



Evaluating the densities and distribution of root-lesion nematodes (*Pratylenchus* spp.) in wheat grown in Canterbury, New Zealand

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Abstract Species of root-lesion nematode (*Pratylenchus* spp.) are associated with significant reductions in wheat yield in wheat-growing regions around the world. Of these, *Pratylenchus thornei* and *P. neglectus* are known to cause the highest damage to the Australasian wheat industry. New Zealand is known to produce high wheat yields on a per-hectare basis yet little research has been conducted to date to determine the effects of *Pratylenchus* spp. on the production of wheat in New Zealand. Therefore, as the first step towards filling this knowledge gap, the current research focused on conducting surveys to determine the population densities and distribution of *Pratylenchus* spp. in wheat-growing regions in Canterbury, South Island, New Zealand. Surveys were conducted at ten selected sites that were geographically distinct from each other. At six of the ten sites, lesion nematode populations were reported to be above the recorded Australian threshold of 2000 nematodes per kg of soil. In Australia, it's been recorded that around 50% yield reductions can occur in intolerant wheat varieties when population densities reaches this number. Differences in population density within each location was also observed indicating the uneven distribution of lesion nematodes within a field. Morphological measurements of the nematodes collected from multiple sites during this study confirmed the presence of *P. thornei* and *P. neglectus* in Canterbury wheat-producing areas indicating a potential threat to the New Zealand wheat industry by root-lesion nematodes. Further studies need to be conducted to fully understand the situation and to develop management strategies to mitigate threats from nematodes.

Keywords *Pratylenchus neglectus*, *Pratylenchus thornei*, population density, wheat, morphology, soil properties

INTRODUCTION

Wheat (*Triticum aestivum*) is the most widely grown crop globally, occupying 22% of the total cultivated area in the world (Leff et al. 2004). However, due to the increasing world population, the production of major staple crops needs to be increased by 2-3% each year to meet projected demand (Hawkesford et al. 2013). In the last 30 years, the area planted in wheat has not increased (FAO 2019). Assuming no future increase in area planted in wheat, the only way to meet demand is to increase yield per unit area. New Zealand wheat production is very efficient and has been recognised as the second largest wheat producer in terms of yields per unit area (FAO 2019). However, New Zealand wheat production currently represents only ~ 0.001% of the global total. To achieve higher yields per unit area, farmers are in a constant battle with a range of abiotic and biotic factors. Pathogens and pests are major yield-

limiting factors affecting crop production both in terms of quantity and quality. A range of pests damage wheat but plant-parasitic nematodes are known to cause significant losses in wheat yield around the world. In many parts of the world, two genera of nematodes *Pratylenchus* spp. and *Heterodera* spp. are known to cause the largest yield losses and are currently recognised to be economically importance globally (Johnson et al. 2008, Nicol et al. 2011). Lesion nematodes (RLN) belong to the genus *Pratylenchus* and consist of over 40 different species. They are migratory endoparasitic nematodes that penetrate the plant root cells and migrate through and out from the root tissue. These nematodes feed entirely on the root cortex and reproduce and lay eggs within plant root tissue. Root lesion nematodes feed on woody, herbaceous and row crops and the *Pratylenchus* genus has a worldwide distribution. Of the 40 *Pratylenchus* species currently reported, 11 are

currently been identified in New Zealand: *P. penetrans*, *P. partensis*, *P. coffeae*, *P. projectus*, *P. crenatus*, *P. vulnus*, *P. thornei*, *P. neglectus*, *P. nanus*, *P. tatea* and *P. minutus*.

Pratylenchus neglectus and *P. thornei* are species commonly associated with significant yield losses in wheat (Smiley & Nicol 2009). *Pratylenchus thornei* has been reported to result in yield losses of 37%, 50%, 70% and 85% in Mexico, the USA, Israel, and Australia, respectively (Armstrong et al. 1993; Ortiz-Monasterio & Nicol 2004; Smiley et al. 2005). In Australia, it has been estimated that *P. neglectus* causes an average annual loss of AUD\$73 million to the wheat industry (Murray & Brennan 2009).

Since 1997, limited research has been done to understand the association between wheat crop and the lesion nematodes but none has evaluated the potential threats of plant pathogenic nematodes to the New Zealand wheat industry. Twenty-five years ago, a study by Knight et al. (1997) indicated the presence of *Pratylenchus* spp. (including *P. thornei*) associated with wheat. However, the aforementioned authors did not investigate the population densities, distribution, or provide any indication of the economic importance of *Pratylenchus* spp. to the industry. So, the overall aims of the current study were to: (a) fill some knowledge gaps that exist in New Zealand; and (b) provide an insight on the effects of root-lesion nematodes to New Zealand wheat industry.

The specific objectives of this preliminary research were to: i) evaluate the population densities of root-lesion nematodes in Canterbury wheat-production areas; ii) identify the dominant root-lesion species present in wheat production areas; and iii) determine the relationship between root-lesion nematodes densities and soil texture.

MATERIALS AND METHODS

Sampling for nematodes

A total of 10 geographically distant sites, 5 from the Selwyn district (including 2 from Lincoln) and 5 from the Ashburton district of Canterbury, South Island, New Zealand were selected for this survey (Figure 1). Around 100-150 composite soil samples were collected from the selected 10 sites during the summer periods of 2019 and 2021 due to the high abundance of nematodes during warmer weather as detected during preliminary sampling. Sampling was done in two different years due to restrictions in personnel movement as a result of the COVID-19 pandemic. Fields smaller than 5 hectares were visually divided into two sections and fields larger than 10 hectares were divided into 4 quadrants as described by Overstreet et al (2002) with modifications. Within each block, 5 geo-referenced composite samples were collected in a double zigzag pattern (Overstreet et al 2002). At each geo-referenced sampling point, 12-15 soil cores averaging around 50 g each, were randomly collected using a 2-cm diameter soil corer (Oakfield Model LS, USA) up to a depth of 30 cm within a 4-m radius from the sampling centre to represent 1 geo-referenced composite sample. This method of sampling has been proven to be accurate in representing nematode distribution within an area with high resolution (Overstreet et al. 2014; Anon 2022a). Each composite sample was placed in a labelled plastic bag and transported to the laboratory at room temperature then stored in a refrigerator at 4°C for further use. The soil analyses were conducted on composite samples for each location due to time and funding limitations.



Figure 1 Selected wheat fields for soil sample collection for nematode and soil texture analysis from Selwyn district (green dots) and Ashburton district (orange dots) in Canterbury New Zealand.

Nematode extraction

The centrifugal sugar flotation method described by Jenkins (1964) was used to extract nematodes from the soil. The method is as follows: Each composite sample was thoroughly mixed by hand, breaking soil clumps inside the sample bags to produce a homogeneous sample. A sub-sample of 200 g from each composite sample were used for nematode extraction. The 200 g soil sample was placed in a 9-L plastic bucket and half-filled with tap water. The soil was then thoroughly mixed with water while stirring. After thorough stirring, the mixture was allowed to stand for about 15 - 30 seconds before decanting through nested 100- μm and 38- μm mesh sieves. This process was repeated three times for each sub-sample. The residue on the 38- μm sieve was collected in a beaker, and the remaining soil was discarded. The soil residue collected from the 38 μm sieve was then poured into a 50-mL centrifuge tube. This was achieved by rinsing the content in the beaker with a little water each time, picking up most of the soil residue, until the centrifuge tube was filled up to 50 mL. The tube was then placed in the centrifuge and spun at 1700 rpm for 5 minutes. After centrifugation, the water was discarded, and the tube containing the soil pellet was filled up to 50 mL with a concentrated sugar solution (454g/litre of water). The soil residue and the sugar solution were thoroughly mixed using a spatula. Next, the tube was placed in the centrifuge at 1700 rpm for 1 minute. After the second centrifugation, the supernatant was poured onto a 38- μm mesh sieve, and the sugar solution was rinsed with water to remove excess sugar from the nematodes. The suspension containing the nematodes was then collected in a 100-mL specimen cup from the sieve and kept refrigerated at 4°C until further use. For nematode analysis, a 10-mL nematode suspension was poured into a nematode counting dish. During microscopic observations, specimen cups were kept outside to bring them to room temperature before transferring the nematode extract into specialised nematode counting dishes. The counting dish has an area of 22.63 cm², with half of the area consisting of four equally sized counting lines. *Pratylenchus* spp. in all four lines were counted and multiplied by 2 to obtain an estimate of the number of nematodes per 100 g. Nematodes were observed, and the numbers were counted using an inverted microscope (Olympus CKx54) at 40 \times magnification.

Nematode identification

Around 100 female individuals of *Pratylenchus* spp. from the collected samples were identified to species level using morphological descriptions. *Pratylenchus thornei* and *P. neglectus* were identified using De Man's values (Corbett 1973; Fortuner 1977; Townshend & Anderson 1976). Female nematodes were identified as those specimens having a vulva and were utilised to obtain measurements. Female nematodes are typically considered economically important because females feed on plants throughout their life cycle, whereas mature males typically leave the root system after sexual dimorphism. Measurements were taken using Olympus cellsens image software (Olympus Corporation, Tokyo, Japan) for morphologically distinct groups of female lesion nematodes having different tail

shapes, stylet appearance and body length from two selected sites in Lincoln. The average value for each measurement was obtained from 10 to 20 female root-lesion nematode individuals from each morphologically distinct group, as conducting measurements with a lower sample size could potentially lead to inaccuracies. Morphological measurements included, total body length (L), stylet length (SL), value 'a' (body length/greatest width) and value 'V' (position of the vulva with respect to total body length). Images of nematodes were taken at 100 \times and 600 \times ocular using the inverted microscope, and Olympus SC 180 camera system (Olympus Corporation, Tokyo, Japan). The measurements obtained were then compared with previously published data by Sher & Allen (1953) and Loof (1960).

Soil texture analysis

Soil texture was analysed using the micro-pipette method described by Miller & Miller (1987). From each composite sample, 2 g of air-dried soil with 40 mL of a dispersant {10 mL 1M NaOH and 10 mL of 5% (NaPO₃) per L} were placed in a 50 mL centrifuge tube and shaken overnight on a rotary shaker at 50 rpm. After the tubes were removed from the shaker, each tube was shaken by hand before placing it in an incubator at 30°C for 2 hours. Clay content was determined by pipetting 2.5 mL of suspension at a 2.5 cm depth from the surface of the suspension. The clay suspension was then dried in a weighing dish at 105°C and weighed to nearest 0.1 mg. Sand content was analysed by collecting the sand from the remaining soil suspension left in the tube by sieving through a 53 μm sieve. The collected sand from the sieve was then dried in a weighing dish at 105°C. The weight of silt content was estimated by subtracting the weight of the clay and the weight of the sand from the initial 2 g soil weight. This method allows for a precise determination of the silt content. The 2 g soil weight was carefully measured using a high-precision scale to ensure accurate calculations. Appropriate soil texture for each sample was determined by using a soil texture triangle (Anon 2023).

Statistical analyses

A simple linear regression analysis was done on soil texture and nematode numbers for the survey data using Genstat (20th Edition) statistical software.

RESULTS and DISCUSSION

Pratylenchus spp. were observed to be the predominant plant-parasitic nematode species associated with Canterbury wheat, constituting more than 95% of the total. The remainder of the nematodes were mainly free-living nematodes, with less than 1% being spiral nematodes.

Population densities

The population densities of *Pratylenchus* spp. exhibited significant variability within and among the surveyed sites throughout the two-year research sample collection period (Table 1; Figure 2). Nematode density is known to correlate with climatic conditions, soil moisture content, and nutrient availability (Siebert et al. 2020; Noe & Barker 1985; Nisa

Table 1 Densities of *Pratylenchus* spp. populations in soils of 10 wheat grown locations in Selwyn and Ashburton districts, Canterbury, New Zealand.

Site Location ^a	Coordinates	Sampling month and year	Number of <i>Pratylenchus</i> spp. individuals/Kg soil ^b		Average soil type
			Mean	Range	
Lincoln (Leadleys Rd)	-43.601022,172.516930	Dec-19	658	100-1,760	Silt
Lincoln (Birchs Rd)	-43.607432,172.512493	Nov-19	1016	20-2,280	Silt Loam
Lincoln (Barnes Rd)	-43.624378,172.529580	Nov-19	229	0-480	Silt Loam
Greendale (Hollands Rd)	-43.601295,172.124169	Jan-19	780	0-2,160	Silt Loam
Leeston (Andersons Rd)	-43.758202,172.263756	Jan-19	221	40-560	Silt Loam
Dorie (Dobsons Ferry Rd)	-43.916506,172.105105	Jan-19	980	160-2,000	Silt
Dorie (Corbetts Rd)	-43.912978,172.116346	Jan-19	1416	240-3,600	Silt
Dorie (Mainwarings Rd)	-43.884355,172.087222	Nov-21	4880	2080-7840	Silt
Chertsey (Dromore Hatfield Rd)	-43.782365,171.938223	Nov-21	2120	320-3520	Silt Loam
Wakanui (Beach Rd E)	-43.998404, 171.844513	Dec-21	1120	640-1920	Silt Loam

^a Soil samples were collected from geographically distant wheat fields in Canterbury district of New Zealand.

^b *Pratylenchus* spp. population for each location was determined from 10-16 composite samples made up from 12-15 soil cores.

et al. 2022; Bakonyi et al. 2007). Sampling at different time intervals and from various fields would have had a direct impact on the variability in *Pratylenchus* spp. populations observed in the current study. Further studies need to

be conducted to address these aspects to gain a deeper understanding of how these attributes influence nematode densities and their overall impact on wheat performance in the presence of nematodes.

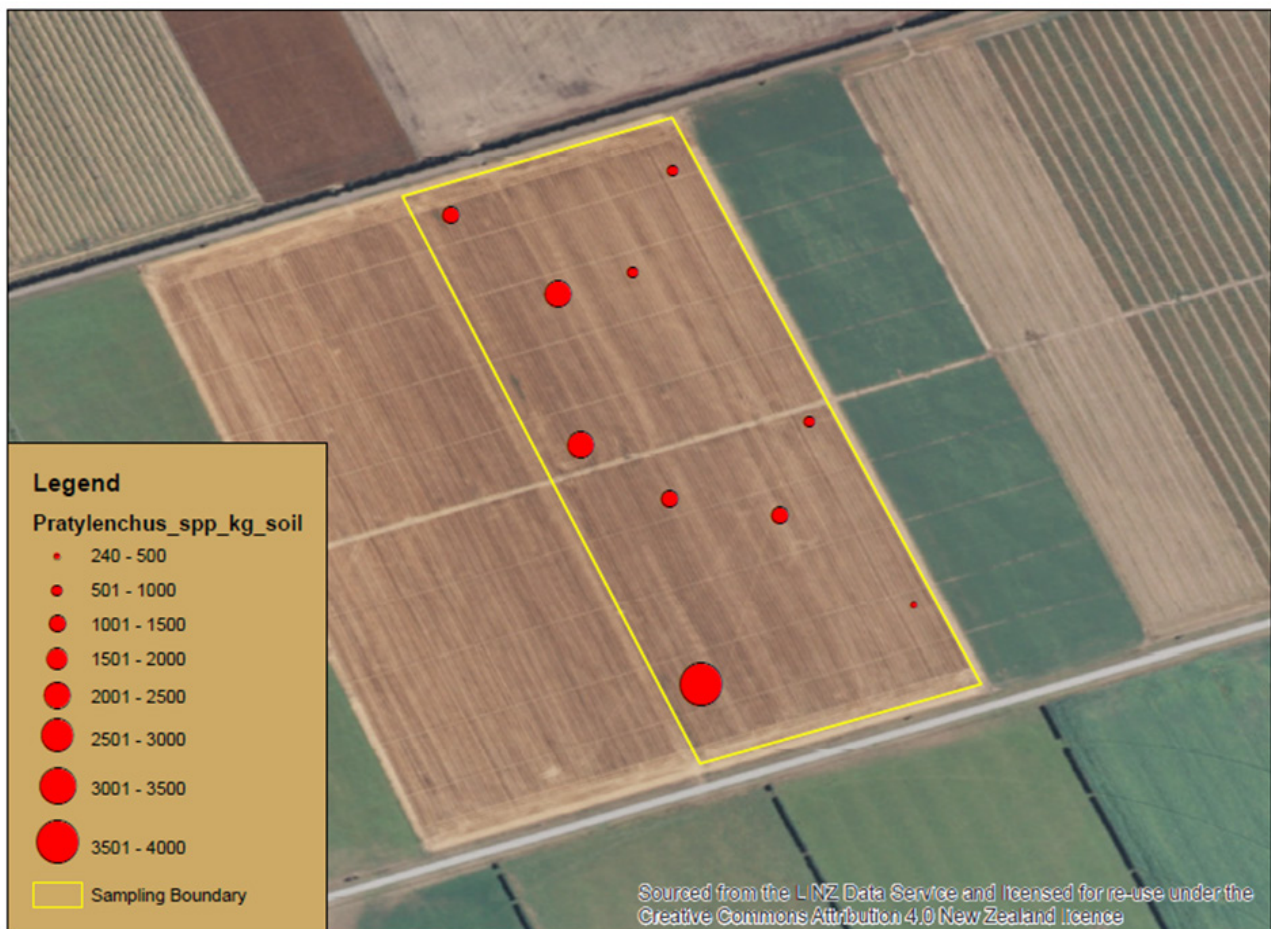


Figure 2 Uneven distribution of *Pratylenchus* spp. across a 10.05 ha field in Dorie, Ashburton district, Canterbury. The diameter of the red circles represents the population density of *Pratylenchus* spp./kg of soil.

Previous reports have indicated that 2,000 *Pratylenchus* spp./kg of soil can cause substantial yield losses in susceptible wheat cultivars grown in the USA or Australia (Anon 2022c; Thomson et al. 2008; Smiley 2015; Thompson et al. 2020) but an economic threshold for *Pratylenchus* spp. is not available for wheat in New Zealand due to lack of research. Among the 10 sites surveyed, 6 sites had high densities of nematodes exceeding 2,000 *Pratylenchus* spp./kg of soil in some areas of specific fields (Table 1). The highest population within a field was recorded from a field in Dorie with 7,840 *Pratylenchus* spp./kg of soil indicating a potential threat of these nematodes in susceptible wheat crops. The sampling methods used in this survey indicated an uneven distribution of the nematode populations within each field that was consistent with previous reports (Overstreet et al. 2014; Anon 2022a).

Species identification

Morphological analysis of around 100 female individuals of *Pratylenchus* spp. from the collected samples confirmed the presence of *P. thornei* and *P. neglectus* (Figure 3). Measurements of: total body length (L; μm); body length/greatest width at the vulva (a); position of the vulva with respect to total body length (V); and stylet length (SL; μm) were consistent with the De Man's measurements recorded by Loof (1960) and Sher & Allen (1953). The average values for each measurement from around 50 female individuals were as follows: for *P. thornei*, L (μm) = 538.8 ± 59.8 , a = 28.7 ± 1.5 , V = 77.4 ± 1.2 , and SL (μm) = 17.8 ± 0.6 ; for *P. neglectus*, L (μm) = 463.8 ± 28.9 , a = 26.9 ± 1.4 , V = 81.3 ± 1.1 , and SL (μm) = 18.3 ± 0.5 . Previous studies conducted by Vanstone et al. in 1998 and Thompson et al. in 2008 highlighted the substantial reduction in wheat yield caused by these two species in susceptible wheat cultivars on a global scale. To date, there is a lack of information regarding the susceptibility of commonly cultivated wheat varieties and their yield performance when affected by *Pratylenchus* spp. in New Zealand.

Due to the habitat preferences of most plant-pathogenic nematodes, their reproduction and pathogenicity are directly linked to soil physical and chemical properties (Xavier et al. 2014; Kularathna et al. 2014; Overstreet et al. 2014). The uneven distribution of nematodes in paddocks has been associated with changes in soil physicochemical properties (Overstreet et al. 2014). According to the work by Shukla et al. (2008), the reproduction of root-lesion nematodes shows a strong correlation with soil texture. Their findings suggest that sandy clay loam soils are more preferable than sandy loam soils for lesion nematode reproduction.

The results of the current study, however, found no correlation between nematode densities and soil types (Figure 4). The soils found at all sampling sites were predominantly silt loam soils, which differ from the soils that Shukla et al. (2008) found to have a positive correlation. To thoroughly comprehend the effects of soil texture on *Pratylenchus* species in New Zealand, sampling should be conducted in regions with various soil textures or under controlled environments exposing nematodes to a variety of soil textures.

Further work

The results of the current study have indicated the coexistence of two nematode species in the fields examined (Tables 2 and 3) and provides a good starting point to generate data on density through time at the same point including relative ratio of the two species. The variation in nematode species composition and density in the collected soil samples emphasises the necessity for further investigation. Understanding the influence of soil physicochemical properties on nematode species composition, density, and their distribution within a field over time is therefore crucial.

Apart from soil texture, other physicochemical properties of soil have been linked with nematode reproduction and pathogenicity (Kularathna et al. 2014). Therefore, a broader analysis of soil physicochemical properties will be needed to better understand the relationship between root-lesion

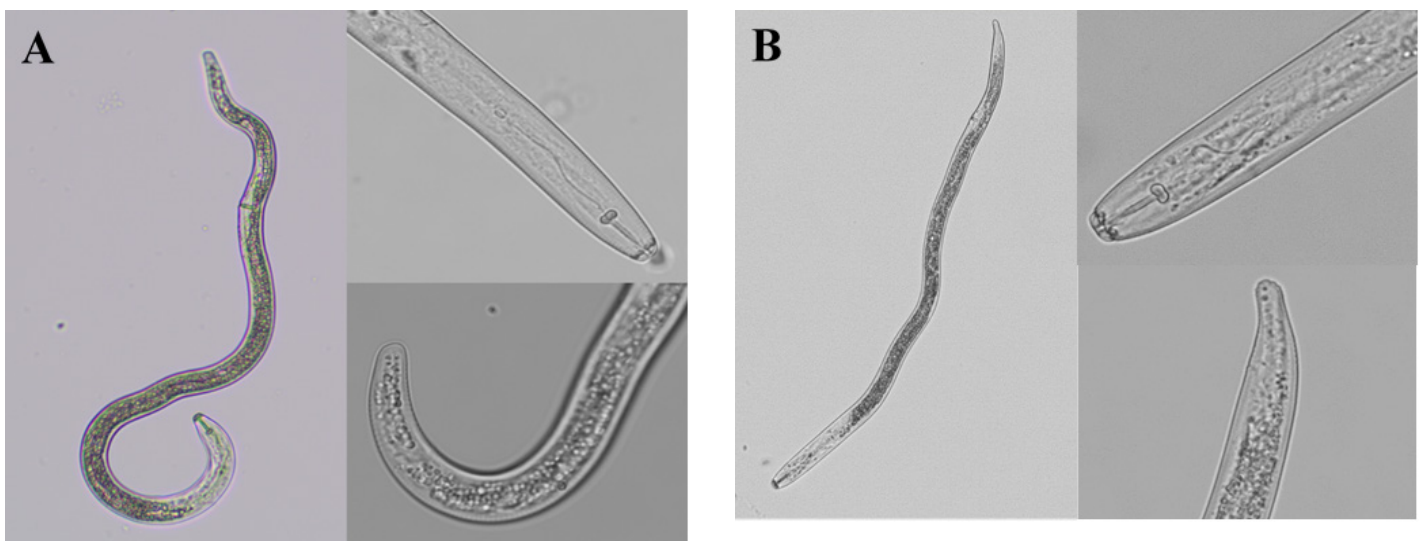


Figure 3 *Pratylenchus thornei* (A) and *Pratylenchus neglectus* (B) specimens collected from survey samples. Left, Female adult body at 100X magnification. Upper right, adult female head at 600X magnification. Lower right, adult female tail at 600X magnification.

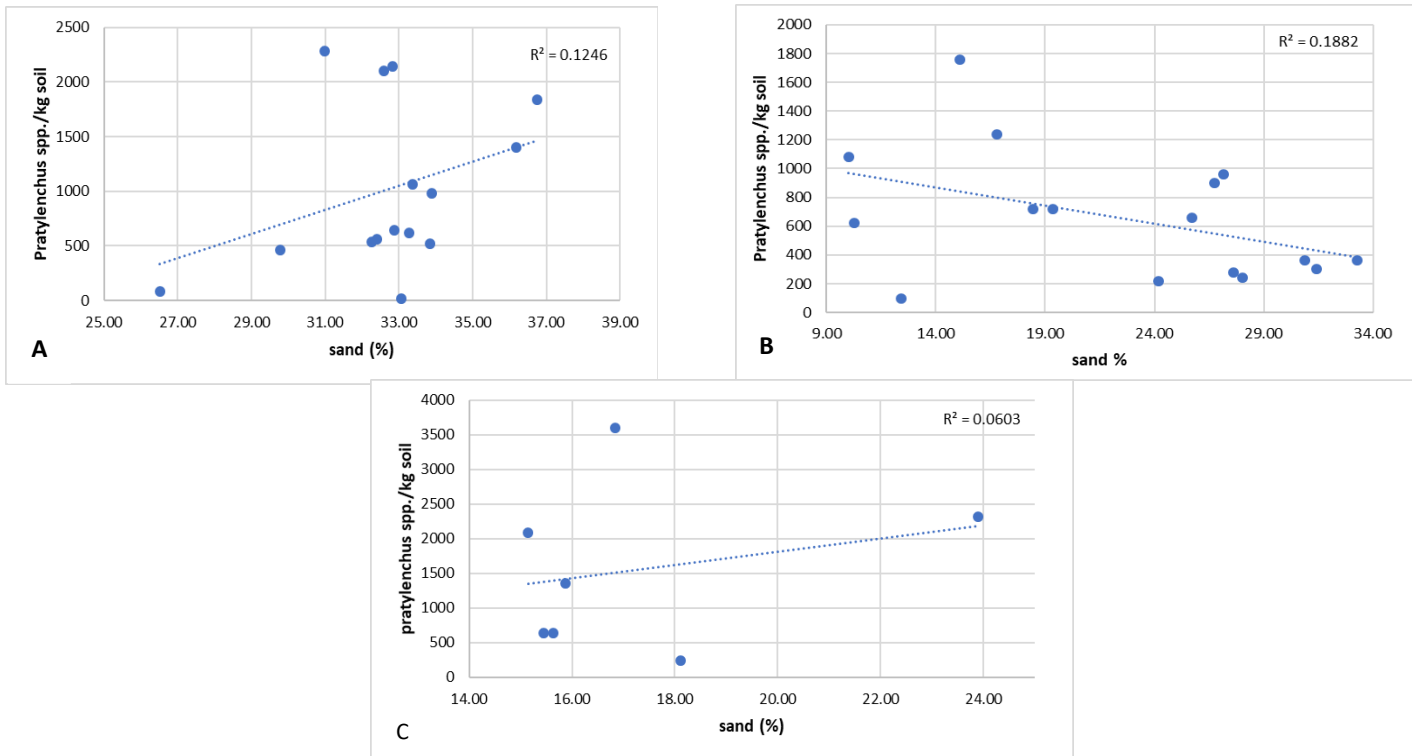


Figure 4 Linear regression analyses of the interaction between soil sand content and *Pratylenchus* nematode densities/kg in three fields A - Birchs Road, Lincoln, Selwyn district; B – Leadleys Road, Lincoln, Selwyn district; C – Corbetts Road, Dorie Ashburton district.

nematode distribution, abundance, reproduction, and pathogenicity with soil properties.

The current survey had provided evidence of *P. thornei* and *P. neglectus* co-existing in Canterbury wheat fields in high densities. Previous reports have indicated that managing the issues caused when both species are present is extremely challenging and will need years of research to develop an efficient management strategy (Anon 2022b; Anon 2022c). This finding indicates the extreme importance of further work to derive effective future nematode-management practices.

CONCLUSIONS

The results of this research confirmed the presence of *P. thornei* and *P. neglectus* species in Canterbury wheat-producing areas. In some locations, nematode density exceeded the threshold of 2,000 *Pratylenchus* spp./kg of soil, which has caused substantial yield losses in susceptible cultivars grown in the USA or Australia. Therefore, the results indicate a potential threat to the New Zealand wheat industry by root-lesion nematode species. Determining the effect of soil texture on root-lesion nematode abundance was outside the scope of the current study, so further work

Table 2 Morphometric data of female *Pratylenchus thornei* from two sites in Selwyn district, Canterbury, New Zealand, and their comparison with Commonwealth Institute of Helminthology descriptions (Loof 1960; Sher & Allen 1953).

Measurements ^a	Current site 1	Current site 2	Previously published data	
	Lincoln (Leadleys Road)	Lincoln (Birchs Road)	Sher & Allen (1953)	Loof (1960)
L (µm)	548.9 ± 56.8 (472.8-653.1)	528.6 ± 62.8 (449-677.3)	450-770	408-708
a	28 ± 1.9 (24.6-31.7)	29.4 ± 1.2 (27.2-31.3)	26-36	25.3-36.4
V	77.2 ± 1.3 (75.4-80.7)	77.7 ± 1.1 (75.6-79.4)	73-80	74.4-79
SL (µm)	18 ± 0.6 (17.3-19.4)	17.6 ± 0.5 (17-18.5)	17-19	15-19

^aMeasurements: **(L)** total body length; **(SL)** stylet length; **(a)** body length/greatest width at the vulva; and **(V)** position of the vulva with respect to total body length.

Table 3 Mean morphometric data of female of *Pratylenchus neglectus* from Selwyn district, Canterbury, New Zealand (ranges in parentheses), and their comparison with Commonwealth Institute of Helminthology descriptions (Loof 1960; Sher & Allen 1953).

Measurements ^a	Current site 1	Current site 2	Previously published data	
	Lincoln (Leadleys Road)	Lincoln (Birchs Road)	Sher & Allen (1953) ^b	Loof (1960)
L (µm)	448.1 ± 10.3 (439.5-459.5)	479.6 ± 47.5 (406.4-532)	310-550	312-588
a	26.3 ± 0.2 (26.1-26.5)	27.6 ± 2.6 (24.6-32.1)	18-25	16.5-32
V	81.2 ± 0.6 (80.6-81.7)	81.4 ± 1.7 (78.7-83.9)	80-88	75.5-86.6
SL (µm)	18.4 ± 0.8 (17.5-19)	18.2 ± 0.3 (17.8-18.5)	16-18	15-19

^a Morphological measurements include: **(L)** total body length; **(SL)** stylet length; **(a)** body length/greatest width at the vulva; and **(V)** position of the vulva with respect to total body length.

^b Numbers in bold indicate where the current data exceed the value range previously published by Sher and Allen (1953).

needs to be conducted to fully understand the effects of soil physicochemical properties on root-lesion nematodes. The information gathered from this research would be extremely valuable when developing management strategies using precision agricultural methods.

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