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Prevalence of *Verticillium* spp. and *Pratylenchus* spp. in Commercial Potato Fields in Atlantic Canada

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Abstract

A survey of New Brunswick (NB) and Prince Edward Island (PEI) potato fields in crop rotation phase prior to potato production was conducted in fall (October and November) between 2017 and 2021. A total of 113 and 126 fields for NB and PEI, respectively, were surveyed with 20 to 35 fields each year tested in each province. Root lesion nematodes (RLN, *Pratylenchus* spp.) were detected in 99 and 98% of the fields for NB and PEI, respectively, and two root lesion nematode species, *P. crenatus* and *P. penetrans*, were identified in both provinces from 2017 to 2021. Based on 2019 and 2020 results, all surveyed fields in NB and PEI were detected with *P. crenatus*, while only 29 and 43% of the fields in NB and PEI were detected with *P. penetrans*, respectively. *P. crenatus* accounted for 96 and 89% of the populations for NB and PEI, respectively, while *P. penetrans* accounted for 4 and 11% in commercial fields, respectively. In a single in-depth sampled experimental field with a history of severe potato early dying complex in 2018 in NB, *P. crenatus* accounted for 88% and *P. penetrans* was 12%. *Verticillium dahliae* was detected in 94 and 92% of potato fields in NB and PEI, respectively. All isolates obtained from potato cv. "Russet Burbank" in a baiting trial were *V. dahliae*, belonging to two lineages. *V. albo-atrum* was detected in a few fields at very low level, except two fields in NB where *V. albo-atrum* was predominating over *V. dahliae*. Rotation crops did not affect *V. dahliae* population densities for NB and PEI, and did not affect RLN population in NB, but significantly affected RLN in PEI. Fall green cover crop did not affect the populations of RLN and *V. dahliae* in PEI. The present study revealed that the potato pathogenic root lesion nematode *P. penetrans* was present in less than 50% of surveyed fields and accounted for around 10% of root lesion nematode population in NB and PEI, and *V. dahliae* was the dominant species and was present in greater than 90% of surveyed fields in both provinces.

Keywords Pathogen survey · Rotation crops · Cover crops · Root lesion nematodes · Potato early dying disease · Wilt

Introduction

Potatoes (*Solanum tuberosum* L.) are the primary cash crop grown in the provinces of New Brunswick (NB) and Prince Edward Island (PEI) in Atlantic Canada. The two provinces produced 35.5% of potatoes in Canada in 2022 (Statistics Canada 2022). Despite efforts made to improve potato yield over the years, potato yield improvement has been slower than other regions of North America, largely due to potato diseases, declining soil organic matter and changing climatic conditions (Powelson and Rowe 1993; Nyiraneza et al. 2017; Zebarth et al. 2021a, b). Potato early dying disease complex, one of the soil-borne diseases affecting this crop, is endemic in Atlantic Canada and is considered to be a primary yield limiting factor by potato industry, which could cause up to 50% yield loss in heavily infested fields

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(Powelson and Rowe 1993). Fungal pathogens *Verticillium* spp. are the primary disease agents causing potato early dying; the root lesion nematode (RLN, *Pratylenchus penetrans*) infecting potato roots synergistically exacerbates the severity of the potato early dying (Powelson and Rowe 1993; Rowe et al. 1985; Bowers et al. 1996). Other pathogens, such as black dot caused by *Colletotrichum coccodes, Rhizoctonia solani*, *Fusarium* spp. and bacterial blackleg (*Pectobacterium* spp.), could contribute to the potato early dying complex if they are present in the field under favorable conditions (Powelson and Rowe 1993).

Historically, *V. albo-atrum* was found to be the primary agent causing Verticillium wilt or potato early dying in NB and PEI. Kimpinski et al. (1998) reported that *V. albo-atrum* was detected in 37 potato fields surveyed in 1991 in NB, at an average population of 350 propagules g^{-1} of dry soil, while *V. dahliae* was found in 17 fields at less than one propagule g^{-1} of dry soil. In PEI, *V. albo-atrum* was considered the sole pathogen causing potato Verticillium wilt before the survey conducted by Celetti and Platt in 1985 who found that *V. dahliae* was detected in 20 out of 36 surveyed fields at a maximum of 2.9 microsclerotia g^{-1} of dry soil, while *V. albo-atrum* was predominant in the soil (Celetti and Platt 1987; Mahuka et al. 1999). Recently, *V. dahliae* was reported to be the predominant species causing Verticillium wilt in ten fields in PEI, six fields in Nova Scotia (Borza et al. 2018, 2019) and two fields in NB (Chen et al. 2022). However, the pathogen species occurrence and abundance in large scale commercial potato fields in Atlantic Canada had not been determined. Knowing what pathogens and their population densities in commercial fields is crucial for decision-making on potato early dying disease management.

Three root lesion nematode species were reported in NB and PEI (Yu 2008), of which *Pratylenchus penetrans and P. crenatus* are common in the two provinces. In a survey done in 1990 and 1991 in NB, Kimpinski et al. (1998) reported that root lesion nematodes were present in majority of the fields, with *P. crenatus* being more prevalent than *P. penetrans*. In PEI, *P. crenatus* and *P. penetrans* were frequently detected in fields, with the population density of *P. penetrans* usually higher than *P. crenatus* (Kimpinski 1979, 1987; Willis et al. 1976). The population density and species frequency of the root lesion nematodes in commercial fields in Atlantic Canada were not well determined since the surveys done more than three to four decades ago (Kimpinski 1979; Kimpinski et al. 1998).

The objectives of this study were (1) to identify the species of *Verticillium* and root lesion nematodes (*Pratylenchus* spp.) and quantify their population densities at rotation phase in fall before commercial potato production, and (2) to examine the impact of rotation and fall green cover crops on pathogen population density. The study used a survey approach which targeted potato fields representing major potato production regions in NB and PEI, Canada, from 2017 to 2021.

Materials and methods

Field Selection and Climate Data Collection

The survey was conducted in the fall of 2017, 2019, 2020 and 2021 in commercial potato fields in NB and PEI. A total of 113 and 126 fields were surveyed in NB and PEI, respectively. Twenty to 35 fields each year were selected for sampling in NB and PEI from the main potato production regions in NB and PEI (Fig. 1). Samples were collected from fields which were planted to rotation crops prior to potatoes in the following year. Information on crop species of each field was collected by interviewing the growers (Tables 7 and 8). Most of the fields surveyed in PEI were under a minimum of 3-year rotation prior to potatoes, and a subset of the surveyed fields were grown with green cover crops in early fall after rotation crops were harvested, including spring barley, spring oats, daikon radish, and brown mustard. The fields surveyed in NB were under a minimum of 2-year rotation prior to potatoes and no fall green cover crop data were collected. None of the fields were fumigated and all fields were managed by growers with their standard practices. The PEI soils had similar pH to the NB soils, 6.0 and 5.9, respectively, but differed significantly in soil physical properties. In average over the 80 fields in NB and 91 fields in PEI, sand represented 51% in NB soils and 62% in PEI soils, clay represented 11% in NB and 9.2% in PEI. Climate data were obtained from the Environment and Climate Change Canada weather station at Woodstock, NB, for NB and Charlottetown Airport for PEI (https://climate.weather.gc.ca/historical_data/search_historic_data_e.html, accessed November 20, 2023). In cases when the climate data were missing at the Woodstock weather station, the records at the Fredericton International Airport weather station were used.

Soil Sampling

Soil samples were collected at each field between October and November each year after the rotation crops were harvested or crop residues were incorporated into soil and green cover crops were planted in some fields in NB and PEI. Soil borne pathogen distributions were mostly heterogeneous, i.e., patchy distributed within a field, and were influenced by host species and environmental conditions (Nielsen et al. 2010; Taylor et al. 2005). Most inoculum of *V. dahliae* and *Pratylenchus* spp. were present in the top 0–30 cm depth of soil (Montasser et al. 2015; Ben-Yephet and Szmulewich

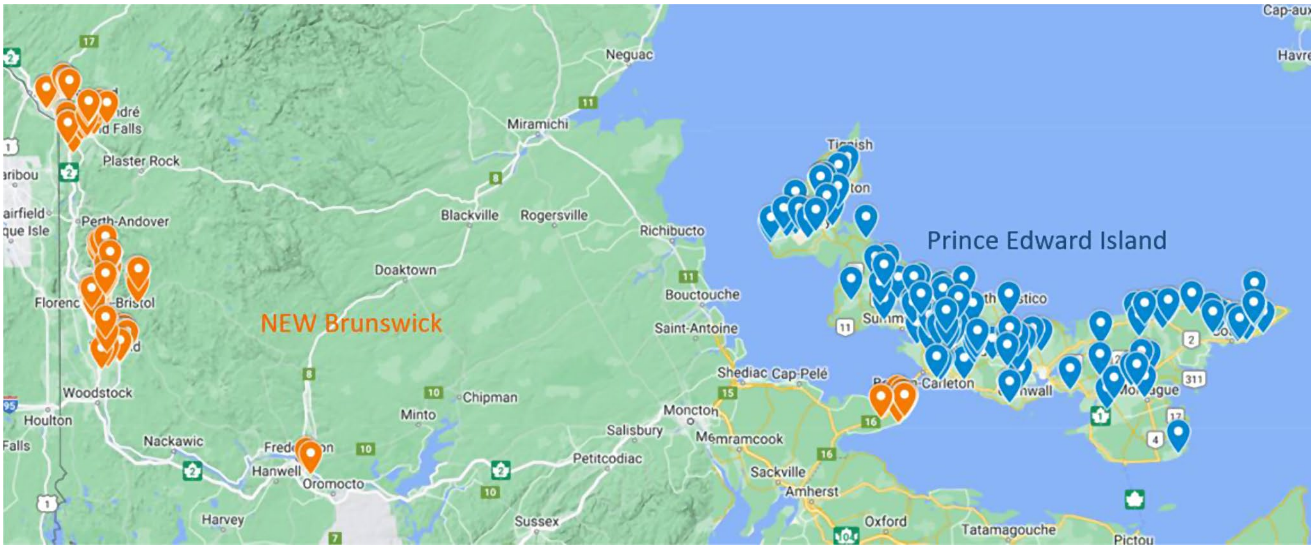

Fig. 1 Distributions of surveyed fields in main potato production regions in the province of New Brunswick (113 fields, orange) and Prince Edward Island (126 fields, blue), Canada, from 2017 to 2021

1985; Gudmestad et al. 2007). Therefore, the sampling sites in each field were selected considering the topography of the field for representation. One composite soil sample was collected from 0 to 25 cm depth in each field using a 6 - cm diameter Dutch augur. Each composite soil sample consisted of 50 cores of sub-samples obtained following a "zigzag" pattern while travelling through the field. For a field greater than 5 ha, a transect of approximately 5 ha of the field was sampled for NB sampling. For PEI sampling, if the field was greater than 15 ha, approximately half of the field was sampled.

The soil cores were placed in a plastic bucket and were mixed thoroughly to form a composite soil sample. The composite soil samples were kept in a plastic bag placed in a cooler with ice pads during sampling and transportation before being stored at 4 °C. In the laboratory, the composite soil sample was divided into four portions for species identification and quantification of *Verticillium* spp. and root lesion nematodes, for soil property characterization, and for a greenhouse trial to induce Verticillium wilt of potato cv. "Russet Burbank" for *Verticillium* isolate collection (Arseneault et al. 2023).

Quantification of Root Lesion Nematodes and *Verticillium* Spp

This survey was a part of a national project with participation of growers in each province. It was not technically feasible and pragmatic to use the same laboratory to quantify the pathogens from different provinces. Therefore, the pathogen quantification of the soil samples from different provinces would follow the growers' practice. For the root lesion nematode quantification, the NB potato growers typically send their soil samples to the Agriculture and Food Laboratory (AFL) Services at University of Guelph, Guelph, Ontario, while the PEI growers were used to sending their soil samples to the Potato Quality Institute Inc. (PQI), located in Charlottetown, PEI. Both service providers used the modified Baermann pan method for extraction and quantification of nematodes (Townshend 1963). Following the growers' practice, approximately 500 g each of wet soil sample from NB was shipped to AFL and 500 g each of wet soil sample from the PEI was shipped to PQI within 48 h after collection for root lesion nematode quantification. The soil samples were kept at 4 °C before shipping and quantification. Wet soil subsamples were used for nematode quantification and another set of wet soil subsamples were oven dried at 70 °C for 24 h for reporting the nematode quantity as number of nematodes kg^{-1} of dry soil. Approximately 300 g from each soil sample was sent to the Agricultural Certification Service Inc. (Fredericton, NB, Canada) for quantification of *V. dahliae*, *V. albo-atrum* and *V. tricorpus* by probe-based quantitative real-time PCR (qPCR) using primers and probes targeting the *elf1α* gene of each species as detailed in Nyiraneza et al. (2021). The soil samples were air-dried and ground to powder before testing. Preliminary results using the soil samples taken from NB and PEI in 2019 showed that the *V. tricorpus* was not present in any of the soil samples, therefore, only *V. dahliae* and *V. albo-atrum* were quantified for the soil samples.

Root Lesion Nematode Species Identification

Composite soil samples taken from 20 commercial fields in fall 2019 and one field from the research farm of the Fredericton Research and Development Centre, located in Fredericton, NB, in 2018, and from 42 fields in the fall of 2019 and 2020 from PEI were used for root lesion nematode isolation and species identification. The single field from the research farm had a history of potato trials and severe PED was observed over the years. It was intensively sampled at 23 sampling sites, and nine soil cores were taken from each site to form a composite sample to investigate the species composition of root lesion nematodes and their population densities. Following Baermann pan's extraction, up to 16 individual nematodes with morphological characteristics compatible with the *Pratylenchus* genus were handpicked from each soil sample. DNA was extracted from individual nematodes using the sodium hydroxide method (Boucher et al. 2013). The mitochondrial COI gene was then amplified using the primers COI-JB5 and COI-F7b-Prat with the PCR conditions detailed in Ozbayrak et al. (2019). The sequences obtained by Sanger sequencing were compared to reference sequences from known species in the BOLD and NCBI databases using BLAST to identify most probable species.

Verticillium Species Identification

To collect *Verticillium* isolates pathogenic to potatoes, a baiting trial was conducted in a greenhouse in Saint-Jean-Sur-Richelieu Research and Development Centre (SJSRRDC), Quebec. Five to 6 L of each soil sample were shipped to the SJSRRDC from NB and PEI and were kept at 4 °C before use. Potato cv. "Russet Burbank" was grown in the soil samples collected from NB and PEI in the fall of 2019 and 2020, with temperature of 23/18°C day/night, and a 16/8 h light/dark cycle. Fifty-one and 63 *Verticillium* isolates were collected and subjected to whole genome sequencing for genetic characterization and species identification (Arsenault et al. 2023). The diversity (h) of *V. dahliae* was calculated as follows,

$$h = \frac{n}{n-1}\left(1 - \sum p_i^2\right)$$

where p_i is the haplotype or lineage frequency of each haplotype or lineage in the sample and n is the sample size (Harris and DeGiorgio 2017; Nei and Tajima 1981).

Statistical Analyses

The raw data of root lesion nematodes and *V. dahliae* were grouped based on the survey year, the crop species / groups and the presence of fall cover crops for analysis of variance. Log(10 + x) was used to transform the RLN counts and *Verticillium* DNA to meet the requirements of the ANOVA (Vrain et al. 1996). Since these soil samples were collected over multiple years, from different farms with different agronomical practices and growing with different crops, datasets failed in normality test using Shapiro-Wilk test. Non-parametric one way analysis of variance on ranks using Kruskal-Wallis H test was performed to determine the median difference among the groups. Pairwise multiple comparison procedure, Dunn's method was used to determine the difference between the groups. All statistical analyses and graphing were performed using SigmaPlot 14.5 (Systat Software, Inc). Since the survey data were asymmetric and skewed with outliers, median together with the interquartile range (IQR), 25% and 75%, were used to describe the central tendency of the datasets (Gosselin 2021; Habibzadeh 2017).

Results

Climate Conditions

The climate data for the NB and PEI were obtained from Environment and Climate Change Canada (https://climate.weather.gc.ca/index_e.html). The mean daily air temperatures during the crop season (May to October) in NB for 2017 and 2021 were 0.8 to 1.2 °C higher than the long-term (1981–2010) average of 14.1 °C and the years 2019 and 2020 were slightly lower than the long-term average (Table 1). The total precipitation during the study period was 60, 87, 55, and 108% of the long-term average total precipitation (577 mm) for 2017, 2019, 2020 and 2021, respectively (Table 1). Three of the four years received 45 to 13% less precipitation than the long-term average total precipitation.

In PEI, the mean daily air temperature at the Charlottetown Airport climate station during the crop season (May to October) for 2017, 2020 and 2021 were 0.8 to 1.2 °C higher than the long-term (1981–2010) average of 13.9 °C, and the year 2019 was slightly cooler than the long-term average (Table 2). The total precipitation during the crop season was 96, 109, 63 and 112% of the long-term average of total precipitation (573.5 mm) for 2017, 2019, 2020 and 2021, respectively (Table 2).

The overall temperatures among the years during the study period in NB and in PEI were mostly higher than the long-term average and the total precipitation was much less in three of the four years in NB and was mostly equivalent or higher than the long-term average in PEI. The year 2020 brought drought conditions in Atlantic Canada, where NB received 55% and PEI 63% of normal precipitation. The

Table 1 Average daily mean air temperature and total precipitation during May to October growing season in Woodstock, New Brunswick between 2017 and 2021 in comparison with the long-term (1981–2010) average. Climate data were obtained from the Environment and Climate Change Canada weather station at Woodstock, and Fredericton International Airport, NB (https://climateweathergcca/historical_data/search_historic_data_ehtml)

	Average air temperature (°C)					Total precipitation (mm)				
Month	2017	2019	2020	2021	1981–2010	2017	2019	2020	2021	1981–2010
May	10.9	8.9	8.3	10.4	10.9	133.4	58.2	61.8	77.5	94.2
June	16.3	15.2	16.7	17.9	16.3	68.8	111.6	28.6	68.7	91.0
July	18.7	19.9	20.3	16.9	19.0	37.8	37.2	27.1	116.8	100.2
Aug	17.8	17.7	18.6	20.0	18.4	38.0	85.6	36.0	79.1	100.6
Sept	16.4	12.3	13.5	14.4	13.2	57.2	97.0	18.9	220.5	95.7
Oct	11.5	7.9	6.4	9.7	6.6	13.6	112.4	143.6	58.4	95.3
Average or total	15.3	13.7	14.0	14.9	14.1	348.8	502.0	316.0	621.0	577.0

Table 2 Average daily mean air temperature and total precipitation during May to October growing season in Charlottetown, Prince Edward Island between 2017 and 2021 in comparison with the long-term (1981–2010) average. Climate data were obtained from the Environment and Climate Change Canada weather station at Charlottetown Airport, PEI (https://climateweathergcca/historical_data/search_historic_data_ehtml)

	Average air temperature (°C)					Total precipitation (mm)				
Month	2017	2019	2020	2021	1981–2010	2017	2019	2020	2021	1981–2010
May	9.5	6.7	9.1	8.5	9.2	160.4	82.5	70.3	107.3	91.0
June	15.5	14.2	16.1	17.4	14.5	85.0	152.1	29.5	66.8	98.8
July	18.7	19.1	19.2	17.6	18.7	68.8	34.5	44.6	134.1	79.9
Aug	18.3	19.8	19.7	20.5	18.3	127.5	72.0	30.9	31.8	95.7
Sept	15.4	13.2	14.9	16.1	14.1	73.9	166.5	95.9	226.8	95.9
Oct	11.5	8.3	8.9	10.4	8.3	37.4	117.2	89.1	73.7	112.2
Average or total	14.8	13.6	14.7	15.1	13.9	553.0	624.8	360.3	640.5	573.5

average temperature of the four years was similar between NB (14.5 °C) and PEI (14.6 °C), but the average precipitation was higher in PEI (554 mm) than in NB (447 mm). The climate data indicated that there existed a wide range of soil moisture conditions in the survey fields in Atlantic Canada.

Population Density of Root Lesion Nematodes in NB and PEI

As the root lesion nematode quantification was performed by two different service providers, variations other than experimental errors were expected. Direct comparison of nematode population densities between NB and PEI was not appropriate. The root lesion nematodes (*Pratylenchus* spp.) were detected in 99 and 98% of fields in the fall of 2017, 2019, 2020, and 2021 in NB and PEI, respectively. The RLN in rotation phase ranged from 20 to 11,536 counts kg^{-1} of dry soil among the NB samples during the survey years, but one sample taken from a field where potato was grown in 2020 had 27,489 counts kg^{-1} of dry soil in NB. In PEI, the RLN ranged from 289 to 34,445 counts kg^{-1} of dry soil in crop rotation phase. Normality test indicated that the data varied significantly from the normal distribution pattern for both NB and PEI datasets ($p < 0.001$) (Fig. 2). The median of RLN ranged from 841 (interquartile range or IQR: 369–1700) to 1680 (IQR: 1150–3260) counts kg^{-1} of dry soil for the NB samples and 2240 (IQR: 1480–4160) to 7719 (IQR: 4056–13,215) counts kg^{-1} of dry soil for the PEI samples during the survey years. The population density of RLN differed significantly among the survey years in both provinces (Table 3).

Root Lesion Nematode Species and Their Frequency in NB and PEI

Based on the mitochondrial COI gene sequences of the samples collected in 2019 and 2020 from the commercial fields and one research farm field in 2018, the nematodes with morphological characteristics compatible with the *Pratylenchus* genus were identified at the species level. Two root lesion nematode species, *P. crenatus* and *P. penetrans*, were detected from the fields in NB and PEI. All surveyed fields were detected with *P. crenatus* in NB and PEI. 25% of the NB fields in 2019 were detected with *P. penetrans*, and 48% and 35% of the fields in PEI were detected with *P. penetrans* in 2019 and 2020, respectively.

The population frequency of *P. crenatus* and *P. penetrans* in commercial fields was determined based on nematode samples combined from subsamples of up to 16 individuals per field. Among the 221 root lesion nematodes taken from the 20 commercial fields in 2019 in NB, 4% were *P. penetrans* and 96% were *P. crenatus*. In PEI, *P. penetrans* accounted for 11% and *P. crenatus* for 89% among the 398 samples taken from 42 commercial fields in 2019 and 2020.

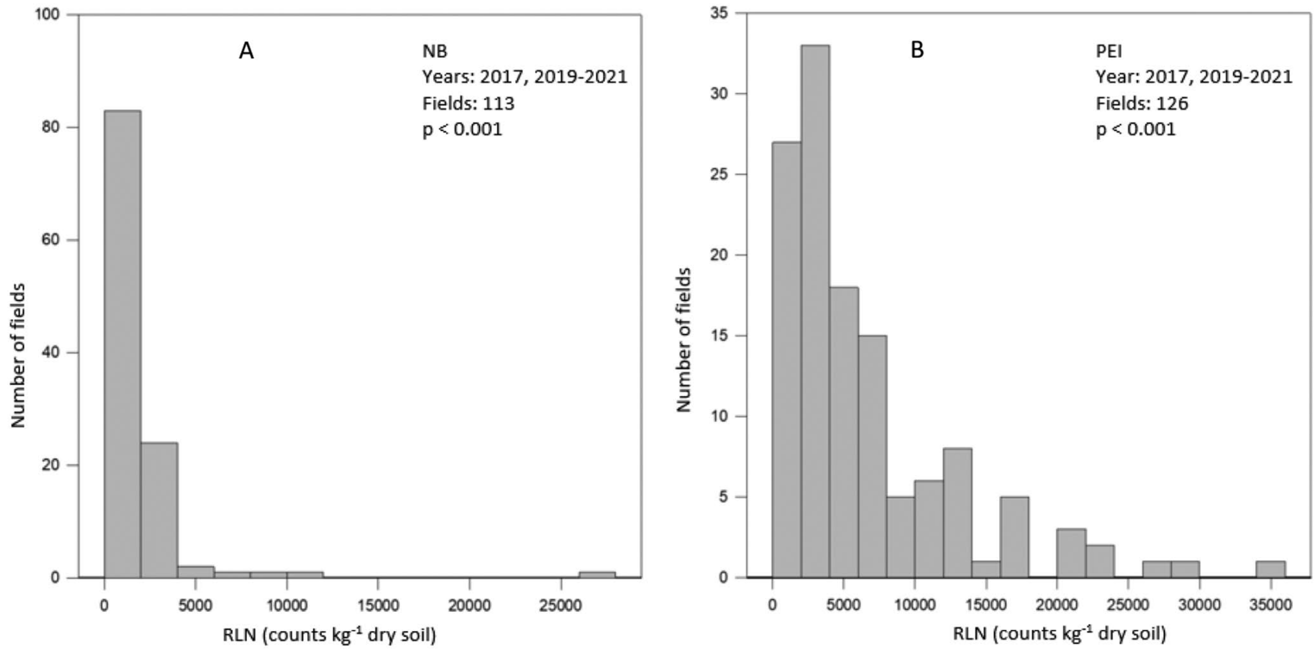

Fig. 2 Number of fields with different population densities of root lesion nematodes (*Pratylenchus* spp., counts kg^{-1} of dry soil), determined from the samples collected in fall after rotation crops in commercial potato fields in NB (**A**) and PEI (**B**), Canada from 2017 to 2021. The distribution of fields with different population densities of root lesion nematodes varied significantly from the normal distribution in both NB and PEI

Table 3 Population density of root lesion nematodes (*Pratylenchus* spp., counts kg^{-1} of dry soil) and *Verticillium* spp. (pg DNA g^{-1} of dry soil) in commercial potato fields, sampled in fall after rotation crop cycle between 2017 and 2021 in NB and PEI, Canada

	New Brunswick				Prince Edward Island			
Year	Number of Fields	*Pratylenchus* spp.[1]	*V. dahliae*[2]	*V. albo-atrum*	Number of Fields	*Pratylenchus* spp.	*V. dahliae*	*V. albo-atrum*
Fall 2017	33	1680 (1150–3260) a	122 (60–166) a	ND[3]	35	2240 (1480–4125) b	107 (53–159) b	ND
Fall 2019	20	1020 (355–1915) ab	84 (76–124) ab	0 (0–5)	30	7719 (4055–13,215) a	284 (162–354) a	0 (0–0)
Fall 2020	30	951 (387–1821) b	77 (30–135) ab	0 (0–0)	31	6326 (3585–13,451) a	217 (95–349) a	0 (0–0)
Fall 2021	30	948 (261–2005) b	57 (19–87) b	0 (0 -0.6)	30	3485 (2053–6454) b	60 (21–133) b	ND
p - value		0.02	0.001	0.414		< 0.001	< 0.001	0.596

1. Root lesion nematodes of NB samples were quantified by Agriculture and Food Laboratory (AFL) Services at University of Guelph, Ontario, and the PEI samples were quantified by Potato Quality Institute Inc. (PQI), located in Charlottetown, PEI

2. The population densities of *Pratylenchus* spp., *V. dahliae* and *V. albo-atrum* were presented in median. Values inside the parenthesis are interquartile range (IQR): 25 – 75%

3. ND = not determined. Numbers followed by the same letters in the same column are not significantly different

For an in-depth estimation of root lesion nematode species frequency, 228 individual root lesion nematodes were taken from the Research Farm field at the Fredericton Research and Development Centre in 2018, 12% belonged to *P. penetrans* and 88% to *P. crenatus* (Table 4). Evidently, *P. penetrans* accounted for a small portion of the root lesion nematode population in commercial fields in Atlantic Canada.

Population Density of *Verticillium* Species in NB and PEI

The *Verticillium* species-specific quantitative PCR determined that *V. dahliae* was predominant over *V. albo-atrum* in both PEI and NB commercial fields. In NB, *V. dahliae* was detected in 94% (106 out of 113) of the fields, ranging from 8 to 770 pg DNA g^{-1} of dry soil among the fields, except one field where the potato was planted and the density was 1872 pg DNA g^{-1} of dry soil. Eighty perecent of

Table 4 Root lesion nematode species and their frequencies in NB from 2018 and 2019, and in PEI, Canada from 2019 and 2020

			Frequency (%) of fields with		Frequency (%) of RLN		
Province	Year	Number of Fields[1]	*P. crenatus*	*P. penetrans*	RLN	*P. crenatus*	*P. penetrans*
NB	2018	1	-	-	228	88	12
	2019	20	100	25	221	96	4
	Total	21	100	29	449	92	8
PEI	2019	25	100	48	274	87	13
	2020	17	100	35	124	94	6
	Total	42	100	43	398	89	11

1: The single field in 2018, located at the Fredericton Research and Development Centre in NB, was a research farm field with a history of severe PED and was sampled in 23 sampling sites (6 × 10 m), each composite sample was made of nine sub-samples. The in-depth assessment of the frequency of root lesion nematodes in this single field in 2018 complemented to the survey results done in commercial fields in NB and PEI, Canada, in this study

the fields were detected with *V. dahliae* above 35 pg DNA g^{-1} of dry soil. *V. albo-atrum* was detected in 21% (17 out of 80) of the fields, ranging from 2 to 191 pg DNA g^{-1} of dry soil. Two fields were detected where *V. albo-atrum* was predominant over *V. dahliae*. Normality test indicated that the data varied significantly from the normal distribution pattern for both *V. dahliae* and *V. albo-atrum* datasets for NB and PEI ($p < 0.001$) (Fig. 3). The median of *V. dahliae* ranged from 57 (IQR: 19–87) to 122 (IQR: 60–166) pg DNA g^{-1} of dry soil among the survey years (Table 5) and the median of *V. albo-atrum* was 0 (IQR: 0–5) pg DNA g^{-1} of dry soil between 2019 and 2021. In PEI, *V. dahliae* was detected in 92% (116 out of 126) of the fields, ranging from 8 to 504 pg DNA $g^{-1}$of dry soil among the samples. 85% of the fields were detected with *V. dahliae* above 35 pg DNA g^{-1} of dry soil. *V. albo-atrum* was detected in 8% (5 out of 61) of the fields, ranging from 3 to 11 pg DNA g^{-1} of dry soil (Fig. 3). The median of *V. dahliae* ranged from 60 (IQR: 21–133) to 284 (IQR: 162–354) pg DNA g^{-1} of dry soil among the survey years. The median of *V. albo-atrum* was 0 (IQR: 0–0) pg DNA g^{-1} of dry soil in 2019 and 2020. The population densities of *V. dahliae* differed significantly among the survey years in both provinces (Table 5), and was significantly different between NB and PEI ($p = 0.011$).

Genetic Characterization of *Verticillium Dahliae* in NB and PEI

A total of 114 isolates were obtained from a baiting trial by growing "Russet Burbank" in NB and PEI soils in the greenhouse. The whole genome sequencing of these isolates confirmed that the isolates were *V. dahliae*, consisting of two lineages. Lineage two dominated over lineage one. Among the 51 isolates from NB, 20% of the isolates belonged to the lineage 1 and 80% belonged to the lineage 2, and among the 63 isolates from PEI, 24% of the isolates belonged to the linaege 1 and 76% belonged to the lineage 2. Lineage 1 had two haplotypes and lineage 2 had 6 haplotypes. The haplotype diversity for the NB and the PEI populations of *V. dahliae* was 0.67 and 0.74, respectively, and the lineage diversity was 0.32 and 0.37 for NB and PEI, respectively (Table 5).

Effect of Green Cover Crops and Rotation Crops on Pathogen Population Density

Green cover crops grown in early fall did not significantly affect the population density of *Pratylenchus* spp. and *V. dahliae* in PEI and no data was collected for NB (Table 6).

In NB, the rotation crops consisted of five crop species or groups. Barley or oat underseeded red clover and ryegrass or grass underseeded red clover accounted for 31 and 44%, respectively. Ryegrass, barley and sudangrass accounted for 13, 5, and 6%, respectively (Table 7). In PEI, the rotation crops in the surveyed fields consisted of 14 crop species / groups. The most common crop group was the grass and legume mix, accounting for 39%, followed by sudangrass, wheat, ryegrass, alfalfa, red clover, mustard, peas, radish, pearl millet, barley and multi-species mix. Buckwheat and soybean had only one field each (Table 8). Apparently, more diverse rotation crops were used in PEI than in NB. In NB, rotation crops did not significantly affect the population density of root lesion nematodes and *V. dahliae* (Table 7), however, rotation crops had significant effect on population density of root lesion nematodes in PEI (Table 8). Though the post-hoc test after ANOVA did not identify the groups with significant differences, numerically lower root lesion nematode densities were observed for the groups with five or more fields (namely sudangrass, wheat, mustard, peas, and radish), and higher densities were associated with grass/legume mix, alfalfa and red clover. Rotation crop groups did not have significant effect on the population density of *V. dahliae* in PEI (Table 2).

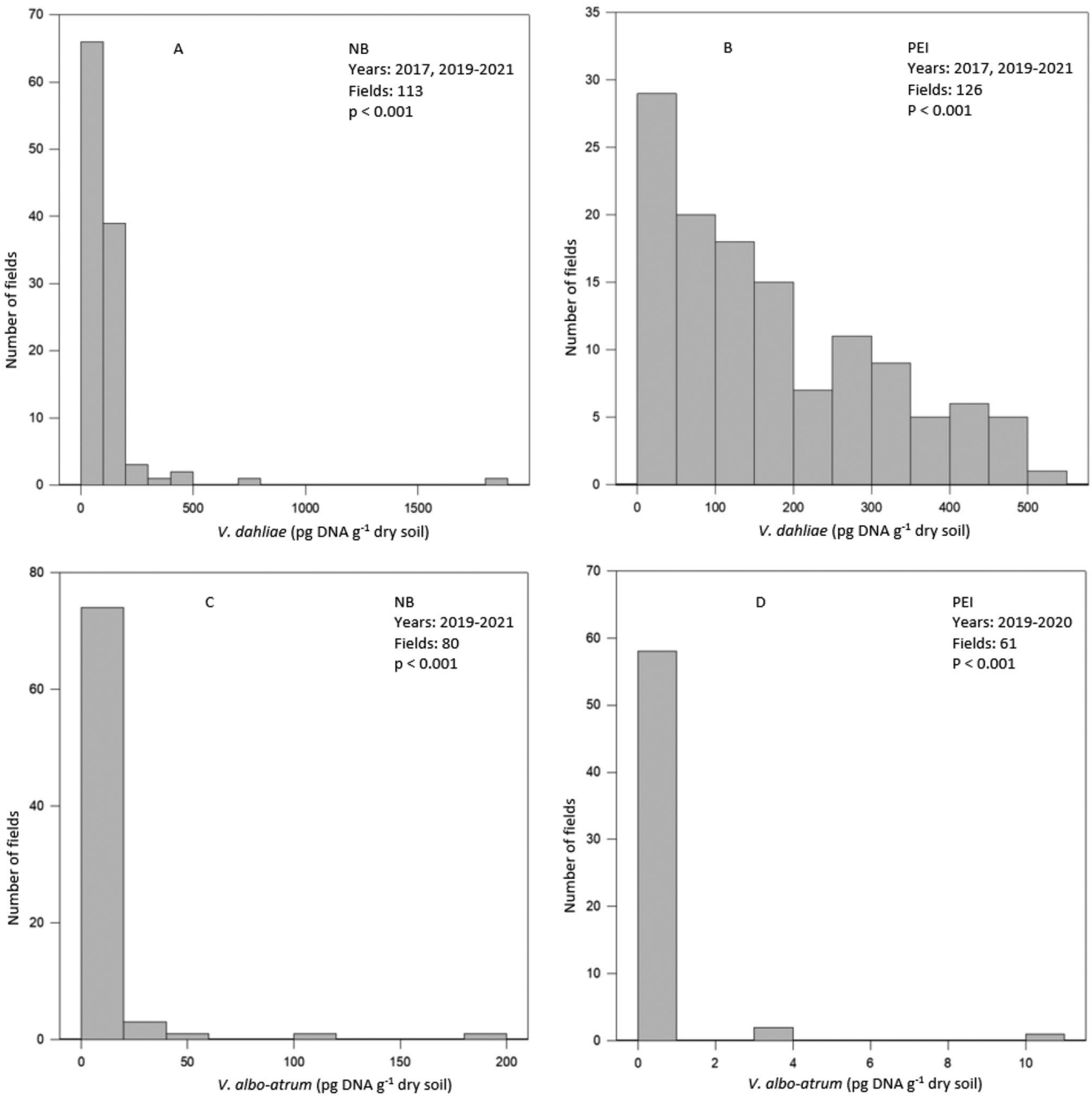

Fig. 3 Number of fields with different population densities (pg DNA g^{-1} of dry soil) of *Verticillium dahliae* and *V. albo-atrum*, determined from the samples collected in fall after rotation crops in commercial potato fields in NB and PEI, Canada from 2017 to 2021. The distribution of fields with different population densities of *V. dahliae* and *V. albo-atrum* varied significantly from the normal distribution in both NB and PEI. (**A**), population densities of *V. dahliae* for NB fields; (**B**), population densities of *V. dahliae* for PEI fields; (**C**), population densities of *V. albo-atrum* for NB fields; (**D**), population densities of *V. albo-atrum* for PEI fields

Discussion

Potato early dying disease complex is endemic and one of the most important potato yield limiting factors in Eastern Canada (Chen et al. 2022). Since the last surveys conducted by Kimpinski (1987), Celetti et al. (1987) and Kimpinski et al. (1998) in NB and PEI, no comprehensive studies were reported on the population dynamics of root lesion nematodes and *Verticillium* spp. in Atlantic Canada. Recently, Borza et al. (2018) reported that *V. dahliae* was the main pathogen causing potato Verticillium wilt in PEI based on a small-scale survey. The present study comprehensively surveyed the population density of root lesion nematodes and *Verticillium* spp. in the main potato production regions

Table 5 Genetic diversity of *Verticillium dahliae* isolates collected from potato cultivar "Russet Burbank" as a baiting plant in a greenhouse trial grown in soil samples collected from NB and PEI, Canada

		Lineage 1[a]		Lineage 2						Genetic diversity	
Province	Number of Isolates	1_2	1_3	2_1	2_2	2_3	2_4	2_5	2_6	h[b]	H[c]
NB	51	1	9	1	1	8	27	0	4	0.67	0.32
PEI	63	1	14	3	1	11	27	2	4	0.74	0.37
Total	114	2	23	4	2	19	54	2	8	0.71	0.35

[a]: Lineage 1 corresponding to vegetative compatibility group (VCG4A) and lineage 2 corresponding to VCG4B (Arseneault et al. 2023). [b]: haplotype diversity, [c]: lineage diversity

Table 6 Effect of fall green cover crops on population densities of *Pratylenchus* spp. (counts kg^{-1} of dry soil) and *Verticillium dahliae* (pg DNA g^{-1} of dry soil) in PEI, Canada from 2019 to 2021. No data on fall green cover crops were collected for NB

Green cover crops	Number of fields	*Pratylenchus* spp.	*V. dahliae*[1]
No	77	5699 (2959–12,697)	168 (72–301)
Yes	14	4478 (2372–7750)	158 (16–335)
p - value		0.296	0.64

[1:]Population densityies of *Pratylenchus* spp. and *V. dahliae* were expressed in median. Values inside the parenthesis are interquartile range (IQR): 25 – 75%

Table 7 Effect of rotation crop groups on population densities of *Pratylenchus* spp. (counts kg^{-1} of dry soil) and *Verticillium dahliae* (pg DNA g^{-1} of dry soil) in NB, Canada. Data were collected in the fall of 2019, 2020 and 2021 after rotation crop cycle

Crop group	Number of fields	*Pratylenchus* spp.	*V. dahliae*[1]
Barley or oat underseeded red clover	24	1154 (405–2157)	87 (62–133)
Barley	4	1806 (726–3303)	54 (38–71)
Ryegrass or grass underseeded red clover	34	1120 (483–1927)	62 (24–107)
Ryegrass	10	615 (73 -1628)	108 (51–122)
Sorghum sudangrass	5	368 (197–1159)	80 (14–210)
P - Value		0.233	0.193

[1:]Population densities of *Pratylenchus* spp. and *V. dahliae* were expressed in median. Values inside the parenthesis are interquartile range (IQR): 25 – 75%

Table 8 Effect of rotation crop groups on population densities of *Pratylenchus* spp. (counts kg^{-1} of dry soil) and *Verticillium dahliae* (pg DNA g^{-1} of dry soil) in PEI, Canada. Data were collected in the fall of 2017, 2019, 2020 and 2021 after rotation crop cycle

Crop group	Number of fields	*Pratylenchus* spp.	*V. dahliae*[1]
Grass/Legume Mix	49	6267 (3950–13,269)	106 (33–260)
Sudangrass	13	2949 (1493–4804)	221 (146–314)
Wheat	12	2627 (1360–5220)	181 (84–217)
Ryegrass	10	4155 (2130–6884)	191 (34–366)
Alfalfa	9	6884 (2204–12,286)	77 (33–173)
Red Clover	8	7975 (3501–25,355)	115 (22–376)
Mustard	5	1460 (1020–2430)	108 (66–269)
Peas	5	1820 (653–8352)	141 (54–382)
Radish	5	2388 (1085–5203)	168 (103–375)
Pearl millet	3	4114 (1812–4807)	197 (143–493)
Barley	3	1775 (400–2616)	63 (59–354)
Multi-species mix	2	2437 (1920–2954)	143 (106–180)
Buckwheat	1	3080 (-)	73 (-)
Soybean	1	21,817 (-)	111 (-)
P - value		< 0.001	0.455

[1:] Population densities of *Pratylenchus* and *V. dahliae* were expressed in median. Values inside the parenthesis are interquartile range (IQR): 25 – 75%

in NB and PEI during 2017 and 2021 in crop rotation phase prior to potato production. The fields surveyed well represented the diversity of rotation crops, different topographies of fields, various grower practices and different climatic conditions among the survey years in NB and PEI.

Pratylenchus spp. were the dominant plant parasitic nematodes in both provinces, and the population density of *Pratylenchus* spp. was dynamic over time. The average population densities of root lesion nematodes in soil samples were estimated at 1400 (samples collected during August 1979) and 900 counts kg^{-1} of dry soil (collected in early September of 1990 and 1991) from NB potato fields (Kimpinski 1987) and 1700 counts kg^{-1} dry soil (collected during August 1984) in potato fields of PEI (Kimpinski et al. 1998). In the present study with samples collected during October and November, the median population densities of root lesion nematodes ranged from 948 to 1680 counts kg^{-1} of dry soil in NB and 2160 to 7719 counts kg^{-1} of dry soil in PEI. The population densities seem similar with the previous surveys for NB, but the densities appear to be higher than the previous surveys for PEI. The high density of root lesion nematodes in commercial fields in PEI may come along with the widespread use of some rotation crops which are favorable hosts for root lesion nematodes, such as red clover as green manure crop over the years (Kimpinski and Thompson 1990; Kimpinski 1987; Carter et al. 2003).

Because these survey results were obtained in different laboratories, and there exist differences in sampling techniques, sampling time, cropping history and other variations in quantifying the nematodes, and the inaccessibility of the old survey datasets, these survey datasets could not be used to perform a sound statistiscal analysis for comparison, any conclusions drawn from the datasets of different surveys should be cautious.

In this study, *P. crenatus* was detected in nearly all fields in both NB and PEI, while *P. penetrans* was detected in less than 50% of the fields. Among 221 and 398 individual root lesion nematodes from commercial fields sequenced, only 4 and 11% of root lesion nematodes were identified as *P. penetrans* for NB and PEI, respectively. The in-depth analysis of 228 individual root lesion nematodes from the single research farm field at the Fredericton Research and Development Centre found that 12% of the samples were *P. penetrans* and 88% were *P. crenatus*. This confirmed the survey results obtained that *P. penetrans* accounted for a small portion of the root lesion nematode populations in commercial fields in Atlantic Canada. These results differed from the previous studies where the frequency of occurrence of *P. crenatus* was twice as high as for *P. penetrans* in NB, while the frequency of occurrence of *P. penetrans* was about five times as great as for *P. crenatus* in PEI (Kimpinski 1987; Kimpinski et al. 1998). Evidently, *P. crenatus* is currently predominating and the proportion of *P. penetrans* in the total root lesion nematode populations reduced in the two provinces, compared to the previous surveys. The reduction of *P. penetrans* could be partially attributable to use of root lesion nematode–suppressive rotation crops in recent years, such as forage pearl millet (*Pennisetum glaucum* L.), sorghum sudangrass, mustard as a biofumigant crop, and ryegrasses in the region (Bélair et al. 2004; Chen et al. 2022; Everts et al. 2006; Neupane and Yan 2023; Taning et al. 2023). Use of nematode-suppressive rotation crops should be promoted in PED management, particularly in Atlantic Canada as the use of soil fumigants is not common. The differences between historical nematode species demographics and the results of these surveys may also reflect advancements in species identification using DNA genotyping.

Though *P. penetrans* accounted for a small portion of the root lesion nematode population, it is the pathogenic species reported to interact synergestically with the main pathogen *V. dahliae* causing potato early dying (Orlando et al. 2020; Powelson and Rowe 1993; Bowers et al. 1996). Studies showed that 0.38 to 2 nematodes of *P. penetrans* g^{-1} of soil could cause significant potato yield reduction in Canada (Kimpinski and McRae 1988; Olthof and Potter 1973; Riedel et al. 1985; Olthof 1986), and the damage thresholds depended on potato cultivars and other environmental factors, such as soil texture, temperature, and moisture (Orlando et al. 2020; Castillo and Vovlas 2007). On the other hand, *P. crenatus* did not interact with *V. dahliae* causing potato early dying (Riedel et al. 1985). Therefore, accurate identification of root lesion nematodes at species level is crucial for early dying disease management. Morphology-based quantification may compromise data accuracy because of difficulties in determining the identity of the juvenile nematodes and similar species. Molecular approach could be a better alternative to the morphology-based method, giving its faster and potential of high reproducibility. Several real-time PCR methods were developed based on the internal transcribed spacer (ITS) of rDNA for identification and quantification of root lesion nematode species (Goto et al. 2011; Oliveira et al. 2017; Sato et al. 2007, 2010; Yan et al. 2013), however, the high variability of ITS sequences in *Pratylenchus* spp. increased the risk of obtaining false-positive and false-negative reactions, and gene copy number differences among the species and at different developmental stages of the nematodes could result in inaccuracy quantification. Further development of qualitative and quantitative molecular methods using single copy genes and rigorous test of the reproducibility should be done in different laboratories before their implementation (Orlando et al. 2020).

V. albo-atrum was considered the main pathogen causing potato Verticillium wilt in Atlantic Canada before Celetti et al. (1987) reported that *V. dahliae* was a new pathogen causing potato Verticillium wilt and the population density was much lower than *V. albo-atrum* in PEI. Kimpinski et al. (1998) found that *V. albo-atrum* was detected in all 37 surveyed fields with the average population of 350 propagules g^{-1} of dry soil, and *V. dahliae* was detected in 46% of surveyed fields at very low populations, usually lower than one progagule g^{-1} of dry soil in NB potato fields. Borza et al. (2018) reported that *V. dahliae* was the only pathogen causing potato Verticillium wilt in 10 surveyed fields in PEI. The present survey corroborated the finding of Borza et al. (2018) that *V. dahliae* is the main pathogen causing potato early dying in all potato fields in PEI, though *V. albo-atrum* was occasionally detected in a few fields at very low population level. In NB, *V. dahliae* is now ubiquitously distributed among the potato fields causing Verticillium wilt, and *V. albo-atrum* was under undetectable levels in majority of the samples. While it is likely that there has been some shift in the *Verticillium* population over the past three decades, it is also likely that advances in species identification in the intervening years may also play a factor. It is possible that *Verticillium* classified as *V. albo-atrum* in previous studies may have belonged to other similar *Verticillium* species that are more easily accounted for using improved species identification techniques (Wang et al. 2022).

The population densities of *V. dahliae* in over 80% of the surveyed fields were above 35 pg DNA g^{-1} of dry soil in both provinces. In a greenhouse trial with "Russet Burbank" grown in sterile soil, the inoculum density of *V. dahliae* at 35 pg DNA g^{-1} of dry soil could result in 50% tuber yield reduction compared to the control (Mathuresh Singh, Tyler MacKenzie, and Dahu Chen, unpublished). This implies that the population densities in majority of the commercial potato fields in NB and PEI reached a level that can cause significant yield reduction even without the presence of other pathogens involving in potato early dying disease complex. *V. dahliae* was also found to have a wider distribution and higher incidence than *V. nonalfalfae* (former *V. albo-atrum* group 1) in the tested fields growing potato and strawberry while *V. albo-atrum* sensu stricto (former *V. albo-atrum* group 2) was not identified in any of the samples in Nova Scotia (Borza et al. 2019). Evidently, *V. dahliae* is the main pathogen causing potato early dying and wilt in other crops in Atlantic Canada.

The replacement of *V. albo-atrum* by *V. dahliae* in Atlantic Canada appears to be coincide with the global climate warming over the years and with the different optimum temperatures for growth and virulence of *Verticillium* species. By examining the global changes in daily maximum temperatures from 1950 to 2004, Brown et al. (2008) found that Canada and Eurasia had positive trends where daily maximum temperatures typically warmed by 1 to 3°C since 1950. The average temperature during the cropping season (May to October) from 2017 to 2021, except 2019, increased 0.8–1.2°C compared to the 30-year average from 1981 to 2010 in Atlantic Canada (https://climate.weather.gc.ca/index_e.html). Verticillium wilt caused by *V. albo-atrum* is distributed in cooler climatic regions during the growing season (Powelson and Rowe 1993). In the study of heterokaryon incompatibility and interspecific hybridization between *V. albo-atrum* and *V. dahliae*, Typas (1983) found that altering the temperature had a marked effect on both the morphology and the nutritional marker ratio of the culture, which could easily become predominantly *V. albo-atrum* resting mycelium and auxotrophic at 18–19°C, or *V. dahliae* microsclerotial and auxotrophic at 28–29°C. *V. dahliae* had a higher temperature range for growth and pathogenicity than *V. albo-atrum* (Smith 1965). *V. albo-atrum* was most virulent at 17–21°C, while *V. dahliae* was most virulent at 21–30°C (Jabnoun-Khiareddine et al. 2006). If the temperature continues to increase with the global warming, the climate conditions would favor the pathogen growth and pathogenicity and exacerbate the PED severity. Aside from the climate changing, other factors such as potato varieties, rotation crops and rotation cycles, and other soil management practices may play roles in *Verticillium* species replacement during the past years.

Crop rotation and using of green cover crops in fall are sustainable good practices in agriculture, which improve soil health, prevent soil erosion, and increase crop productivity (Nadeem et al. 2019; Khakbazan et al. 2023), however, they had varied effects on potato diseases in succeeding crop season (Johnston et al. 1994). Larkin et al. (2010) studied the long-term effect of 2-year crop rotation using different crop species on potato diseases, tuber yield, and soil microbial community in a plot trial over 10 years. They found that different crop species and fall green cover crops had distinct effect on soil microbial community, tuber yield and disease severity. Common scab and black scurf were significantly reduced after growing canola and rapeseed but not soybean. However, Verticillium wilt on potato increased and became prominent in all rotation plots over the time, though significant difference in incidence was detected among the rotation crops with the barley having the lowest incidence of potato Verticillium wilt. Kimpinski et al. (1992) found that the most recent crop species, but not crop sequence, affected the root lesion nematode population. In the previous survey in NB, Kimpinski et al. (1998) found that the previous crop species did not affect the population densities of *Pratylenchus* spp. and *V. albo-atrum*. Not only crop species affected the disease severities, the length of rotation cycle significantly affected disease severities (Peters et al. 2004). In the present study, the crop species / groups used for rotation had significant effect on root lesion nematodes for PEI ($p < 0.001$) but not for NB. No significant difference was detected for the population density of *V. dahliae* among the crop species / groups in both provinces. The fall green cover crops did not affect populations of RLN and *V. dahliae* in PEI, but they have been increasingly adopted by growers in PEI as a measure to prevent soil erosion and carry-over residual soil nitrate in recent years. While the choice of rotation crop species / groups is likely to affect the variance of the pathogens, other variables such as grower practices, climate conditions and different service providers for quantification would also affect the detection of the variance among the groups. The imbalanced crop group size (from 1 to 49 for PEI and 4 to 34 for NB) could compromise the statistical power to detect the variance among the groups (Rusticus and Lovato 2019). It is important to note that the population density in this study was determined using qPCR, which quantify all types of *V. dahliae* present in the field soils disregarding their races and vegetative compatibility groups (VCGs).

V. dahliae population structure is complex and has significant effect on crop disease management (Chen et al. 2021). With different tools used in the studies, different subpopulations on population structures could be determined. *V. dahliae* were differentiated into at least three pathogenic races based on the response of host differentials (Dobinson et al. 1996; Usami et al. 2017; Vallad et al. 2006), and the species could also classified into multiple clonal lineages or haplotypes, or VCGs based on molecular markers or the compatibility of nutritional deficiency mutants (Bhat et al. 2003; Jimenez-Gasco et al. 2014; Dung et al. 2013; Short et al. 2015). Host specialization of *V. dahliae* has been found in various crop species, such as mint, potato, olive and cocoa, and the gene flow between the subpopulations was restricted (Dung et al. 2013; Baroudy et al. 2019; Resende et al. 1994). Though the clonal lineages do not always correspond to VCGs or races (Chen et al. 2021), the populations of *V. dahliae* infecting potatoes for NB and PEI consisted of two lineages, with lineage two, corresponding to VCG4B, predominating the lineage one, corresponding to VCG4A, in both provinces (Arseneault et al. 2023). In North America, VCG4A is more aggressive than VCG4B causing PED (Omer et al. 2000). Because these isolates were obtained in a baiting experiment using potato cv. "Russet Burbank", the genetic diversity of *V. dahliae* in this study might be underestimated, it is important to determine the overall genetic diversity of *V. dahliae* in Eastern Canada including isolates infecting other crop species and other cultivars in the potato production system and determine their roles in potato early dying disease complex.

Based on the present study, it can be concluded that two root lesion nematode species, *P. crenatus* and *P. penetrans* are currently the primary plant parasitic root lesion nematodes, and that the potato pathogenic species *P. penetrans* is present at low levels in potato fields in NB and PEI. However, its impact on potato yield reduction cannot be ignored since it interacts synergistically with *V. dahliae*, exacerbating the PED severity. *V. albo-atrum* is either absent or at undetectable levels in majority of the fields, *V. dahliae* is ubiquitous in potato fields and is the main pathogen causing potato early dying with or without other pathogens in Atlantic Canada. The potato early dying is a soil borne disease. The management of potato early dying disease should focus on reduction of pathogen inoculum densities in soil by using integrated disease management approaches, including cultural, biological and chemical. Use of disease– suppressive rotation crops would reduce the pathogen load in the soil. Use of resistant potato cultivars is the most economic and effective tactic in controlling diseases; consequently, use of cultivars showing improved levels of resistance to Verticillium wilt in comparison to standard cultivars (i.e., "Russet Burbank") has significantly increased in recent years (Prince Edward Island potato news 2024). Potato vine post-harvest management should be considered as an important measure in PED management. Numerous microsclerotia of *V. dahliae* are present in potato vascular systems. Currently the vines are routinely incorporated into soil, significantly increasing inoculum density to next round of disease cycle. An alternative approach to manage potato vines after harvest should be developed in order to reduce the initial inoculum levels. Though crop rotations with disease-suppressive crop species could reduce many soil borne pathogen population densities and reduce disease severity, they cannot, by themselves, control early dying disease (Rowe and Powelson 2002; Larkin et al. 2010). Optimized crop rotation should be used in conjunction with other crop, soil and fertility management approaches to improve soil health and reduce pathogen population density in order to achieve more sustainable potato production.

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Declarations

Ethical Approval The authors declare that the experiments comply with the current laws of Canada.

Conflict of Interest The authors have no conflict of interest.

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