agronomy

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A New Method for Single-Plant Selection of Wheat Genotypes for Tolerance and Resistance to the Root-Lesion Nematode *Pratylenchus thornei* by Low-Density Sowing

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Abstract

A new method of selecting wheat genotypes for tolerance and resistance to *Pratylenchus thornei* could enhance early-generation selection in wheat-breeding programs. Currently, the tolerance of fixed genotypes is determined in field experiments at a *P. thornei*-infested site, and resistance indices are determined by inoculated glasshouse experiments. For early-generation selection from segregating populations, resistance screening is limited to assessing single plants for resistance only using glasshouse experiments. The objective of this study was to develop a novel method that evaluates a single plant for both tolerance and resistance by using low density (LD) sowing in the field. Four replicated LD (1, 4, 16 and 32 plants/m^2) field experiments evaluated 14 or 15 fixed wheat genotypes over two growing seasons in a field with damaging population densities of *P. thornei* (>2500 *P. thornei*/kg soil). To check the validity of these experiments, a linear regression analysis was performed for each experiment between the single plant grain yield and the population density of *P. thornei* with the published tolerance and resistance indices derived from multiple field and glasshouse experiments, respectively. Tolerance was best determined by the grain yield of each single plant grown at a density of 16 plants/m^2 in 2021 (R^2 = 0.63, $p < 0.001$) and 4 plants/m^2 in 2022 (R^2 = 0.79, $p < 0.001$), when compared to published results of tolerance indices assessed by grain yield from plots grown at 100 plants/m^2. Resistance was best determined from the final population density of *P. thornei* in the soil and roots under each single plant when grown at a density of 4 plants/m^2 in 2021 (R^2 = 0.73, $p < 0.001$) and 1 plant/m^2 in 2022 (R^2 = 0.54, p = 0.001), when compared to published resistance indices derived from multiple glasshouse resistance experiments. This study demonstrated that LD can be used to effectively identify individual plants with both tolerance and resistance to *P. thornei*, with single-plant ultra-low densities (ULD) between 1 and 4 plants/m^2 being the most suitable. The advantage of using ULD sowing in the field for segregating populations of wheat over single plant glasshouse resistance screening experiments is the ability to simultaneously screen plants for tolerance to *P. thornei*.

Keywords: *Pratylenchus thornei*; tolerance; resistance; wheat; plant density; field; breeding; new method

Academic Editor: Isabel Luci Pisa Mata da Conceição

Received: 20 June 2025
Revised: 17 July 2025
Accepted: 7 August 2025
Published: 26 August 2025

Citation: Robinson, N.A.; Sheedy, J.G.; Zwart, R.S.; Owen, K.J.; Lin, J.; Thompson, J.P. A New Method for Single-Plant Selection of Wheat Genotypes for Tolerance and Resistance to the Root-Lesion Nematode *Pratylenchus thornei* by Low-Density Sowing. *Agronomy* **2025**, *15*, 2049. https://doi.org/10.3390/agronomy15092049

1. Introduction

Plant breeding is crucial to sustainably increase global production of broadacre crops [1]. For pathogens like the root-lesion nematode *Pratylenchus thornei*, breeding genetic tolerance and resistance traits into wheat (*Triticum aestivum*) genotypes is the most viable option for minimizing related yield losses [2]. These two traits are independently determined [3]. A tolerant genotype yields well when grown in soils with damaging populations of nematodes, while a resistant genotype inhibits the reproduction of the nematode [4,5]. By combining resistance with tolerance to *P. thornei* in the one wheat genotype, its yield can be greater than genotypes that are tolerant only [6]. Measuring grain yield at the regional level or in the target population of environments is desirable in breeding programs [7]. Similarly, tolerance to *P. thornei* is best evaluated by grain yield in the field [8] where the plants are subjected to relevant regional environmental stresses [2,9,10]. Currently, general tolerance to *P. thornei* is measured by the grain yield of the genotypes on sites with damaging population densities (>2000 *P. thornei*/kg soil) of *P. thornei* [2]. Recently, non-destructive and high-throughput in-crop vegetative assessments, namely, visual tolerance rating and normalized difference vegetation index (NDVI), have been shown to be predictive of tolerance assessed by grain yield [11,12]. The studies above [2,11,12] used yield plots on a site with initial population densities > 2000 *P. thornei*/kg soil in replicated experiments to assess the tolerance of fixed genotypes by sowing viable seeds at a rate of 100 seeds/m^2 and harvesting the mature grain from whole plots of area ~10 m^2.

Pratylenchus thornei is commonly found in many wheat-growing areas worldwide [13–15]. In the subtropical grain region of eastern Australia, *P. thornei* is found in 70% of the fields sampled, and 30% of the fields have population densities that exceed the estimated threshold of 2000 *P. thornei*/kg soil for yield reduction [14], with an annual wheat production loss of AUD 31M [16]. The three major factors that have favored the widespread distribution of *P. thornei* throughout the region are (i) extensive production over the last 50 years of susceptible broadacre crops, namely winter crops like wheat, barley (*Hordeum vulgare*) and chickpea (*Cicer arietinum*) and summer crops like mung bean (*Vigna radiata*) and black gram (*V. mungo*) [17]; (ii) the lack of chemical control options [2]; and (iii) the persistence and survival mechanisms of *P. thornei* in soils [18,19].

Wheat yield losses due to *P. thornei* of up to 65% of intolerant wheat genotypes in Australia [2] and costing USD 8–20 per hectare in the Pacific Northwest of the USA [20] have been reported. To counteract the impact of *P. thornei* in the subtropical grain region of eastern Australia, growers sow wheat genotypes that are tolerant, thus minimizing yield loss. In this region in 2024, 52% of the genotypes available for growers have high levels of tolerance to *P. thornei* [21], with yield loss of only 0–15% in infested fields [2]. Conversely, 10% of available genotypes are intolerant, incurring yield losses of 45–60% in infested fields [2,21]. The availability of tolerant wheat genotypes that are suitable for production for this region is essential and has resulted from Australian wheat breeders actively incorporating sources of genetic tolerance into their wheat genotypes.

For determination of resistance to *P. thornei*, both field [20,22,23] and environment-controlled glasshouse methods [22,24–26] have been used. A resistance index produced from a comprehensive series of glasshouse experiments was shown to be highly predictive of field resistance [22]. These replicated experiments involved growing single plants in pots inoculated with *P. thornei* in an environmentally controlled glasshouse for 16 weeks, extracting the nematodes from roots and soil, and enumerating *P. thornei* microscopically [12]. This method can be used to screen fixed genotypes [22] or plants within segregating populations [26] for resistance to *P. thornei*. Other studies have shown that wheat genotypic expression of resistance to *P. thornei* is highly correlated between different glasshouse methods used in Queensland and South Australia [25]. Thompson et al. [2,22] detailed separate

methods through which to advance genetic gain by assessing tolerance and resistance in the field, or resistance only in the glasshouse, but not selecting for both traits on a single plant simultaneously; this is potentially a very useful tool for early-generation selection in plant breeding or other segregating populations.

Ultra-low-density (ULD) sowing is a field-based method used in plant breeding programs [27,28] where individual genotypes, grown as individual plants, are given sufficient space so that there is no competition between neighboring plants [27–33]. This not only allows individual plants to grow without competition from neighbors, thereby supplying access to similar amounts of resources (water, nutrients, light) [34], but also provides an opportunity to visually assess each plant more easily and to select the superior plants [32]. It also facilitates research with a minimal number of seeds and gives a greater seed return from individual plants [35], but it does require a greater land area [34] compared to higher plant densities. An alternate hypothesis is that the main benefit of ULD sowing is to select yield components, rather than yield itself (ref. [36] cited in ref. [37]). It is recognized that although yield is the most desirable trait to select, it is generally difficult to do because of the interaction of the genotype with the environment and management [38].

The current methods used to determine tolerance [2] and resistance [22] are highly valuable methods that assist wheat breeding programs and research and continue to support the grain industry in minimizing *P. thornei*-related losses. However, neither method is appropriate for evaluating both traits on a single plant simultaneously. Often, phenotyping for many plant traits is a bottleneck, due to costs and labor, that impedes genetic advancement of crops [39–42]. It was our objective to identify new methods that would relieve this bottleneck by simultaneously selecting tolerance and resistance at the single-plant level. To do this, we used fixed genotypes (F∞) with a range of tolerance and resistance reactions to *P. thornei* [2,22] to determine the effect of different plant densities on the characterization of a genotype's tolerance and resistance. Our objectives were (a) to determine the optimum density at which single plants could be grown to select tolerant (as assessed by grain yield) and resistant (as assessed by final population densities of *P. thornei*) wheat genotypes from within a segregating population, and (b) to investigate whether the in-crop normalized difference vegetation index (NDVI) and visual tolerance rating (VTR) of the single plants could be used to expedite the process of selecting tolerant plants.

2. Materials and Methods

2.1. Field Site

The field site used is located 50 km WNW of Toowoomba, Queensland, Australia (24.464° S, 151.426° E) and has been extensively used for research determining the impact and the management of *P. thornei* in broadacre cropping. The field site is managed as four strips, each ~60 m wide by 600 m long, in a four-year rotation so that population densities of *P. thornei* are uniform and there is >2000/kg soil prior to the sowing of the experimental crop (Table 1). The population densities are achieved by growing a wheat genotype that is susceptible to *P. thornei* in the year prior to the experiments.

The soil type at the site is a cracking Black Vertosol [43] of the Waco series [44], which can store 288 mm of plant available water (PAW) in the soil profile to a depth of 1.8 m [19]. To avoid unnecessary loss of moisture from the soil, the site is managed using conservation tillage, with stubble retention and herbicidal control of weeds both in crop and during fallow periods, resulting in minimal soil disturbance but with strategic tillage as necessary for control of recalcitrant weeds [45]. The field site is rainfed, relying on in-crop rainfall and stored soil water reserves to grow the winter crops. Rainfall was measured onsite (2013 only) and nearby (~3 km north of the field site) in other years, as accessed online

from the OzForecast website (Formartin DDCGI weather station; 151.4° E, 27.4° S) [46] and is presented as cumulative rainfall (mm).

Table 1. The rotational cropping history of the field site for *Pratylenchus thornei* research located northwest of Toowoomba, Queensland, Australia, for the year when the experiments were conducted in this study and the immediately preceding year.

Year	Strip 1	Strip 2	Strip 3	Strip 4
2012	Wheat	Sorghum	Experiments	Fallow
2013	Experiments	Fallow	Sorghum	Wheat
2020	Wheat	Sorghum	Experiments	Fallow
2021	Experiments	Fallow	Sorghum	Wheat
2022	Sorghum	Wheat	Fallow	Experiments
2023	Fallow	Experiments	Wheat	Sorghum
2024	Wheat	Sorghum	Experiments	Fallow

2.2. Characterisation of the Field Site for Pratylenchus thornei and Plant Available Water

The initial population densities of *P. thornei* and the PAW were determined each year prior to sowing the experiments. A handheld pneumatic soil corer was used to push thin-walled steel tubes of 45 mm diameter to a soil depth of 90 cm at multiple locations in a grid pattern covering the experimental strip. Each soil core was cut into three depth segments, 0–30 cm, 30–60 cm and 60–90 cm, which were sealed in separate plastic (PVC) bags for each sampling location.

In the laboratory, the soil cores collected from the field were each processed by hand into an aggregate size of <10 mm, and each sample was mixed thoroughly. Subsamples of ~100 g and ~150 g of soil were taken for determination of gravimetric soil water content (GWC) and *P. thornei* population density, respectively.

The 100 g subsamples of soil were dried at 105 °C for 48 h in a forced draught oven to determine the GWC. The plant available water (PAW; mm) that accounts for soil bulk density (BD) and the wilting point of the soil (or crop lower limit, CLL) was calculated by the method described in Thompson et al. [22].

Nematodes were extracted from the soil samples using a modified Whitehead tray method [47,48] at 22 °C. After 48 h, nematodes were collected from the Whitehead tray on a sieve of 200 mm diameter with a pore–mesh aperture of 20 µm and concentrated in ~15 mL water. These samples were stored at 4 °C prior to enumerating *P. thornei* microscopically. From the ~15 mL nematode sample, *P. thornei* were identified [49] and counted in a 1 mL subsample using a gridded slide [50] (Chalex Corporation, Portland, OR, USA) at 40 and 100× magnification under a compound microscope (Olympus BX50, Tokyo, Japan). The population density of *P. thornei* was expressed as *P. thornei*/kg oven dry soil equivalent.

2.3. General Management and Naming Convention of Field Experiments

Regionally recommended best management practices were implemented to manage all the field experiments. This included the use of registered herbicides at label rates to control weeds, and registered fungicides to control foliar diseases when required. Two types of experiments were used in this investigation, namely plot density (PD) and single-plant (SP) experiments. The experimental coding is as follows: The first two digits indicate the year of testing; the following two letters indicate the type of experiment; and for SP experiments, the final two numbers indicate plant density per m^2. For example, 21SP16 refers to the experiment being conducted in 2021, which assessed the tolerance and resistance of a single plant (SP) when grown at a plant density of 16 plants/m^2 (16). The details of these experiments are provided in Table 2, and a list of the genotypes with their respective

published tolerance and resistance ratings [2,22] used in both types of experiments is provided in Table 3.

Table 2. Details of plant density (PD) and single-plant (SP) experiments, including the year, experiment code, number of genotypes assessed, plant density, the rotation strip number (from Table 1), and sowing date for each experiment.

Year	Experiment Descriptions			Rotation Strip No.	Sowing Date
	Experiment Code	Genotypes (*n*)	Density (Plants/m^2)		
2013	13PD	9	20, 40, 60, 80, 100	1	28 June 2013
2021	21SP04	14	4	1	27 July 2021
	21SP16	14	16	1	27 July 2021
	21SP32	14	32	1	27 July 2021
2022	22PD	9	20, 40, 60, 80, 100	4	15 July 2022
	22SP01	15	1	4	27 June 2022
	22SP04	14	4	4	23 June 2022
	22SP16	13	16	4	23 June 2022
	22SP32	14	32	4	23 June 2022
2024	24SP01	15	1	3	19 June 2024

Table 3. The tolerance and resistance ratings to *Pratylenchus thornei* for the wheat genotypes (from [2,22]) that were evaluated as single plants (SPs) and in plant density (PD) experiments.

Genotype	Tolerance Rating [a]	Resistance Rating [b]	Experiments
Cobalt	T	S	SP
Crusader	MI	S	SP
Cunningham	MI-I	S	SP
EGA Gregory [c]	T-MT	MS-S	PD, SP
EGA Hume	I	S	SP
EGA Stampede	VI	S-VS	PD, SP
EGA Wylie	T-MT	MS-S	PD
Gatcher	VI	S-VS	SP
Gauntlet	MT	MR-MS	SP
Gladius	I-VI	S-VS	SP
GS50a	MT-MI	R-MR	SP
Kennedy	MT-MI	S-VS	PD, SP
Lang	MI	S	PD
Lincoln	VI	S-VS	SP
QT8447	T-MT	MR	PD, SP
Strzelecki	I	S-VS	PD, SP
Suntop [d]	T	MR-MS	PD, SP
Sunvale	MT	MS-S	PD

[a] T = tolerant; I = intolerant; M = moderately; V = very (sourced from [2]). [b] R = resistant; S = susceptible, M = moderately; V = very (sourced from [22]) [c] EGA Gregory was omitted from 22SP16 due to seed impurity [d] Suntop was grown in 22SP01 and 24SP01 only.

2.4. Plant Density (PD) Experiments

The plant density (PD) experiments were conducted in 2013 and 2022 (13PD, 22PD) (Table 2). These two experiments each had the same nine wheat genotypes (Table 3), sown at the same five planting densities (20, 40, 60, 80 and 100 seeds/m^2) and replicated three times in randomized blocks. The recommended plant density for this region is 100 plants/m^2. To establish the different densities, a weight of seed, relative to the prescribed density, was calculated and packaged into individual seed packets. The seed was sown with a cone planter at ~5 cm depth in three rows, 25 cm apart and 5 m long. StarterZ fertilizer (Incitec Pivot, Southbank, Melbourne, Australia) at 40 kg/ha (supplying 8 kg P/ha and

1 kg Zn/ha) was applied beside the seed, prior to closing the seed trench with press wheels. In 2013, nitrogen (N) fertilizer, that is, urea at 200 kg/ha, supplying 92 kg N/ha, was drilled into the soil at ~5 cm depth approximately one month prior to sowing. In 2022, urea at 120 kg/ha, supplying 55 kg N/ha, was applied to the soil surface and incorporated into the soil during the sowing operation. When the plots matured, grain was harvested with a mechanical harvester and weighed. A grain subsample of measured weight (~100 g) was taken from each harvested plot and dried in a fan-forced oven at 80 °C for 48 h and then reweighed to calculate grain moisture content. Each plot weight was adjusted to 12% grain moisture equivalent.

2.5. Single Plant (SP) Experiments

Eight single plant (SP) field experiments were conducted between the years 2021 and 2024 at four different plant densities to assess wheat genotypes as single plants for their tolerance and resistance to *P. thornei* (Tables 2 and 3). Each of these experiments comprised the same 14 genotypes as treatments, with the additional wheat genotype Suntop included in the 22SP01 and 24SP01 experiments. In 2021, three experiments were sown, namely 21SP04, 21SP16, and 21SP32. In 2022, four experiments were sown, repeating the experiments from 2021, plus the additional experiment, 22SP01. In 2024, 22SP01 was repeated as 24SP01. Therefore, each experiment was grown in two separate years, with each experiment having four replications of the treatments (genotypes).

The site had urea (120 kg/ha) and StarterZ (40 kg/ha) mechanically drilled into the ground about two weeks prior to sowing the experimental seed by hand. Additional to the herbicide applications, manual hand weeding was required to control weeds in these experiments. The desired density for each experiment was obtained by using a gridded layout. The grid dimensions for these experiments were 1.0 × 1.0 m, 0.5 × 0.5 m, 0.25 × 0.25 m, and 0.25 × 0.125 m for the experiments SP01, SP04, SP16, and SP32, respectively. At each intersection, three seeds of each genotype were hand sown into moist soil at a depth of 50 mm. Seeds for each genotype were generally sourced from the same seed increase. At the approximate two-leaf stage, excess plants were removed so that one plant remained at each position. At crop maturity, the entire above-ground plant was cut and removed, leaving the crown and roots in the soil. The plants were dried in a forced draught oven at 80 °C for 48 h and weighed to determine biomass (g/plant). Heads were removed from the dried plant, then threshed by machine to obtain the grain. The grain was dried at 80 °C for 48 h and weighed to determine grain yield (g/plant). After each plant was harvested in the field, a single soil core was taken by placing a 45 mm diameter soil sampler over the remaining crown of each plant and collecting a sample of soil and roots to 30 cm depth. The single soil core from under each plant was sealed in a plastic (PVC) bag and stored at 4 °C until processing.

2.6. In-Season Visual Tolerance Ratings (VTRs) and Normalized Difference Vegetation Index (NDVI) of the Single Plants

Individual plants were assessed for a visual tolerance rating twice (VTR1 and VTR2) during the growing season at 121 and 130 days after sowing (DAS), respectively, using the one-to-nine ordinal scale as reported in Robinson et al. [12] and reproduced in Table S1. The rating scale ranges over nine categories from an intolerant genotype (score of one) which is chlorotic, with severely reduced tillering and head development, to a tolerant genotype (score of nine), which has no symptoms of chlorosis or growth impediment ([12]; Table S1). For assessment of NDVI, a Trimble® Greenseeker [51] (Trimble Inc., Sunnyvale, CA, USA) was positioned directly above each plant. The Greenseeker button was depressed for three seconds per plant, and the average NDVI recorded before advancing to the next plant.

These assessments were repeated at five assessment times (80, 86, 99, 113 and 130 DAS) for all plants and for all the experiments grown in 2022 (22SP01, 22SP04, 22SP16 and 22SP32).

2.7. Statistical Software

The initial experimental site characterization, the PD and the SP experiment and the SP experiments were all analyzed using Genstat 23rd Edition [52].

2.7.1. Statistical Analysis for the Initial Experimental Site Characterization

A $\log_e(x + 1)$ transformation of number of *P. thornei*/kg soil was applied to ensure homogeneity of variance, and values from each of the three depth increments of each core were combined to represent the mean population density for 0–90 cm soil depth. The core mean population density (0–90 cm) was analyzed by one-way ANOVA, where each experimental year was the treatment, and the site mean population density for each of the years was presented as $\log_e(x + 1)$ *P. thornei*/kg soil 0–90 cm. The back-transformed means (BTM; 0–90 cm) for *P. thornei*/kg soil were also calculated for each year. Plant available water (PAW; 0–90 cm) was determined for each core and analyzed by one-way ANOVA, where each experimental year referred to a treatment. The mean PAW is presented for each year of the experiments. A Bonferroni test ($p < 0.05$) was used to determine significant differences between experimental years for $\log_e(x + 1)$ *P. thornei*/kg soil and PAW.

2.7.2. Statistical Analysis of Plant Density Experiments (PD)

Analysis of variance was performed for grain yield for each of the nine genotypes for each of the five plant densities. Linear regression analysis was used to determine the significance of the relationships of the genotype × density means between 20, 40, 60 and 80 viable seeds/m^2, with the corresponding genotype mean when sown with the standard 100 viable seeds/m^2.

2.7.3. Statistical Analysis of Single-Plant Experiments (SP)

A $\log_e(x + 1)$ transformation of the *P. thornei*/kg soil data was used to ensure homogeneity of variance before the ANOVA for each of the eight experiments. An analysis of variance was also conducted on grain yield for all the experiments. The five NDVI assessment times and the two visual tolerance ratings for the 2022 experiments were analyzed using an ANOVA and the relationships of their means, with published values for genotype tolerance [2] were determined by regression analysis.

Linear regression analysis was used to determine the significance of the relationships of our results for tolerance based on grain yield and resistance based on *P. thornei* population density using single plants and published values for genotype tolerance [2] and resistance [22].

3. Results

3.1. Initial Pratylenchus thornei Population Densities, Plant Available Water (PAW), and In-Crop Rainfall for Each of the Experimental Years

The initial mean *P. thornei* population densities for the research site ranged from a minimum of 2588/kg soil (0–90 cm) in 2021 to a maximum population density of 7777 *P. thornei*/kg soil in 2013 (Table 4). In all experimental years, the initial population densities of *P. thornei* exceeded the damaging threshold of 2000 *P. thornei*/kg soil. The mean PAW (0–90 cm) for the research site ranged from 121 mm in 2021 to 200 mm in 2022. The highest and lowest total rainfalls from soil sampling to 31 October (termed in-crop rainfall) were 249 mm in 2022 and 128 mm in 2024.

Table 4. The mean population density of *Pratylenchus thornei* and plant available water (PAW) in the soil profile and the in-crop rainfall for each year the experiments were conducted.

Year	*P. thornei*/kg Soil 0–90 cm			Plant Available Water 0–90 cm		Rainfall (mm) [d]
	$\log_e(x+1)$ [a]	s.e.m [b]	BTM [c]	Mean (mm) [a]	s.e.m [b]	
2013	8.96 [b]	0.21	7777	164 [ab]	16.38	157
2021	7.86 [a]	0.12	2588	121 [a]	9.46	180
2022	8.83 [b]	0.15	6835	200 [b]	11.59	249
2024	8.75 [b]	0.11	6310	131 [a]	9.09	128
ANOVA	F prob	*l.s.d* [e]		F prob	*l.s.d*	c.v. [f]
Year	>0.001	0.43		>0.001	16.75	4.9%

[a] Different letters denote means that are significantly different for $\log_e(x+1)$ *P. thornei* kg/soil and PAW (mm) between years ($p < 0.05$); [b] standard error of the mean; [c] back-transformed mean; [d] rainfall from soil sampling to Oct 31; [e] least significant difference; [f] coefficient of variance.

3.2. The 2013 and 2022 Plant Density Experiments on High Population Densities of Pratylenchus thornei

In the 2013 plant density experiment, there were significant ($p < 0.001$) treatment effects for genotype and density (viable seeds/m^2) but not for the interaction of genotype × density (Table 5). The mean grain yield (kg/ha) increased as plant density increased.

Table 5. The mean yield (kg/ha) for each wheat genotype when sown at five different seeding densities (viable seeds/m^2) in 2013 on a field site with a high population density of *Pratylenchus thornei*.

Genotype	Seeding Density (Viable Seeds/m^2)					Genotype
	20	40	60	80	100	Mean
EGA Gregory	2393	2707	3241	3300	3470	3022
EGA Stampede	405	584	789	754	792	665
EGA Wylie	2853	3651	3777	3754	3903	3588
Kennedy	1362	1959	2296	2410	2202	2046
Lang	1398	1452	1581	1763	2211	1681
QT8447	3655	3901	4197	4130	4077	3992
Strzelecki	910	1361	1060	1679	1177	1237
Suntop	2664	3219	3321	3804	3923	3386
Sunvale	2661	2968	3198	3516	3463	3161
Density mean	2034	2422	2607	2790	2802	2531
ANOVA	F prob	*l.s.d* [b]				
Genotypes	<0.001	240.1				
Density	<0.001	179				
Geno × dens	ns [a]					

[a] ns: non-significant; [b] least significant difference.

The plot grain yields when sown at 20, 40, 60 and 80 viable seeds/m^2 (13PD) were strongly and positively correlated with the plot grain yields (kg/ha) of the 100 viable seeds/m^2 plot ($R^2 = 0.93$ to 0.95; $p < 0.001$) (Figure 1). Despite the reduction in the number of viable seeds sown, the grain yields obtained for each of the genotypes at the lower sowing densities remained predictive of the genotype yield when sown at the regionally recommended density of 100 viable seeds/m^2.

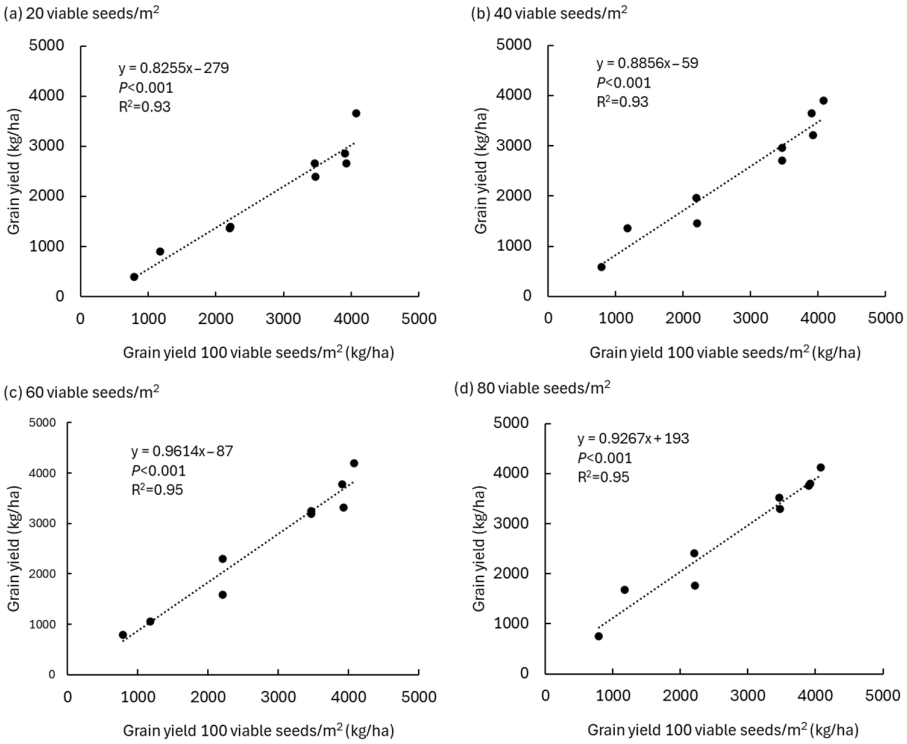

Figure 1. The relationships between mean grain yield (kg/ha) of nine wheat genotypes (n = 9) grown at four lower sowing densities of (**a**) 20 (**b**) 40 (**c**) 60 and (**d**) 80 viable seeds/m^2 with their yield when grown at the standard rate of 100 viable seeds/m^2 on a site with high population densities of *Pratylenchus thornei* at Formartin, Queensland, Australia in 2013.

When the plant density experiment was repeated in 2022, again there were significant ($p < 0.001$) treatment effects for genotype and density (viable seeds/m^2) but not for the interaction of genotype × density (Table 6). Generally, the mean grain yield (kg/ha) increased as plant density increased from 20 to 80 viable seeds/m^2 treatments.

Table 6. The mean yield (kg/ha) for each wheat genotype when sown at five different seeding densities (viable seeds/m^2) in 2022 on a field site with a high population density of *Pratylenchus thornei*.

Genotype	Seeding Density (Viable Seeds/m^2)					Genotype
	20	**40**	**60**	**80**	**100**	**Mean**
EGA Gregory	4230	5241	5144	5080	5199	4979
EGA Stampede	2231	2307	2231	2968	2758	2499
EGA Wylie	3621	4162	4145	4559	4613	4220
Kennedy	3207	3644	4093	4775	3895	3923
Lang	2773	3129	3390	3462	3791	3309
QT8447	4808	5830	6651	6097	6299	5937
Strzelecki	2827	3525	3747	4156	4176	3686
Suntop	4310	5069	5888	6116	6108	5498
Sunvale	3944	4677	4214	4200	4529	4312
Density mean	3550	4176	4389	4601	4597	2531
ANOVA	F prob	*l.s.d* [b]				
Genotypes	<0.001	437.9				
Density	<0.001	326.4				
Geno × dens	ns [a]					

[a] ns: non-significant; [b] least significant difference.

The plot grain yields when sown at 20, 40, 60 and 80 viable seeds/m^2 (13PD) were strongly and positively correlated with the plot grain yields (kg/ha) of the 100 viable seeds/m^2 plot ($p < 0.001$) (Figure 2). The strongest relationship ($R^2 = 0.96$) and the weakest relationship ($R^2 = 0.88$) were obtained at 60 and 20 viable seeds/m^2, respectively. Like the 2013 experiment, when the number of viable seeds sown were decreased, the grain yields obtained for the genotypes at the lower sowing densities remained predictive of the regionally recommended sowing density 100 m viable seeds/m^2. The positive linear regressions from the 2013 and 2022 plant density experiments show the plot yields obtained at densities as low as 20% of the regionally recommended density were suitable and predictive of a genotype's tolerance response to *P. thornei*.

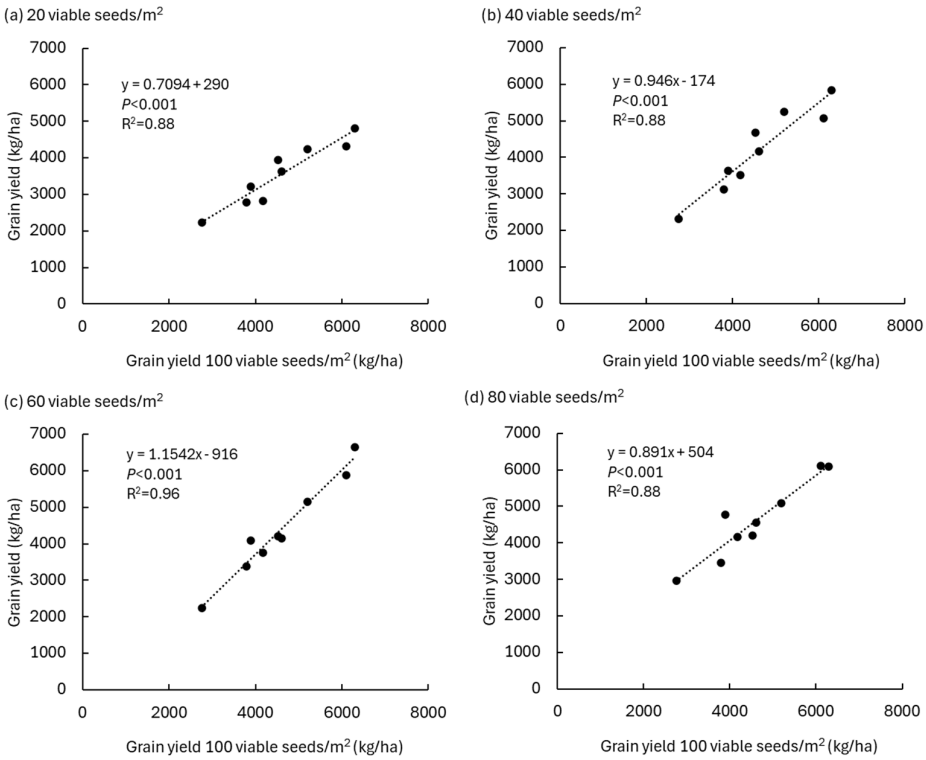

Figure 2. The relationships between mean grain yield (kg/ha) of nine wheat genotypes grown at four lower sowing densities of (**a**) 20 (**b**) 40 (**c**) 60 and (**d**) 80 viable seeds/m^2 with their yield when grown at the standard rate of 100 viable seeds/m^2 on a site with high population densities of *Pratylenchus thornei* in Formartin, Queensland, Australia in 2022.

3.3. Single-Plant Assessment of Tolerance to Pratylenchus thornei at 1, 4, 16 and 32 Plants/m^2

The mean genotype yield of the single plants grown at different plant densities in 2021 (4, 16 and 32 plants/m^2) and 2024 (1 plant/m^2 only) were all significantly correlated ($p < 0.05$) with the predicted genotype yield from multiple *P. thornei* tolerance experiments [2] (Figure 3, Table S2). Although the linear regressions were positive and statistically significant for all the experiments, the strength of these relationships varied, with the strongest relationship occurring when the density was 16 plants/m^2 (21SP16; $R^2 = 0.63$; $p < 0.001$) (Figure 3c) and the weakest when the density was increased to 32 plants/m^2 (21SP32; $R^2 = 0.30$; $p = 0.024$) (Figure 3d). In terms of the mean genotype grain yield that was produced from a single plant, the yields decreased as the density of the plants increased, with the yields being 22–132 g, 19.5–64.6 g, 4.7–33.3 g and 2.6–25.3 g for 1 plant/m^2 (24SP01), 4 (21SP04), 16 (21SP16) and 32 (21SP32) plants/m^2, respectively.

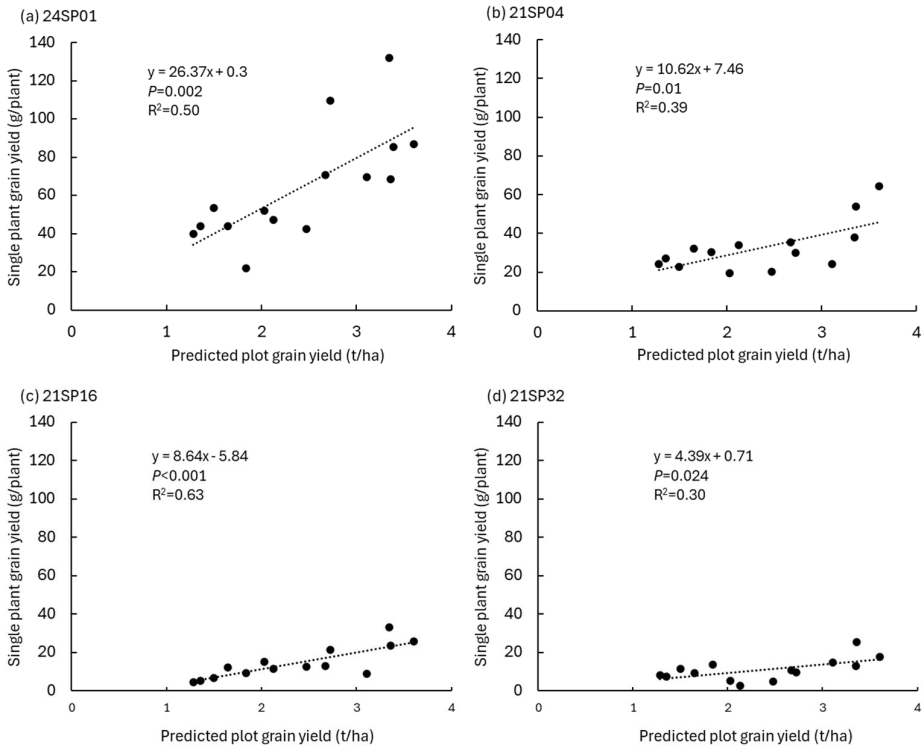

Figure 3. The mean grain yield (g/plant) for the different wheat genotypes when sown at densities of (**a**) 1 plant/m^2 in 2024, (**b**) 4, (**c**) 16, and (**d**) 32 plants/m^2 in 2021 compared to the *Pratylenchus thornei* tolerance yield prediction from plot grain yield from Thompson et al. [2].

The mean genotype yields of the single plants grown at four different densities in 2022 (1, 4, 16 and 32 plants/m^2) were all significantly correlated ($p < 0.001$) with the predicted genotype yield reported from multiple *P. thornei* tolerance experiments [2] (Figure 4, Table S3). The strength of these relationships varied, with the strongest relationship occurring when the density was 4 plants/m^2 (22SP04; $R^2 = 0.79$; $p < 0.001$) (Figure 4b) and the weakest when the density increased to 32 plants/m^2 (22SP32; $R^2 = 0.61$; $p < 0.001$) (Figure 4d). The mean genotype grain yield produced from a single plant trended the same as the three 2021 and 2024 experiments, where the plant yields decreased as the density of the plants increased, with the yields being 29.4–162.1 g, 21.8–107.6 g, 3.8–40.8 g and 3.7–32.4 g for 1 plant/m^2 (22SP01), 4 (22SP04), 16 (22SP16) and 32 (22SP32), plants/m^2, respectively.

Single Plant Assessment for Tolerance to *Pratylenchus thornei* Using Normalized Difference Vegetation Index and Visual Tolerance Ratings in 2022

There were significant genotype treatment effects ($p < 0.05$) for NDVI at each assessment time when the single-plant density was 1 plant/m^2 (22SP01), 4 (22SP04, except NDVI_2) and 16 (22SP16, except NDVI_4 and NDVI_5) plants m^2 (Tables 7 and S4). When the plant density increased further to 32 plants/m^2 (22SP32), the fourth assessment time (NDVI_4) was the only time to have significant genotype treatment effects. However, when assessed by VTR, there were significant ($p < 0.001$) genotype treatment effects at both assessment times and for all single-plant densities. When the experiments were sown at 1 plant/m^2 (22SP01) and 4 plants/m^2 (22SP04) and regardless of the assessment time, the correlation coefficients between NDVI or VTR with the *P. thornei* yield tolerance predictions [2] were greater than $R^2 = 0.66$ ($p \leq 0.007$) and all were highly significant. Where the experiments had higher single-plant densities of 16 and 32 plants/m^2, the correlation coefficients were generally lower compared to the two other experiments at lower plant densities (1 plant/m^2 and 4 plants/m^2). This trend was not observed with VTR, as all the

single-plant densities were highly and significantly correlated ($p < 0.001$) with the yield tolerance predictions of Thompson et al. [2], regardless of the assessment time. In terms of assessment time, the NDVI of the single plant was most highly correlated with the predicted *P. thornei* yield tolerance [2] at DAS99 ($R^2 = 0.80$) and DAS113 ($R^2 = 0.89$) for 1 plant/m^2 (22SP01) and 4 plants/m^2 (22SP04), respectively.

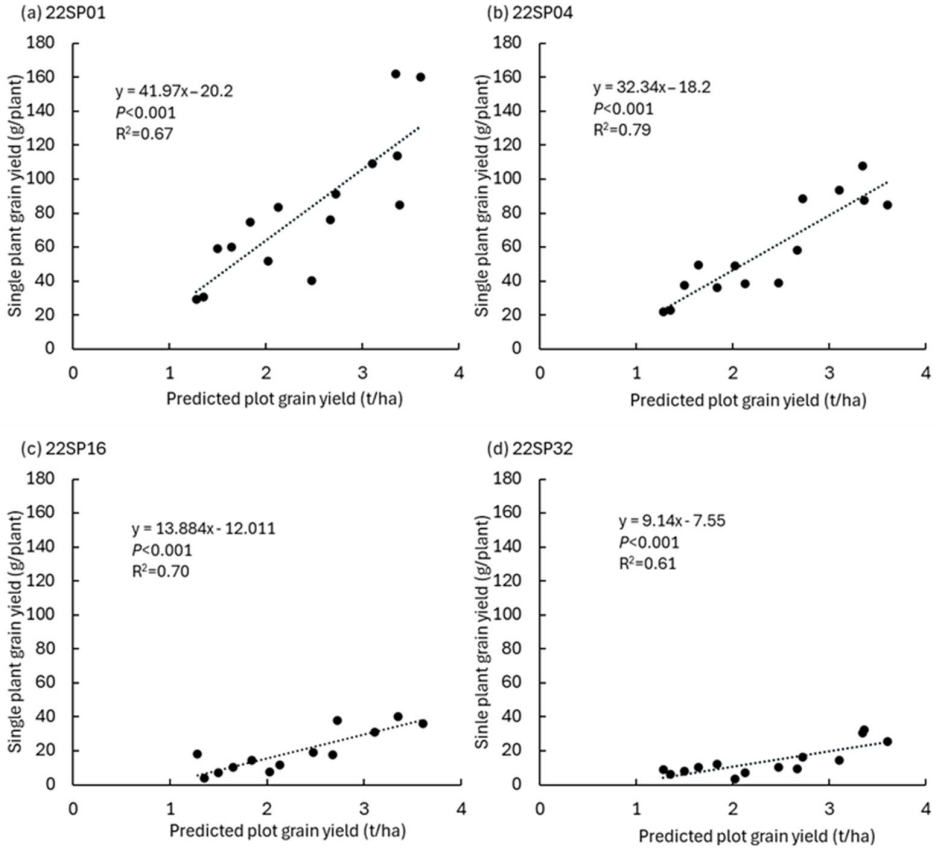

Figure 4. The relationship between mean grain yield (g/plant) for the different wheat genotypes when sown at densities of (**a**) 1, (**b**) 4, (**c**) 16, and (**d**) 32 plants/m^2 in 2022 with the *P. thornei* tolerance yield predictions from plot grain yield from Thompson et al. [2].

Table 7. The correlation coefficients (r) between the tolerance yield prediction [2] with either the genotype mean for normalized difference vegetation index (NDVI) or tolerance ratings (VTRs) on various days after sowing (DAS) for four densities of single plants in 2022.

Experiment		**22SP01**		**22SP04**		**22SP16**		**22SP32**	
Genotypes (*n*)		**15**		**14**		**13**		**14**	
Assessment	**DAS**	**r**	***p***	**r**	***p***	**r**	***p***	**r**	***p***
NDVI_1	80	0.73	0.002	0.69	0.006	0.54	0.059	0.50 [a]	0.066
NDVI_2	86	0.66	0.007	0.77 [a]	0.001	0.46	0.117	0.42 [a]	0.139
NDVI_3	99	0.80	<0.001	0.85	<0.001	0.45	0.123	0.34 [a]	0.233
NDVI_4	113	0.73	0.002	0.89	<0.001	0.37 [a]	0.212	0.77	0.001
NDVI_5	130	0.77	<0.001	0.75	0.002	0.71 [a]	0.007	0.24 [a]	0.408
VTR_1	121	0.87	<0.001	0.86	<0.001	0.83	<0.001	0.90	<0.001
VTR_2	130	0.92	<0.001	0.95	<0.001	0.85	<0.001	0.81	<0.001
Yield [b]		0.83	<0.001	0.90	<0.001	0.85	<0.001	0.80	<0.001

[a] The NDVI means for each genotype at the individual assessment times were not significantly different within the experiment. [b] The tolerance grain yield prediction from plot grain yield from Thompson et al. [2].

3.4. Single-Plant Assessment for Resistance to *Pratylenchus thornei* at 1, 4, 16 and 32 Plants/m^2

There was a significant ($p < 0.001$) genotype treatment effect for *P. thornei*/kg soil when plants were grown at 1 plant/m^2 (24SP01) and 4 plants/m^2 (21SP04) and when plants were grown at 16 plants/m^2 (21SP16; $p = 0.025$) (Table S2). There was no significant genotype treatment effect when the plants were grown at the highest density of 32 plants/m^2 (21SP32). The final population densities of *P. thornei* when grown at the three plant densities in 2021 (21SP04, 21SP16 and 21SP32) and 2024 (24SP01 only) were all correlated ($p < 0.05$) with the predicted final population densities as reported in Thompson et al. [22] (Figure 5). The strongest relationship was when the plants were grown at 4 plants/m^2 (21SP04; $R^2 = 0.73$; $p < 0.001$) (Figure 5b), and the weakest was when the plants were grown at 1 plant/m^2 (24SP01; $R^2 = 0.37$; $p = 0.01$) (Figure 5a). When comparing within the 2021 season only, the next weakest relationship was when plants were grown at 32 plants/m^2 (21SP32). The range of genotype means in final $\log_e(x + 1)$ *P. thornei* population densities with back-transformed means (BTM) in parentheses was 8.37–10.1 (4317–24,250 *P. thornei*/kg soil) when the plants were grown at 1 plant/m^2 in 2024 (24SP01). As for the three experiments grown in 2021, the experiment where the plant density was 4 plants/m^2 (21SP04) had the lowest minimum and maximum genotype means (7.7–9.75 $\log_e(x + 1)$ *P. thornei*/kg soil; 2295–17,153 BTM). As for the highest minimum and maximum genotype means, this occurred when the density was 32 plants/m^2 (21SP32; 9.34–10.33 $\log_e(x + 1)$ *P. thornei*/kg soil; 11,991–30,610 BTM).

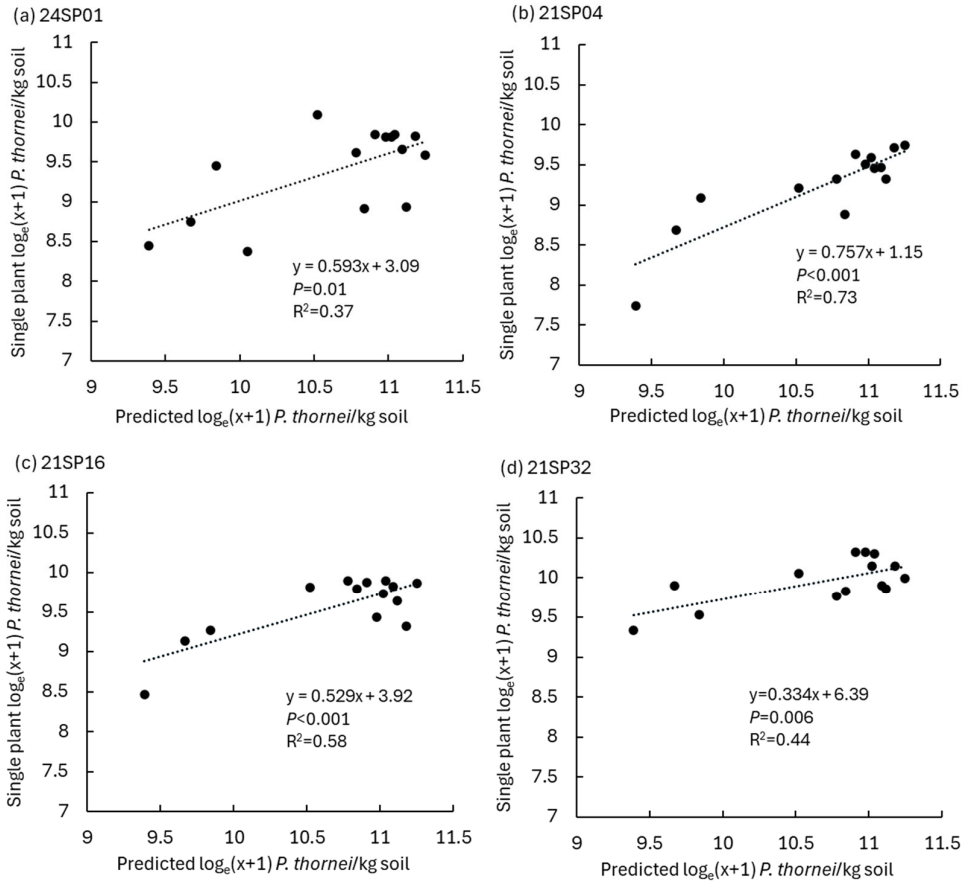

Figure 5. The mean final population density ($\log_e(x + 1)$) of *Pratylenchus thornei* for the different wheat genotypes when sown at densities of (**a**) 1 plants/m^2 in 2024, (**b**) 4, (**c**) 16, and (**d**) 32 plants/m^2 in 2021 compared to the resistance prediction (predicted $\log_e(x + 1)$ *P. thornei*/kg soil) from Thompson et al. [22].

For the 2022 experiments, the genotype treatment effect for *P. thornei*/kg soil was highly significant ($p < 0.001$) for the lowest two plant densities of 1 plant/m^2 (22SP01) and

4 plants/m^2 (22SP04). It was not significant when the plants were grown at the higher densities of 16 (22SP16) and 32 plants/m^2 (22SP32) (Table S3). The final population densities of *P. thornei* for the wheat genotypes at three plant densities in 2022 (excluding the highest density (22SP32)) were all correlated ($p < 0.05$) with the predicted final population densities as reported in Thompson et al. [22] (Figure 6). The strongest relationship was when the experiment was grown at the lowest plant density of 1 plant/m^2 (22SP01; $R^2 = 0.54$; $p = 0.001$) (Figure 6a), and the weakest relationship was when the experiment was grown at the highest plant density of 32 plants/m^2 (22SP32; $R^2 = 0.19$; $p = 0.069$) (Figure 6d). Unlike the 2021 single-plant experiments, there was no trend of increasing final *P. thornei* population densities with increasing plant density. The lowest plant density (22SP01) had both the second lowest minimum (7.29 $\log_e(x + 1)$ *P. thornei*/kg soil; 1462 BTM) and the highest maximum (9.90 $\log_e(x + 1)$ *P. thornei*/kg soil; 19,870 BTM) final genotype mean *P. thornei* population density. The 4 plants/m^2 experiment (22SP04) had the lowest mean genotype final *P. thornei* population density (7.21 $\log_e(x + 1)$ *P. thornei*/kg soil; 1355 BTM).

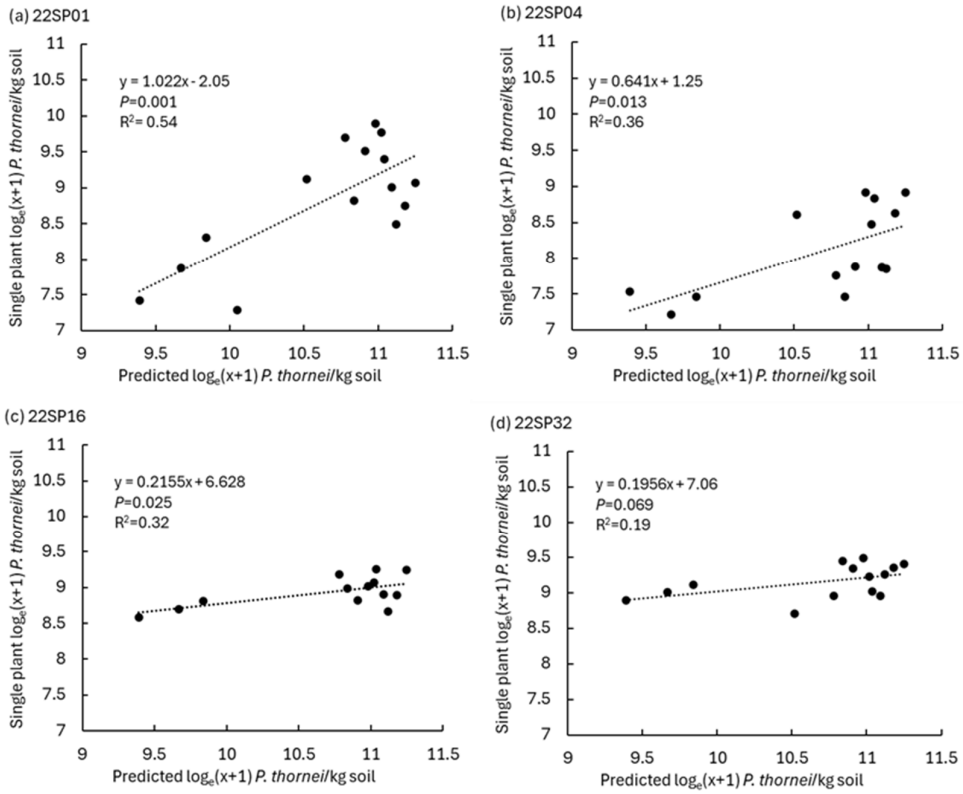

Figure 6. Relationships between mean final population density of *Pratylenchus thornei* ($\log_e(x + 1)$) for the different wheat genotypes when sown at densities of (**a**) 1, (**b**) 4, (**c**) 16, and (**d**) 32 plants/m^2 in 2022 compared to the resistance prediction (predicted $\log_e(x + 1)$ *P. thornei*/kg soil) from Thompson et al. [22].

4. Discussion

For the first time, ultra-low-density sowing has been shown to be an effective field-based approach for characterizing single wheat plants of different genotypes for tolerance and resistance to *P. thornei*. This method can provide researchers and wheat breeders an effective high-throughput phenotypic platform to select for both traits simultaneously, potentially from within segregating breeding populations.

4.1. Tolerance Stability at Lower-than-Industry-Recommended Plant Densities

The two plant density experiments (13PD and 22PD) demonstrated that reducing the plant density of yield plots by 20–80% of the recommended density for commercial production in the subtropical grain region of eastern Australia (100 plants/m^2) resulted in grain yields being highly correlated ($p < 0.001$) with the recommended higher density. Reducing plant density may cause different genotypes to exhibit a range of compensatory responses [53] such as increased tiller number, grain size, and grain number. However, the present study demonstrated the over-riding effect of *P. thornei* on yield, even when the plant density is reduced for yield plot evaluation. For compensatory mechanisms to function, water and nutrients must be accessible [54]. However, the root damage caused by *P. thornei* restricts the uptake of available water and nutrients [55] by intolerant genotypes. This can prevent intolerant genotypes from benefiting from the increased land area per plant and therefore the greater resources available per plant. Our results demonstrated that lowering the plant density to 20 plants/m^2 maintained consistent ranking of tolerance to *P. thornei*. Determining tolerance by yield plots with reduced plant densities may be an attractive option for screening breeding lines with minimal amounts of seed required on the proviso that all the lines were sown to achieve the same plant density. The over-riding effect of *P. thornei* on yield when yield plots were grown at reduced plant densities suggests that even lower densities could be used, whereby a single plant sown at LD could be evaluated for tolerance to *P. thornei*.

4.2. Evaluating Tolerance and Resistance at the Single-Plant Level Using Low-Density Sowing

To further improve selection efficiency, we investigated four planting densities that could be used for selecting both tolerance and resistance to *P. thornei* in single plants. Fasoula and Fasoula [31] detail a 'honeycomb' design for ULD sowing. Although there are small yield and precision improvements in using the 'honeycomb' design over the traditional grid design [56], we opted to use a grid layout, which is more practical and scalable when using current mechanized precision seeders to sow experiments [57]. With the further improvement in seed singulation and accurate seed placement with mechanised precision seeders [34] and the improvement in honeycomb design constructs and analysis [57], there is scope to efficiently upscale the experiments presented in this study. The moving replication constructs within honeycomb designs address underlying soil variability across the field site [35] and would be beneficial in fields with variable population densities of *P. thornei*.

The genotypes included in each of the experiments were chosen based on their known tolerance [2] and resistance [22] responses to *P. thornei*. These genotypes were used to assess the range of reactions for both traits, allowing us to determine the most effective by linear regression analysis with the published indices for tolerance and resistance to *P. thornei* of the various genotypes. It is noteworthy that the application of LD sowing is intended to select superior plants from within segregating populations and not to replace the approaches described by Thompson and colleagues [2,22] that provide information to the industry pertaining to commercial varietal decisions for growers (see below).

4.3. Ultra-Low-Density Sowing for the Selection of Genotypes with Tolerance to Pratylenchus thornei

Overall, the linear regressions showed stronger relationships in 2022 compared to 2021 and 2024 for tolerance of *P. thornei* among the four plant densities. This effect was most likely due to the greater population density of *P. thornei*, PAW, and in-crop rainfall in 2022 than in 2021 (6835 and 2588 *P. thornei*/kg; PAW 200 and 121 mm; rainfall 249 and 180 mm, respectively). This supports the conclusion made by Thompson et al. [2] that tolerance

is best discriminated amongst different genotypes when high population densities of *P. thornei* are present at sowing and the PAW is also high.

To date, the tolerance of wheat genotypes to *P. thornei* has been determined using field plots when grown at 100 plants/m^2, by using VTR, NDVI or grain yield [2,11,12]. In these field plots, tolerance is measured averaged over a total of ~800 plants, rather than a single plant when using LD sowing. However, with ULD, the opportunity for phenotypic expression of the single plant is elevated [58], aiding the selection of tolerant genotypes. To maximise this expression, our study was conducted at a field site dedicated to *P. thornei* research, with high and uniform population densities of *P. thornei* and no other major biotic stresses [2,11,12] and where yield losses of up to 65% due to *P. thornei* are common in intolerant genotypes [2]. Tolerance to *P. thornei* is a major yield determinant of wheat genotypes on sites with high population densities of this nematode species. This is supported by the results of a factor analytical multi-environment trial analysis of 29 field experiments that revealed the first principal axis, or tolerance axis, accounted for a high 84% of the genotype environmental variation [2]. To capitalise further on the expression of tolerance, the experiments were sown later in the recommended window, aligning approximately with the mean second sowing time of 30 June as reported by Robinson et al. [12]. The symptoms of *P. thornei* intolerance, such as yellowing of the lower leaves and poor canopy biomass, are exacerbated in later sowings and are most likely due to the warmer soils that promote *P. thornei* reproduction [12,18]. By adopting this later sowing time, the VTR and NDVI of the canopies are more likely to be highly predictive of tolerance when measured by grain yield [12]. As such, the in-season VTRs of the experiments were highly predictive ($p < 0.001$) of published tolerance ratings by Thompson et al. [2]. Our study demonstrates that researchers can identify plants on the tolerance spectrum using VTR on a one-to-nine scale, making visual tolerance assessments a viable method for selecting tolerant genotypes from a segregating population.

An alternative and objective method for assessing tolerance is to use NDVI instead of VTR [12]. In two recent studies, a handheld Greenseeker™ sensor was deployed to measure the NDVI of wheat genotypes when grown in field plots for tolerance studies [11,12]. However, using this sensor to measure individual plants for tolerance is likely to be limited by its field of view (FOV), which must exclude the canopy of any neighbouring plants. At a height of 61 cm above the canopy, the Greenseeker® has an FOV of 25 cm [51]. This is not an issue when plants are grown at densities of 1 or 4 plants/m^2, as there is limited interference from neighbouring plants. However, at densities of 16 plants/m^2 and 32 plants/m^2, there is overlap of plant material, resulting in NDVI readings from multiple plants, or genotypes. Another important factor is the timing of NDVI assessment. For experiments with plant densities of 1 or 4 plants/m^2, assessing individual plants between 99 and 133 days after sowing (DAS) provided accurate tolerance measurements. This assessment window by NDVI was slightly later than that described by Robinson et al. [11] and may better reflect the slower maturation of single plants due to lack of inter-plant competition and thus increased resource availability compared to plants grown at field plot density.

4.4. Ultra-Low-Density Sowing for the Selection of Genotypes with Resistance to Pratylenchus thornei

The relative resistance of the genotypes determined in the glasshouse is predictive of the resistance of those genotypes when grown in field experimental plots [22]. The advantages of assessing resistance in the glasshouse are control over the initial population density of *P. thornei*, superior environmental control of abiotic factors, and elimination of other pests and diseases that might limit plant growth and nematode reproduction [22]. However, a method of assessing genotype tolerance in glasshouse experiments has not

been reported, thereby requiring a field-based approach if both tolerance and resistance are to be determined for single plants.

In our study of resistance to *P. thornei* based on single plants grown in the field in different LD populations, we compared final nematode population densities with predicted *P. thornei* population densities for the same genotypes derived from 22 combined glasshouse experiments [22]. These replicated glasshouse experiments involved growing plants in 70 mm square pots with root containment which were then inoculated with pure cultures of *P. thornei* and maintained as single plants in controlled environments for 16 weeks [22,25]. In 2021, the experiment sown at 4 plants/m^2 (21SP04) showed the strongest regression relationship ($R^2 = 0.73$, $p < 0.001$) with the resistance predictions published by Thompson et al. [22]. For 2022, the strongest regression relationship ($R^2 = 0.54$, $p < 0.001$) with the resistance predictions was observed when experiments were sown at 1 plant/m^2. In both years, as plant densities increased (16 and 32 plants/m^2), the regression relationships with the published predictions decreased, indicating that these higher LD levels are less predictive of resistance. These weaker regression relationships likely resulted because the soil samples contain roots invading from the neighbouring plants, thereby confounding individual plant resistance levels. The reduced slope of the regression line with increasing plant density indicated that closer plant proximity probably resulted in greater contamination by neighbouring roots, which reduced the accuracy of higher LD in estimating the resistance of a genotype. To prevent competition for resources among neighbouring plants, the optimal plant densities need to be 1 to 1.2 plants/m^2 [28] or 1.4 plants/m^2 [59], but no more than 4 plants/m^2 [38]. This suggests that in our experiments with plant densities of 1 and likely 4 plants/m^2, the roots belong exclusively to the sown genotype, unlike the higher densities of 16 and 32 plants/m^2.

In addition, the vertical distribution of *P. thornei* in the soil profile can vary widely [60]. This variability was not captured in the 30 cm soil depth sample collected to determine resistance at any LD in this study. Furthermore, the initial population density may also influence the reproduction rate of *P. thornei*, where rates are lower when the initial population densities are higher [10,61]. However, in the present study context, the intrinsic rate of population increase that is wheat genotype-specific [62] is the major determinant of final *P. thornei* population densities on this site, which is managed in order to provide relatively uniform initial *P. thornei* population densities. As mentioned previously, the initial population densities of *P. thornei* can be controlled more effectively and repeatably in controlled glasshouse environments [25] rather than in the field. Regardless of the above, resistance was predicted along with tolerance for the single plants when sown at ULD across the different years. This further reiterates that LD sowing is not a substitute for routine resistance screening; rather, it is a method of potentially improving the selection of resistant progeny from within a breeding population when grown in the field.

4.5. Ultra-Low-Density Sowing for Dual Selection of Tolerance and Resistance to Pratylenchus thornei

The dual selection for tolerance and resistance to *P. thornei* at the single-plant level is a distinct advantage of using ULD sowing, and it potentially allows the identification of superior genotypes from within a segregating population

For tolerance, we reported earlier that the linear regressions with the published tolerance predictions were stronger in 2022 than 2021 [2]. It needs to be noted that the 2021 experiments were grown under atypical site conditions compared with the previous studies of Robinson et al. [12] and Thompson et al. [2]. In these two studies, the mean initial population densities (0–90 cm) were ~4900 *P. thornei*/kg soil (eight years) [12] and ~4850 *P. thornei*/kg soil (15 years) [2], both being almost double that of the population density for the 2021 experiments (2588 *P. thornei*/kg soil). In comparison, the initial pop-

ulation density was 6835 *P. thornei*/kg soil in 2022. Regardless, the linear regression was the strongest when the experiment was sown at 4 plants/m^2 (22SP04; R^2 = 0.79) in 2022, while the second strongest was 16 plants/m^2 (22SP16; R^2 = 0.70), the same density that was the strongest in 2021 (21SP16; R^2 = 0.63). It should also be noted that LD remained effective across the three very contrasting years (2021, 2022, 2024) indicating LD sowing to be robust across the environments. In a typical year at this field site (mean population density of ~4900 *P. thornei*/kg soil [2,12]), the expectation would be that the strength of the linear regressions with the published tolerance predictions [2] would exceed the regressions observed in the 2021 experiments.

For resistance, the linear regressions between the population density of *P. thornei* per single plant and the predicted population density [22] were all significantly correlated ($p < 0.05$, except for 22SP32), although in both years where the plants were grown at 32 plants/m^2, there was no significant genotype treatment effect. The linear regressions of the 2021 grown experiments were greater than the regressions in 2022. One plausible reason for this is that the stronger regressions in 2021 compared to 2022 were a byproduct of the lower initial population densities of *P. thornei*, as observed by Fanning et al. [9], in whose study lower initial densities resulted in greater *P. thornei* reproduction rates. As for the sole 2024 experiment (24SP01), it would be reasonable to expect that the linear regression would be weaker than the 2021 experiments given a comparable initial *P. thornei* population density to the 2022 experiments. The initial population density of *P. thornei* in field experiments is dynamic and heavily influenced by many seasonal and genetic factors (soil temperature, rainfall, and susceptibility of the first-year wheat). Controlling the initial population densities is a significant challenge, but by using a long-term field site dedicated to *P. thornei* research and characterizing the site for the initial *P. thornei* population density and PAW, in-season rainfall records and the inclusion of check genotypes with known levels of tolerance and resistance will assist wheat breeders in using ULD sowing. Our results suggest that *P. thornei* has an over-riding impact on different wheat genotypes, and deploying ULD at densities of 4 plants/m^2 could be an effective platform for selecting for tolerance using yield, NDVI, visual tolerance scores, and for resistance using final *P. thornei* population densities.

The additional benefit of this approach is that selection for agronomic traits can be made, which is particularly useful when unadapted genotypes are used as donors to introduce alleles for resistance and/or tolerance. There has been significant progress in identify *P. thornei*-resistant genes from unadapted and novel genetic backgrounds [20,26] and incorporating these into current commercial genotypes [63]. However, introducing genetic resistance from novel unadapted sources may also bring undesirable traits [64] that contribute to poor agronomy that limits yield production. Fischer [34] and Fasoula & Fasoula [32] propose using ULD for early-generation selection to select more productive germplasm. The method we propose could potentially be used to select plants that are tolerant and resistant to *P. thornei* and could assist backcrossing programs to retain both these traits in adapted genotypes, provided the initial *P. thornei* population density is high (>2500 *P. thornei*/kg soil) and relatively even, such as on the managed site used in these studies.

To streamline the platform, plants can be selected for tolerance during the growing season using NDVI (113 DAS) or VTR (130 DAS), and for agronomic attributes, before being grown to maturity and harvested. Subsequently, only the plants selected for tolerance would be soil-sampled at plant harvest to determine their resistance. The most tolerant and resistant progeny could then be advanced. Once the lines were genetically fixed (around F5 generation), replicated field yield and glasshouse experiments could be conducted to accurately confirm their levels of tolerance and resistance, respectively. If seed availability

is limited from the F4 selected single plant, the replicated field experiment can be sown at a reduced plant density (20 plants/m^2) to confirm tolerance.

We propose an approach that combines the benefits of ULD with traditional robust methods to streamline the development of resistant and tolerant genotypes from segregating generations through to homozygous wheat genotypes that are resistant and tolerant to *P. thornei*. Although this initial investigation used homozygous genotypes of known tolerance and resistance reactions to *P. thornei*, the next phase of experimentation should apply this approach to breeding populations that are segregating for tolerance and resistance to *P. thornei*.

5. Conclusions

This study demonstrated that ULD sowing of single plants between 1 and 4 plants/m^2 is a novel method that can help to identify *P. thornei*-tolerant and -resistant genotypes at the single-plant level. If the objective of the experiment is to select for tolerance only, then increasing the density to 16 plants/m^2 would also suffice if land area is limited. The in-season assessment of the canopies by NDVI and VTR was also highly predictive of the tolerance reaction of wheat genotypes to *P. thornei* and could potentially be integrated into experiments to select for tolerance in-season when the plant densities are between 1 and 4 plants/m^2. By sowing single plants at ULD, breeders and researchers can potentially select for both tolerance and resistance from within segregating breeding populations and thereby complement breeding programs to develop new genotypes with both traits. The development and commercial use of wheat genotypes that are resistant and tolerant to *P. thornei* increases the grain yield of these genotypes when grown in *P. thornei*-infested fields and reduces the population densities of *P. thornei* to minimize against yield loss of subsequent crops.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy15092049/s1, Table S1. The system used to visually recognize and to score the symptoms of intolerance of wheat genotypes to *Pratylenchus thornei*, as sourced Robinson et al. 2024 [12]. Table S2. The mean grain yield (g/plant) and final population density of *Pratylenchus thornei* ($\log_e(x + 1)$) for the different wheat genotypes when sown at densities of 1 plant/m^2 in 2024, and 4, 16 and 32 plants/m^2 in 2021 on a field site with a high initial population density of *P. thornei*. Table S3. The mean grain yield (g/plant) and final population density of *Pratylenchus thornei* ($\log_e(x + 1)$) for the different wheat genotypes when sown at densities of 1, 4, 16 and 32 plants/m^2 in 2022 on a field site with a high initial population density of *P. thornei.* Table S4. The mean normalized difference vegetation index (NDVI) and visual tolerance rating (VTR) at various days after sowing (DAS) for four densities of single plants in 2022 on a field site with a high initial population density of *P. thornei*.

Author Contributions: Conceptualization, N.A.R., R.S.Z. and K.J.O.; methodology, N.A.R., J.L. and J.G.S.; formal analysis, N.A.R. and J.P.T.; investigation, N.A.R.; resources, N.A.R.; data curation, N.A.R.; writing—original draft preparation, N.A.R.; writing—review and editing, N.A.R., R.S.Z., K.J.O., J.G.S. and J.P.T.; visualization, N.A.R.; supervision, R.S.Z., K.J.O. and J.P.T.; project administration, R.S.Z. and K.J.O.; funding acquisition, R.S.Z., N.A.R. and J.P.T. All authors have read and agreed to the published version of the manuscript.

Funding: The Grains Research and Development Corporation (GRDC) supported and co-funded this research (grant numbers: USQ00019, USQ1907-002SAX) together with the Queensland Department of Agriculture and Fisheries (QDAF) through the Broadacre Cropping Initiative (BACI). The first author was also funded through the UniSQ Domestic PhD Scholarship program and received support from the Australian Government Research Training Program (RTP) Scholarship.

Data Availability Statement: Data is contained within the article or Supplementary Materials.

Acknowledgments: The authors would like to thank Jack Crimean (Queensland Department of Primary Industries) for the operation of field machinery and the Gwynne family for access to their property and their long-term support for our research.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

Abbreviation	Definition
ANOVA	Analysis of variance
BD	Bulk density
BTM	Back-transformed mean
CLL	Crop lower limit
CV	Coefficient of variance
DAS	Days after sowing
eBLUP	Empirical best linear unbiased prediction
FOV	Field of view
GRDC	Grains Research and Development Corporation
GWC	Gravimetric water content
MET	Multi-environment trial
LD	Low density
NDVI	Normalized difference vegetation index
PAW	Plant available water
PD	Plant density experiment
RLN	Root lesion nematode
SP	Single-plant experiment
UAV	Unmanned aerial vehicle
ULD	Ultra-low-density
VTR	Visual tolerance rating

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