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Investigating the Impact of Root-Lesion Nematodes (*Pratylenchus thornei*) and Crown Rot (*Fusarium pseudograminearum)* on Diverse Cereal Cultivars in a Conservation Farming System

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Abstract: In two experiments on a farm practicing conservation agriculture, the grain yield of a range of wheat cultivars was significantly ($p < 0.001$) negatively related to the post-harvest population densities of *Pratylenchus thornei* in the soil profile to 45 cm depth. In a third and fourth experiment with different rotations, methyl bromide fumigation significantly ($p < 0.05$) decreased (a) a low initial population density of *P. thornei* in the soil profile to 90 cm depth and (b) a high initial population of *P. thornei* to 45 cm depth, and a medium level of the crown rot fungus, *Fusarium pseudograminearum*, at 0–15 cm depth to a low level. For a range of wheat and durum cultivars, grain yield and response to fumigation were highly significantly ($p < 0.001$) related to (a) the *P. thornei* tolerance index of the cultivars in the third experiment, and (b) to both the *P. thornei* tolerance index and the crown rot resistance index in the fourth experiment. In the latter, grain yield was significantly ($p < 0.001$) positively related to biomass at anthesis and negatively related to percentage whiteheads at grain fill growth stage. One barley cultivar was more tolerant to both diseases than the wheat and durum cultivars. Crop rotation, utilizing crop cultivars resistant and tolerant to both *P. thornei* and *F. pseudograminearum*, is key to success for conservation farming in this region.

Keywords: root-lesion nematode; *Pratylenchus thornei*; crown rot; *Fusarium pseudograminearum*; wheat; durum; barley; soil fumigation; DNA based diagnosis

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1. Introduction

Root-lesion nematodes (*Pratylenchus thornei* Sher and Allen, 1953 and *P. neglectus* (Rensch, 1924) Filipjev and Schuurmans Stekhoven, 1941) are a serious threat to wheat (*Triticum aestivum* L.) production in Australia [1,2], where they have been estimated to cause losses valued at AU$123 M/annum [3]. These nematode species are also of global importance to wheat production in many other countries [4,5]. In the subtropical grain region of eastern Australia, also referred to as the northern grain region, *P. thornei* was found to be more widespread, and in higher population densities, than *P. neglectus* [6]. In this region, wheat and other grain crops are grown mainly on vertisols [7]. The high clay contents and deep soil profiles of vertisols can store soil water during fallow periods, which is an important factor for successful dryland cropping in this semi-arid environment [8].

Wheat was the dominant crop in rotations when root-lesion nematodes were first recognised as a problem in the Darling Downs area of the subtropical grain region, in the late 1970s [1]. Since then, there have been substantial changes in the farming systems used by growers in the region with a greater diversity of crops grown, and adoption of conservation farming measures including stubble retention, reduced tillage, soil testing to guide fertiliser use and monitoring of soil water to aid sowing decisions (Table 1).

Table 1. Change in farming practices on the Darling Downs of Queensland.

Agronomic Practice	Traditional Systems	Conservation Farming Systems
Crop rotations	• Continuous wheat, or 3 years of wheat then 1–2 years sorghum (*Sorghum bicolor*)	• Less wheat, and more summer crops and other winter crops
	• No grain legumes	• Grain legumes
Stubble management	• Stubble burnt	• Stubble retained
Tillage practices	• Full tillage • Random traffic	• Reduced tillage • Controlled traffic
Fertiliser practices	• None or little fertiliser use	• N fertiliser used based on deep soil testing • P and Zn fertilisers used based on topsoil testing
Soil water monitoring	• None, or monitoring by probing	• Soil coring to assess plant-available water before sowing

Better balanced rotations now include other cereals, coarse grains, particularly sorghum (*Sorghum bicolor* (L.) Moench), pulses and oilseeds. However, it has become apparent that some of these rotational crops can also host *P. thornei* [9].

Traditional farming practices were to burn stubble soon after harvest and mechanically till fallow land to control weeds. These practices have now been largely replaced by conservation tillage practices in which stubble is retained and weed control is largely with herbicides [10]. Conservation tillage practices have been shown to result in less erosion of the clay soils of this region [10,11], and to achieve better water infiltration into the soil profile. Better water infiltration occurs because the retained stubble protects the soil surface from raindrop impact, which causes soil disaggregation, resulting in decreased water penetration. In addition, mechanical tillage disrupts continuous macropores from root and earthworm channels and can form plough pans, which restrict water percolation. Conservation tillage practices work best to increase water infiltration and consequent grain yield when combined with controlled traffic to minimise the impact of farm machinery on soil compaction [12]. Conservation tillage practices also provide better conditions for soil biota than traditional tillage practices [13]. However, higher population densities of *P. thornei* were detected in the topsoil layer with zero tillage than with mechanical tillage of a vertisol in the long-term Hermitage Fallow Management Experiment [14]. *Pratylenchus thornei* was found to cause poor yield response to the extra stored soil water in the conservation tillage treatment by an intolerant wheat cultivar compared to a tolerant barley (*Hordeum vulgare* L.) cultivar [15]. Subsequent selection of a wheat cultivar for sowing each year that is the most *P. thornei*-tolerant cultivar available commercially at the time has resulted in wheat yields that better reflect the available water stored in the soil profile with the various stubble and tillage management treatments in the Hermitage Fallow Management Experiment [16]. It has been considered that continued retention of stubble in conservation agriculture might contribute to organic matter-mediated general suppression of *P. thornei* in the soil [17], thereby counteracting other environmental factors in conservation agriculture that promote reproduction and survival of *P. thornei*.

Cultivars of wheat and other susceptible crop species vary in their reaction to *P. thornei*, as characterized by levels of resistance and tolerance. There are various ways to characterize the resistance and tolerance of nematodes and fungal pathogens [18]. Here, we define a resistant cultivar as one that can retard nematode reproduction in its roots, resulting in lower final nematode population densities than a susceptible cultivar. Resistance of cereal cultivars to *P. thornei* can be determined under field conditions [19] or under more controlled conditions in the glasshouse [20]. Tolerance is best determined under the field conditions of the region where the crop is grown, so that relevant environmental conditions can interact with the nematode parasite and the host cultivar to result in a relevant yield outcome [21,22]. Here, we define a tolerant crop cultivar as one that can grow and yield

well in nematode-infested fields in comparison with an intolerant cultivar [22–25]. Both resistance and tolerance are quantitative traits and such information about commercial cultivars is provided to growers in the northern grain region on a nine-level ordinal rating system [26,27] derived from multi-experiment analysis of quantitative data [20,22]. Inheritance of resistance to *P. thornei* in wheat is polygenic with additive gene action [28–30]. Because *P. thornei* builds up to a lesser extent in the roots of resistant than susceptible wheat cultivars during the growing season, this can result in grain yield differences between cultivars [20]. Cultivars can also possess independent non-specific genetic factors [21] by which they yield better (tolerance) or worse (intolerance) than expected from their levels of resistance. The combined effects of such resistance factors and tolerance factors on the grain yield of wheat cultivars have been captured in a single tolerance index derived from a multi-environment trial analysis of grain yield on *P. thornei*-infested field sites [22].

Another major disease of winter cereals in the subtropical grain region of eastern Australia is crown rot caused by the fungus *Fusarium pseudograminearum* O'Donnell and T. Aoki (syn. *F. graminearum* Group 1), which has been estimated to cause losses of AU$79 M/annum Australia wide [3]. The incidence of this disease has increased with the change to conservation agriculture, and it is now the most important soil/stubble-borne disease, followed by root-lesion nematodes, as gauged by specific DNA levels in soil samples from Australian grain regions [31]. During its parasitic phase, *F. pseudograminearum* colonises the stem bases of wheat plants, resulting in browning that extends with the infection up the stem during the growing season. The disease is most severe when rainfall of 25 to 50 mm occurs within 6 to 8 weeks of plant emergence followed by a dry spring [32]. Interaction between water availability in the soil and the effects of the crown rot fungus reducing water conductance in the xylem can result in bleached heads, known as whiteheads or deadheads, that contain shriveled or no grain at the normal grain filling stage. Klein et al. [33] found that the loss of potential wheat yield was better correlated with percentage of whiteheads (r = 0.89) than with incidence of stem basal browning (r = 0.59) or infection with *Fusarium pseudograminearum* determined by plating stubble pieces on a selective agar medium (r = 0.58). Between crops, the fungus survives in stubble located above the soil surface or incorporated in the soil [34]. Consequently, it is unsurprising that burning stubble soon after harvest, compared with retaining stubble throughout the fallow period, results in a reduction in crown rot in the following crop [35]. Therefore, the change in farming practices towards stubble retention was seen as a contributing factor to an increase in the prevalence of crown rot as an issue for growers in the region [36,37]. However, the better storage of soil water with stubble retention can offset the interaction between crown rot disease and limited water supply in reducing yield [32]. Ratings of resistance to crown rot of cultivars of wheat and durum (*Triticum turgidum* L.) for growers [27,38] are provided on an ordinal scale of 1 (very susceptible) to 9 (resistant), derived using a formula incorporating both incidence (based on tiller numbers) and severity of browning of stem bases of mature plants from field experiments [32].

The 'National Soil Fumigation Project' was established in 2001 by the Grains Research and Development Corporation (GRDC) of Australia to assess the potential for increasing wheat production through removal of known and unknown biological constraints in the soil by methyl bromide fumigation. Methyl bromide was regarded as an effective broad-spectrum biocide with good soil penetration properties, which was available for use then, but has now been phased out, along with other ozone depleting organohalides, as part of the Montreal Protocol. Two types of trials were envisaged: (1) farm trials with small replicated plots imposed on farmer-managed fields, and (2) research trials with strips of fumigant imposed across established research experiments investigating management treatments such as crop rotation, type of tillage and stubble treatment. Although the program was nationally coordinated, participants were given the flexibility to investigate issues that they considered important using methods considered appropriate for their region. As part of this program, we used methyl bromide fumigation to quantify the effects of *P. thornei* on yield loss in wheat on a farm where the best conservation agriculture practices

were in use. Methyl bromide presented some advantages over other fumigants, such as chloropicrin or methyl isothiocyanate liberators such as dazomet that can considerably change the concentrations of available nutrients in soil [39,40], complicating interpretation of responses. Additionally, because methyl bromide has a vapour density of 3.3, it presented the opportunity for control of *P. thornei* occurring in vertisols deeper in the soil profile than 30 cm, which was the limit of effectiveness of the nematicide aldicarb in a previous study [41]. Aldicarb was the most effective of nematicides previously tested for control of *P. thornei* in vertisols in this region [40,42]

This paper reports an investigation into the yield responses of a range of cereal cultivars to *P. thornei* occurring in fields with best management practices for conservation agriculture, and their yield responses where *F. pseudograminearum* naturally co-occurred with *P. thornei* at damaging levels.

2. Materials and Methods

2.1. Field Experiments

Four field experiments were conducted over several years on a commercial grain farm near Macalister (Lat. 27.03° S, Long. 151.07° E; elevation 337 m), Australia. The farm combined good crop rotational practices with conservation tillage practices (zero-till, stubble retention, controlled traffic), as well as pre-plant soil sampling for monitoring of soil water and deep nitrate for best sowing decisions and fertiliser management practice. The soil at the site is a Black Vertosol [43] of the Bongeen soil type [44] containing 60% clay. Experiments 1 and 2 were designed to assess the grain yield of a range of wheat breeding fixed lines and named cultivars in the year 2001. Following the observation of poor growth of some cultivars known to be intolerant to *P. thornei*, the soil under a number of cultivars in both experiments was sampled in layers to 150 cm deep after harvest to characterize final *P. thornei* population densities. In this paper, we will refer to all fixed lines as 'cultivars', whether named for commercial release or with code numbers. The term cultivar here means 'cultivated variety' derived from plant breeding, and is used rather than 'genotype', a term best confined to genetic studies [45]. Experiments 3 and 4 were conducted to assess a range of cereal cultivars (11 bread wheat and one durum in Experiment 3 in 2002; and 16 bread wheat, three durum and one barley in Experiment 4 in 2003) for response to soil fumigation with methyl bromide. The land for the experiments had 92 kg N/ha applied as urea by the grower in March of each year. The cropping histories from farm records of the land used for the respective experiments are given in Table 2.

Table 2. Cropping histories of the field sites used for the experiments. Cultivar names are in parentheses.

Experiments 1 and 2	Experiment 3	Experiment 4
2001 Wheat cultivar experiments	2002 Fumigation experiment	2003 Fumigation Experiment
2000–2001 Black gram (*Vigna mungo* cv. Regur) 1999 Wheat (Hybrid Mercury)	2001 Chickpea (*Cicer arietinum*, mixed cultivars Jimbour, Kaniva, Howzat and Barwon)	2002 Chickpea (Howzat) 2001 Wheat (Kennedy) 2000–2001 Black gram (Regur)
1998 Barley (Skiff)	2000 Barley (Tallon)	1999 Wheat (Hybrid Mercury)
1997–1998 Sorghum (Buster MR)	1998–1999 Maize (*Zea mays* C76)	1998 Barley (Skiff)
1996–1997 Sorghum (Buster MR)	1997–1998 Sorghum (Buster MR)	1997–1998 Sorghum (Buster MR)
1995–1996 Sorghum (Buster MR)	1996–1997 Sorghum (Buster MR)	1996–1997 Sorghum (Buster MR)

2.2. Experiments

All experiments were conducted with three replicates of treatments in randomised complete blocks. In Experiments 1 and 2, the treatments were wheat cultivars laid out in the field as a row: column design [46]. In Experiments 3 and 4, the treatments comprised a full factorial of cereal cultivars by soil fumigation with two levels (nil and fumigated). Plot dimensions were 8 m long × 7 seed rows of 25 cm cut back to 6.5 m for harvest in

Experiments 1 and 2, and 8 m long × 7 rows of 25 cm with plots spaced 2.25 m apart and cut back to 5 m for harvest in Experiments 3 and 4. In Experiments 3 and 4, plots to be fumigated were trenched on all sides to 20 cm depth, covered with thick polyethylene tarpaulin, which was then sealed in the trench with soil. The soil was fumigated with methyl bromide (0.91 kg/10 m^2) by a commercial operator using the hot gas method [47]; tarpaulins were removed after 4 days. After 14 days, the soil was sampled and after 17 days, the experiments were sown. The various cereal cultivars were sown through the cone of a seed drill. Weighed quantities of seed, calculated from kernel weights and germination percentages, were sown for a target population of 100 viable seeds/m^2. For Experiments 1 and 2, fertiliser applied at sowing was urea supplying 55 kg N/ha as a side dressing, and with 40 kg/ha Starter Z (Granulock Z, Incitec Pivot) supplying 3.7 kg N/ha, 8.2 kg P/ha, 1.6 kg S/ha, and 1.0 kg Zn/ha applied in the seed drill rows. For Experiments 3 and 4, fertiliser applied at sowing in the seed drill rows was 75 kg/ha Starter Z supplying 7.0 kg N/ha, 15.4 kg P/ha, 3.0 kg S/ha, and 1.9 kg Zn/ha.

Grain from all experiments was harvested when ripe (Zadoks growth stage Z93 [48]) with a combine harvester (Kingaroy Engineering Works, Kingaroy, Australia). Grain moisture percentage was determined by drying a 100 g subsample in a forced draught oven at 80 °C for 2 days. Grain yield was expressed in kg/ha at 12% moisture equivalent, calculated from harvested grain weight/plot and grain moisture percentage.

2.3. Soil Sampling

Deep soil coring of Experiments 1 and 2 was undertaken in early February following harvest of wheat cultivars in late November. Deep soil coring of Experiments 3 and 4, was undertaken in July at 15 days after the tarpaulins for fumigation were removed. Cores of 4.5 cm diameter were taken at four evenly spaced intervals from the plots using an hydraulically operated rig. The cores were divided into depth intervals of 0–15, 15–30, 30–45, 45–60, 60–90, 90–120, and 120–150 cm. The four cores from each plot were bulked in respective depth intervals, and sealed in plastic bags for transport to the laboratory where they were stored at 4 °C pending processing for analysis. The soil samples were manually broken into pieces <1 cm, mixed, and subsamples were taken for determination of soil water and nematode population densities, and for chemical analyses.

2.4. Soil Water

Soil gravimetric water content (GWC) was determined on a 100 g subsample dried in a forced draught oven at 105 °C for 2 days. Volumetric water content (VWC) was calculated from GWC and pre-determined bulk densities (BD) for each soil layer. Plant-available water (PAW) at sampling was calculated from VWC and pre-determined crop lower limits (CLL) for wheat in depth intervals, then summed over the profile to 150 cm using the following Equations:

$$\mathrm{GWC}(\%) = 100 * (\mathrm{FWt} - \mathrm{DWt})/\mathrm{Dwt} \quad (1)$$

$$\mathrm{VWC}(\%) = \mathrm{GWC} * \mathrm{BD} \quad (2)$$

$$\mathrm{PAW}(\mathrm{mm}) = (\mathrm{VWC} - \mathrm{CCL}) * \text{soil layer thickness } (\mathrm{cm})/10 \quad (3)$$

where GWC = gravimetric water content (%), FWt = fresh weight, DWt = oven dry weight, VWC = volumetric water content (%), BD = bulk density (g/mL), PAW = plant-available water (mm), CLL = crop lower limit for wheat (%).

Bulk densities and crop lower limits for the soil had been determined using methods described by Dalgliesh and Foale [49]. The bulk densities used for the various depth intervals were 1.1 for 0–15; 1.11 for 15–30, 30–45 and 45–60; 1.13 for 60–90; 1.15 for 90–120; and 1.18 for 120–150 cm (N. Dalgliesh pers. comm.). The crop lower limits used for the various depth intervals were 28% for 0–15 and 15–30; 29% for 30–45 and 45–60; 30% for 60–90; 33% for 90–120; and 35% for 120–150 cm (N. Dalgliesh pers. comm.).

2.5. Pratylenchus Thornei, other Nematode Species and Fusarium Pseudograminearum

Nematodes were extracted using the Whitehead tray method [50] from a 150 g field-moist subsample of soil in a constant temperature room at 22 °C for 48 h. Nematodes in the extract were collected on a 20-µm mesh sieve and concentrated into ~15 mL water then stored in a specimen vial at 4 °C until counting. Nematodes were counted in a 1-mL Peters slide [51] (Chalex Corporation, Portland, Oregon, USA), with a compound microscope (Olympus BH-2, Tokyo, Japan) at ×40 and ×100 magnifications. *Pratylenchus thornei* were identified morphologically [52], and counts were expressed as number of *P. thornei*/kg soil (oven dry equivalent).

Subsamples of 300 g of undried soil were tested for nematode and fungal pathogens by the Root Disease Testing Service (RDTS) of the South Australian Research and Development Institute (SARDI). Quantitative PCR assays using species-specific rDNA sequences for the nematode species *P. thornei, P. neglectus* and the cereal cyst nematode *Heterodera avenae* (Wollenweber 1924), and the fungus *F. pseudograminearum* were applied to DNA extracted from the bulk soil [53]. Results for this DNA based method were expressed in equivalent number of nematodes/g of soil from prior calibrations of DNA extracted from known numbers of nematodes [53], and in pico grams of *F. pseudograminearum* DNA/g soil.

2.6. Soil Chemical Properties

For Experiments 3 and 4, subsamples of 150 g of field moist soil were extracted with 1 N KCl for determination of nitrate-nitrogen and ammonium-nitrogen (Code no. 7C2, [54], and results were expressed as mg N/kg dry soil. These values were converted to kg/ha by multiplying by the appropriate bulk density and depth interval in decimetres, then summing over the profile to 150 cm depth. Other subsamples of 100 g were dried in a forced draught oven at 40 °C for 4 days, then ground to pass a 4 mm sieve. These samples were analysed for (a) Colwell [55] bicarbonate-extractable phosphorus (Code No. 9B2) [54]; (b) DTPA-extractable zinc [56] with analysis by atomic absorption spectrometry (Code 12A1) [54], and pH of 1 soil: 5 water suspension (Code No. 4A1) [54]. Duplicate undried soil samples (equivalent of 20 g oven-dry weight) were moistened to 56% moisture content (equivalent to pF 2) in sterile glass jars (60 mm diameter × 70 mm high), sealed with polythene film and incubated at 30 °C for 28 days. After incubation, one sample was extracted with 100 mL of 2N potassium chloride, and analysed for ammonium-nitrogen and nitrate-nitrogen as described above. The other incubated sample was dried at 40 °C and analysed for phosphorus, zinc and pH as described above.

2.7. Crop Measurements

In Experiment 3, plant biomass was determined at stem elongation (growth stage Z32 [48]. Plants were counted in two 0.5 m lengths of row selected at random from the middle rows of the plots, and then plants were cut at ground level and removed in paper bags. The plant biomass was dried in a forced draught oven at 65 °C for 4 days, and after weighing, was ground for chemical analyses.

In Experiment 4, marked differences between plots in the growth of plants was observed at late stem elongation (Z35), with symptoms of *P. thornei* damage evident in a number of the cultivars. Plots were rated on a 1 to 6 scale for symptoms of crop yellowing, stunting, and poor canopy closure (Table 3).

Table 3. Rating system of plant growth and symptoms at late stem elongation (Z35) in Experiment 4.

Growth Rating	Description of Plants
1	stunted with marked leaf chlorosis and necrosis
2	severe lower leaf chlorosis, reduced tillering, and poor canopy closure
3	lower leaf chlorosis, reduced biomass, and ~50% canopy closure of inter-row space
4	green with some lower leaf chlorosis, and ~60% canopy closure of the inter-row space
5	green with ~80–90% leaf closure of the inter-row space
6	dark green with full canopy closure of the inter-row space.

Plant biomass was determined at anthesis (Z65) [48] by cutting two quadrats of 1 m × 2 rows at approximately one third the distance into the plots from each end. Biomass was dried at 80 °C in a forced draught oven for 4 days before weighing. Whiteheads were evident in the plots at the grain filling stage (Z75–80) [48] and these whiteheads were on culms with basal stem browning indicative of crown rot. The percentage of whiteheads on a whole plot basis was estimated visually.

2.8. Plant Chemical Analysis

The dried plant material taken at Z32 in Experiment 3 was ground and subsamples were subjected to micro-Kjeldahl digestion before autoanalysis for nitrogen [57] and phosphorus [58]. Other subsamples were subjected to di-acid digestion (nitric-perchloric [59]), then analysed for zinc by atomic absorption spectrometry. Bromine concentrations were determined in other subsamples by x-ray fluorescence analysis.

2.9. Statistical Analyses

All soil and plant data were analysed by analysis of variance (ANOVA) and where the *F* test was statistically significant, Fishers protected least significant difference (l.s.d.) at $p = 0.05$ was used to compare treatments. Nematode count data were transformed by ln(x+1) and percentage whiteheads by arcsine$\sqrt{\%}$ before statistical analysis for conformation to normal distributions. Linear regression analysis was used to relate grain yield of wheat cultivars to final population density of *P. thornei* in the soil profile to 45 cm for Experiments 1 and 2. Correlation coefficients between grain yield and other measures of plant performance during the growing season were calculated for Experiment 4. Multiple regression was used in Experiment 4 to relate grain yield to growth rating or biomass at anthesis and percentage whiteheads (transformed by arcsine $\sqrt{\%}$). Multiple regression was used to relate grain yield from nil-treated soil, or percentage change in grain yield with soil fumigation, as response variates, to indices for tolerance to *P. thornei* [22] and resistance to crown rot ([38], G.B. Wildermuth pers. comm.) as explanatory variates for wheat and durum cultivars in Experiments 3 and 4. The *P. thornei* tolerance index used was derived from independent multi-experiment analysis based on predicted grain yield (t/ha) of wheat and durum cultivars grown on *P. thornei*-infested field sites [22]. The crown rot resistance index used was an ordinal rating system of 1 (very susceptible) to 9 (resistant) based on stem browning from independent field experiments ([38], G.B. Wildermuth pers. comm.). Statistical analyses were conducted in Genstat [60].

2.10. Rainfall

The monthly and annual rainfalls relevant to the experiments are given in Table 4, in comparison with average long-term monthly and annual rainfalls from the nearest Bureau of Meteorology (BOM) site. For both years in which the fumigation Experiments 3 and 4 were conducted there was little rainfall in May, but approximately 50 mm in June, which moistened the soil for subsequent effective fumigation and sowing of the experiments in July. For Experiment 3, there was substantial rainfall in August for nodal root development, and some rainfall in October for grain set, whereas in Experiment 4, there was some rainfall in August for nodal root development and a substantial amount in October for grain development.

Table 4. Rainfall in experiment years compared with long-term averages (1972–2021) of Bureau of Meteorology (BOM site no. 41469) records from Macalister (4 km from experiment site).

		Rainfall (mm)												
Experiment No.	**Year**	**J**	**F**	**M**	**A**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**Total**
Exps 1 and 2	2001	32	135	41	54	14	0	21	8	18	83	119	44	568
Exp 3	2002	58	60	88	1	5	48	0	61	7	30	22	92	472
Exp 4	2003	30	108	150	50	14	51	13	18	8	89	22	154	707
Macalister BOM		83	75	57	33	42	31	31	28	32	65	69	92	636

3. Results

3.1. Experiment 1

The final population densities of *P. thornei*, occurring in layers in the soil profile to 120 cm deep after harvest of wheat cultivars in Experiment 1, are shown in Figure 1a as ln(*P. thornei*/kg soil), with the back-transformed means in Figure 1b. In ANOVA, there were highly significant effects of cultivar ($p < 0.001$) and soil depth ($p < 0.001$), and a significant cultivar x depth interaction ($p = 0.03$). Greatest population densities were after Strzelecki in the 15–30 and 0–15 soil layers with 23,500 and 21,500 *P. thornei*/kg soil, respectively. In comparison, the population densities in unplanted soil at these depths were 5600 and 5100, respectively. Other cultivars with a relatively higher population density in the 15–30 cm layer were Sunco, Petrie and Gregory, whereas cultivars Batavia and Cunningham had relatively high population densities in both 0–15 and 15–30 cm depth intervals, and Giles and Sunvale had relatively higher population densities somewhat deeper in the 15–30 and 30–45 cm layers. Overall, cultivars Hartog and QT8447 had the lowest population densities in the soil profile, not much greater than the unplanted soil.

There was a wide range in grain yield from 730 kg/ha for Batavia to 2410 kg/ha for QT8447 in Experiment 1 (Figure 2a). Other higher yielding cultivars were Sunvale, Baxter, Hartog, Gregory and Giles, while other lower yielding cultivars were Cunningham, Strzelecki, Petrie, and Sunco. There was a highly significant linear regression relationship between grain yield and *P. thornei* population density in the 0–15 cm layer ($R^2 = 0.603$, $p = 0.003$, d.f. = 9). This relationship increased in significance as greater depths were taken into consideration to reach a maximum at 0–45 cm ($R^2 = 0.734$, $p < 0.001$, $n = 9$) (Figure 2b,c). The regression relationships then decreased in significance gradually with successive depths to become $p = 0.006$ at 0–120 cm and fell ultimately to $p = 0.056$ at 0–150 cm.

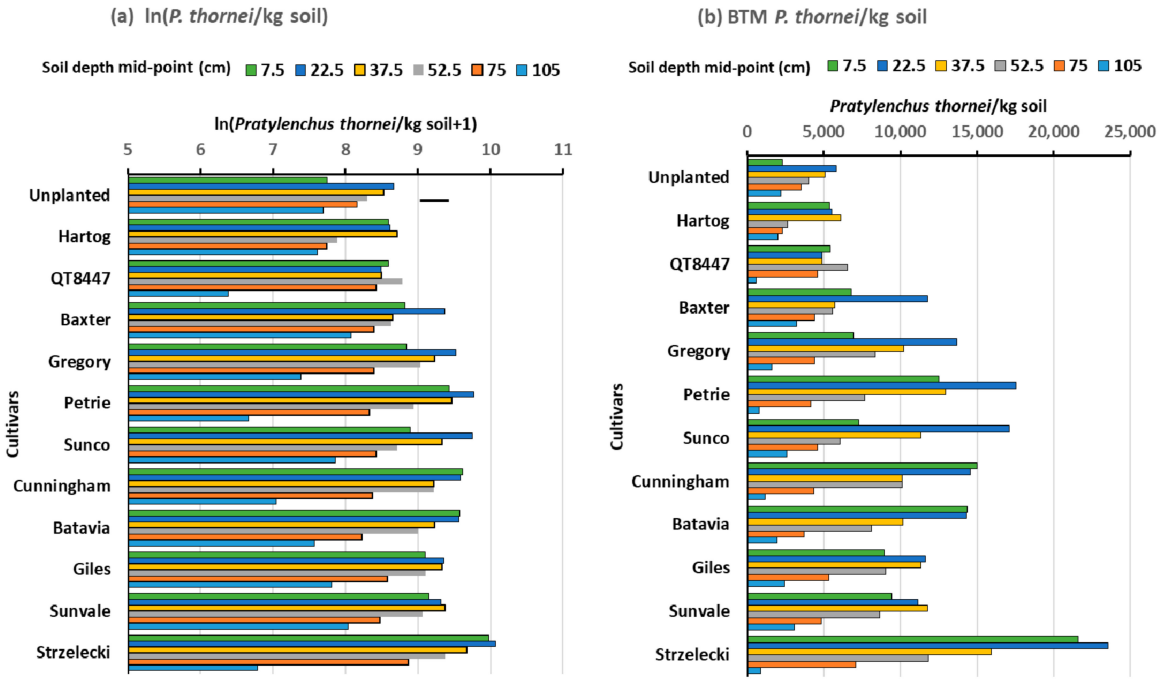

Figure 1. Population densities of *Pratylenchus thornei*, in depth intervals of 0–15, 15–30, 30–45, 45–60, 60–90 and 90–120 cm in the soil profile, after harvest of 11 wheat cultivars in Experiment 1, (**a**) mean values for ln(*P. thornei*/kg soil+1) from ANOVA with bar marker representing l.s.d. ($p = 0.05$) for cultivar x depth interaction, (**b**) back-transformed values of number of *P. thornei*/kg soil. Cultivars are arranged in ascending order of mean *P. thornei* population density.

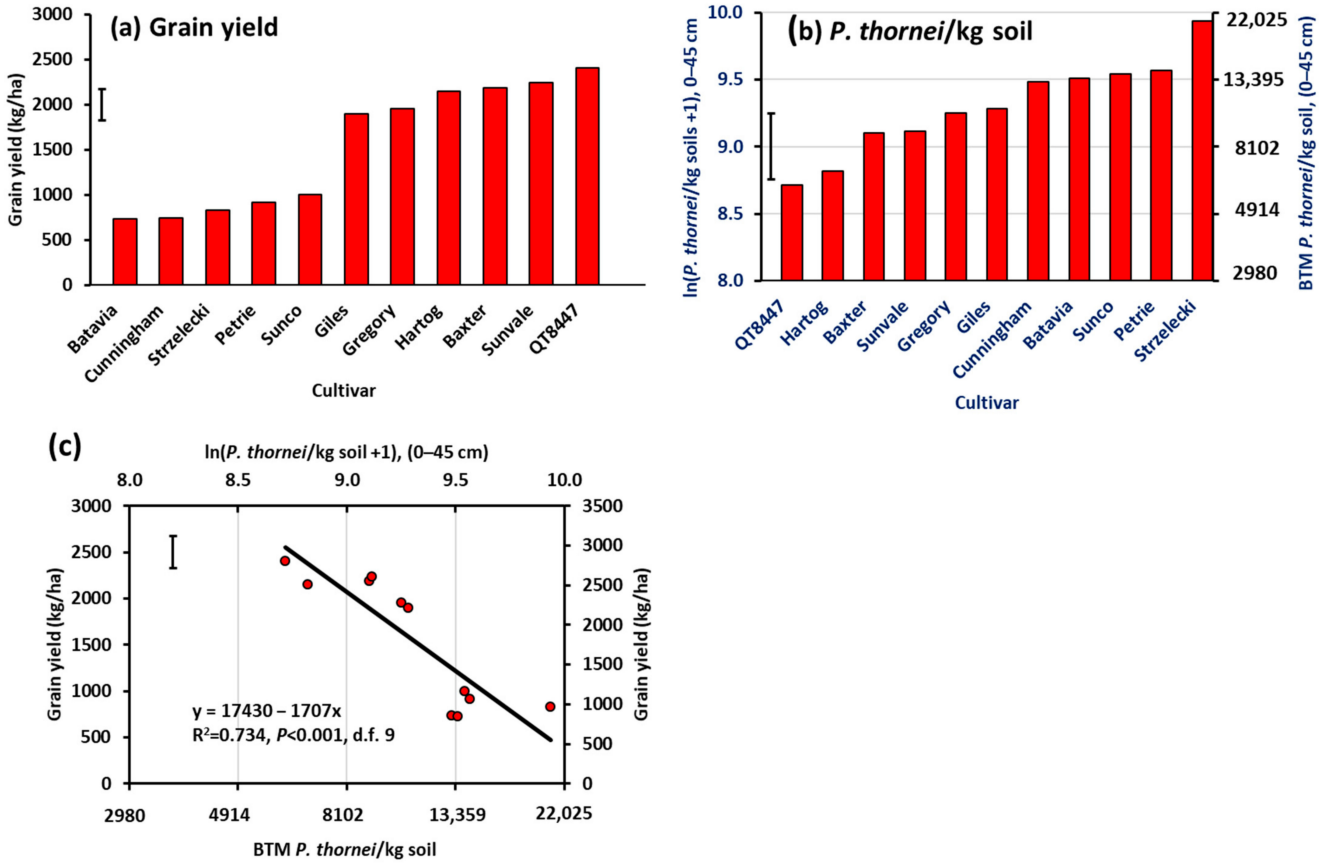

Figure 2. (**a**) Grain yield of 11 wheat cultivars in Experiment 1, (**b**) mean ln(*P. thorneiI*/kg soil +1) of final population densities in the soil profile to 45 cm depth after harvest, and (**c**) regression relationship between yield and *P. thornei* population density. Cultivars are in ascending order of value on the *Y*-axis in (**a**,**b**). BTM = back-transformed mean. Bar markers represent the l.s.d. ($p = 0.05$).

3.2. Experiment 2

The final population densities of *P. thornei* in layers in the soil profile to 120 cm deep, after harvest of the wheat cultivars in Experiment 2, are shown in Figure 3a as ln(*P. thornei*/kg soil) with the back-transformed means in Figure 3b. In ANOVA, there were highly significant effects of cultivar ($p < 0.001$) and soil depth ($p < 0.001$), but a non-significant interaction. Greatest population densities were in the 15–30 and 0–15 layers with respective averages across all cultivars of 12,250 and 11,150 *P. thornei*/kg soil. Average population density then diminished further down the profile to be 8300 at 30–45, 4930 at 45–60, 4000 at 60–90 and 1170 at 90–120. Greatest population densities at any depth in the soil profile were 27,580 after Janz and 26,130 after QT8368, both at 15–30 cm (Figure 3b). Additionally, over the whole soil profile to 120 cm, these two cultivars had the highest average population densities with 10,800 *P. thornei*/kg soil for QT8368, and 10,400 for Janz. Lowest average population densities were for QT8620 with 2290 *P. thornei*/kg soil, and for QT9050 with 3420 *P. thornei*/kg soil, compared with unplanted soil with 820 *P. thornei*/kg soil (Figure 3b).

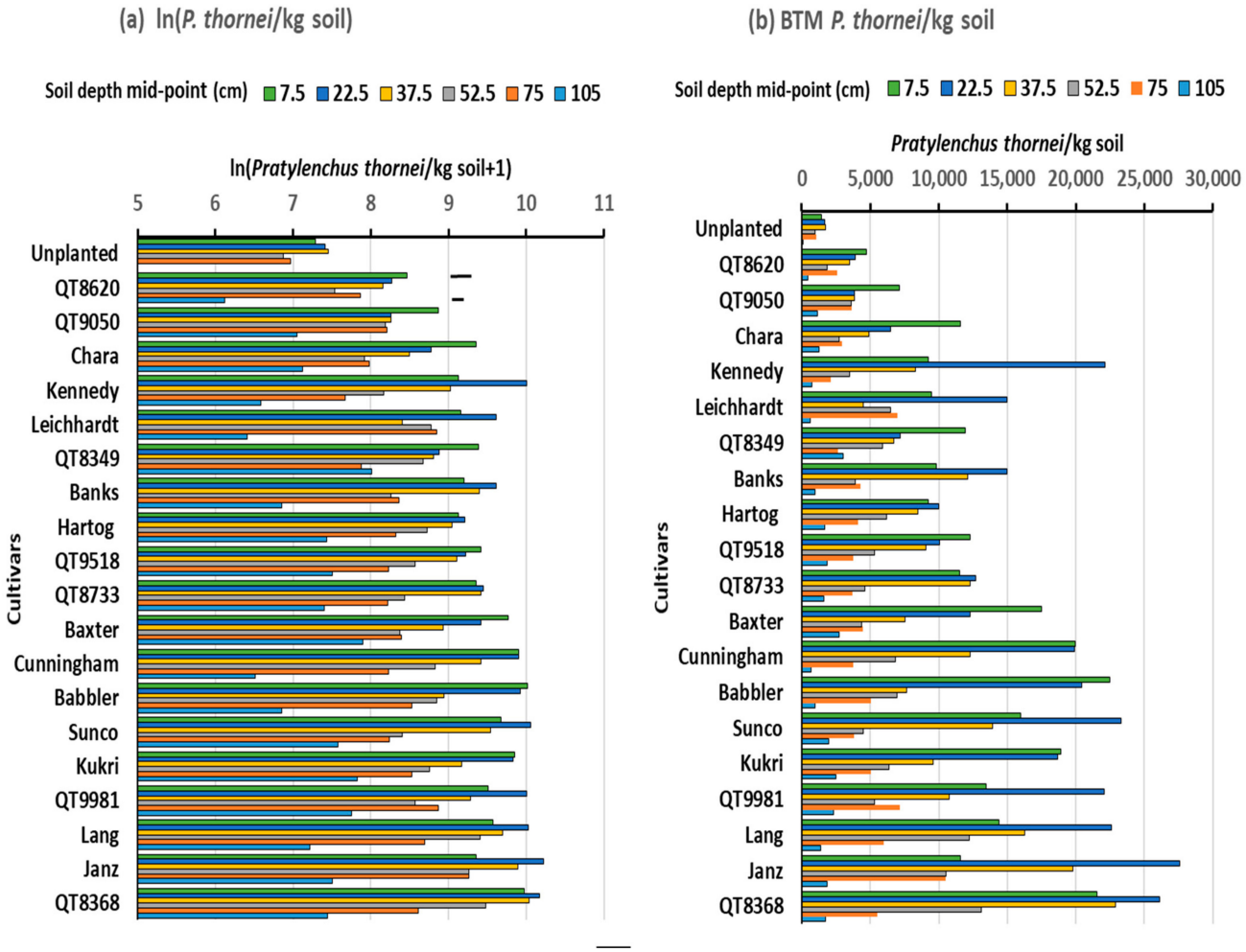

Figure 3. Population densities of *Pratylenchus thornei* in the soil profile in depth intervals of 0–15, 15–30, 30–45, 45–60, 60–90 and 90–120 cm after harvest of 19 wheat cultivars in Experiment 2, (**a**) mean values for ln(*P. thornei*/kg soil+1) from ANOVA with bar markers ($p = 0.05$) for main effects of cultivar and soil depth, (**b**) back-transformed values of number of *P. thornei*/kg soil. Cultivars are in ascending order of mean *P. thornei* population density.

Grain yield ranged from the least for Banks of 920 kg/ha up to the most for Chara of 3210 kg/ha in Experiment 2 (Figure 4a). Other cultivars with higher yields were QT8620, Hartog, QT8349, and Baxter. Other cultivars with lower yields were QT8368, Babbler and Sunco. There was a significant linear regression relationship between grain yield and *P. thornei* population density in the 0–15 cm depth ($R^2 = 0.268$, $p = 0.013$, $n = 17$), which became highly significant when greater depths in the soil profile were taken into account. The regression relationships between grain yield and population densities of *P. thornei* in the soil profile for 0–45 cm depth had the greatest percentage of variation explained ($R^2 = 0.531$, $p < 0.001$, $n = 17$) (Figure 4b,c) compared with other profile depths.

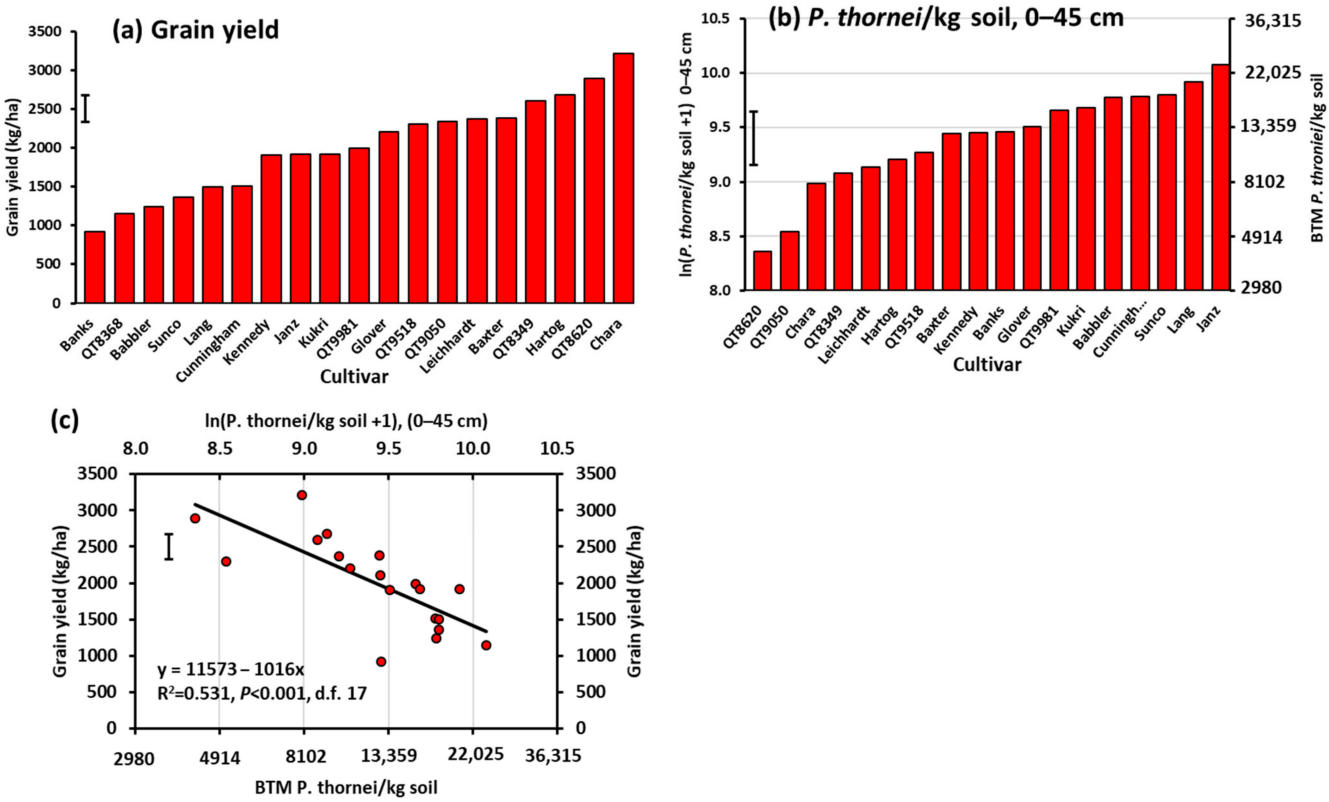

Figure 4. (**a**) Grain yield of 19 wheat cultivars in Experiment 2, (**b**) mean ln(*P. thorneiI*/kg soil +1) of final population densities in in the soil profile to 45 cm depth after harvest, and (**c**) regression relationship between yield and *P. thornei* population density. Cultivars are in ascending order of value on the *Y*-axis in (**a**,**b**). BTM = back-transformed mean. Bar markers represent the l.s.d. (p = 0.05).

3.3. Experiment 3.

The soil before sowing Experiment 3 contained a low population density of *P. thornei* in the topsoil (1100/kg soil), increasing with depth to a peak of 2400/kg soil in the 60–90 cm layer (Figure 5). Fumigation resulted in 100% reduction in the population density of *P. thornei* at all depths to 45 cm, 74% reduction in the 45–60 cm layer and 23% reduction in the 60–90 cm layer (Figure 5).

Results for nematodes in the topsoil (0–15 cm) using DNA quantification were the equivalent of 3500 ± 630 *P. thornei*/g soil, n = 15, with no *P. neglectus* or *H. avenae* detected. There was a very low level of *F. pseudograminearum* with 7.0 ± 3.25 pico g DNA/g soil, n = 15, in the nil-treated topsoil, and 1.3 ± 0.99 pico g DNA/g soil, n = 15, in the fumigated topsoil.

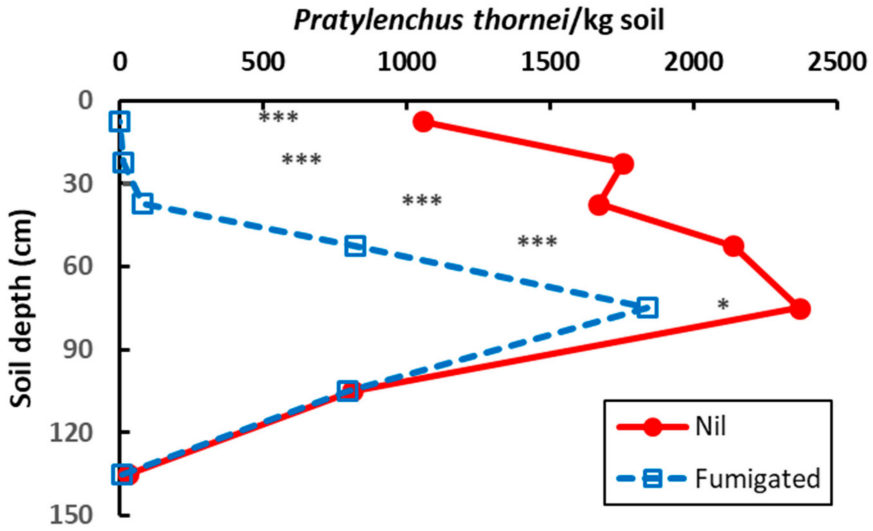

Figure 5. Effects of fumigation with methyl bromide on population density of *Pratylenchus thornei* in the soil profile before sowing Experiment 3. Points are the back-transformed values from means of ln (x + 1) transformations (n = 15), where significant differences between transformed means for nil and fumigated treatments at the respective depth intervals are indicated as * = $p < 0.05$ and *** = $p < 0.001$.

There was no significant difference between fumigated and nil-treated soil in the distribution of soil water in the profile to 150 cm with both treatments having a volumetric water content well above the crop lower limit (Figure 6a). The total quantity of plant-available water in the whole soil profile (0–150 cm) of fumigated soil (124 ± 3.3 mm) was very similar to nil-treated soil (117 ± 2.4 mm). There was no significant difference between fumigated and nil-treated soil in the distribution of nitrate-nitrogen in the profile (Figure 6b), or in the total quantity of nitrate-nitrogen in the soil profile (0–150 cm) between fumigated soil (151 ± 13.4 kg NO_3–N/ha) and nil-treated soil (150 ± 12.5/ha).

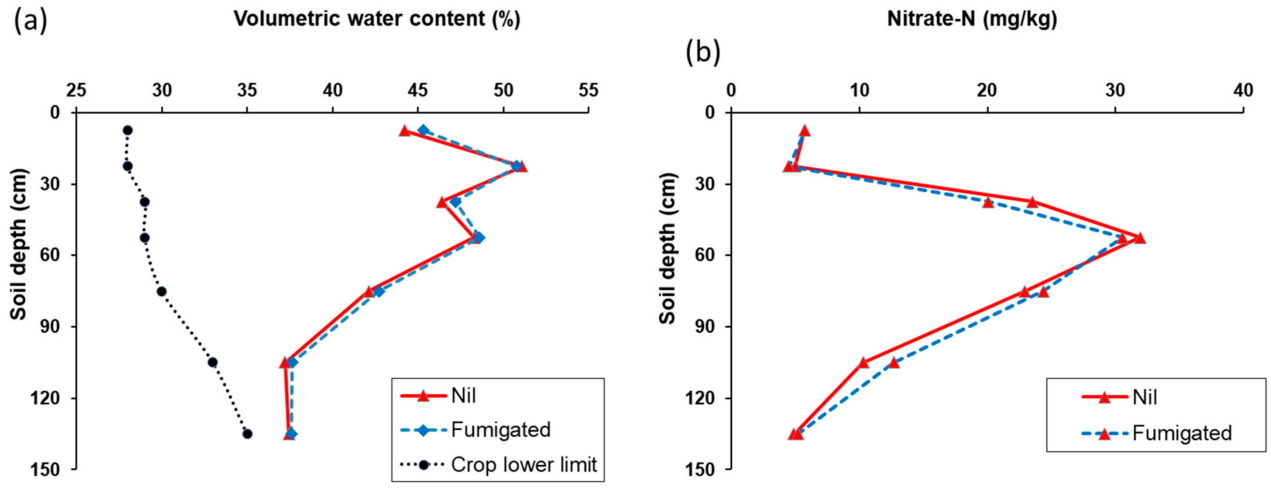

Figure 6. (**a**) Volumetric water content (%), and (**b**) nitrate-nitrogen concentration (mg/kg) in the soil profile from fumigated and nil treatments before sowing Experiment 3. There were highly significant differences due to soil depth for both soil water and nitrate nitrogen, but not due to fumigation or interaction of fumigation and soil depth in ANOVA.

Chemical analysis of soil samples taken before sowing indicated that methyl bromide fumigation resulted in a small increase in ammonium-nitrogen (Table 5). Phosphorus and zinc concentrations were both 3 to 4 times greater in the 0–15 cm layer than in the 15–30 cm layer (Table 5). There was slightly less available phosphorus and slightly more

available zinc in the fumigated than in the nil-treated soil at 15–30 cm. After incubation, the ammonium-nitrogen in the untreated soil had increased, but not in the fumigated soil, and the nitrate had decreased more so in the nil-treated soil than in the fumigated soil (Table 5). After incubation, available phosphorus and zinc were slightly lower in fumigated soil than in nil-treated soil, whereas there was no difference in soil pH.

Table 5. Effect of fumigation on soil chemical properties in Experiment 3, before and after incubation for 28 days. Values are the means of n = 15, with SE in parentheses, n = 15.

Soil Depth (cm)	Fumigation	NH_4–N (mg/kg)	NO_3–N (mg/kg)	[a] P (mg/kg	[b] Zn (mg/kg)	[c] pH
Pre-incubation 0–15	nil	1.83 (0.65)	16.50 (0.78)	17.81 (1.55)	0.67 (0.06)	8.07 (0.05)
	fumigated	2.90 (0.41) [1]	16.80 (0.67)	17.86 (1.33)	0.80 (0.09)	8.19 (0.05)
15–30	nil	1.21 (0.92)	15.48 (0.52)	4.86 (0.40)	0.27 (0.04)	8.44 (0.04)
	fumigated	2.80 (1.16)	12.80 (0.31)	3.90 (0.33)	0.23 (0.03)	8.61 (0.02)
Post-incubation 0–15	nil	8.24 (0.04)	5.70 (0.17)	14.91 (1.02)	0.76 (0.10)	8.11 (0.06)
	fumigated	2.01 (0.02)	10.56 (0.17)	13.77 (0.93)	0.52 (0.06)	8.14 (0.06)

[a] P (Colwell bicarbonate extraction [55]; [b] Zn (DTPA extraction [56]; [c] pH (1 soil: 5 water).

There was no significant difference in the number of plants/m^2 at Z32 (stem elongation) due to either cultivar or fumigation in ANOVA with an overall mean of 69.0 plants/m^2. There was a range of biomass production at Z32 among the nil-treated cultivars with a two-fold increase from Banks to Wollaroi (Figure 7a). Soil fumigation resulted in increased biomass of all cultivars, there being a significant fumigation x cultivar interaction (Figure 7a). The percentage response to fumigation in biomass ranged from least for the cultivar Hartog (35%) to greatest for QT10162 (119%). The nitrogen concentration of the biomass (Figure 7b) differed significantly between cultivars, but differences due to fumigation were not significant. There were significant differences between cultivars in phosphorus concentration of the biomass, and fumigation decreased phosphorus concentration with no interaction between fumigation and cultivars in ANOVA (Figure 7c). Overall, fumigation increased zinc concentration of the biomass (Figure 7d). There were significant differences between cultivars for zinc concentration, with no interaction between fumigation and cultivar. QT9050 had the lowest zinc concentration while the durum wheat Wollaroi had the highest.

Because the wheat in the fumigated plots was observed to have a slight bronzed appearance and tip necrosis of the lower leaves, the dried plant material obtained at Z32 was analysed for bromide. There was a highly significant effect of fumigation on bromide concentration with a mean value of 5284 mg Br/kg for wheat from the fumigated plots, compared with 97 mg Br/kg from the nil plots. There were no significant cultivar or fumigation x cultivar interaction effects in ANOVA of bromide concentration.

There was an exponential relationship between the percentage response to fumigation of dry weight per plant and the percentage response to fumigation of plant P concentration (Figure 8). This indicates that (1) cultivars that responded most negatively in P concentration to fumigation were those that responded least positively to fumigation in dry weight per plant, and (2) cultivars that responded most positively in P concentration to fumigation were those that responded most positively in dry matter to fumigation.

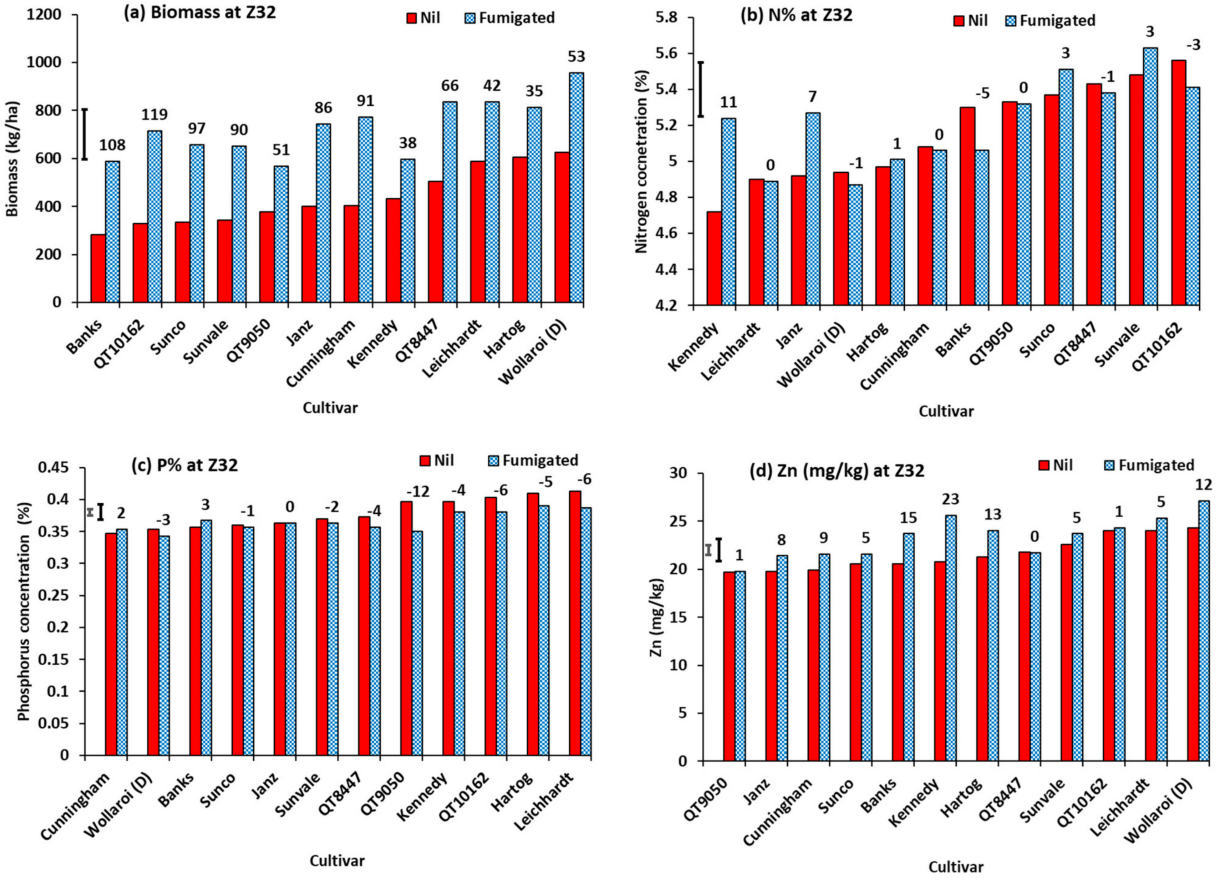

Figure 7. Response of wheat and durum cultivars at stem elongation (Z32) to soil fumigation with methyl bromide in Experiment 3, (**a**) plant biomass, (**b**) N concentration, (**c**) P concentration and (**d**) Zn concentration. Bar markers represent the l.s.d. for (**a**) fumigation x cultivar interaction, (**b**) cultivar main effect and (**c**,**d**) main effects of fumigation and cultivar. (D) signifies a durum cultivar. Each graph is presented with nil-treated cultivars in ascending order of *Y*-axis value. Values above bars are the percentage response to fumigation.

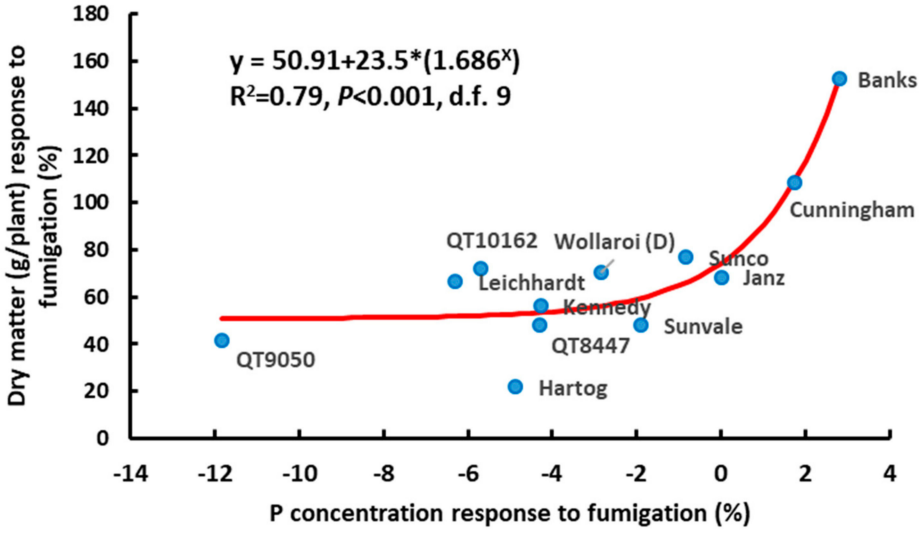

Figure 8. Relationship between percentage response to fumigation of plant dry matter at Z32 (stem elongation) and percentage response to fumigation of phosphorus concentration in Experiment 3 of wheat and durum (D) cultivars.

There was a significant interaction between cultivars and soil fumigation in grain yield (Figure 9). Without fumigation, grain yield ranged from 965 kg/ha for Banks up to 2354 kg/ha for QT9050. The percentage response to soil fumigation ranged from 71% for Banks down to 5% and 7% for Leichhardt and QT9050, respectively. No crown rot browning of stem bases or whiteheads was evident in the crop. In multiple regression analysis, both grain yield of the nil treatment, and percentage response to fumigation of grain yield, were highly significantly related to the *P. thornei* tolerance index, but not to the crown rot resistance index as shown in the following Equations.

$$\text{Ynil} = -182 + 702(PtTI{**}) - 9.3(\text{CR n.s.}),\ \text{R}^2 = 0.56,\ p = 0.01,\ \text{df} = 9 \quad (4)$$

$$\text{Y\%resp} = 1.225 - 0.393(PtTI{***}) - 0.04(\text{CR n.s.}),\ \text{R}^2 = 0.64,\ p = 0.004,\ \text{df} = 9 \quad (5)$$

where Ynil = grain yield (kg/ha) from nil treatment; Y%resp = percentage response to soil fumigation of grain yield; *PtTI* = the *P. thornei* tolerance index in t/ha; and CR = the crown resistance index on ordinal scale of 1 to 9. Significance of coefficients indicated ** = $p < 0.01$, *** = $p < 0.001$, n.s. = non-significant.

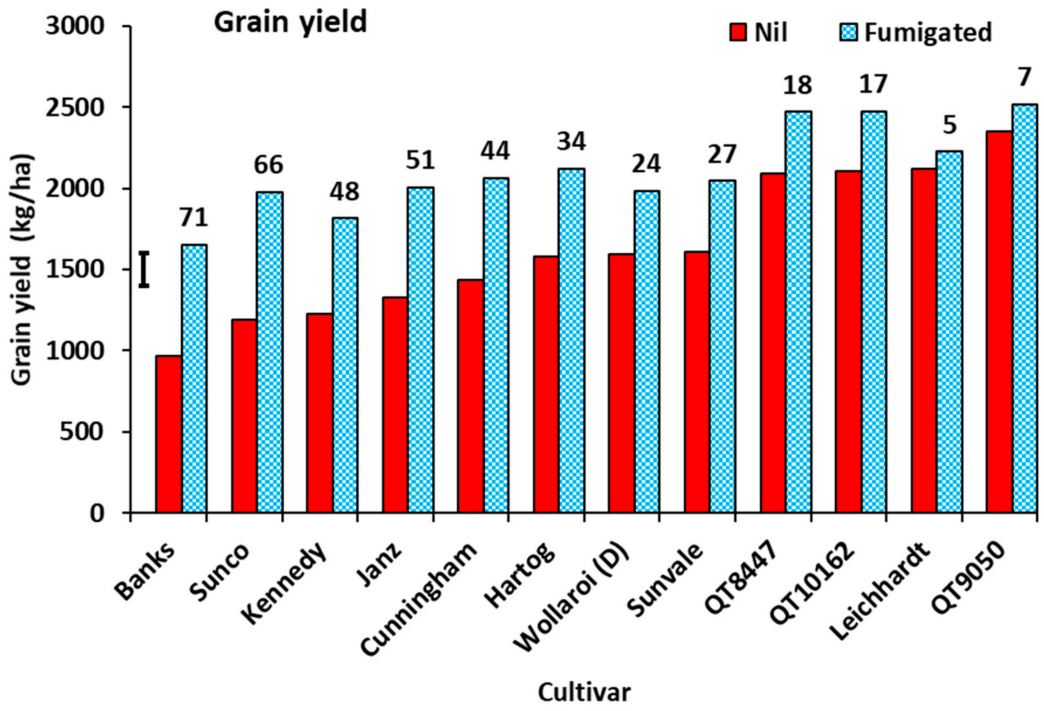

Figure 9. Grain yield of wheat and durum cultivars in response to soil fumigation with methyl bromide in Experiment 3. The graph is presented with cultivars in ascending order of grain yield from nil treatment. Bar marker represents l.s.d. (p = 0.05) for fumigation x cultivar interaction. (D) signifies a durum wheat cultivar. Values above bars are the percentage response to fumigation.

There was a highly significant relationship (p = 0.002), between percentage response to fumigation of grain yield and percentage response to fumigation in phosphorus concentration in the plant tissue at Z32 (Figure 10).

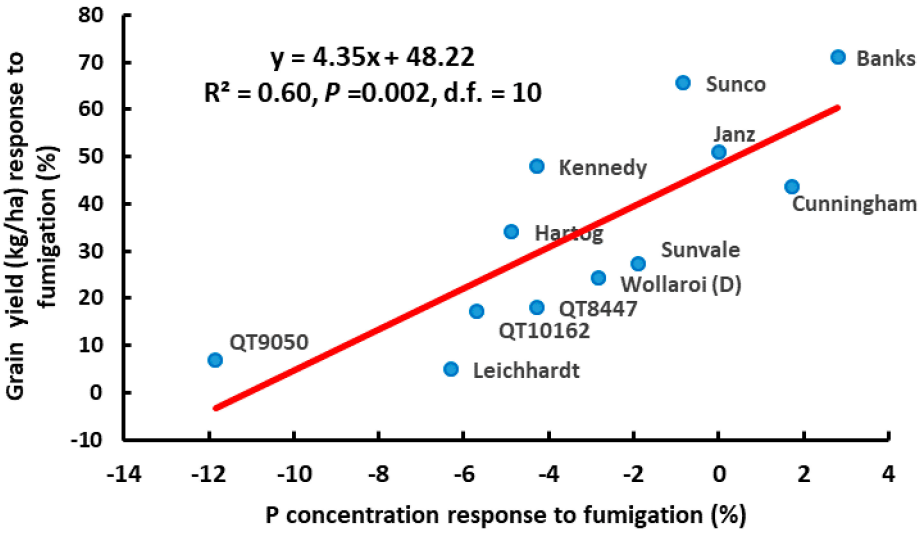

Figure 10. Relationship between response to fumigation of grain yield (kg/ha) and response to fumigation of phosphorus concentration (%) in wheat plants at Z32 (stem elongation) in Experiment 3.

3.4. Experiment 4.

Soil from nil-treated plots before sowing Experiment 4 contained a high population density of *P. thornei* with 7040/kg soil at 0–15 cm and a maximum population density of 12,690/kg soil at 15–30 cm (Figure 11a). The *P. thornei* population density then decreased gradually with depth to 2130/kg soil at 90–120 cm. Fumigation significantly reduced the *P. thornei* population density by 100% at 0–15 cm, 77% at 15–30 cm and 31% at 30–45 cm (Figure 11a). A similar pattern of *P. thornei* population density in the soil profile, and the effect of fumigation on it, was seen in the results for the DNA assay of *P. thornei* (Figure 11b). The equivalent estimated population density by DNA assay was several fold greater than that estimated from counting extracted live *P. thornei*. Mean crown rot DNA was 130 pico g/g soil in the 0–15 cm layer (considered a medium risk for wheat and a high risk for durum; RDTS pers. comm.) (Figure 11c). Fumigation reduced crown rot DNA to 33 pico g/g (considered a low risk for wheat and medium risk for durum; RDTS pers. comm.).

There was no significant difference between fumigated and nil-treated soil in the distribution of soil water in the soil profile (Figure 12a). Similarly, there was no significant difference in plant-available water in the whole soil profile (0–150 cm) between fumigated soil (200 ± 5.3 mm) and nil-treated soil (205 ± 5.3). The distribution of ammonium and nitrate nitrogen in the soil profile is given in Figure 12b. There was significantly greater ammonium nitrogen, but significantly less nitrate nitrogen in the fumigated soil than in the nil-treated soil at 0–15 and 15–30 cm soil depths. There was no significant difference deeper in the profile between fumigated and nil-treated soil. Over the whole soil profile, there was somewhat less nitrate-nitrogen in the fumigated soil (199 ± 9.2 kg N/ha) than in the nil-treated soil (225 ± 11.4 kg N/ha), but more ammonium nitrogen in the fumigated soil (36.0 ± 4.0 kg N/ha) than in the nil-treated soil (26.0 ± 2.0 kgN/ha).

There were no significant differences between fumigated and nil-treated soil for available phosphorus and zinc or of pH (Table 6). There was a marked decrease in available phosphorus and available zinc and an increase in soil pH from the 0–15 cm layer to the 15–30 cm layers (Table 6). After incubation, there was a small amount of ammonium-nitrogen in the fumigated soil, but none in the untreated soil. Nitrate-nitrogen was considerably higher after incubation than before incubation, but with no difference between fumigated and nil-treated soil. There was no significant difference between fumigated and nil-treated soil after incubation for available P or zinc, or for soil pH.

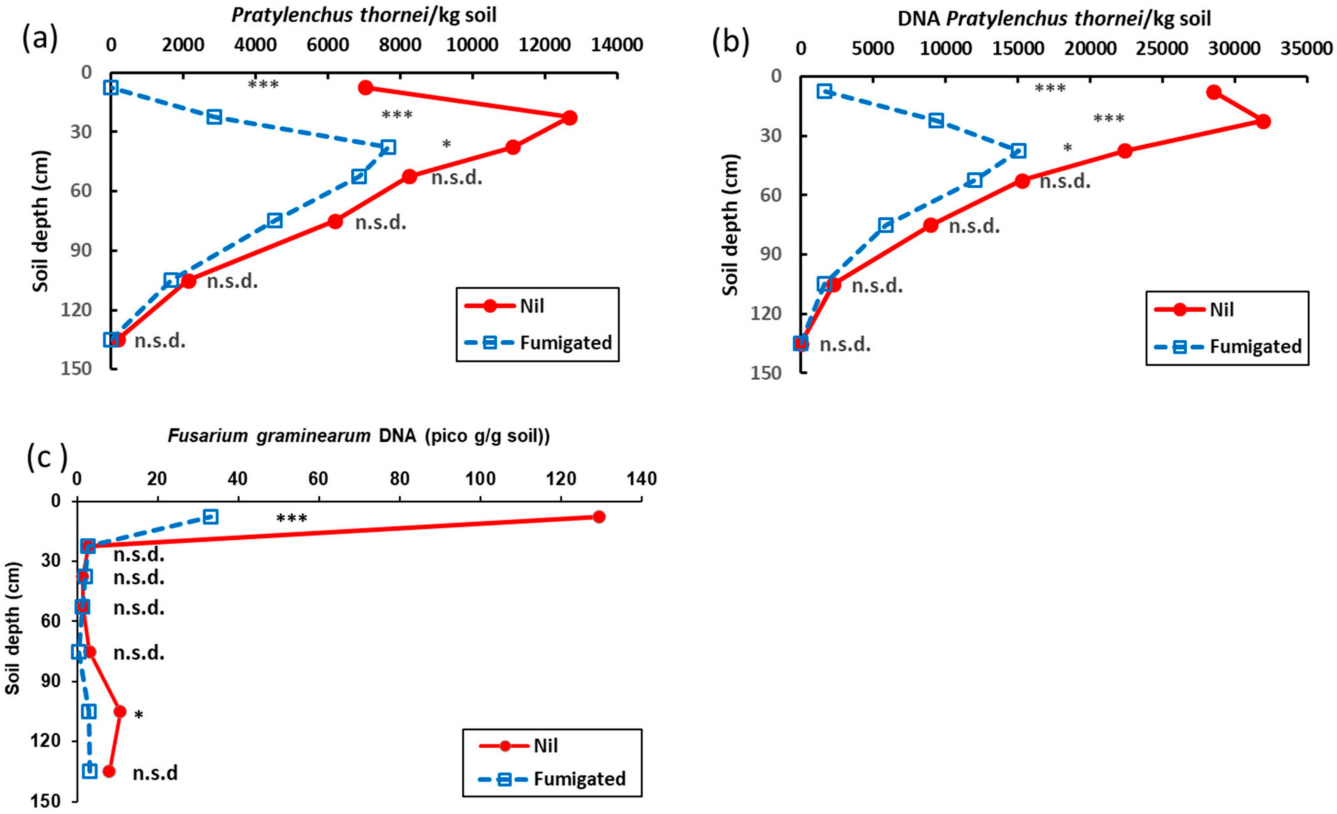

Figure 11. Effect of fumigation with methyl bromide on population densities of *Pratylenchus thornei* in the soil profile before sowing Experiment 4, (**a**) *P. thornei* determined by extraction and counting microscopically, (**b**) *P. thornei* determined by DNA quantification, and (**c**) *Fusarium pseudograminearum* determined by DNA quantification. Points are the back-transformed values from means of ln(x+1) transformations (n = 15), where significant differences between transformed means for nil and fumigated treatments at the respective depth interval are indicated as * = $p < 0.05$, *** = $p < 0.001$.

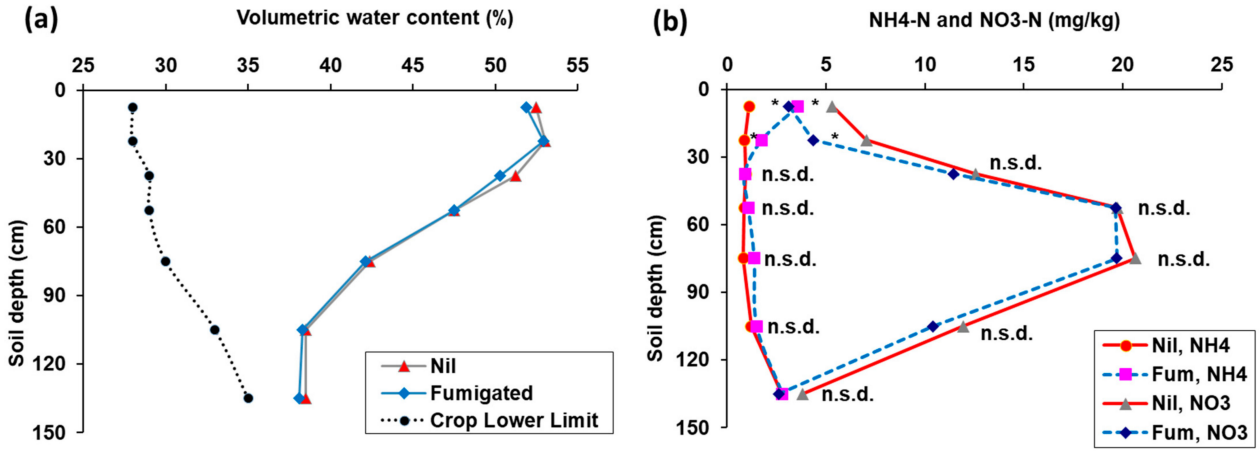

Figure 12. Effect of fumigation with methyl bromide on (**a**) volumetric water content and (**b**) mineral nitrogen (ammonium and nitrate) in the soil profile before sowing Experiment 4. Points are the means (n = 15), where significant difference between nil and fumigated treatments at the respective depth interval is represented as * = $p < 0.05$. There were no significant effects of fumigation or of the interaction of fumigation and soil depth for volumetric water content in ANOVA.

Table 6. Effect of fumigation on soil chemical properties in Experiment 4, before and after incubation for 28 days. Values are the means of n = 15, with SE in parentheses, n = 15.

Soil Depth (cm)	Fumigation	NH_4–N (mg/kg)	NO_3–N (mg/kg)	[a] P (mg/kg	[b] Zn (mg/kg)	[c] pH
Pre-incubation 0–15	nil	1.28 (0.18)	6.11 (0.97)	24.48 (1.55)	1.26 (0.25)	7.61 (0.07)
	fumigated	4.11 (0.83) [1]	3.58 (0.91)	24.30 (1.33)	1.12 (0.14)	7.65 (0.07)
15–30	nil	1.01 (0.00)	8.13 (0.90)	6.48 (0.22)	0.25 (0.05)	8.43 (0.05)
	fumigated	2.03 (0.33)	5.02 (0.92)	3.90 (0.33)	0.23 (0.03)	8.47 (0.04)
Post-incubation 0–15	nil	0 (0)	20.5 (1.37)	24.62 (1.55)	1.39 (0.25)	7.58 (0.07)
	fumigated	1.3 (0.8)	21.2 (1.54)	24.26 (1.38)	1.39 (0.22)	7.60 (0.07)

[a] P (Colwell bicarbonate extraction [55]; [b] Zn (DTPA extraction [56]; [c] pH (1 soil: 5 water).

There were substantial differences in the appearance of the plants at late stem elongation (Z35) in Experiment 4 as shown in Figure 13. Some cultivars showed symptoms of the consequences of *P. thornei* damage to the roots of chlorotic lower leaves, upright appearance of leaves as though drought stressed, with poor canopy closure (e.g., Figure 13a image of cv. Janz in nil-treated soil,). Soil fumigation decreased these symptoms (e.g., Figure 13b, image of cv. Janz in fumigated soil). Other cultivars did not have these symptoms (e.g., Figure 13c, image of wheat line QT9050 in nil-treated soil).

Figure 13. Appearance of wheat plots at Z35 late stem elongation (**a**) *P. thornei*-susceptible cv. Janz soil showing symptoms of lower leaf yellowing, poor canopy closure and low biomass, (**b**) cv. Janz growing on fumigated soil showing reduced, and (**c**) QT9050, a wheat line moderately resistant to *P. thornei*, showing no symptoms.

Ratings of cultivars at late stem elongation(Z35) growing in untreated soil ranged from 2.2 for wheat cv. Strzelecki to 5.8 for barley cv. Binalong (Figure 14a). Other wheat cultivars

with relatively low ratings indicating poor growth were Banks, Petrie, Hume, and Janz, while cultivars with relatively high ratings indicating good growth were QT9050, Baxter, Leichhardt and Hartog. Percentage responses in growth rating to fumigation ranged from zero for Binalong to 44% for Cunningham. There was a general trend that cultivars that had the lowest growth rating with nil treatment had the highest percentage response to soil fumigation (Figure 14a). Biomass at anthesis of the cultivars grown on untreated plots ranged from 2655 kg/ha for Strzelecki to 8445 kg/ha for Binalong (Figure 14b). Other cultivars with low biomass production were Strzelecki, Petrie, Hume, Banks, Lang and Janz, while other cultivars with high biomass production were the durum cv. Kamilaroi, and wheat cultivars Leichhardt, Baxter, Sunvale and QT9050. Generally, cultivars with low biomass production in the untreated soil had the highest percentage response to soil fumigation, while the cultivars with the high biomass production had little or even negative responses to soil fumigation.

The cereal cultivars had a wide range of percentage whiteheads from 0% for barley cv. Binalong to 90.0% for the durum cv. Wollaroi (Figure 15a). The two other durum cultivars, Kamilaroi and Bellaroi, had the next highest with 75.2 and 70.5% whiteheads, respectively. The wheat cultivars ranged from 5.0% whiteheads for Sunco to 50.0% for Kennedy. Grain yield ranged from 1120 kg/ha for durum cv. Wollaroi to 3400 kg/ha for barley cv. Binalong (Figure 15b). Other cultivars with low grain yield were Wollaroi (durum), Strzelecki, Banks, Hume, Kamilaroi (durum), and Petrie. Others with high grain yield were wheat cultivars Sunvale, Baxter, QT9050, Chara and Giles. Generally, cultivars with low yield for nil treatment had high percentage response to soil fumigation, while cultivars with high yield with nil treatment had low or even negative response to soil fumigation.

Biomass and growth rating were strongly correlated with each other and both were correlated with grain yield (Table 7). The percentage of whiteheads was not correlated with either biomass or growth rating, but was strongly negatively correlated with grain yield.

The following equations were obtained from multiple regression analysis relating yield to both growth rating and percentage whiteheads (arcsine$\sqrt{}$% transformations), or to both biomass and percentage whiteheads:

$$Y = 1046 + 327.5(GR * **) - 35.95(WH * **),\ R^2 = 0.832,\ p < 0.001,\ df = 37 \quad (6)$$

$$Y = 1812 + 0.2643(Biom * **) - 30.76(WH * **), R^2 = 0.827,\ p < 0.001,\ df = 37 \quad (7)$$

$$Y = 1373 + 511.1(GR * **) - 34.30(WH * **),\ R^2 = 0.80,\ p < 0.001,\ df = 29 \quad (8)$$

$$Y = 1729 + 0.3259(Biom * **) - 38.01(WH * **),\ R^2 = 0.827,\ p < 0.001,\ df = 29 \quad (9)$$

where Y = grain yield (kg/ha), GR = growth rating (1 to 6), Biom = biomass (kg/ha), and WH = arcsine $\sqrt{}$% whiteheads. Equations (6) and (7) are for 20 cereal cultivars on both nil-treated and fumigated soil. Equations (8) and (9) are for only the 16 wheat cultivars on both nil-treated and fumigated soil. Significance of coefficients indicated: *** $p < 0.001$.

When all cereal cultivars were included in the multiple regression, grain yield was highly significantly related ($p < 0.001$) to both growth rating (GR) and to percentage whiteheads in the crop (WH), the former having a positive coefficient and the latter a negative coefficient (Equation (6)). Similarly, when all cereal cultivars were included in the multiple regression, grain yield was highly significantly related ($p < 0.001$) to both biomass at anthesis (Biom) and to percentage whiteheads in the crop (WH), the former having a positive coefficient and the latter a negative coefficient (Equation (7)). When only wheat cultivars were included in the multiple regression, grain yield was still highly significantly related ($p < 0.001$) to both growth rating (GR) and to percentage whiteheads in the crop (WH), the former having a positive coefficient and the latter a negative coefficient (Equation (8)). Similarly, when only wheat cultivars were included in the multiple regression, grain yield was highly significantly related ($p < 0.001$) to both biomass at anthesis (Biom) and to percentage whiteheads in the crop (WH), the former having a positive coefficient and the latter a negative coefficient (Equation (9)).

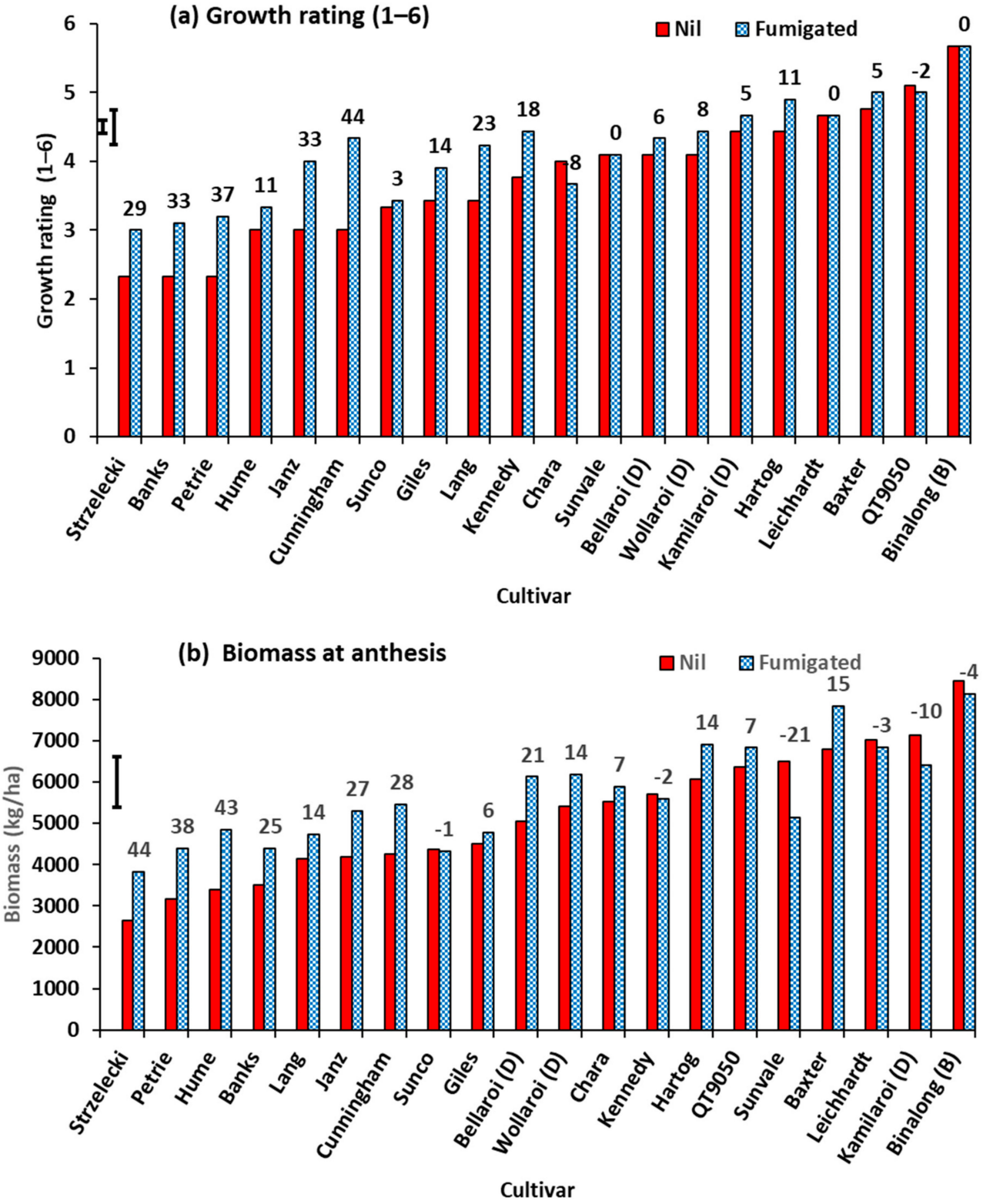

Figure 14. Response of wheat, durum (D) and barley (B) cultivars to soil fumigation with methyl bromide (**a**) growth rating at stem elongation and (**b**) biomass at anthesis. Each graph is presented with cultivars in ascending order of values for nil treatment. Bar markers represent the l.s.d. for (**a**) main effects for fumigation and cultivar, and (**b**) fumigation x cultivar interaction from ANOVA. Values above bars are percentage response to fumigation.

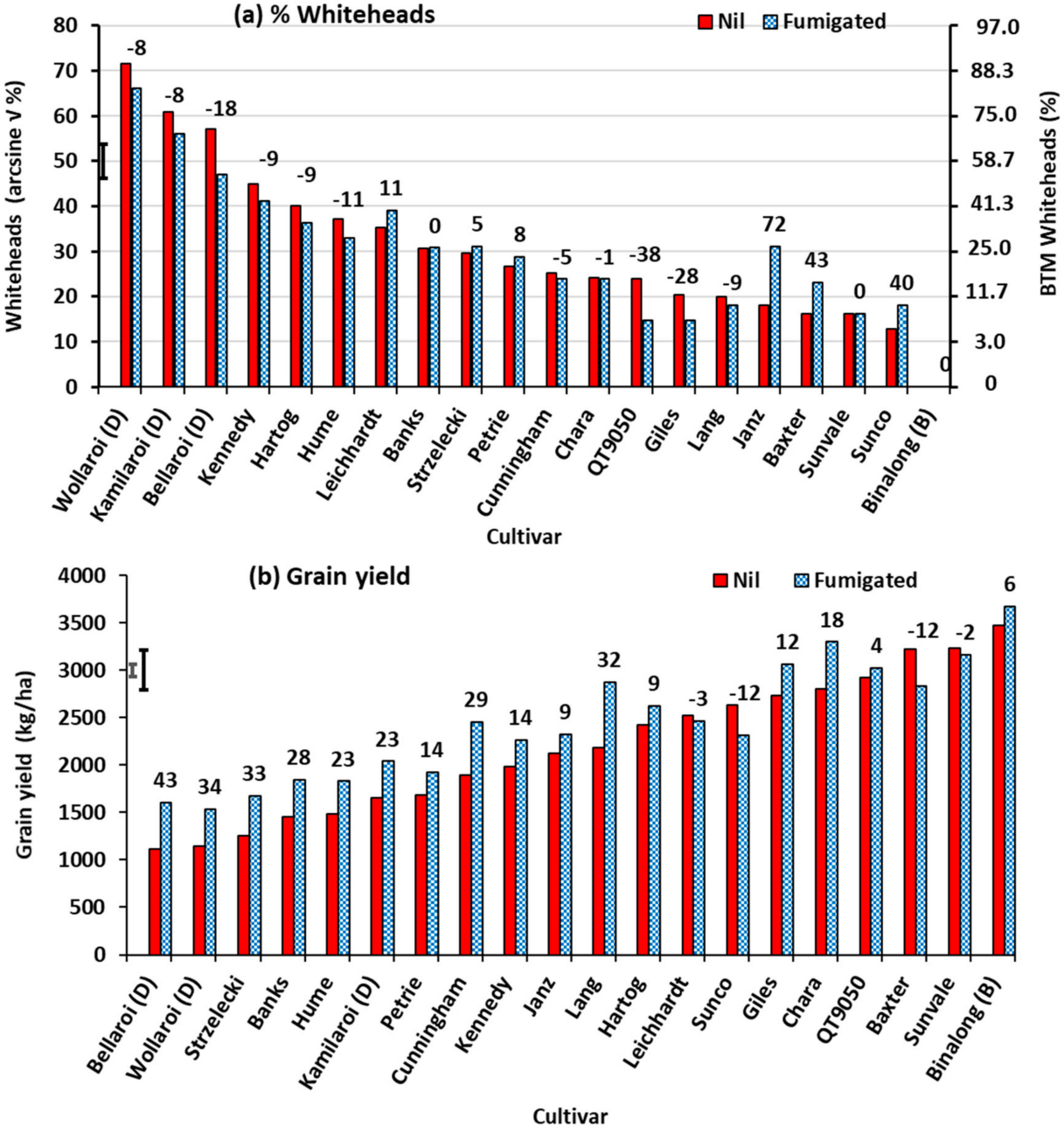

Figure 15. Response of wheat, durum (D,) and barley (B) cultivars to soil fumigation with methyl bromide, (**a**) whiteheads (arcsine $\sqrt{}$%), and (**b**) grain yield. Graph (**a**) is presented in descending order and graph (**b**) in ascending order, respectively of values for nil-treated cultivars. Bar markers represent the l.s.d. (**a**) main effect of cultivar, and (**b**) main effects of fumigation and cultivar from ANOVA. Values above bars are percentage response to fumigation.

Table 7. Correlation matrix between measures of plant growth of all cultivars growing on either nil-treated or fumigated soil (n = 40).

	Growth Rating	Biomass	Whiteheads
Biomass	0.937 ***		
Whiteheads	−0.051 n.s.	−0.031 n.s.	
Grain yield	0.580 ***	0.561 ***	−0.739

*** signifies correlation coefficient significant at $p < 0.001$, n.s. = non-significant. Whiteheads transformed by arcsine $\sqrt{}$%.

The following equations were obtained from multiple regression analysis relating yield with nil treatment, or percentage yield response from fumigation, to both the *P. thornei* tolerance index and the crown rot resistance index:

$$\text{Ynil} = -935 + 707(PtTI{**}) + 368.6(\text{CR}**), \text{R}^2 = 0.616, p < 0.001, \text{ df} = 16 \quad (10)$$

$$\text{Y\%resp} = 70.2 - 8.69(PtTI \text{ n.s.}) - 9.43(\text{CR}**), \text{ R}^2 = 0.535, p < 0.001, \text{ df} = 16 \quad (11)$$

$$\text{Ynil} = -877 + 1039(PtTI***) + 159.8(\text{CR}*), \text{ R}^2 = 0.819, p < 0.001, \text{ df} = 13 \quad (12)$$

$$\text{Y\%resp} = 72.9 - 13.01(PtTI*) - 7.58(\text{CR}*), \text{ R}^2 = 0.512, \text{ } p = 0.004, \text{ df} = 13 \quad (13)$$

where Ynil = grain yield (kg/ha) from nil treatment, Y%resp = percentage response in grain yield to soil fumigation, *PtTI* = the *P. thornei* tolerance index in t/ha [22] and CR = the crown resistance index ordinal scale of 1 to 9 [38]. Equations (10) and (11) are for 19 cultivars (16 wheat and three durum); Equations (12) and (13) are for 16 wheat cultivars. Significance of coefficients indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

When both wheat and durum cultivars were included in the multiple regression, yield from the nil-treated soil was highly significantly related both positively to crown rot resistance rating ($p < 0.001$) and to the *P. thornei* tolerance index ($p = 0.003$) (Equation (10)). The percentage response to fumigation was significantly negatively related to the crown rot resistance index ($p < 0.05$), but not to the *P. thornei* tolerance index, when wheat and durum were considered together (Equation (11)). When only wheat cultivars were analysed, their yield in the nil-treated soil was highly significantly ($p < 0.001$) related to the *P. thornei* tolerance index, and significantly ($p < 0.05$) related to the crown rot resistance index (Equation (12)). The percentage response of the wheat cultivars was significantly ($p < 0.05$) negatively related to both the *P. thornei* tolerance index and the crown rot resistance rating (Equation (13)).

4. Discussion

Wheat is the prime host of *Pratylenchus thornei* and much financial loss has been suffered by wheat growers in the subtropical grain region of eastern Australia from this nematode. Despite the change in this region to conservation agriculture, and the many benefits that it provides compared with traditional agronomic practices, conservation farmers must pay close attention to the management of plant pathogens. Our results show that *P. thornei* can cause major loss in yield of many wheat cultivars in a commercial farming system that utilizes best management practices for conservation agriculture. The results of Experiments 1 and 2 show the difficulty in managing *P. thornei*, where wheat was grown after the grain legume black gram (*Vigna mungo* (L.) Hepper). This pulse crop, along with the closely related host crop mung bean (*Vigna radiata* (L.) R. Wilczek), should be beneficial summer crops in rotations with wheat, but are not so in *P. thornei*-infested fields. These results showed that the final population density of *P. thornei* in the soil profile associated with the various wheat cultivars was a strong explanatory variable of their grain yield, indicating that *P. thornei* was the major cause of the wide range of yield of the wheat cultivars in these experiments. Susceptible to very susceptible cultivars, which produced high population densities of *P. thornei* and suffered substantial yield loss in these experiments, were Strzelecki, Petrie, Banks, QT8368, Batavia, Cunningham, Babbler, and Sunco. Cultivars with this level of susceptibility and intolerance should be avoided in fields that are infested with *P. thornei.* By contrast, cultivars such as QT8447, QT8620 and QT9050, which have moderate resistance to *P. thornei* derived from the resistant selection GS50a [25], produced much lower population densities of *P. thornei* and higher grain yield in Experiments 1 and 2. Other cultivars with relatively lower final population densities of *P. thornei* and higher grain yields were Sunvale, Baxter, Gregory, Giles, Chara, Leichhardt and Glover. Cultivars with at least this level of tolerance should be chosen for sowing in fields that contain *P. thornei.* An additional cultivar that exhibited better tolerance to *P. thornei* based on grain yield in Experiment 3 was QT10162, a reselection from Baxter.

Other cultivars that tolerated *P. thornei* based on biomass at anthesis in Experiment 4 were the barley cultivar Binalong and the durum cultivar Kamilaroi. Binalong also had no whiteheads and loss of grain yield from crown rot. However, this was not the case for Kamilaroi, which had a high percentage of whiteheads from crown rot and suffered much yield loss.

The results of Experiments 1 and 2 also demonstrated clearly that susceptible wheat cultivars build population densities of *P. thornei* in the soil profile to much higher levels in comparison with fallow land, resulting in a detrimental legacy for following crops in the farming system.

The farmer's field used for Experiment 3 had not grown wheat for at least 6 years having had (most recent crop first) chickpea (*Cicer arietinum* L.), barley, maize (*Zea mays* L.) and two crops of sorghum. At that site, the population density of *P. thornei* was lower in the topsoil, but increased with depth in the soil profile, and overall was damaging to wheat yield. Thus, the sequence of crops that had preceded Experiment 3 had resulted in a relatively lower, but not inconsequential, population density of *P. thornei* in the soil profile. All cultivars of grain sorghum that have been tested have proved to be resistant or moderately resistant to *P. thornei* [9,61]. Cultivars of maize are also mainly moderately resistant [9], and while most cultivars of barley and chickpea are hosts of *P. thornei,* they are not as susceptible as many wheat cultivars [62,63]. Nevertheless, the population density of *P. thornei* in Experiment 3 was above the arbitrary damage threshold of 2000/kg soil at any depth in the soil profile [1] or of 1000/kg soil averaged over the profile to 90 cm deep [9]. This was borne out by the substantial responses to soil fumigation at that site in proportion to the intolerance of the various cultivars. There were only traces of *F. pseudograminearum* detected in soil samples before this experiment was sown, because the only winter cereal grown in the previous six years was one barley crop. In accordance with this cropping history, and low soil test for *F. pseudograminearum*, no symptoms of basal stem browning or whiteheads were evident in the wheat or durum cultivars grown in Experiment 3. Furthermore, in multiple regression analysis, grain yield was significantly related to the *P. thornei* tolerance index [22], but not to the crown rot resistance index.

Experiment 4 was grown in a section of the same field in which Experiments 1 and 2 had been conducted. This experiment followed chickpea in the previous winter season, which followed a commercial crop of wheat that was grown at the same time as Experiments 1 and 2. Before sowing Experiment 4, there was a high population density of *P. thornei* in the soil profile, and a moderately high density of *F. pseudograminearum* DNA in the topsoil. During the growing season, there was strong development of the symptoms of *P. thornei* damage evident at late stem elongation, and a reduction in biomass production at anthesis, typical of the effects of *P. thornei* [40,42]. A moderate level of *F. pseudograminearum* occurred in the topsoil before sowing this experiment, despite the crop following chickpea, a non-host of *F. pseudograminearum*. However, the site had grown two wheat crops and one barley crop in the previous 7 years, and *F. pseudograminearum* can survive in stubble pieces after an infected crop for at least 2 years [64]. Crown rot caused damage to the plants in Experiment 4, resulting in the production of whiteheads and reduction in grain yield, particularly in the durum cultivars, but also in the wheat cultivars to varying degrees. The dual effects of *P. thornei* and crown rot on grain yield were evident from the highly significant ($p < 0.001$) positive relationships with growth rating at stem elongation or biomass production at anthesis and the highly significant ($p < 0.001$) negative relationships with percentage of whiteheads in correlation analysis and multiple regression analyses. Similarly, the dual effects of *P. thornei* and crown rot on grain yield or percentage response of grain yield to fumigation, was evidenced by significant ($p < 0.001$) relationships with the *P. thornei* tolerance index [22] combined with the crown rot resistance index [38] as explanatory variates in multiple regression analyses.

Methyl bromide was withdrawn from sale in developed countries in 2005 and from developing countries in 2015 because of environmental concerns [65] While it was a useful product for control of nematodes and other pests and diseases in high value horticultural

crops, it was never considered a potential method for control of nematodes on grain farms in the subtropical grain region of eastern Australia, for economic and logistic reasons. Nevertheless, in our study, it proved to be a valuable research tool killing *P. thornei* in the soil profile to 90 cm in Experiment 3 and to 45 cm in Experiment 4, deeper than the 30 cm previously achieved with the most effective non-volatile nematicide aldicarb [41,42]. Methyl bromide also provided some advantages over other broad-spectrum fumigants. Previously, the fumigants chloropicrin, and methylisothiocyanate liberated from dazomet, caused a marked increase in the quantities of mineral N in a vertisol, as well as increases in available phosphorus and zinc, whereas the non-fumigant nematicides did not [40]. In the present study, methyl bromide caused much smaller changes in mineral nitrogen with an increase in ammonium in the 0–15 and 15–30 cm layers, and a similar decrease in nitrate nitrogen indicating inhibition of nitrification in Experiment 4. These effects were small, however, and the total quantity of mineral N in the profile was similar for fumigated and nil-treated soil. Incubation of topsoil in the laboratory for 28 days resulted in increased nitrate in both nil-treated and fumigated soil with no significant difference between them in Experiment 4. This was a different result from that obtained with the incubated samples from Experiment 3 in which ammonium-nitrogen increased and nitrate decreased substantially on incubation in both treatments, but more so in the nil-treated than in the fumigated soil. It is possible that incubation conditions of Experiment 3 soil promoted ammonification and denitrification. Previously, methyl bromide fumigation was found to double the available phosphorus in a solonised brown soil (Calcarosol) in South Australia [39]. However, there was no significant effect of methyl bromide fumigation on available phosphorus or zinc in the vertisol in our study, either in samples from the field or after incubation in the laboratory for 28 days. Additionally, the profile distribution of soil water and the total quantity of plant-available water in the entire profile before sowing were similar for fumigated and nil treatments. Thus, in our study, there were no marked differences in inorganic nutrients or soil water between fumigated and nil treatments that would complicate interpretation of experimental outcomes.

In Experiment 3, there was a slight bronzing chlorosis and leaf tip necrosis of plants at Z32 in the fumigated treatment, suggesting possible bromide toxicity. In soil, methyl bromide decomposes to methanol and inorganic bromide, which can be toxic to some plant species. Wheat growing in a clay soil in England suffered bromide toxicity appearing as "scorched" plants, containing up to 61,000 mg Br/kg, where methyl bromide had been injected into the soil [66]. The concentration in the plants in fumigated soil in our experiment (average 5284 mg Br/kg) was less than this and may not have been of any consequence to the outcome of the experiment. In Experiment 3, plants at Z32 (early stem elongation) responded markedly in biomass production with a significant fumigation x cultivar interaction and with a range of 35% response to fumigation for Hartog to 119% for QT10162. This was accompanied by a significant decrease in the concentration of phosphorus, and a significant increase in the concentration of zinc in the plant tissues, but no significant effect on nitrogen concentration. The percentage response of dry weight per plant to fumigation was exponentially related to the percentage response of phosphorus concentration to fumigation in the plants at this growth stage, and ultimately in percentage response to fumigation in grain yield

In all four experiments, *P. thornei* was an issue for wheat production, reflecting the difficulty for effective management of this nematode species in the subtropical grain region of eastern Australia. Not only can *P. thornei* build up rapidly in the soil profile on susceptible wheat cultivars, but also its population density can be maintained or increased by cultivars of a range of winter cereals and winter and summer pulse crops [9]. Although population densities of *P. thornei* show a negative decline during fallow periods, this nematode species can survive for many years at damaging population densities in the moist soil profile in fallowed vertisols or during the growth of poor hosts such as sorghum [67] Damaging population densities of *P. thornei* can cause yield loss of susceptible/intolerant wheat cultivars under most seasonal conditions [22], with the greatest differences among cultivars

occurring on sites with the highest population densities of *P. thornei* in the soil profile and the greatest amount of stored soil water at sowing [22]. The yield of a wheat cultivar is dependent on its susceptibility or resistance to reproduction of *P. thornei* in its roots as demonstrated here by the strong negative relationship between final population densities in the soil profile and yield of the cultivars in Experiments 1 and 2. A strong negative relationship was also found between final *P. thornei* population densities determined in multiple glasshouse experiments and grain yield of wheat cultivars in field sites infested with *P. thornei* [20]. However, some cultivars have better tolerance and yield better than would be expected from their level of resistance/susceptibility, and this is captured in the tolerance index based on predicted grain yield from multiple experiments on *P. thornei*-infested sites [22]. It is important for growers with *P. thornei*-infested fields to choose wheat cultivars that have both a high tolerance index to ensure little yield loss and a high resistance index. A high level of resistance not only reduces yield loss in the current crop, but also leaves lower residual population densities of *P. thornei* in the soil profile to infect subsequent crops in the farming system.

Crown rot was a serious issue in Experiment 4, resulting in very high percentages of whiteheads in the durum cultivars, and high percentages in many of the wheat cultivars, but not in the barley cultivar Binalong. Among the wheat cultivars, Sunco had the lowest percentage of whiteheads reflecting its known better level of adult plant resistance to crown rot among commercial wheat cultivars. The extreme susceptibility of durum wheat to crown rot is a limiting factor to durum production on the Darling Downs and elsewhere in the subtropical grain region of eastern Australia. However, there is promise that better levels of crown rot resistance can be transferred from hexaploid wheat to tetraploid durum. Martin et al. [68] showed from a cross between Sunco and the susceptible durum cultivar Wollaroi that equal or better levels of crown rot resistance than that in Sunco could be recovered in tetraploid progeny. Continued breeding is required to improve the level of crown rot resistance not only in durum, but also in bread wheat, and to combine crown rot resistance with resistance and tolerance to *P. thornei* in individual cultivars that are agronomically suitable to the subtropical grain region of eastern Australia. Such cultivars will be of great benefit to all growers, and will enable conservation farmers to more fully capitalise on the extra water stored in the soil profile to increase grain yield compared with traditional agronomic systems [15,16].

5. Conclusions

As demonstrated by the results of these experiments, the root-lesion nematode *P. thornei* and the crown rot fungus *F. pseudograminearum* are major constraints for wheat and durum production in conservation farming systems of the subtropical grain region of eastern Australia. The nematode affects growth early in crop life, resulting in reduced biomass production and potential grain yield. On the other hand, the major yield-limiting effects of crown rot are through the induction of whiteheads that contain no grain at the grain filling stage late in the crop cycle. Because of these modes of action, *P. thornei* causes yield loss under most seasonal conditions, whereas crown rot is most damaging when rainfall in the early part of the growing season is followed by dry conditions later. Both root-lesion nematode and crown rot can individually cause serious yield loss and when both are present at damaging population densities as in Experiment 4, their detrimental effects on yield are both expressed. Under these conditions, the *P. thornei* tolerance index and the crown rot resistance index for wheat and durum cultivars established in separate, independent experiments to this study, are valuable guides to cultivar performance. Continued breeding to combine high levels of resistance and tolerance to both *P. thornei* and *F. pseudograminearum* in individual cultivars of wheat and durum is required to ensure the success of conservation farming with these crops in this region.

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preparation, J.P.T.; writing—review and editing J.P.T. and T.G.C.; All authors have read and agreed to the published version of the manuscript.

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