Australasian Plant Pathology
https://doi.org/10.1007/s13313-025-01050-5

ORIGINAL RESEARCH ARTICLE

Population development, damage assessment, and susceptibility of maize hybrids to root-lesion nematodes (*Pratylenchus neglectus* and *P. crenatus*) under glasshouse conditions

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Received: 19 February 2025 / Accepted: 30 April 2025

Abstract

Root-lesion nematodes, particularly *Pratylenchus neglectus* and *P. crenatus* (PNC), are widely distributed in New Zealand and cause significant damage to maize roots, reducing crop productivity. Despite their economic importance, no comprehensive assessment of commercial maize hybrids' resistance to PNC has been conducted in the country. Significant variation was observed in the nematode reproduction factor (*Rf*) and final population (*Pf*) among hybrids. In Experiment 1 (initial population (*Pi*) = 1250 PNC kg^{-1} soil), *Rf* ranged from 3.1 in hybrid P8500 to 7.1 in hybrid P9127, with *Pf* values ranging from 3863 to 8903 PNC kg^{-1} soil + roots in 45 days. In Experiment 2 (*Pi* = 750 PNC kg^{-1} soil), *Rf* ranged from 18.4 in hybrid P1613 to 37.5 in hybrid P8805, with *Pf* values from 13,784 to 28,426 PNC kg^{-1} soil + roots in 60 days. These results indicate active nematode reproduction and substantial hybrid-dependent variation in host response. Experiment 3 examined the impact of varying initial inoculum densities (500, 1000 and 1500 PNC kg^{-1} soil), showing a dose-dependent increase in *Pf* and corresponding root damage. Susceptible hybrid (P9127) exhibited up to 42% root dry weight and 22% shoot dry weight reductions. This study is the first systematic evaluation of PNC resistance in New Zealand maize hybrids. It identifies P9127 and P8805 as highly susceptible, and P0891, P8500, and P1613 as moderately resistant. These findings offer valuable benchmarks for future breeding and support nematode management in New Zealand.

Keywords Plant-parasitic nematodes · Host resistance · Biotic stress · Biomass · Reproduction factor · Resistance screening · Dry matter loss · Pathogenicity assay

Introduction

Root-lesion nematodes (*Pratylenchus* spp.) cause damage and notably reduce yields in maize (Lewis et al. 1976). Plant-parasitic nematodes (PPN) can be managed in the field using soil nematicides, resistant or tolerant varieties, or non-hosts. PPN, especially root-lesion nematodes, have wide host ranges (Lewis et al. 1976; Hooper and Evans 1993; Batista da Silva 2013; Yan et al. 2016). Hence, implementing a non-host crop to reduce the population densities can be more difficult. Using synthetic chemicals to control the nematodes was reported as toxic to the soil and the ecosystem. Thus, the use of synthetic chemicals against pests has been reduced over the years (Jorgenson 1979; Eddleston and Phillip 2004; Barrett et al. 2021; Tudi et al. 2021). Therefore, implementing nematode-resistant or tolerant crop varieties is an alternative possibility to control the PPN population. For alternative crops, resistant or tolerant

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Published online: 07 May 2025

varieties have been implemented, with soybeans having over 100 varieties exhibiting these traits against the soybean cyst nematode (Tylka et al. 2019). In contrast, commercially available maize hybrids with resistance to *Pratylenchus* spp. remain limited (Batista da Silva 2013). Within New Zealand, recent surveys indicated that *Pratylenchus* spp. especially PNC is widely distributed in maize fields (Thiruchchelvan et al. 2024a).

There are more than 40 maize hybrids available in New Zealand from different seed-producing companies (Corsonmaize 2023; Pioneer 2023). Maize hybrids have been developed to address biotic and abiotic stresses tailored to New Zealand conditions (Pioneer 2023). Most hybrid traits are well described by breeders with information regarding yield, plant and agronomic characteristics, grain quality, food-grade, and recommendations for the establishment of the population conveyed (Corsonmaize 2023; Pioneer 2023). Before the release of maize hybrids, testing for disease expression for the most common maize diseases in New Zealand, such as Northern leaf blight, rust, eyespot, head smut, *Fusarium* ear rot, *Diplodia* ear rot, *Gibberella* ear rot, and anthracnose stalk rot, is undertaken. Most maize hybrids in New Zealand exhibit moderate to high tolerance to pathogens (Pioneer 2023).

The abundance of *Pratylenchus* spp. in New Zealand maize fields, with over 1000 individuals kg^{-1} of soil found in 30% of fields, and more than 500 individuals kg^{-1} in 65% of fields sampled (Thiruchchelvan et al. 2024b), was higher than the threshold levels reported in other parts of the world (Niblack 2009, 2014; Thompson et al. 2010; Batista da Silva 2013; Simon 2015; Simon et al. 2018a; Chowdhury et al. 2020; Han et al. 2021; Thapa et al. 2023). Therefore, it is important to identify maize hybrids that allow nematode reproduction to remain below these threshold levels (Batista da Silva 2013). The challenge lies in the adaptability of maize hybrids with nematode-resistant traits to adapt to varying environmental conditions (Waudo and Norton 1986). Screening of existing maize hybrids, which are resistant to the nematodes, is cost-effective and gives a practical opening compared to the production of entirely new resistant hybrids against nematodes (Batista da Silva 2013). Therefore, this research aims to address this gap by comparing the reproductive abilities and pathogenicity of the mixed population of PNC on 15 selected maize hybrids in New Zealand.

Materials and methods

Maize hybrids

Fifteen commercially available maize grain hybrids in New Zealand were obtained from Pioneer Seed Company to assess the pathogenicity and reproduction potential of PNC. These hybrids were selected based on their suitability to grow in all the maize-growing regions in New Zealand, popularity among the maize growers, and high yields. A list of the hybrids and basic characteristics used in the experiments is shown in the Supplementary Table 1.

Nematode culture and potting soil

Root-lesion nematodes of PNC-naturally infested soil were collected from a maize field in Dorie, Canterbury, New Zealand (43°53’04.0"S 172°04’33.4"E) for use in experiments 1 to 3 of this study. Approximately 600 to 750 kg of field soil (topsoil to a depth of 15 to 20 cm) was collected from the site using clean shovels in plastic containers (40 L capacity) and stored at room temperature (20 ± 2 °C) for up to three days before processing. To ensure homogeneity, the soil was initially mashed to break big clods and promote even mixing. This step was crucial for improving the efficiency of subsequent manual removal of stones and plant debris, which could interfere with sieving and nematode distribution. The soil was then sieved using a 5 mm mesh aperture (Lincoln University-designed sieving machine, New Zealand). Sieved soil was spread on the ground and mixed manually using a shovel at least three times to obtain a homogenized mixture. The processed soil (~ 500 L) was piled on the ground to a depth of ~ 10 cm, spanning 1 m in width and 4–5 m in length. To determine the initial PNC population, three composite samples were collected using a 2 cm diameter hand soil corer (OAKFIELD Apparatus, USA). Soil cores were taken in a random zig-zag pattern across the pile, with the corer inserted at a 45-degree angle to ensure representative sampling throughout the soil profile. Species identification of the PNC population present in the soil was performed using both morphological and molecular methods, following the protocol described by Thiruchchelvan et al. (2024d). Based on the confirmed identification and nematode counts, sterilized soil (double-pasteurized at 81 °C to eliminate all nematodes) was added as needed to adjust the initial PNC population density for each experiment.

Glasshouse experiments

Three pot experiments (Experiments 1–3) were conducted in the Plant Nursery and Greenhouse at Lincoln University,

New Zealand, to evaluate the response of maize hybrids to PNC. All experiments were arranged in a completely randomized design (CRD) under controlled glasshouse conditions, with full details on pot size, potting soil properties, experimental duration, initial nematode inoculum, fertilizer application, watering schedule, and experimental design for each experiment provided in Supplementary Table 2. In Experiment 1, an interim screening of 15 maize hybrids was conducted using adjusted PNC populations with sterilized soil to a uniform density of 1250 PNC kg^{-1} of soil. Experiment 2 was a long-standing screening, where hybrids were grown for 60 days in naturally infested field soil. Due to low nematode abundance in the field's soil, the inoculum was adjusted to 750 PNC kg^{-1}. Experiment 3 evaluated pathogenicity and damage levels of PNC on three selected maize hybrids, highly susceptible (P9127), moderately susceptible (P8666) and moderately resistant (P8500), at four inoculum levels (0, 500, 1000, and 1500 PNC kg^{-1}). In all experiments, two seeds per pot were sown, with one plant retained after 7 days. Fertilizer (Osmocote® Exact Standard 3–4 M, ICL, 16% N, 3.9% P, 10% K + trace elements) was applied at 3 g L^{-1} of soil. Watering was done every other day at rates specified in Supplementary Table 2. The population of the PNC in the maize roots and soil was determined separately for each replicate, as described in the section on nematode extraction. For Experiment 3, final plant height, dry root and shoot weights were measured. All roots (including the roots after nematode extraction) were carefully separated from the soil through manual washing and sieving (3 mm and 4 mm mesh), dried at 65 °C for 3 days (BINDER GmbH oven), and weighed to determine dry matter.

Nematode extraction, identification, and parameter estimation

Nematodes were extracted from 100 g soil samples using the sieving-centrifugal-sugar flotation method (Jenkins, 1964). Soil was mixed with 1 L of water, stirred, and passed through nested sieves (150 µm and 38 µm) (Glenammer, UK) three times. The material on the 38 µm sieve was centrifuged at 576 × g for 5 min, resuspended in 45% sucrose solution, and centrifuged again at 576 × g for 1 min. The nematodes were collected by rinsing the sieve with water and stored in 50 mL specimen bottles. Root-lesion nematodes were also extracted from 5 g of maize fresh roots using a modified centrifugal-sugar-flotation method described by Thiruchchelvan et al. (2024c). Roots were blended in 150 mL of water, sieved through 250 µm and 38 µm sieves, and the retained material in sieve 38 µm was centrifuged with 45% sucrose solution. The nematodes were collected by rinsing the sieve and stored in 50 mL specimen bottles. Nematode species were identified using an inverted compound microscope (Olympus CKX53, Japan) at 40× magnification. Identification to the genus level was based on the morphological keys of Fortuner (1988) and Mai et al. (1996). The extracted nematodes were stored at 4 °C for further analysis.

To assess the population dynamics of PNC, several key parameters were measured as described in Chowdhury et al. (2022). The initial population (*Pi*) was defined as the number of PNC individuals inoculated per pot at the experiment's onset. The final population (*Pf*) was determined by quantifying the total number of PNC kg^{-1} of soil, including those within root tissues, using the formula: *Pf* (kg^{-1}) = (PNC in total root tissue per plant per pot + PNC in total pot soil) / total weight of pot soil per pot (kg). The number of PNC (adults and juveniles) in root tissue per plant was calculated by multiplying the PNC g^{-1} of fresh roots by the total fresh root weight (Supplementary Table 3). The reproduction factor (*Rf*), indicating the nematode's multiplication rate, was calculated as the ratio of the final to the initial population ($Rf = Pf / Pi$). To evaluate the distribution of PNC within the plant system, the proportion of nematodes in roots was calculated as a percentage of the total PNC population per pot: PNC in roots (%) = (Total PNC in roots / Total PNC per pot) × 100. Additionally, the population density ratio (%) was assessed to compare PNC densities among different hybrids relative to the most susceptible hybrid (used as a susceptibility check), using the formula: PNC density ratio (%) = (PNC per hybrid / Highest PNC per hybrid (susceptibility check) × 100. All parameters encompassed PNC populations present in both root tissues and soil unless specified otherwise. The ranking for each hybrid was considered into four classes, Resistant = R (final population density of PNC < 25% of the susceptible check (hybrid P9127); Moderately Resistant = MR (26 to 50%); Moderately Susceptible = MS (51 to 75%); and Susceptible = S (76%) (Smiley et al. 2014; Chowdhury et al. 2022).

Statistical analysis

The statistical analyses were conducted using GenStat® 23rd Edition software. Before analysis, all data were checked for normality using the distribution test with Normal Q-Q plots. Data were transformed using square root or log10 transformations to meet the normality assumption. Nematode data from Experiments 1 and 2 were analysed using one-way ANOVA. Subsequently, post hoc Bonferroni tests were performed at a 95% confidence level. Experiment 3 data were analysed using a two-way ANOVA adjusted for covariates. Post hoc Bonferroni tests were then conducted with 95% confidence intervals. Covariates, including root dry weight, shoot dry weight, and plant height, were incorporated into the analysis of nematode data, such as extracted PNC from

5 g roots and 100 g soil, and total PNC kg^{-1} of soil to minimize residual effects. Furthermore, plant growth parameters, such as root, shoot dry weights, and plant height, were used as covariates in the analysis of plant growth parameters.

Results

Experiment 1: interim screening of the maize hybrids against PNC

The evaluation of 15 maize hybrids revealed significant differences ($p < 0.0001$) in their susceptibility to PNC (Table 1). The final PNC population per kg of soil (including roots) (*Pf*) showed a 2.2-fold variation among hybrids, demonstrating the strong influence of host genotype on PNC reproduction. P9127 emerged as the most susceptible hybrid with the highest *Pf* (8903 PNC kg^{-1} soil + roots), while P8500 and P0891 showed the lowest populations (3863 and 4033 PNC kg^{-1} soil + roots, respectively) (Table 1).

Analysis of nematode distribution patterns in roots and soil revealed distinct colonization strategies among hybrids (Table 1). While P1477W allocated 64% of its nematode population to root tissues, the highest proportion observed, P9127 showed the opposite trend, with only 32% in roots, suggesting greater soil-based proliferation. These differences were reflected in both root (180–337 PNC/5 g) and soil (158–489 PNC/100 g) extraction counts, confirming hybrid-specific variations in PNC habitat preference.

The *Rf* results paralleled these trends, with all hybrids supporting PNC multiplication ($Rf > 1$) but to varying degrees (Table 1). P9127 showed the highest reproductive success ($Rf = 7.1$), while P8500 and P0891 exhibited the most restricted reproduction ($Rf = 3.1$ and 3.2, respectively).

Based on population density ratios relative to P9127 (considered as a susceptibility check), hybrids were classified into three susceptibility groups. The susceptible category (S) included P9127, P0547, and P7524 (79–100%), which all maintained $Pf > 7000$ PNC kg^{-1} soil + roots. Moderately resistant hybrids (MR) P0891 and P8500 (43–45%) showed the lowest PNC (< 4100), while the remaining hybrids (53–75%) were classified as moderately susceptible (MS) (Table 1).

Experiment 2: long-term susceptibility evaluation of the maize hybrids against PNC

The extended 60-day evaluation of 15 maize hybrids revealed significant differences ($p < 0.0001$) in their response to PNC infestation (Table 2). Hybrid P8805 emerged as the

Table 1 Reproduction of *Pratylenchus neglectus* and *P. crenatus* on 15 maize hybrids and their susceptibility ratings. (Experiment 1 in 45 days after planting)

Hybrids	[a]PNC Extracted		[b]Pf (kg^{-1})	[c]Rf	[d]PNC in Roots (%)	[e]PNC Density (%)	[f]Rating
	Roots (5 g)	Soil (100 g)					
P0362	283 ± 33 abc	223 ± 33 ab	5745 ± 520 ab	4.6 ± 0.4 ab	53	65	MS
P0547	337 ± 36 c	307 ± 26 abc	7235 ± 518 bc	5.8 ± 0.4 bc	47	81	S
P0891	180 ± 8 a	178 ± 15 ab	4033 ± 255 a	3.2 ± 0.2 a	46	45	MR
P1253	218 ± 19 abc	223 ± 42 ab	4948 ± 565 ab	4 ± 0.5 ab	48	56	MS
P1477W	285 ± 11 abc	158 ± 24 a	5265 ± 493 ab	4.2 ± 0.4 ab	64	59	MS
P1613	199 ± 21 ab	213 ± 31 ab	5058 ± 556 ab	4 ± 0.4 ab	50	57	MS
P7524	316 ± 36 bc	330 ± 27 bc	7058 ± 389 bc	5.6 ± 0.3 bc	41	79	S
P8000	284 ± 38 abc	275 ± 34 ab	5945 ± 412 ab	4.8 ± 0.3 abc	42	67	MS
P8333	261 ± 16 abc	275 ± 31 ab	5495 ± 460 ab	4.4 ± 0.4 ab	38	62	MS
P8500	206 ± 12 ab	166 ± 17 ab	3863 ± 297 a	3.1 ± 0.2 a	46	43	MR
P8666	256 ± 19 abc	274 ± 53 ab	5558 ± 789 ab	4.4 ± 0.6 ab	41	62	MS
P8805	275 ± 21 abc	320 ± 50 abc	6445 ± 701 abc	5.2 ± 0.6 abc	43	72	MS
P9127	310 ± 33 bc	489 ± 44 c	8903 ± 616 c	7.1 ± 0.5 c	32	100	S
P9400	250 ± 15 abc	250 ± 20 ab	5655 ± 332 ab	4.5 ± 0.3 ab	44	64	MS
P9721	291 ± 32 abc	289 ± 53 ab	6410 ± 803 abc	5.1 ± 0.6 abc	47	72	MS

a- PNC-*Pratylenchus neglectus* and *P. crenatus* were extracted using the centrifugal-sugar-flotation-method for soil (Jenkins, 1964) and root (Thiruchchelvan, et al. 2024). Means having same letter in a column is not significantly different according to the Bonferroni post hoc test at 95% confidence interval. Numbers are rounded as full number, and all the means ± standard error of mean (untransformed data) from 12 replicates

b- *Pf*- PNC final population

c- *Rf* - Reproduction factor

d- PNC in roots- PNC proportion in roots to the total PNC population

e- PNC density- Proportion of the population density to the susceptibility check

f- R-Resistant; MR- Moderately Resistant; MS-Moderately Susceptible; S-Susceptible (compared to the susceptibility check)

Table 2 Reproduction of *Pratylenchus neglectus* and *P. crenatus* on 15 maize hybrids and their susceptibility ratings. (Experiment 2 in 60 days after planting)

Hybrids	[a]PNC Extracted		[b]Pf (kg^{-1})	[c]Rf	[d]PNC in Roots (%)	[e]PNC Density (%)	[f]Rating
	Roots (5 g)	Soil (100 g)					
P0362	4525 ± 262 ef	179 ± 22 ab	27,352 ± 1550 c	36.5 ± 2 cd	93	96	S
P0547	2522 ± 209 abc	220 ± 32 abc	16,534 ± 1696 ab	22.1 ± 2 ab	86	58	MS
P0891	2711 ± 140 abcd	145 ± 12 a	16,298 ± 1001 ab	21.7 ± 1 ab	91	57	MS
P1253	2762 ± 188 abcd	178 ± 11 ab	16,671 ± 1111 ab	22.2 ± 1 ab	89	59	MS
P1477W	2302 ± 199 ab	213 ± 16 abc	16,076 ± 1396 ab	21.4 ± 2 ab	86	57	MS
P1613	1953 ± 214 a	174 ± 12 ab	13,784 ± 1285 a	18.4 ± 2 a	86	48	MR
P7524	3211 ± 321 bcd	247 ± 44 abc	19,225 ± 1822 ab	25.6 ± 2 ab	87	68	MS
P8000	3634 ± 288 de	323 ± 38 c	20,763 ± 1202 b	27.7 ± 2 bcd	84	73	MS
P8333	3220 ± 133 bcd	201 ± 25 abc	16,816 ± 674 ab	22.4 ± 1 ab	88	59	MS
P8500	3591 ± 242 cde	213 ± 27 abc	19,212 ± 1178 ab	25.6 ± 2 ab	89	68	MS
P8666	2927 ± 177 bcd	161 ± 10 ab	15,654 ± 1207 ab	20.9 ± 2 ab	89	55	MS
P8805	4476 ± 200 f	271 ± 34 bc	28,426 ± 2061 c	37.5 ± 3 d	90	100	S
P9127	3491 ± 242 cde	241 ± 30 abc	22,058 ± 1165 c	27.8 ± 2 bcd	88	96	S
P9400	2983 ± 145 bcd	186 ± 15 ab	18,150 ± 1131 ab	24.2 ± 2 ab	89	64	MS
P9721	2584 ± 208 abcd	221 ± 15 abc	15,610 ± 1125 ab	20.8 ± 2 ab	85	55	MS

a- PNC-*Pratylenchus neglectus* and *P. crenatus* were extracted using the centrifugal-sugar-flotation-method for soil (Jenkins, 1964) and root (Thiruchchelvan et al. 2024a). The mean values with the same letter in a column are not significantly different according to the Bonferroni post hoc test at a 95% confidence interval. Numbers are rounded as full numbers, and all the means ± standard error of mean (untransformed data) were from 12 replicates

b- *Pf*- PNC final population

c- *Rf* - Reproduction factor

d- PNC in roots- PNC proportion in roots to the total PNC population

e- PNC density- Proportion of the population density to the susceptibility check

f- R-Resistant; MR- Moderately Resistant; MS-Moderately Susceptible; S-Susceptible (compared to the susceptibility check)

most susceptible, supporting the highest *Pf* (28426 PNC kg^{-1} soil + roots) and root colonization (4476 PNC/5 g roots), followed closely by P0362 (*Pf* = 27352). In contrast, P1613 showed the lowest infestation levels (*Pf*-13784 PNC kg^{-1} soil + roots; 1953/5 g roots). The *Rf* ranged from 18.4 (P1613) to 37.5 (P8805), with 14 of 15 hybrids exceeding *Rf* = 20, indicating substantially greater nematode multiplication than observed in the 45-day experiment.

A striking shift in nematode distribution occurred over time, with root colonization increasing to 85–93% of the total populations (vs. 32–64% in Experiment 1). This suggests progressive nematode migration to root tissues as the plants mature. Resistance classification identified three susceptibility groups: P8805, P0362, and P9127 as susceptible (S; 96–100% density ratios); P1613 as the only moderately resistant (MR; 48%) hybrid; and the remainder as moderately susceptible (MS; 55–73%) (Table 2).

Experiment 3: pathogenicity and damage level of PNC

Nematode reproduction

The pathogenicity study of PNC on three maize hybrids (P8500, P8666, P9127) revealed significant differences in nematode reproduction under varying inoculation levels ($p < 0.0001$, Table 3). Root nematode counts increased with higher inoculation densities, ranging from 822 to 896 per 5 g at 500 PNC soil to 2017–2086 at 1500 PNC kg^{-1} soil. Similarly, soil nematode populations showed a dose-dependent response, increasing from 120 to 160 per 100 g of soil at the lowest inoculation to 450–600 at the highest level ($p < 0.0001$).

Reproduction factors remained consistently high across all treatments (8.5–10.7), with no significant differences between inoculation levels ($p = 0.703$) or among hybrids ($p = 0.634$) (Table 3). However, the distribution pattern of nematodes between roots and soil changed noticeably with inoculation pressure. At lower densities (500 PNC kg^{-1}), 74–75% of the total population resided in roots, while at the highest inoculation (1500 PNC kg^{-1}), this proportion decreased to 56–65%, indicating a shift toward greater soil colonization under high infestation pressure.

Final nematode populations showed a strong positive correlation with inoculation levels, increasing from 4898 to 5089 PNC kg^{-1} at 500/kg to 12,764–14,048 at 1500 PNC kg^{-1} ($p < 0.0001$). The hybrid P9127 at 1500 PNC kg^{-1} supported the highest population (14048 PNC kg^{-1}) (Table 3), confirming its status as the most susceptible genotype in this experiment.

Table 3 Reproduction of *Pratylenchus neglectus* and *P. crenatus* on 3 maize hybrids and their susceptibility ratings under different nematode inoculation levels (Experiment 3 in 60 days after planting)

Hybrid	[a]PNC Levels	[b]PNC Extracted		[c]*Pf* (kg^{-1})	[d]*Rf*	[e]PNC in Roots (%)	[f]PNC Density (%)
		Roots (5 g)	Soil (100 g)				
P8500	500	824 ± 123 a	125 ± 13 a	4898 ± 384 a	9.8 ± 0.8 a	74	35
	1000	1739 ± 104 b	221 ± 22 bc	9396 ± 798 b	9.4 ± 0.8 a	75	67
	1500	2017 ± 226 b	456 ± 99 def	13,154 ± 1809 c	8.8 ± 1.2 a	65	94
P8666	500	896.2 ± 83 a	155 ± 21 ab	5210 ± 572 a	10.4 ± 1.1 a	70	37
	1000	1843 ± 181 b	323 ± 24 cde	10,657 ± 1105 b	10.7 ± 1.1 a	67	75
	1500	2086 ± 174 b	518 ± 68 ef	12,764 ± 1012 c	8.5 ± 0.7 a	59	91
P9127	500	822 ± 118 a	146 ± 14 ab	5089 ± 532 a	10.2 ± 1.1 a	67	36
	1000	1602 ± 176 ab	234 ± 16 bcd	8763 ± 865 b	8.8 ± 0.9 a	72	62
	1500	2032 ± 242 b	602 ± 72 f	14,048 ± 1511 c	9.4 ± 1.0 a	56	100

a- Inoculation rate to the pot (Number of PNC inoculated per kg of soil)

b- PNC- *Pratylenchus neglectus* and *P. crenatus* were extracted using the centrifugal-sugar-flotation-method for soil (Jenkins, 1964) and root (Thiruchchelvan, et al. 2024a). Means having same letter in a column is not significantly different according to the Bonferroni post hoc test at 95% confidence interval. Numbers are rounded as full number, and all the means ± standard error of mean (untransformed data) were from 10 replicates

c- *Pf*- PNC final population

d- *Rf* - Reproduction factor

e- PNC in roots- PNC proportion in roots to the total PNC population

f- PNC density- Proportion of the population density to the susceptibility check

g- R-Resistant; MR- Moderately Resistant; MS-Moderately Susceptible; S-Susceptible (compared to the susceptibility check)

Table 4 Damage level of *Pratylenchus neglectus* and *P. crenatus* on 3 maize hybrids and their yield reduction under different nematode inoculation levels (Experiment 3 in 60 days after planting)

Hybrid	[a]PNC Levels	[b]Height (cm)	[c]Height Reduction (%)	[b]Dry Weight (g)		[c]Dry Weight Reduction (%)	
				Shoot	Root	Shoot	Root
P8500	0 - Control	176.8 ± 7.3ab	-	41.98 ± 1.6a	6.00 ± 0.5d	-	-
	500	169.4 ± 7ab	4	35.55 ± 1.3a	4.80 ± 0.2bcd	15	20
	1000	173.3 ± 6.4ab	2	35.69 ± 1.9a	3.99 ± 0.3abcd	15	33
	1500	164.5 ± 5.5ab	7	33.12 ± 2.4a	4.26 ± 0.5abcd	21	29
P8666	0 - Control	177.9 ± 5ab	-	38.39 ± 1.6a	5.30 ± 0.5 cd	-	-
	500	174.2 ± 8.7ab	2	37.93 ± 1.8a	3.98 ± 0.3abcd	1	25
	1000	164.7 ± 8.2ab	7	35.37 ± 1.2a	3.82 ± 0.2abc	8	28
	1500	154.1 ± 6.3a	13	34.58 ± 1.5a	3.45 ± 0.2a	10	35
P9127	0 - Control	190.9 ± 3.3b	-	42.48 ± 1.5a	5.75 ± 0.4 cd	-	-
	500	190.9 ± 4.1b	0	35.36 ± 1.7a	4.06 ± 0.4abc	17	29
	1000	178.8 ± 6.4ab	6	35.30 ± 1.7a	3.32 ± 0.2ab	17	42
	1500/	177.1 ± 6.8ab	7	33.18 ± 1.5a	3.00 ± 0.2ab	22	39

a- Inoculation rate to the pot (number of PNC inoculated per kg of soil)

b- Means having the same letter in a column is not significantly different according to the Bonferroni post hoc test at a 95% confidence interval. All the means ± standard error of mean (untransformed data) were from 10 replicates

c- The reduction percentage of each PNC level was calculated compared to the control. Reduction percentage = 100 - (Height or weight of the hybrid/height or weight of the control of each hybrid) × 100

Damage level

The evaluation of three maize hybrids (P8500, P8666, P9127) revealed significant nematode-induced effects on plant growth parameters ($p < 0.05$, Table 4). Root dry weights differed significantly among hybrids ($p = 0.009$) and showed a strong response to inoculation level ($p < 0.0001$), though no significant interaction was observed between these factors ($p = 0.547$). P9127 exhibited the greatest sensitivity with root weight reductions of 29–42% compared to controls, followed by P8666 (25–35%) and P8500 (20–33%). For shoot parameters, dry weight showed no significant variation among inoculation levels ($p = 0.531$) or between hybrids ($p = 0.154$), with no interaction effect ($p = 0.146$). However, shoot weight reductions reached 21% for P8500, 10% for P8666, and 22% for P9127 (Table 4).

Plant height measurements showed significant variation between hybrids ($p<0.0001$) but no significant effect of inoculation level ($p=0.094$) or interaction ($p=0.480$). Despite the lack of statistical significance for nematode effects, all inoculated plants were shorter than their respective controls (Table 4), suggesting a consistent but indirect impact on plant height.

Discussion

This study addresses the reproductive abilities of PNC and the susceptibility of available maize hybrids in New Zealand. Using resistance or tolerance of host crops to PPN is an important management strategy (Batista da Silva 2013). In New Zealand, no studies have been done to evaluate the resistance of commercial hybrids to PPN, especially for *Pratylenchus* species. Screening for resistant hybrids or inbred lines is the first step to providing information on resistant traits that can be used for breeding programs (Waudo and Norton 1986; Timper et al. 2007; Batista da Silva 2013). Across the three experiments, the interaction between maize hybrids and PNC provided valuable insights into nematode infestation levels and maize susceptibility.

This study evaluated the mixed population of the PNC naturally infested soil. The identity of these species was confirmed by both morphology and molecular methods, as reported by Thiruchchelvan et al. (2024d). Similarly, mixed populations of PNC were used previously for the evaluation of soybean cultivars under glasshouse trials (Elhady et al. 2019). However, previous studies, including Thompson's (2008) work with wheat, have demonstrated that crop cultivars may exhibit resistance to one *Pratylenchus* species while being susceptible to another. This study used a mixed population of PNC, which introduces potential variability in our findings, so the single-species related susceptibility studies are warranted in future. Also, naturally infested soil has been used by many researchers for the screening bioassays under glasshouse conditions (Batista da Silva 2013; Biela et al. 2016; Forge et al. 2000; Chowdhury et al. 2022). Like the results of the current study, Batista da Silva (2013) noted inconsistencies in their pathogenicity and reproduction results using 15 maize cultivars, including the hybrids and inbred lines, tested against *P. neglectus* in the glasshouse using the naturally infested soil. In contrast, the current studies have utilised a mixture of naturally infested field soil and sterilized soil. Sterilized soil with artificial inoculation of a single species of nematode grown in culture could ensure that the single nematode species in the soil infest the plants (Elhady et al. 2019). However, in this study, potting soil was prepared to mimic the biotic and abiotic characteristics of field soil with minimum changes. The abundance of PNC in the soil mixture used in the current study was over 98% of the total PPN, which is higher than the abundance percentage of 95% root-lesion nematodes used in a similar experiment (Smolik and Evenson 1987). As previously indicated, repeated soil mixing and sampling procedures were used to obtain a uniform distribution of nematodes throughout the experimental units (Biela et al. 2016; Chowdhury et al. 2022).

The goal of the first experiment was to test the invasion of PNC into 15 maize hybrids and their reproduction within a short period using field soil. Another aspect of this trial was to select three hybrids with three different levels of susceptibility for subsequent experiments. The initial nematode inoculum of 1250 PNC kg^{-1} soil was used. This number is known to be above the threshold level for maize and cereals as reported by other authors (Niblack 2009; Thompson et al. 2010; Tylka et al. 2011; Simon et al. 2018a). The duration of the experiment was 45 days, which is approximately a minimum of two cycles of the *Pratylenchus* life cycle (Castillo and Vovlas 2007). The second experiment duration was determined by consulting previous research that was comparable to our duration-extended 60-day trials (Batista da Silva 2013).

The susceptibility levels of maize and other crops are frequently ranked and evaluated using *Rf* (Schmitt and Barker 1981; Batista da Silva 2013; Chowdhury et al. 2022). In the first experiment, the *Rf* of PNC was between 3.1 and 7.1, whereas in the second experiment, it was between 18.4 and 37.5. The prolonged period may have contributed to the root-lesion nematodes' greater *Rf*, as noted by Inomoto (2011). Additionally, as the *Rf* is dependent on the initial inoculum levels, the resistance's ranking will have an impact on it. This information, based on the *Rf*, will be the baseline for the subsequent research. However, in the third trial of this investigation, the *Rf* did not demonstrate any significant variations between the initial inoculum levels (500, 1000, and 1500 PNC kg^{-1} of soil) or their interaction, nor did it indicate any differences between the tested hybrids, P8500, P8666, and P9127. According to the *Rf* of nematodes at 45 days (0.4 kg of soil), these three hybrids were selected from the first experiment with three distinct levels of susceptibility. Furthermore, at 60 days in the third experiment, the *Rf* of nematodes in these three hybrids was not significant. The inconsistent nematode reproduction and hybrid susceptibility rankings observed in our experiments align with previous reports of variable *Pratylenchus* responses in maize (Waudo and Norton 1986; Smolik and Evenson 1987; Timper et al. 2007; Inomoto 2011; Kagoda et al. 2011; Batista da Silva 2013). This variability likely reflects: (1) differences in initial nematode densities between trials (1250 vs. 750 PNC kg^{-1} soil); (2) potential shifts in the *P. neglectus* to *P. crenatus* ratio, given known species-specific

resistance (Thompson 2008); and (3) inherent challenges of using naturally infested soils (Biela et al. 2016; Chowdhury et al. 2022). Similar inconsistencies occurred in soybean trials with varying inoculum levels (Chowdhury et al. 2022), confirming this as a common methodological challenge. While multiple *Pratylenchus* species infect maize (Castillo and Vovlas 2007; Subbotin et al. 2008; Nicol et al. 2011), our findings demonstrate that resistance screening requires controlled multi-experiment approaches to account for natural population fluctuations.

Consequently, Chowdhury et al. (2022) proposed the use of a susceptibility check to rate the susceptibility level. Therefore, the rating related to the population density in the susceptibility check was used to rate susceptibility. Based on these results, it can be concluded that hybrid sensitivity to PNC increases with time after planting, regardless of the nematode inoculum levels, hybrids, or amount of potting soil used. Importantly, the study demonstrated that nematode reproduction increased over time, suggesting that prolonged exposure intensifies susceptibility. The difference in days between Experiment 1 and 2 was sufficient to allow at least one additional generation of PNC (Castillo and Vovlas 2007), which likely contributed to the observed population increases. However, accounting for the life stages of PNC (e.g., eggs, juveniles, adults) could provide a more detailed understanding of population dynamics and host responses. Similar to Thompson et al. (2015), who demonstrated that *P. thornei* populations in wheat followed exponential growth patterns before stabilizing or declining, our results show time-dependent population growth influenced by initial inoculum levels and host resistance traits. The observed increase in nematode populations up to 60 days in susceptible maize hybrids mirrors the growth patterns reported in wheat, where maximum populations were typically reached by 16 weeks before potential decline (Thompson et al. 2015). This parallel suggests that the exponential growth model may also apply to PNC in maize systems, though the specific time thresholds likely vary by host-nematode combination. However, further studies are warranted to confirm these results extensively.

Previous studies show that *Pratylenchus* spp. caused moderate damage to maize (Smolik and Evenson 1987; Kleynhans et al. 1996). *Pratylenchus* spp. infestations reduce root dry weight, as evidenced by our results, which show decreases in biomass of the hybrids up to 42% in P9127, 35% in P8666, and 33% in P8500 when compared to the control. These root dry weight reductions were greater than the 9–21% weight reduction reported by McDonald and van den Berg (1993). It's interesting to note that, in contrast to the control, shoot dry weight losses range from 21 to 22% in P8500 and P9127 to 10% in P8666. This was despite a significant fall in root dry weight. This implies that infestations of PNC may not always have the same impact on shoot growth as they do on overall biomass. Notably, there were no significant variations in plant height when compared to the control group, suggesting that infestations of PNC could mainly impact below-ground biomass rather than above-ground growth. Similar outcomes against *P. zeae* and *P. brachyurus* in maize have been reported by McDonald and van den Berg (1993). These baseline findings suggest there is an immediate requirement to assess the maize hybrids against the root-lesion nematodes extensively in New Zealand to understand this fully. This could be achieved by examining all commercially available hybrids in New Zealand for their grain yield losses with different levels of root-lesion nematodes, specific species, and field-level studies to estimate yield losses.

The experiments collectively highlight the complexity of the interactions between maize hybrids and root-lesion nematodes. Experiments 1 and 2 provided a broad overview of nematode susceptibility among 15 hybrids and inquired meaningfully into specific varieties, identifying susceptible and resistant hybrids. Experiment 3, focusing on three selected hybrids, contributed insights into the impact of nematode inoculation levels on both roots and soil biomass. The selection of hybrids based on susceptibility levels highlights the potential for targeted nematode management strategies and crop improvement. Overall, these findings contribute valuable understandings into the complexities of PNC infestation on maize hybrids, highlighting the need to consider both root and shoot parameters in assessing plant responses to nematode stress. Intensive future studies related to the search for resistant maize hybrids to the root-lesion nematodes among the available hybrids in New Zealand will be the priority for the immediate solution. At the same time, it is important to screen the possible root-lesion nematode-resistant parental lines or inbred lines to develop the new hybrids in the future. As per the recent surveys, there were 5 root-lesion nematodes associated with maize fields across the three regions (Canterbury, Waikato, and Manawatu-Whanganui regions) (Thiruchchelvan et al. 2024d). Therefore, searching for the hybrids or inbred lines and testing against the regional-specific root-lesion nematode species could reduce the yield losses in maize production. Even though developing resistant or tolerant hybrids against the root-lesion nematodes is a lengthy process, it would possibly give scientists an environmentally friendly and cost-effective nematode management option to minimize existing yield losses due to root-lesion nematode damage.

Conclusions

This study provides an understanding of the susceptibility of 15 commercially available maize hybrids to PNC, filling an important knowledge gap in understanding the host status against PNC in New Zealand. The findings highlight significant variation in nematode reproduction and pathogenicity among hybrids, with P9127 and P8805 identified as highly susceptible, while P8500, P0891 and P1613 exhibited moderate resistance. Importantly, the study demonstrated that nematode reproduction increased over time, suggesting that prolonged exposure intensifies susceptibility. Future studies should focus on further characterizing the genetic and physiological mechanisms underlying resistance, screening additional maize hybrids and parental lines for nematode tolerance and validating these findings under field conditions. Additionally, nematode species-specific and region-specific studies are necessary to assess hybrid resistance against local nematode populations, ensuring effective and sustainable nematode management strategies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13313-025-01050-5.

Acknowledgements The authors sincerely thank Richard Brent, nursery manager at Lincoln University, for his valuable assistance during the glasshouse experiments. We also extend our gratitude to the team at Pioneer Agricultural Company for their support and generous provision of free seeds for the research.

Author contributions NT, MK, RM, LC, and SC conceived and planned the research. NT conducted the experiments and research work. All authors contributed to the interpretation of the results. NT took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions
This work was funded by a PhD scholarship under the AHEAD operation (Round 03-2020, grant number AHEAD/Ph.D./R3/Agri/463) and by research funds from Lincoln University, New Zealand (grant number 3601/AGLS/45401/1145841).

Data availability All related data are included in this article.

Declarations

Ethics approval Not applicable.

Conflict of interest No conflict of interest was declared.

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