter), b (body length/distance from anterior to esophageal-intestinal valve), c (body length/tail length), c′ (tail length/tail diameter at anus or cloaca), e* (vulva anus distance to tail length ratio), and V% (% distance of vulva from anterior) were also determined. For morphological examination, individuals were hand-picked and transferred to water or glycerol glass slide mounts and gently heat killed (Golden, 1990). Measurements were performed on digital images obtained using 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) software.

Nematodes of all developmental stages were also obtained from garlic bulbs (provided by Drs. Mary Ruth McDonald and Michael Celetti, University of Guelph, Guelph, Canada; designated *D. dipsaci*^{C58}) infested with *D. dipsaci* and used in this study for reference comparisons of morphological characters. Although garlic is grown in the Canadian prairies, there have been no reports of infestation with *D. dipsaci*. We were unable to find any plant parasitic nematodes of economic concern from sampling bulbs and soil or the largest garlic grower in Manitoba.

Molecular identification by PCR-ITS-RFLP: Ditylenchus specimens recovered from pea grain and *C. arvense* samples (Tables 1,4,5) were stored in dimethyl sulphoxide, disodium EDTA, and saturated NaCl solution (DESS) (Yoder et al., 2006) and saturated NaCl solutions frozen at -15°C. An individual nematode was rinsed several times in autoclaved distilled water with a last rinse occurring overnight. Then the individual nematode was transferred to 250-µl sized PCR tubes containing 10-µl double-distilled water. Extraction buffer (Viagen, Biotech, Inc., Los Angeles, CA) was added to the 10-µl of water, and the nematodes were disrupted for 90 sec using the end of a flame modified glass Pasteur pipette attached to a microhomogenizer (IKA RW 14, Sigma-Aldrich, Oakville, Canada). DNA extraction was performed with adding 2 µl of Proteinase K (600 µg ml^{-1}) to each tube and placement for 60 min at 60°C and then for 10 min at 94°C.

For each extraction of a single nematode, the ITS (ITS1 + 5.8S + ITS2) of the rRNA gene was amplified by PCR using TW81 and AB28 primers (Chizhov et al.,

Table 1. Density of recovered *Ditylenchus* from pea grain harvest samples (harvest sample) in 2009 and repeated on their separated pea grain (pea alone) and weed seeds plus debris (weeds alone).

Province	Field coding	Harvest sample (nematodes kg^{-1})	Pea alone (nematodes kg^{-1})	Weeds alone (nematodes g^{-1})
Alberta	13-6	0	0	4
Alberta	33-6	0	0	636
Alberta	58-3	1,333	0	0
Saskatchewan	12-8	3	0	0
Saskatchewan	41-5	7	0	3
Saskatchewan	44-5	107	0	0
Saskatchewan	46-4	0	0	22
Saskatchewan	76-5	166	0	0
Saskatchewan	77-9	10	0	0
Manitoba	81	0	0	827
Manitoba	84	1,076	0	0

2010). DNA extraction solution (1 to 3 µl) and 250 nM of each primer were added to attain a PCR amplification total volume of 25 µl using a premixed master solution (Dream Taq Green, Thermo Scientific, Waltham, MA). The amplification reaction had a preheating step at 94°C for 3 min; 35 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 1 min; and a final extension at 72°C for 10 min.

For RFLP analysis, 3 µl of amplification product was digested with one of five restriction enzymes (*Bsh1236*I, *Hinf*I, *Msp*I, *Rsa*I, and *Taq*I) in a buffer stipulated by the supplier (Fermentas, Thermo Scientific Inc., Waltham, MA). Each digestion reaction consisted of 2 µl of 10X reaction buffer, 0.2 µl of acetylated BSA (10 μg^{-1} µl), 8 µl of direct PCR product, and 1 µl of restriction enzyme (10 μg^{-1} µl) and increased to a total volume of 15 µl with double-distilled water. The digestion mixture was incubated at 37°C in a water bath for 1 to 3 hr. Digested DNA fragments were separated on buffered (0.5% TAE) 1% agarose gel containing 0.5 µl of 10,000X GelRed (Biotium, Hayward, CA) dye and visualised on a UV transilluminator (GBox, Syngene, Cambridge, UK).

Molecular identification by PCR-ITS-RFLP of *Ditylenchus* species was possible on nematodes recovered from weed seeds plus debris from pea harvest grain samples in 2009 (58-4 and 76-5), *C. arvense* from Field 84 and RS2 in 2010, and *C. arvense* from C11F18 and C11F11 in 2011. *Ditylenchus* recovered from other pea and *C. arvense* samples did not yield usable DNA for PCR-ITS-RFLP analysis. Nematodes were also obtained from the garlic bulbs infested with *D. dipsaci* from Ontario (*D. dipsaci*^{C58}) and Quebec (provided by Gilbert Gérard of le ministère de l'Agriculture, des Pêcheries et de l'Alimentation du Québec; designated *D. dipsaci*^{PQ}) used in this study for reference comparisons of the RFLP pattern. *Ditylenchus* nematodes were also obtained from a rhizome segment of *C. arvense* from Russia.

Results

Frequency of Ditylenchus in pea grain harvest samples: Growers provided one pea grain harvest sample per field with the majority of the growers supplying a sample from more than one field. The harvest samples were scattered throughout the geographical area for growing field pea in the Canadian prairies except for the Peace River area of northwestern Alberta (Fig. 1). A total of 328 pea grain harvest samples (293 yellow and 35 green) were received from 86 growers in 2009, whereas 210 samples (172 yellow and 38 green) were received from 65 growers in 2010. The number of pea grain harvest samples by province was 392 in Saskatchewan, 122 in Alberta, and 24 in Manitoba over the two years of the study.

Ditylenchus was recovered from seven pea grain harvest samples in 2009 (Table 1). The recovery of *Ditylenchus* numbers ranged from 3 to 1,333 nematodes kg^{-1} for the positive harvest samples. Upon examination of recovered nematodes in dishes by stereo microscopy, it was apparent individuals of *Ditylenchus* were localized around weed seeds. Thus, the nematode extraction was repeated for all pea grain harvest samples in 2009 but for weed seeds plus debris and pea grain separately. *Ditylenchus* was not recovered from pea grain alone but was taken from weed seeds plus debris alone for one sample previously positive for the initial analysis on the pea grain harvest sample (41-5) and four samples not previously positive on the pea grain harvest (13-6, 33-6, 46-4, 81; Table 1). The recovery of *Ditylenchus* ranged from 3 to 8,270 nematodes g^{-1} for weed seeds plus debris. Thus a total of 11 fields were positive for *Ditylenchus*

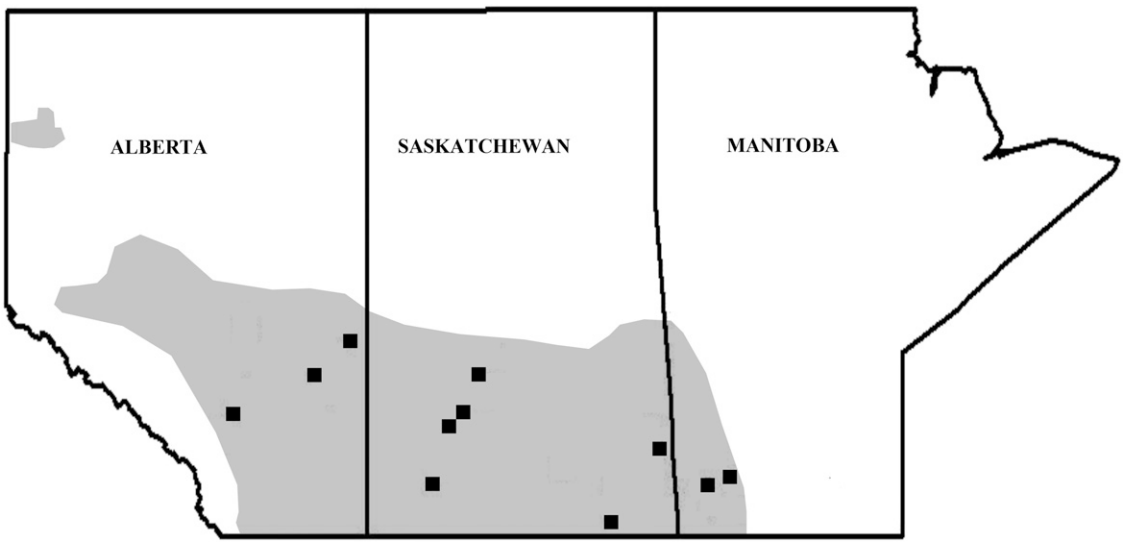

Fig. 1. Location in Canadian prairie provinces of positive fields (squares) for *Ditylenchus* in pea grain harvest samples given in Table 1. Also shown is the region of field pea production (gray area).

TABLE 2. Recent cropping history of fields positive for *Ditylenchus* in pea grain harvest samples given in Table 1.

Field Coding	Pea variety (color) 2009	Previous crop[a] 2008	2007	2006
13-6	Eclipse (yellow)	S.Wheat	Canola	S.Wheat
33-6	Polstead (yellow)	Barley	Canola	S.Wheat
58-3	Profi (yellow)	–[b]	–	–
12-8	CDC Golden (yellow)	S.Wheat	Canola	Canary
41-5	CDC Leroy (yellow)	S.Wheat	Canola	Triticale
44-5	CDC Golden (yellow)	S.Wheat	Y.Pea	S.Wheat
46-4	CDC Golden (yellow)	Lentil	DurumW.	Y.Pea
76-5	CDC Golden (yellow)	DurumW.	Lentil	DurumW.
77-9	CDC Golden (yellow)	S.Wheat	Fallow	Canola
81	CDC Golden (yellow)	S.Wheat	Canola	S.Wheat
84	Eclipse (yellow)	Barley	Canola	W.Wheat

[a] S.Wheat = Spring wheat, DurumW. = durum wheat, W.Wheat = winter wheat, and Y.Pea = yellow pea.
[b] Information not available from the producer.

in 2009. The recovery of the nematode estimated for extraction directly of pea grain harvest sample weed seeds plus debris and pea grain separately ranged from 2 to 1,333 nematodes kg^{-1} harvest sample (Table 1). In 2010, *Ditylenchus* was not recovered from any of the samples analyzed separately for pea grain and weed seeds and debris. Across the two years, 11 of 538 or 2% of fields were positive for *Ditylenchus*.

Of the fields that were positive for *Ditylenchus*, six, three, and two were positive from Saskatchewan, Alberta, and Manitoba provinces, respectively, with the fields distributed throughout the pea-growing region (Fig. 1). All positive samples were from fields planted with yellow and not green field pea, although only 73 of 538 fields examined were of the later type. Of the fields that were positive for *Ditylenchus*, six contained pea variety CDC Golden, two contained Eclipse, and one each contained CDC Leroy, Polstead, and Profi (Table 2). The varieties CDC Golden, Eclipse, Polstead, Profi, and Leroy accounted for 36%, 10%, 3%, 1%, and <1%, respectively, of total pea grain harvest samples received in 2009 and 2010 (data not shown). Pea grain harvest samples of varieties CDC Mozart, CDC Meadow, Cutlass, and Delta were not positive for *Ditylenchus* despite accounting for 13%, 12%, 7%, and 7%, respectively, of total samples received (data not shown). Wheat [spring wheat and Durum (*Triticum durum* Desf.)] had been grown in the previous year to yellow pea in six of 10 fields positive for *Ditylenchus* (Table 2). Of all samples provided, wheat was grown most in the year previous to pea being in 66% of the fields whereas barley (*Hordeum vulgare* L.) and canola were grown in 14% and 8% of the fields, respectively. Noticeably, canola was absent in being the preceding crop in fields positive for *Ditylenchus* (Table 2). Two years before having grown yellow pea in 2009, six of the 10 positive fields with known crop history had been planted with canola and one each with yellow pea, lentil, durum wheat, and fallow (Table 2).

Weed contaminants: Seeds of six weed and one additional crop species were identified from the pea harvest samples. In 2009, 62 of 328 samples contained weed seeds: 39 with *C. arvense*, 13 with sow thistle (*Sonchus oleraceus* L.), five with wild buckwheat (*Polygonum convolvulus* L.), three with wild oat (*Avena fatua* L.), and one each with black medic (*Medicago lupulina* L.) and marsh cudweed (*Gnaphalium uliginosum* L.; Table 3). Similar weed species were also observed in 2010 but with the inclusion of volunteer canola seeds in three samples. In 2010, 92 of 210 samples contained weed seeds: 32 with wild oats, 31 with *C. arvense*, 16 with sow thistle, six with black medic, and two with marsh cudweed (Table 3). The average weight of *C. arvense* seed kg^{-1} of pea harvest sample in 2009 was less than half (0.3 g) of that from 2010 (0.7 g). Further, the recovered

TABLE 3. Number of pea grain harvest samples containing weed seeds and the mean density and range of weed seeds by species in samples obtained in 2009 (n = 328) and 2010 (n = 210).

Weed species	2009 Samples with weeds	2009 Mean (range) seeds kg^{-1}	2010 Samples with weeds	2010 Mean (range) seeds kg^{-1}
Cirsium arvense	39	0.7 (0.03-14.6)	31	0.3 (0.03-1.6)
Avena fatua	3	0.6 (0.2-1.0)	32	4.9 (0.4-20.9)
Sonchus oleraceus	13	0.2 (0.02-1.0)	16	0.3 (0.03-2.1)
Fallopia convovulus	5	5.2 (0.1-32.8)	2	4.2 (0.8-7.5)
Medicago lupulina	1	26.9	6	7.7 (0.6-19.9)
Gnaphalium uliginosum	1	0.05	2	1.1 (0.03-2.2)
Brassica spp.	0	–	3	3.9 (2.5-5.2)

TABLE 4. Incidence, mean density, and range of *Ditylenchus* in *C. arvense* shoots and soil, and pea shoots collected from Manitoba pea fields and nearby road side locations in 2010.

		Incidence (%)[b]			Mean (Range) *Ditylenchus* density		
Location	Plant[a]	Stems+leaves	Inflorescences	Soil[c]	Stems+leaves (nematodes g^{-1})	Inflorescences (nematodes g^{-1})	Soil (nematodes 100 g^{-1})
Field coding							
81	*C. arvense*	80	100	80	3 (1-11)	67 (1-249)	5 (3-9)
F2	*C. arvense*	40	20	60	3 (1-12)	321 (1,603)	8 (5-30)
F3	*C. arvense*	80	80	0	26 (1-103)	447 (1-2,221)	0
84	*C. arvense*	80	100	40	14 (2-47)	47 (4-209)	23 (18-98)
Average		70	75	45	12	221	9
F2	Y.Pea	NA	NA	nd	0	NA	nd
F3	Y.Pea	NA	NA	nd	0	NA	nd
Average		0	NA	nd	0	NA	nd
Road side							
RS1	*C. arvense*	NA	NA	nd	2	4	nd
RS2	*C. arvense*	NA	NA	nd	50	3	nd
Average		NA	NA	nd	26	4	nd

Numbers of nematodes in each pea field is an average of five plants or five soil samples analyzed. Only one plant each from the road side location was analyzed.
[a] Y.Pea = yellow pea.
[b] % incidence determined on the number of nematode-infested plants to the total plants analyzed.
[c] nd = not determined.

C. arvense inflorescences were visually smaller in appearance in 2010 compared with 2009 (data not presented).

Why pea harvest samples from 2009 but not 2010 contained *Ditylenchus* is not known. For the City of Regina, in the center of the pea-growing region, the growing season (May-August) of 2010 was slightly warmer with average daily air temperature of 15.0°C compared with 13.9°C in 2009 (Regina RCS Station, Environment Canada). However, precipitation was almost double for the growing season of 2010 (311 mm) compared to 2009 (155 mm). Little is known about the host-parasite interaction of *C. arvense* and *D. weischeri*, so we are unable to explain how rainfall may affect presence of the nematode in shoots.

Surveys of Cirsium arvense: Cirsium arvense shoots collected from all four fields planted with or previously planted with pea and two road side locations in 2010 in Manitoba were heavily infested with *Ditylenchus* (Figure 1B). Across the fields, 70% of the *C. arvense* shoots were infested with *Ditylenchus*. Inflorescences and stem plus leaves of *C. arvense* were both heavily infested with the nematode (Table 4). Averaged across the fields, the nematode density in stem plus leaves and inflorescences were 12 and 221 nematodes g^{-1}, respectively. One of two shoots of *C. arvense* collected from each road side location was positive for the nematode with mean numbers across the two locations being 26 and 4 g^{-1} in stems plus leaves, and inflorescences, respectively. Of the four fields, soil collected below the *C. arvense* shoots in three of the fields was also positive for the presence of *Ditylenchus* (range 3 to 98 100 g^{-1}).

For the 2011 samples of *C. arvense* shoots collected from commercial fields in the prairie provinces, 15 of 22 fields contained shoots positive for *Ditylenchus* (Table 5). All but one of the 13 field samples in Manitoba were positive for *Ditylenchus*, whereas three of five field samples from Saskatchewan were positive but none of the four field samples from Alberta were positive (Table 5). Again, the recovery of *Ditylenchus* was greater from inflorescences than from stem plus leaves with the average density in samples from positive fields being 244 and 30 g^{-1}, respectively, or across all fields sampled, 144 and 19 g^{-1}, respectively.

TABLE 5. Density of *Ditylenchus* nematodes recovered from stems plus leaves and inflorescences of *C. arvense* shoots from commercial fields in the Canadian prairie provinces in 2011.

			Nematodes g^{-1}	
Field coding	Province	Crop[a]	Stem + leaves	Inflorescences
CT11F1	MB	Soybean	11	0
CT11F2	MB	Soybean	24	1,275
CT11F4	MB	Soybean	2	-[µ]
CT11F5	MB	Soybean	4	10
CT11F6	MB	Soybean	5	1,171
CT11F7	MB	Alfalfa	22	266
CT11F8	MB	G.Pea/oat	17	1
CT11F9	MB	Y.Pea	180	77
CT11F10	MB	Y.Pea	1	36
CT11F11	MB	Y.Pea	7	30
CT11F12	MB	Y.Pea	2	76
CT11F13	MB	Y.Pea	0	0
CT11F14	MB	Y.Pea	0	2
CT11F15	SK	Y.Pea	0	0
CT11F16	SK	Y.Pea	0	0
CT11F17	SK	Y.Pea	<1	9
CT11F18	SK	Y.Pea	139	193
CT11F19	SK	Y.Pea	6	29
CT11F20	AB	Y.Pea	0	0
CT11F21	AB	Y.Pea	0	0
CT11F22	AB	Y.Pea	0	0
CT11F23	AB	Y.Pea	0	0
Average			19	144

[a] Y.Pea = yellow pea, G.Pea = green pea.

TABLE 6. Morphometric measures of adult nematode indices for *Ditylenchus* obtained from *C. arvense* and D. *dipsaci* from garlic obtained in this study (all measurements in μm).

Measure	*D. weischeri* ♀	*D. weischeri* ♀[a]	*D. dipsaci*[PQ] ♀	*D. dipsaci*[C58] ♀	*D. dipsaci* ♀[a]	*D. weischeri* ♂	*D. weischeri* ♂[a]	*D. dipsaci*[PQ] ♂	*D. dipsaci*[C58] ♂	*D. dipsaci* ♂[a]
Number examined	10	23	10	8	23	10	25	10	8	22
L	1,202 (1,049-1,355)	1,545 (1,371-1,619)	1,225 (1,210-1,240)	1,555 (1,532-1,578)	1,392 (1,250-1,708)	1,249 (1,244-1,254)	1,433 (1,281-1,578)	1,118 (1,110-1,125)	1,500 (1,400-1,625)	1,362 (1,201-1,473)
Body maximum width	30 (27-33)	37 (32-44)	25.3 (23.6-27.0)	33 (32-35)	35 (31-45)	23.9 (21.9-26.0)	26 (23-29)	25 (24-26)	33 (30-35)	28 (24-34)
Body width at anus	15.2 (14.4-16.0)	17 (14-19)	17 (15-19)	21 (20-22)	20 (17-23)	15.9 (14.8-17)	14 (13-16)	18 (17-20)	21 (20-21)	16 (14-18)
a	39.9 (38.8-41.0)	40.6 (35.5-44.8)	44.7 (44.4-45.0)	54 (45-63)	40.0 (31.3-45.5)	52 (48-56)	54.3 (50.1-60.4)	44.7 (43.2-46.3)	46.5 (46.4-46.6)	44.4 (39.7-53.5)
b	6.7 (6.2-7.2)	8.2 (7.0-9.0)	5.8 (5.4-6.1)	6.2 (6.1-6.3)	7.1 (6.4-8.2)	6.6 (6.4-6.8)	7.4 (6.3-8.2)	5.7 (5.4-6.0)	6.8 (6.5-7.1)	6.9 (6.3-7.8)
Tail length	58 (53-62)	65 (54-84)	89 (80-98)	89 (88-91)	93 (85-103)	62 (58-66)	61 (50-73)	96 (90-102)	86 (83-89)	87 (80- 97)
c	20.8 (19.7-21.8)	23 .2 (18.3-28.1)	12.8 (12.4-13.1)	17.4 (17.3-17.4)	15.1 (13.5-19.5)	20.2 (19.0-21.4)	23.4 (18.4-26.4)	11.7 (11.0-12.3)	17.5 (16.8-18.2)	14.9 (13.9-16.3)
c′	3.7 (3.6-3.8)	3.7 (2.9-4.8)	5.2 (5.1-5.3)	4.3 (4.1-4.4)	4.8 (4.2-5.5)	3.85 (3.8-3.9)	4.2 (3.5-5.0)	5.2 (5.1-5.2)	4.2 (4.1-4.2)	5.3 (4.7-6.2)
e*	2.75 (2.7-2.8)	3.0 (2.4-3.8)	1.75 (1.6-1.9)	1.8 (1.7-1.9)	1.7 (1.4-2.1)	NA	NA	NA	NA	NA
Stylet length	10.0 (8.5-11.5)	11.0 (9-13)	11.3 (10.0-12.5)	8.7 (7.5-9.8)	11 (10-12)	8.5 (8-9.1)	11 (9-13)	12.3 (12-12.5)	9.7 (9.0-10.5)	11 (10-12)
V%	81.2 (82.3-80.0)	83 (81-85)	79 (78-79)	77 (75-79)	82 (80-86)	NA	NA	NA	NA	NA
Spicule length	NA	NA	NA	NA	NA	24.5 (23.0-26.0)	21 (20-24)	21 (20-22)	29 (28-30)	26 (22-28)
Gubernaculum length	NA	NA	NA	NA	NA	6.35 (5.30-7.40)	7 (6-9)	7.1 (7.0-7.2)	5.4 (5-5.8)	9 (8-11)
Esophagus length	177 (169-186)	184 (170-203)	195 (192-198)	249 (240-258)	194 (175-208)	187 (179-196)	194 (176-214)	174 (168-180)	223 (195-250)	189 (159-207)
Distance anterior terminus to:										
Median bulb	73 (69-77)	71 (65-75)	70 (66-75)	76 (73-80)	73 (67-78)	71 (69-72)	75 (67-86)	62.5 (60-65)	76 (74-77)	73 (68-85)
Hemizonid	117 (115-119)	137 (127-145	–	137 (137-138)	144 (127-158)	130 (125-135)	139 (128-145)	117 (113-122)	132 (129-135)	142 (131-151)
Excretory pore	123 (109-137)	143 (133-150)	–	165 (161-168)	153 (140-168)	145 (140-150)	143 (130-150)	119 (115-123)	159 (158-160)	151 (136-162)
Vulva	972 (864-1,080)	1,240 (1,132-1,308)	965 (960-970)	1,202 (1,154-1,250)	1,144 (1,020-1,475)	NA	NA	NA	NA	NA
Vulva to anus distance	160 (146-175)	194 (172-240)	162 (159-165)	169 (165-173)	150 (132-175)	NA	NA	NA	NA	NA
Postvulval uterine sac length	91 (70-111)	121 (101-150)	61 (53-68)	82 (80-83)	83 (70-100)	NA	NA	NA	NA	NA
Bursa length	NA	NA	NA	NA	NA	58 (50-65)	65 (48-78)	64 (55-73)	67 (61-73)	82 (62- 97)
Tail length without bursa	NA	NA	NA	NA	NA	19 (18-20)	21 (12-30)	40 (30-50)	18 (15-20)	29 (25-34)

[a] *D. weischeri* from *C. arvense* and *D. dipsaci* from *Allium cepa* (Chizhov et al., 2010).

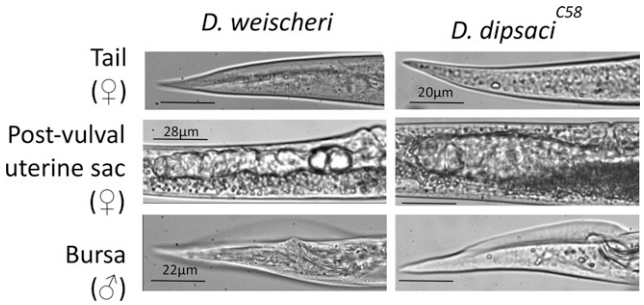

FIG. 2. Micrographs of tail, postvulval uterine sac and bursa regions of *Ditylenchus* from *C. arvense* in Manitoba (*D. weischeri*), and garlic obtained from Ontario (*D. dipsaci*^{C58}). Scale length is similar within a row.

Cirsium arvense spreads mainly through vegetative reproduction from the roots and also through sectioning and dispersal of root fragments by tillage equipment (Tiley, 2010). Sexual reproduction and seed production does occur but as *C. arvense* is a dioecious plant, seed production depends largely on successful pollination and contributes to long-distance dispersal in this species (reviewed by Moore, 1975). Larson et al. (2005) observed germination of *C. arvense* seeds varied from 7% to 47%. Other studies have also observed a wide range in germinability in *C. arvense* (reviewed in Moore, 1975). The impact of *D. weischeri* in contributing to the variation in seed germination of this plant is unknown though certainly interesting in light that inflorescences were observed in this study to contain a high density of the nematode (maximum 2,221 g^{-1}).

Morphological identification: Ditylenchus females from *C. arvense* shoots had shorter body width at anus, tail length, esophagus length, and smaller indices a and c′ than those for *D. dipsaci* females from garlic (Table 6). In contrast, *D. dipsaci* females from *C. arvense* had larger indices for c, e, and V and longer postvulval uterine sac length (Table 6; Fig. 2). *Ditylenchus* males from *C. arvense* had shorter body width at anus level, tail, stylet and bursa lengths and smaller index, c′ than those of *D. dipsaci* from garlic (Table 6; Fig. 2). In comparison, the males of *D. dipsaci* had larger indices a and c than those of *Ditylenchus* from *C. arvense* (Table 6).

Ditylenchus adults obtained in the present study from *C. arvense* shoots were generally morphologically similar to adults of *D. weischeri* from *C. arvense* described by Chizhov et al. (2010) from Russia; however, they differed from the Russian population by smaller body sizes and other morphometrical characters. Our morphometric analysis confirmed that *D. weischeri* can be distinguished from *D. dipsaci* by several diagnostic features: shorter tail in adults, larger index c, and longer postvulval uterine sac.

Molecular identification: The ITS-PCR-RFLP diagnostic patterns obtained in the present study for *D. weischeri* from *C. arvense* in Russia (Fig. 3A), samples of *Ditylenchus* from pea grain harvest and *C. arvense* samples (Fig. 3B) were similar to that published for *D. weischeri* (Chizhov et al. 2010) from Russia. The restriction profiles for individual *Ditylenchus* nematodes were obtained from pea grain harvest (58-3 and 76-5) and from *C. arvense* (84, C11F11, C11F18, and RS2) samples were identical and, thus, confirmed that the *Ditylenchus* species found in these samples belonged to the same species, *D. weischeri*. The ITS-PCR-RFLP pattern of individual nematodes of *D. dipscaci*^{C58} and *D. dipsaci*^{PQ} (Fig. 3C) differed from those of *D. weischeri* in Figure 3A and B.

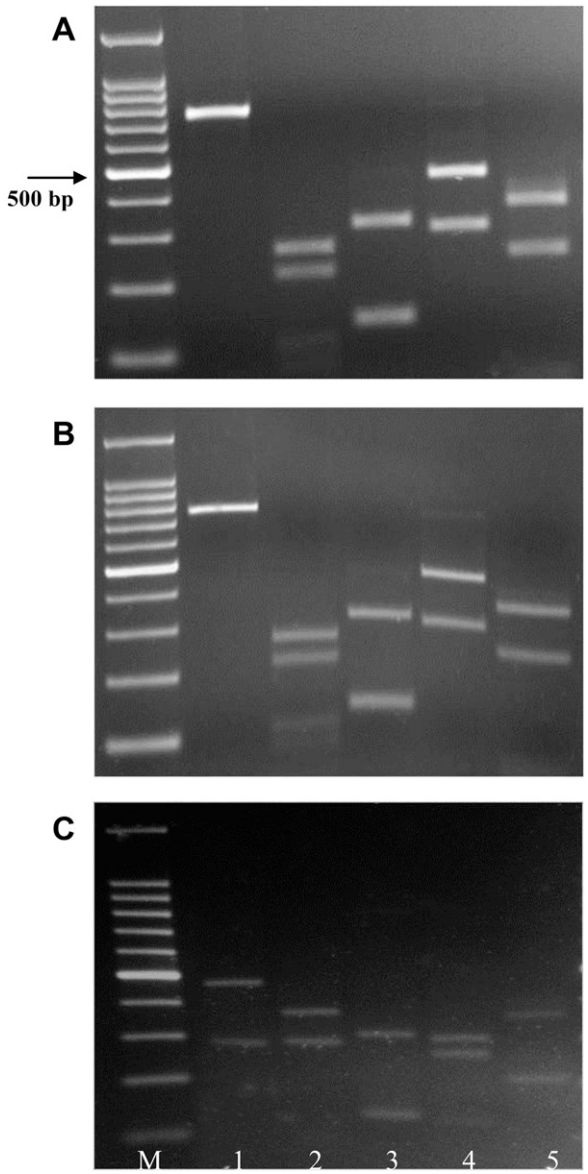

FIG. 3. Representative ITS-PCR-RFLP diagnostics pattern for *Ditylenchus*: (A) from *C. arvense* obtained from Russia and consistent with being *D. weischeri*; (B) obtained from pea grain harvest samples 58-3 and 76-5, and *C. arvense* from samples 84, C11F11, C11F18, and RS2, and also consistent with being *D. weischeri*, and (C) pattern obtained for samples of garlic from Ontario (*D. dipsaci*^{C58}) and Quebec (*D. dipsaci*^{PQ}) and consistent with being *D. dipsaci*. Lanes are M, 100 bp DNA marker ladder (Promega, Madison, WI) and restriction digests: 1, *Bsh*1236I; 2, *Hinf* I; 3, *Msp*I; 4, *Rsa*I; and 5, *Taq*I. Example patterns shown are for analysis of a single nematode.

Crop history: There is likely no link between canola being grown 2 years previous to many of the harvest pea samples positive for *Ditylenchus* in 2009. Canola or pea following each other in a cropping sequence is not recommended because of disease buildup of white mold, *Sclerotinia sclerotiorum* (Lib.) de Bary (Boland and Hall, 1994). Further, Leeson et al. (2005) found similar midseason presence of *C. arvense* in canola and cereal fields across the Canadian prairies.

Weed seeds and debris: It is important to note that pea harvest samples were analyzed in the current study. Export shipments of pea by large exporting nations usually occur in bulk container ships. The presence of weeds seeds plus debris, including those of *C. arvense*, may differ between harvest samples and the export commodity. During the bulk-handling chain, weed seeds and debris can be removed from the pea seed. However, foreign matter specifications of shipping contracts allow the reintroduction of screening debris, including weed seeds, collected at port facilities during the loading of container ships. Thus, the frequency of occurrence of *D. weischeri* in bulk pea shipments may differ from that determined in this study.

Discussion

This study identified the species of *Ditylenchus*, based on morphologic and molecular characters, in pea harvest samples and *C. arvense* shoots from pea fields to be *D. weischeri* and not *D. dipsaci*. The occurrence of *D. weischeri* in pea harvest samples was low, about 2% of samples. Moreover, the presence of *D. weischeri* was associated with the presence of weed seeds and debris in pea harvest samples. The perennial weed, *C. arvense*, was commonly found in pea harvest samples and was also found to be readily infested by *D. weischeri* in shoots obtained from Manitoba and Saskatchewan. Pea harvest samples positive for *D. weischeri* were scattered throughout the pea-growing area of Prairie Canada. Unlike *D. dipsaci*, *D. weischeri* is not considered a nematode pest of economic importance. Therefore, its presence is not a concern for trade of pea.

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