CSIRO PUBLISHING

Crop & Pasture Science, 2014, **65**, 227–241
http://dx.doi.org/10.1071/CP13295

Wheat biomass and yield increased when populations of the root-lesion nematode (*Pratylenchus thornei*) were reduced through sequential rotation of partially resistant winter and summer crops

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Abstract. The root-lesion nematode, *Pratylenchus thornei*, can reduce wheat yields by >50%. Although this nematode has a broad host range, crop rotation can be an effective tool for its management if the host status of crops and cultivars is known. The summer crops grown in the northern grain region of Australia are poorly characterised for their resistance to *P. thornei* and their role in crop sequencing to improve wheat yields. In a 4-year field experiment, we prepared plots with high or low populations of *P. thornei* by growing susceptible wheat or partially resistant canaryseed (*Phalaris canariensis*); after an 11-month, weed-free fallow, several cultivars of eight summer crops were grown. Following another 15-month, weed-free fallow, *P. thornei*-intolerant wheat cv. Strzelecki was grown. Populations of *P. thornei* were determined to 150 cm soil depth throughout the experiment. When two partially resistant crops were grown in succession, e.g. canaryseed followed by panicum (*Setaria italica*), *P. thornei* populations were <739/kg soil and subsequent wheat yields were 3245 kg/ha. In contrast, after two susceptible crops, e.g. wheat followed by soybean, *P. thornei* populations were 10 850/kg soil and subsequent wheat yields were just 1383 kg/ha. Regression analysis showed a linear, negative response of wheat biomass and grain yield with increasing *P. thornei* populations and a predicted loss of 77% for biomass and 62% for grain yield. The best predictor of wheat yield loss was *P. thornei* populations at 0–90 cm soil depth. Crop rotation can be used to reduce *P. thornei* populations and increase wheat yield, with greatest gains being made following two partially resistant crops grown sequentially.

Additional keywords: maize, *Merlinius brevidens*, millet, mungbean, panicum, sorghum, soybean, sunflower.

Received 28 August 2013, accepted 31 January 2014, published online 4 April 2014

Introduction

As a reflection of its broad host range, the root-lesion nematode, *Pratylenchus thornei*, is commonly called the 'cereal and legume root-lesion nematode' (Castillo and Vovlas 2007). Damage caused by this nematode in the root cortex of intolerant–susceptible plants produces symptoms of nutrient and water deficiency (Castillo and Vovlas 2007). *Pratylenchus thornei* has been found on wheat in Syria, Yugoslavia, Mexico, Canada, Israel, Iran, Morocco, Tunisia, Turkey, Pakistan, India, Algeria, Italy and the USA, and significant wheat yield loss has been recorded in several of these countries (Nicol *et al*. 2011). It is a particular problem in the northern grain region of Australia, where it has been identified in ~70% of fields and can reduce yields of intolerant wheat cultivars (*Triticum aestivum*) by >50% (Thompson *et al*. 2008, 2010). The potential annual cost to the wheat industry in this region (assuming a price of AU$239/t wheat) is ~$104 million if left unchecked, or $38 million when current management strategies are taken into account (Murray and Brennan 2009).

The Australian northern grain region is a semi-arid, subtropical area of 6 Mha, extending from northern New South Wales (~32°S) to the Central Highlands of Queensland (~22°S). Rainfall is highly variable (550–880 mm/year, 30% coefficient of variation) with potential evapotranspiration of 1300–2200 mm/year (Webb *et al*. 1997). Both summer and, particularly, winter crops rely on soil water accumulated during fallow periods (Unkovich *et al*. 2009). The region is renowned for valuable, high-protein bread wheat and durum wheat (*T. durum*), with chickpea (*Cicer arietinum*) and barley (*Hordeum vulgare*) also important winter crops. The main summer grain crop is sorghum (*Sorghum bicolor*). Other summer crops grown include cotton (*Gossypium hirsutum*), maize (*Zea mays*), sunflower (*Helianthus annuus*), mungbean (*Vigna radiata*), black gram (*V. mungo*) and soybean (*Glycine max*). There is a small birdseed market for summer-grown millets (*Panicum miliaceum*, *Echinochloa* spp. and *Pennisetum glaucum*) and panicum (*Setaria italica*; also known as foxtail millet), and winter-grown canaryseed (*Phalaris canariensis*) (Unkovich *et al*. 2009).

Current management of *P. thornei* consists of an integrated approach including farm hygiene (controlling water runoff and soil erosion, and cleaning farm machinery to prevent contamination of paddocks) and growing tolerant cultivars of wheat in rotation with *P. thornei*-resistant and non-host crops (Thompson *et al*. 2009; Owen *et al*. 2010). A plant's response to nematodes is classified separately into resistance (ability of the plant to prevent nematode reproduction or development) and tolerance (host response to nematode parasitism measured by the impact of nematodes on plant growth) (Starr *et al*. 2002; Roberts 2002). Resistance can be partial (allowing a low to moderate level of reproduction) or complete (no reproduction); similarly, there is a spectrum of tolerance and intolerance. Notably, summer crops grown in the northern grain region remain poorly characterised for their resistance and tolerance to *P. thornei*, raising the possibility that they could be better used for management of *P. thornei* populations.

This paper describes changes in population densities of *P. thornei* throughout the soil profile over three cropping phases with (*i*) winter crops to establish both high and low populations of *P. thornei*, followed by (*ii*) a range of cultivars of 11 summer crop species, and then (*iii*) an intolerant wheat crop. The impact of residual nematode populations in the soil profile to 90 cm depth on the subsequent wheat biomass and grain yield clearly demonstrated the benefit of growing two partially resistant crops compared with two susceptible crops beforehand.

Materials and methods

Field site

The experiment was conducted over three crop phases within the years 2000–03 at Formartin (27.46401°S, 151.42616°E; 364 m elevation; 70 km west of Toowoomba) on the Darling Downs, Queensland, Australia. The previous cropping history of the experimental site was cotton followed by a 12-month, weed-free fallow, then chickpea followed by an 18-month, weed-free fallow, at which point the experiment was started. The field site is rain-fed. Stubble was left standing after harvest and no-tillage was practised during fallow periods in which weeds were controlled with herbicides. Soil disturbance occurred to a depth of ~70 mm during application of fertiliser and at planting.

Before the experiment (in June, Year 1), *P. thornei* was found throughout the soil profile to 120 cm depth and population densities were greatest between 15 and 60 cm soil depth (range of 2207–3983/kg soil) (Table 1). *Merlinius brevidens* was also detected in low populations throughout the soil profile, with population densities ranging from 17/kg soil at 0–15 cm to 1140/kg soil at 45–60 cm (Table 1). Populations of non-parasitic nematodes (or free-living nematodes) did not exceed 97/kg soil (data not shown). Soil moisture content ranged from 38.2% at 0–15 cm to 48.6% at 45–60 cm (Table 1).

The soil at the site is a haplic, self-mulching, endohypersodic, Black Vertosol (Isbell 1996) of the Waco Series (Beckmann and Thompson 1960). The soil is very deep, contains 56% clay (Dalal *et al*. 1995) and has a very high water-holding capacity (PAWC); for example, average PAWC was 224 mm to 1.8 m for wheat (Hochman *et al*. 2001). Average monthly rainfalls during the experiment and long-term averages from farm records and from the nearest Bureau of Meteorology station (Bowenville; Station Number 041008 27.30°S 151.49°E; 383 m elevation) are listed in supplementary table S1 at the journal's website. In the year before the experiment started, the annual rainfall was 522 mm, and during the 4-year experiment, rainfall was 320, 409, 423 and 430 mm, compared with an average of 572 mm from 65 years of farm records or 634 mm from 114 years of BOM data.

Field experiment and design

A timeline of agronomic practices and sample collection during the experiment is summarised in Table 2. The winter crops planted in Year 1 are referred to as Phase 1 crops; summer crops planted in Year 2 and harvested in Year 3 are referred to as Phase 2 crops; wheat planted in Year 4 is referred to as the Phase 3 crop. The experimental design was row–column with three replicated blocks. The Phase 1 wheat and canaryseed crops were duplicated within each replicate block in preparation for the Phase 2 summer crops in the factorial structure. The treatments were formed by the factorial of Phase 1 wheat or

Table 1. Populations of *Pratylenchus thornei* and *Merlinius brevidens* per kg soil and soil moisture content in the soil profile at 1 month before planting the Phase 1 crops in the field experiment

BTM, Back-transformed mean

Soil depth (cm)	Nematodes/kg dry soil				Soil moisture (%)
	P. thornei		*M. brevidens*		
	ln(*x*+1)	BTM	ln(*x*+1)	BTM	
0–15	4.65	104	2.87	17	38.2
15–30	7.70	2207	4.11	60	45.0
30–45	8.29	3983	5.30	199	48.2
45–60	8.23	3751	7.04	1140	48.6
60–90	5.85	346	4.76	116	46.5
90–120	1.23	2	4.51	90	44.2
120–150	0	0	1.98	6	45.7
l.s.d. ($P = 0.05$) (depth)	1.67		2.7		2.1

Table 2. Summary of agronomic practices and sampling procedures carried out during the 4-year field experiment

Procedure	Phase 1 crops	Phase 2 crops		Phase 3 crop
	Year 1	Year 2	Year 3	Year 4
Soil sampling	8 June	1 Nov.	14 May	9 June
Fertilising	6 May	5 May		5 May
Planting	6 June (wheat and canaryseed)	6 Nov. (summer crops)		17 July (wheat)
Biomass collection			1 Feb. (summer crops)	22 Oct. (wheat)
Harvesting/crop removal	2 Oct. (canaryseed), 6 Nov. (wheat)		15 April (summer crops)	28 Nov. (wheat)

canaryseed × Phase 2 summer crop cultivars, which were grouped into eight crops.

In the first year of the experiment (Phase 1), wheat cv. Cunningham, and canaryseed cv. Moroccan were planted. Wheat cv. Cunningham is very susceptible to *P. thornei*; canaryseed is partially resistant (Sheedy *et al*. 2012; Matthews *et al*. 2013). Each plot was 10 m long by 1.75 m wide planted as seven rows with 0.25-m row spacing. Urea (120 kg N/ha) was applied 3 weeks before planting, and Starter Z (Incitec Pivot) was applied at 35 kg/ha in the furrows to a depth of ~70 mm at planting to supply 4 kg N, 7 kg P and 0.9 kg Zn/ha (similar to regional agronomic practices).

Whole plant shoots of canaryseed were cut at ground level from the plots before flowering to prevent contamination of plots with the fine seed, which is easily dispersed and has summer dormancy. The wheat plots were machine-harvested at maturity.

Following an 11-month, weed-free fallow, 1–16 cultivars of 11 species of summer crops were planted in November (Phase 2) into both former wheat and canaryseed plots. The Phase 2 crops were: black gram, mungbean, maize, panicum, sorghum, soybean, sunflower, Japanese millet (*Echinochloa esculenta*), Siberian millet (*E. frumentacea*), pearl millet (*Pennisetum glaucum*) and white French millet (*P. miliaceum*). The four species of millet (Japanese, pearl, Siberian and white French) were grouped to simplify presentation of results and discussion. The grain crops and the cultivars chosen were representative of those commonly grown in the region. Urea had been applied to the soil 6 months before planting and Starter Z was applied at planting as described previously. The summer crops were planted in four rows with 0.45-m row spacing. Whole plant shoots were sampled to measure biomass from each plot at 3 months after planting. Plant samples of soybean, millets, panicum, mungbean and black gram were randomly collected from two 1-m lengths within the middle rows in two positions. Sorghum, sunflower and maize were sampled by taking five plants from the middle rows within an 8-m section of the middle of the plot. All crops were cut from the trial site at 5 months after planting to ensure uniform treatment of all plots because differences in crop maturity prevented harvest.

Wheat cv. Strzelecki (Phase 3) was planted over the entire experimental site in July in Year 4 following a 15-month, weed-free fallow. Strzelecki was chosen because it is intolerant–very intolerant to *P. thornei* (Lush 2013) and is grown in the northern grain region. Fertiliser and planting were the same as described for the first year of the experiment. Biomass was measured at anthesis, 3 months after planting, from two 1-m lengths within the middle rows in two positions as described previously. Grain from each plot was machine-harvested at maturity.

Soil sampling

Before planting the Phase 1 crops, the experimental site was assessed for nematode populations from nine soil cores collected in a grid pattern. One week before planting the Phase 2 summer crops, soil cores were taken from three Phase 1 wheat and three Phase 1 canaryseed plots selected at random within each block (total of nine plots each of wheat and canaryseed). One month after removal of the Phase 2 summer crops, soil cores were taken from every plot. Prior to planting the Phase 3 wheat, soil cores were taken from plots that had been planted with the Phase 1 wheat.

Soil cores were taken with a hydraulically operated push-tube of 43 mm internal diameter. Three cores were collected from each sampling point or per plot to 150 cm depth on the middle rows of each plot. The soil cores were cut into the following depth intervals: 0–15, 15–30, 30–45, 45–60, 60–90, 90–120, 120–150 cm. The soil from each of the three cores, for each depth interval, was placed in the same plastic bag for storage at 4°C until processing.

Nematode extraction and enumeration, and soil properties

The soil samples were broken manually into <5-mm aggregates, mixed, and a 150-g, field-moist subsample, including accompanying roots, was processed for nematode extraction for 48 h at 22°C by the Whitehead tray method (Whitehead and Hemming 1965). Nematodes were collected on a sieve with 20-µm pore size. *Pratylenchus thornei* and *M. brevidens* were morphologically identified (Siddiqi 1972; Fortuner 1977) and counted in a 1-mL Hawksley slide under a compound microscope at 40× and 100× magnification. Non-parasitic nematodes were counted as a composite of species. Counts were expressed on a dry-soil weight basis after correction for soil moisture content. Soil moisture was determined for each composited soil interval by oven-drying a 100-g subsample at 105°C for 48 h. Number of nematodes per kg dry soil was also calculated for accumulated soil depth intervals (0–15, 0–30, 0–45, 0–60 and 0–90 cm). Reproduction factor was calculated as: (final *P. thornei* population/kg dry soil) ÷ (initial *P. thornei* population/kg dry soil). Nematode populations after the Phase 2 crops are shown for the individual and accumulated soil depth intervals to 90 cm, because below that depth, populations were very low or zero. Soil collected 11 months after harvest of the Phase 1 wheat and canaryseed was analysed for nitrate in 1 N KCl extracts (Rayment and Higginson 1992).

DNA analysis of nematode samples

Duplicate soil samples (500 g each) collected from wheat and canaryseed plots (before planting the Phase 2 summer crops) were submitted for DNA analysis by PreDicta B (SARDI, Urrbrae, S. Aust.) to detect root-lesion nematodes (Ophel-Keller *et al*. 2008). This commercial service extracts total DNA from 500 g of oven-dried soil. Separate rDNA probe sequences specific for *P. thornei* or *P. neglectus* were quantified by TaqMan® MGB real-time PCR (described in Riley *et al*. 2010). Standard curves (determined by adding known numbers of nematodes to soil) were used to convert the number of PCR cycles when fluorescence associated with the amplified DNA crossed the defined threshold to number of nematodes per g soil.

Statistical analyses

Nematode data were transformed by $\ln(x+c)$ where x is number of nematodes per kg dry soil including roots, and c is a constant chosen to stabilise the variances of the residuals across the range of fitted values (Proctor and Marks 1974; Berry 1987). A linear mixed model was fitted including fixed terms for crops, cultivars nested within crops and soil depth. Correlations across soil depths were included in the models where applicable. The REML (residual maximum likelihood) procedure in GENSTAT

(VSN International 2011) algorithm was used to fit the model. Some analyses were extended by partitioning the fixed effects to allow testing of effects within each Phase 2 crop. The REML procedure was chosen so that complex correlations across depths and any heterogeneity could be fitted.

Regression analysis was used to investigate the relationships between the Phase 3 biomass or grain yield of wheat cv. Strzelecki and nematode populations (for accumulated soil depth intervals) and soil water. Predicted grain yield or biomass loss (%) was determined from the regression equations by solving them with the minimum and maximum population densities of *P. thornei* within the range of the data.

All data were analysed using GENSTAT with the significance level set at $P = 0.05$ for all testing.

Results

Nematode populations and soil properties after Phase 1 wheat or canaryseed

Twelve months after harvest of Phase 1 wheat or canaryseed, population densities of *P. thornei* showed a significant soil depth × Phase 1 crops interaction effect ($P \leq 0.05$). The densities were highest following wheat, being 5265/kg soil at 15–30 cm, and mean 3150/kg soil for the accumulated depth of 0–90 cm (Fig. 1). In contrast, there were significantly fewer *P. thornei* following canaryseed, the greatest populations being 1152/kg soil at 30–45 cm, and mean 683/kg soil for 0–90 cm depth. Population densities of *M. brevidens* (356/kg soil) did not differ significantly ($P > 0.05$) between the soil depths or the Phase 1 crops (data not shown). Populations of non-parasitic nematodes differed significantly ($P \leq 0.05$) only between soil depths and ranged from 1468/kg soil at 0–15 cm to 435/kg soil at 60–90 cm (data not shown).

Overall, the soil moisture contents following the Phase 1 canaryseed or wheat showed a significant soil depth × Phase 1 crops interaction effect ($P \leq 0.05$) but the differences between means were similar with respect to their importance. For each soil layer to 150 cm (0–15, 15–30, 30–45, 45–60, 60–90, 90–120, 120–150 cm) for Phase 1 canaryseed and wheat, respectively, soil moisture was 44.9 and 45.1%; 47.4 and 47.3%; 48.7 and 47.4%; 46.8 and 44.9%; 42.6 and 42.2%; 42.2 and 43.3%; 44.9 and 44.8% (l.s.d. 1.1, $P = 0.05$). For the respective soil layers to 150 cm, soil nitrate was 45.2, 81.2, 53.0, 43.8, 32.1, 25.5 and 19.2 mg/kg soil (l.s.d. 4.4, $P = 0.05$); there was no significant effect of Phase 1 crop ($P > 0.05$).

A DNA assay on duplicate samples collected before planting the Phase 2 summer crops detected only *P. thornei.* The pattern of distribution of *P. thornei* in the soil profile determined by the DNA method was similar to the Whitehead tray extraction and microscopy counts (data not shown). There was a significant linear relationship between the Whitehead tray extraction and microscopy assay methods for transformed *P. thornei* data ($R^2 = 0.95$, $P < 0.001$; Fig. 2). Solving the equation from this regression and back-transforming the result showed that populations estimated by the DNA method were generally 2-fold greater than counts from the Whitehead tray extraction.

Phase 2 summer crops

Biomass

Biomass of the Phase 2 mungbean crop after the Phase 1 wheat was 9% lower than after Phase 1 canaryseed (2845 *v.* 3137 kg/ha; l.s.d. 509, $P \leq 0.05$) (data for other crops not shown). There was no significant interaction between the Phase 1 crops and Phase 2

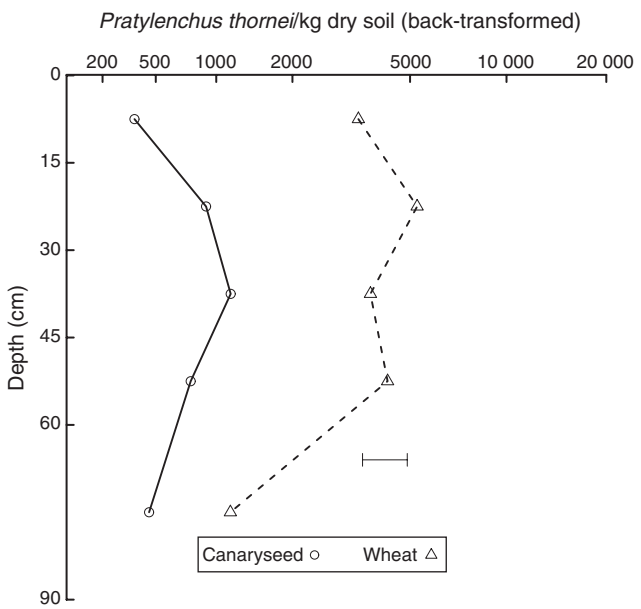

Fig. 1. Populations of *Pratylenchus thornei* per kg soil following a 12-month, weed-free fallow after harvest of the Phase 1 susceptible wheat cv. Cunningham or the partially resistant canaryseed cv. Moroccan. Points are plotted on the transformed scale with back-transformed labels indicated on the horizontal axis. Bar maker: l.s.d. ($P = 0.05$) for soil depth × Phase 1 crop.

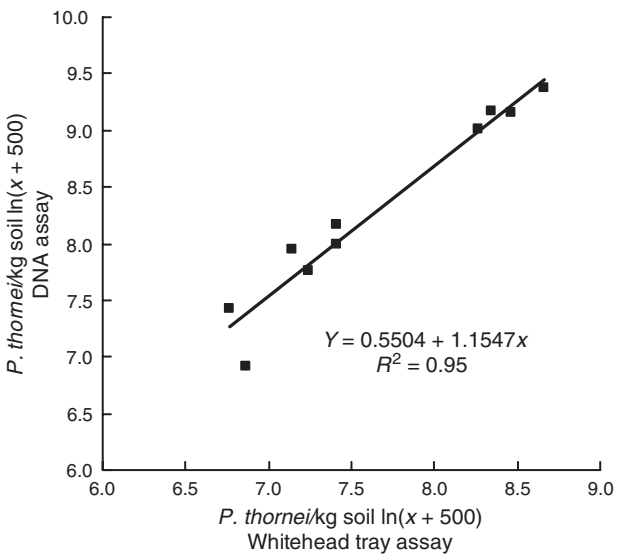

Fig. 2. Relationship between populations of *Pratylenchus thornei* per kg dry soil assessed by a DNA assay (Ophel-Keller *et al.* 2008) and the Whitehead tray extraction and microscopy assay on samples collected following a 12-month, weed-free fallow after harvest of the Phase 1 susceptible wheat or the partially resistant canaryseed. Means of wheat and canaryseed for each soil depth interval to 90 cm were plotted; $P < 0.001$; $n = 10$.

cultivars ($P > 0.05$). For the means of the Phase 2 crop cultivars, averaged over the Phase 1 crops, there were significant differences ($P \leq 0.05$) between cultivars within sorghum (range of 579 g/5 plants for cv. DK 39Y to 1232 g/5 plants for cv. Freedom), millets (range of 378 kg/ha for White French millet to 9070 kg/ha for pearl millet) and panicum (range of 2608 kg/ha for cv. Common panicum to 5736 kg/ha for cv. Panorama) (Fig. 3).

Pratylenchus thornei

Population densities of *P. thornei* at 1 month after removal of the Phase 2 summer crops showed a significant soil depth × Phase 1 crops × Phase 2 crops interaction effect ($P \leq 0.05$). Densities of *P. thornei*, averaged for cultivars within each Phase 2 crop were highest following soybean, mungbean and black gram (Fig. 4). Intermediate populations remained after maize and sunflower and smallest populations after millets, panicum and sorghum. Population densities of *P. thornei* after the Phase 2 summer crops following the Phase 1 wheat at 0–15 cm were in the range 862–10 850/kg soil, compared with 318–6042/kg soil following the Phase 1 canaryseed. Following the Phase 1 canaryseed, greatest populations after black gram were at 0–15 cm, after soybean at 0–30 cm, and after mungbean at 0–45 cm. For the other Phase 2 crops, populations did not differ significantly

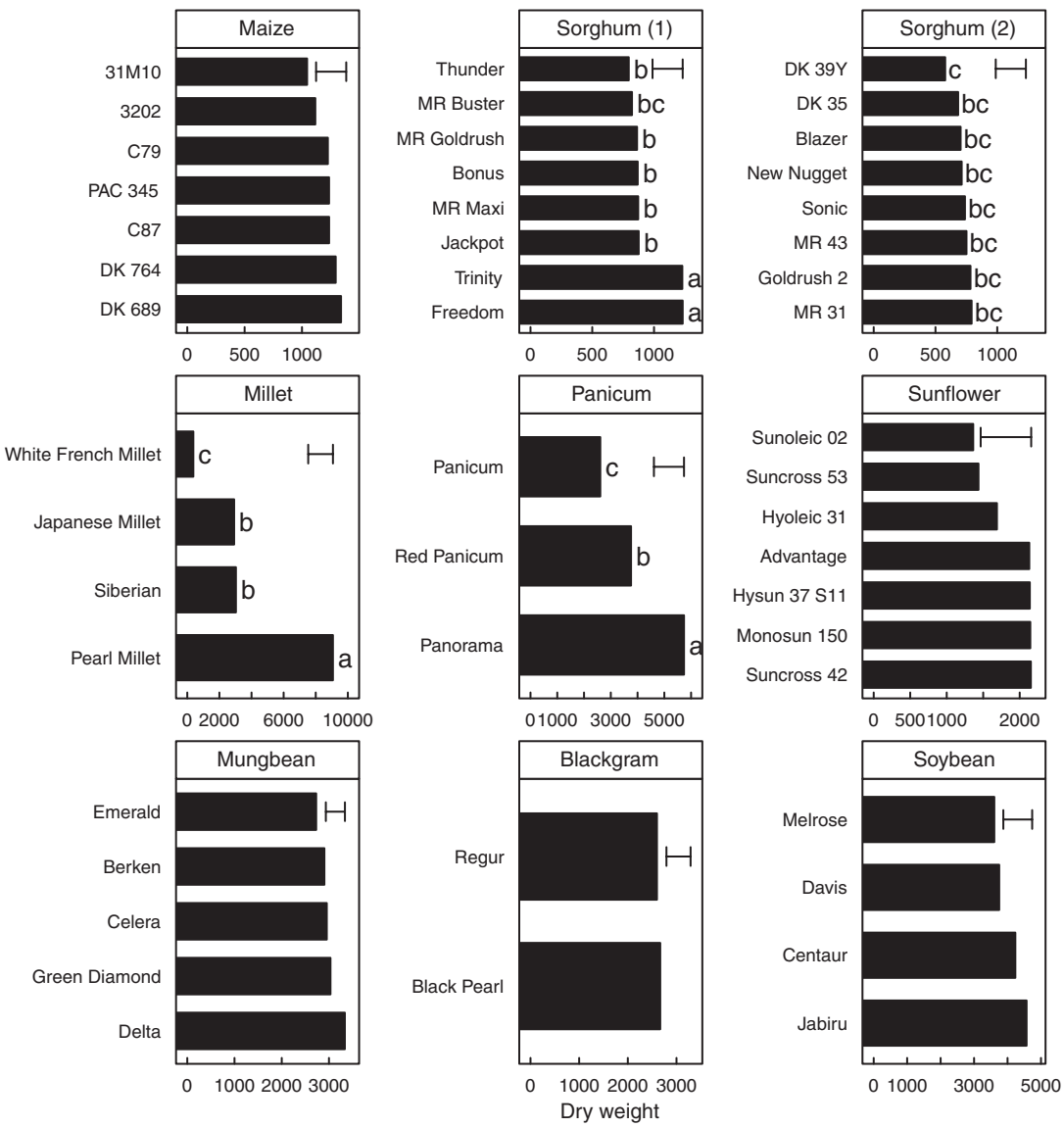

Fig. 3. Biomass of the Phase 2 summer crops at 3 months after planting. Biomass of maize, sorghum and sunflower is the dry weight of five plants (g); for all other crops biomass was calculated as kg/ha. Means of the combined Phase 1 wheat and canaryseed treatments are presented. Differences between cultivars were non-significant except where indicated with letters (means with the same letter are not significantly different at $P = 0.05$). Bar marker, l.s.d. ($P = 0.05$) for cultivars within each crop.

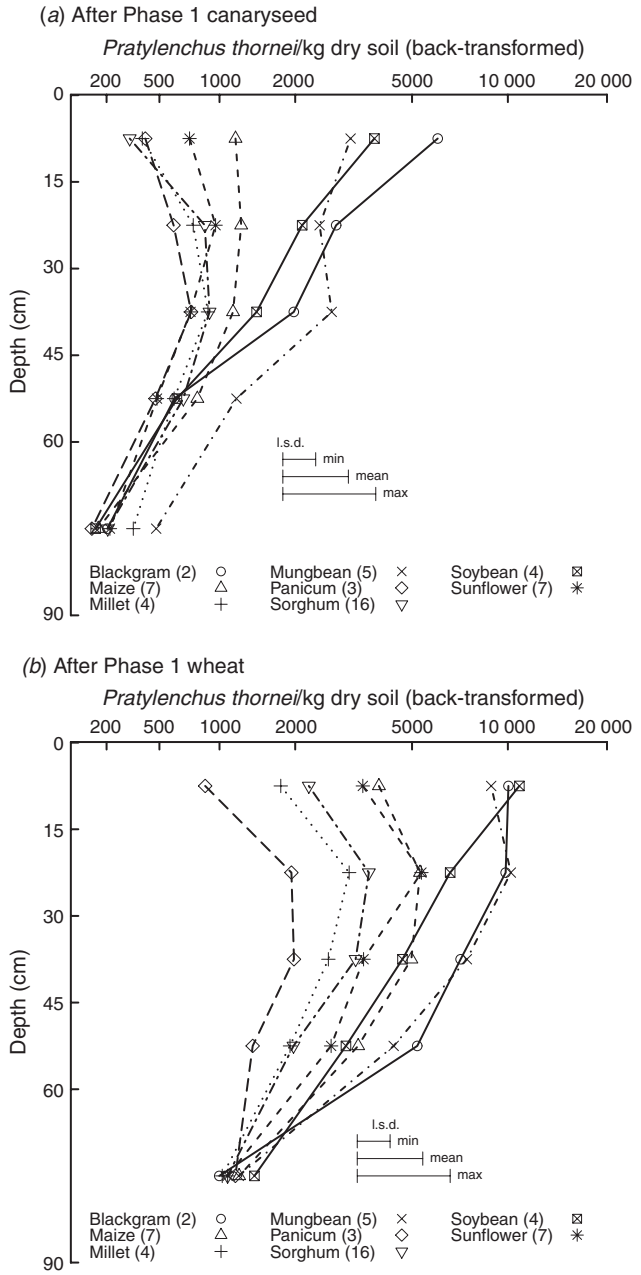

Fig. 4. Populations of *Pratylenchus thornei* per kg soil in the soil profile at 1 month after removal of the Phase 2 summer crops following Phase 1 (*a*) canaryseed or (*b*) wheat. Points are plotted on the transformed scale with back-transformed labels indicated on the horizontal axis. Bar maker: l.s.d. ($P = 0.05$) for soil depth × Phase 1 crop × Phase 2 crops. Maximum l.s.d. for comparing black gram and panicum; minimum l.s.d. for sorghum; mean l.s.d. for all other crops. Values in parentheses after crop names are number of cultivars tested.

throughout the profile. After the Phase 1 wheat, greatest populations after millet were at 15–30 cm, after sorghum at 15–45 cm, after maize and sunflower at 0–60 cm, and after black gram, mungbean and soybean at 0–45 cm. Populations after panicum were similar throughout the profile.

Accumulated *P. thornei* counts over 0–90 cm soil depth showed a significant Phase 1 crops × Phase 2 crops interaction effect ($P \leq 0.05$); the Phase 2 cultivars (within crops) effect was significant ($P \leq 0.05$) but there was no interaction with Phase 1 crops. The greatest population densities of *P. thornei* at 0–90 cm soil depth followed soybean cv. Davis (8099/kg soil), mungbean cv. Green Diamond (7862/kg soil), maize cv. DK 764 (7650/kg soil) and black gram cv. Regur (6931/kg soil) after the Phase 1 wheat. Smallest populations of *P. thornei* followed sorghum cvv. Sonic and DK 39Y (231 and 328/kg soil, respectively), sunflower cv. Hyoleic 31 (311/kg soil) and panicum cv. Panorama (360/kg soil) after the Phase 1 canaryseed (Table 3).

Reproduction factors (RF) of the Phase 2 summer crops were generally greater for cultivars grown after Phase 1 canaryseed than after Phase 1 wheat (Table 3). Crops with low RF (<1.8) included millets, panicum, sorghum and sunflower (except cv. Suncross 42 after Phase 1 canaryseed). The RF for maize cv. DK 764 was relatively high (RF 2.4) following Phase 1 wheat compared with the other maize cultivars. Cultivars of mungbean, black gram and soybean, after both Phase 1 crops, had RF values from 1.2 to 6.2 (except soybean cv. Jabiru following Phase 1 canaryseed, RF 0.7) (Table 3).

Pratylenchus thornei population densities for individual depth intervals to 90 cm for all summer crop cultivars following the Phase 1 canaryseed and wheat crops are given in supplementary table S2. There was a significant soil depth × Phase 2 cultivars interaction ($P \leq 0.05$). Very high population densities were found in soybean cv. Davis (19 921/kg soil at 0–15 cm), mungbean cv. Green Diamond (17 588/kg soil at 15–30 cm) and maize cv. DK 764 (12 615/kg soil at 15–30 cm) after the Phase 1 wheat crop.

Merlinius brevidens *and non-parasitic nematodes*

Densities of *M. brevidens* averaged over 0–90 cm soil depth showed a significant effect for Phase 2 crop cultivars ($P \leq 0.05$) only (supplementary table S3). Populations were smallest after panicum cv. Panorama (300/kg soil) and greatest after Siberian millet (5853/kg soil). Population densities of *M. brevidens* at individual depth intervals showed a significant soil depth × Phase 2 cultivars interaction effect ($P \leq 0.05$) and were lowest after sorghum cv. Bonus and panicum cv. Panorama (226 and 234/kg soil at 0–15 cm, respectively) and highest after Siberian millet (11 532/kg soil at 0–15 cm). For most crop cultivars, population densities of *M. brevidens* were highest at 0–30 cm (supplementary table S4).

Densities of non-parasitic nematodes averaged over 0–90 cm soil depth showed a significant effect of Phase 2 crops or Phase 2 cultivars ($P \leq 0.05$). For the individual soil intervals, there was a significant soil depth × Phase 2 cultivars interaction ($P \leq 0.05$). Most non-parasitic nematodes were found at 0–15 cm but were present at all depth intervals to 90 cm. Populations were greatest after the sunflower cultivars (7701–18 793/kg soil at 0–15 cm or 2531–5067/kg soil at 0–90 cm); for other crop species, populations ranged from 1135/kg soil (sorghum cv. Freedom) to 2326/kg soil (maize cv. C87) at 0–90 cm (supplementary tables S5 and S6).

Table 3. Populations of *Pratylenchus thornei* per kg soil at 0–90 cm soil depth at 1 month after removal of the Phase 2 summer crops following the Phase 1 wheat or canaryseed crop

For each Phase 2 summer crop, cultivars are ranked in ascending order within each Phase 1 crop; BTM, back-transformed mean; RF, reproduction factor = (no. of *P. thornei*/kg dry soil after harvest of Phase 2 crops) ÷ (no. of *P. thornei*/kg dry soil at 1 month before planting, i.e. 603 *P. thornei*/kg dry soil after Phase 1 canaryseed; 3255 *P. thornei*/kg dry soil after Phase 1 wheat)

		Phase 1 canaryseed					Phase 1 wheat		
Crop	Cultivar	ln(x + 500)	BTM	RF	Crop	Cultivar	ln(x + 500)	BTM	RF
Maize	C79	6.97	560	0.9	Maize	DK 689	7.95	2341	0.7
	DK 689	7.12	735	1.2		31M10	8.03	2578	0.8
	PAC 345	7.13	754	1.3		C79	8.17	3028	0.9
	31M10	7.20	840	1.4		3202	8.32	3590	1.1
	C87	7.34	1036	1.7		C87	8.34	3683	1.1
	DK 764	7.48	1269	2.1		PAC 345	8.36	3755	1.2
	3202	7.49	1287	2.1		DK 764	9.01	7650	2.4
Millets	White French	6.83	429	0.7	Millets	White French	7.73	1768	0.5
	Pearl	7.06	663	1.1		Siberian	7.89	2181	0.7
	Siberian	7.13	747	1.2		Japanese	7.92	2247	0.7
	Japanese	7.16	785	1.3		Pearl	8.01	2504	0.8
Panicum	Panorama	6.76	360	0.6	Panicum	Panorama	7.37	1088	0.3
	Red	6.86	453	0.8		Panicum	7.71	1719	0.5
	Panicum	7.00	595	1.0		Red	7.95	2340	0.7
Black gram	Black Pearl	7.69	1688	2.8	Black gram	Black Pearl	8.69	5448	1.7
	Regur	8.05	2625	4.4		Regur	8.91	6931	2.1
Mungbean	Delta	7.65	1607	2.7	Mungbean	Berken	8.38	3846	1.2
	Emerald	7.72	1755	2.9		Delta	8.78	6001	1.8
	Celera	7.80	1930	3.2		Emerald	8.84	6432	2.0
	Berken	7.87	2109	3.5		Celera	8.88	6663	2.0
	Green Diamond	8.14	2944	4.9		Green Diamond	9.03	7862	2.4
Sorghum	Sonic	6.59	231	0.4	Sorghum	MR Maxi	7.72	1748	0.5
	DK 39Y	6.72	328	0.5		Sonic	7.84	2047	0.6
	MR Buster	6.85	447	0.7		MR Buster	7.87	2115	0.6
	Jackpot	6.91	497	0.8		Jackpot	7.88	2141	0.7
	Blazer	6.93	522	0.9		MR 43	7.90	2194	0.7
	MR 43	6.96	558	0.9		DK 39Y	7.92	2260	0.7
	MR Goldrush	6.98	576	1.0		Bonus	7.93	2267	0.7
	New Nugget	6.99	589	1.0		Goldrush 2	7.97	2390	0.7
	DK 35	7.04	645	1.1		New Nugget	7.98	2409	0.7
	Thunder	7.07	671	1.1		Freedom	8.00	2473	0.8
	Freedom	7.08	691	1.1		Trinity	8.03	2569	0.8
	MR Maxi	7.14	759	1.3		Blazer	8.04	2588	0.8
	MR 31	7.14	763	1.3		MR 31	8.05	2632	0.8
	Bonus	7.21	851	1.4		DK 35	8.08	2729	0.8
	Goldrush 2	7.24	894	1.5		Thunder	8.15	2963	0.9
	Trinity	7.36	1078	1.8		MR Goldrush	8.19	3111	1.0
Soybean	Jabiru	6.84	436	0.7	Soybean	Jabiru	8.43	4072	1.3
	Melrose	7.47	1261	2.1		Centaur	8.48	4310	1.3
	Centaur	7.88	2136	3.5		Melrose	8.57	4766	1.5
	Davis	8.35	3713	6.2		Davis	9.06	8099	2.5
Sunflower	Hyoleic 31	6.70	311	0.5	Sunflower	Hyoleic 31	8.05	2617	0.8
	Advantage	6.86	453	0.8		Monosun 150	8.12	2855	0.9
	Sunoleic 02	6.95	545	0.9		Sunoleic 02	8.12	2867	0.9
	Monosun 150	7.03	631	1.0		Suncross 42	8.24	3290	1.0
	Suncross 53	7.12	741	1.2		Advantage	8.27	3411	1.0
	Hysun 37 S11	7.18	806	1.3		Suncross 53	8.28	3428	1.1
	Suncross 42	7.51	1325	2.2		Hysun 37 S11	8.40	3966	1.2

l.s.d. (P = 0.05) (cultivar 0.40)

Soil moisture

Soil moisture at 1 month after removal of the Phase 2 crops showed a significant soil depth × Phase 2 cultivars interaction effect ($P \leq 0.05$). Average values for Phase 1 crops were lowest at 0–15 cm (range from 30.7% for Siberian millet to 39.3% for maize cv. PAC 345) and highest at 15–30 cm soil depth (range from 38.1% for sorghum cv. MR Buster to 47.3% for maize cv. PAC 345). Generally, soil moisture was lowest after sorghum at

Table 4. Populations of *Pratylenchus thornei* per kg soil at 0–90 cm soil depth at 13 months after removal of the Phase 2 summer crops following the Phase 1 wheat and change (%) in populations compared with the previous sampling at 1 month after harvest of the Phase 2 summer crops
Within each Phase 2 summer crop, cultivars are ranked in ascending order; means of the combined Phase 1 wheat and canaryseed crops are shown; BTM, back-transformed mean; number in italics is the mean for the crop.

Crop	Cultivar	ln (*x*+ 500)	BTM	Change (%)	Crop	Cultivar	ln(*x* + 500)	BTM	Change (%)
Black gram	Regur	8.09	2746		Sorghum	MR 43	7.52	1339	
	Black Pearl	8.15	2960			Freedom	7.59	1483	
		8.12	*2851*	*–54*		DK 39Y	7.62	1540	
						Trinity	7.68	1664	
Mungbean	Berken	8.19	3114			MR Maxi	7.74	1808	
	Celera	8.25	3313			Goldrush 2	7.87	2115	
	Delta	8.41	3983			New Nugget	7.93	2268	
	Emerald	8.48	4291			MR Buster	7.94	2301	
	Green Diamond	8.66	5287			Thunder	8.00	2479	
		8.40	*3934*	*–36*		Bonus	8.05	2631	
						Blazer	8.13	2879	
Soybean	Melrose	8.28	3458			MR Goldrush	8.13	2881	
	Jabiru	8.34	3695			Jackpot	8.17	3033	
	Davis	8.48	4313			DK 35	8.19	3086	
	Centaur	8.70	5515			MR 31	8.29	3498	
		8.45	*4182*	*–21*		Sonic	8.38	3850	
							7.95	*2338*	*–3*
Sunflower	Hysun 37 S11	7.87	2124						
	Monosun 150	7.99	2436		Panicum	Panorama	7.58	1464	
	Hyoleic 31	8.09	2770			Panicum	7.78	1884	
	Suncross 42	8.10	2783			Red	7.88	2136	
	Sunoleic 02	8.24	3273				*7.75*	*1811*	*6*
	Advantage	8.28	3433						
	Suncross 53	8.64	5149						
		8.17	*3038*	*–5*	Millets	Siberian	7.64	1580	
						Japanese	7.68	1668	
Maize	31M10	7.79	1916			Pearl	7.76	1846	
	C87	7.87	2123			White French	8.12	2856	
	PAC 345	7.96	2370				*7.80*	*1941*	*–11*
	DK 689	7.97	2380						
	C79	8.07	2690						
	3202	8.16	3009						
	DK 764	8.90	6860						
		8.10	*2806*	*–26*					

l.s.d. ($P = 0.05$) (cultivar 0.73, crop 0.33)

15–45 cm and after maize at 45–90 cm. Soil moisture was highest after millets and panicum at 30–90 cm (supplementary table S7).

Nematode populations and soil moisture before planting the Phase 3 wheat

Prior to planting the Phase 3 wheat, population densities of *P. thornei* at 0–90 cm soil depth (collected only from the Phase 1 wheat plots) were significantly affected by Phase 2 crops or Phase 2 cultivars ($P \leq 0.05$). Densities remained higher following black gram, mungbean and soybean (2851–4182/kg soil at 0–90 cm) and were lowest after panicum and millets (1811 and 1941/kg soil at 0–90 cm, respectively) (Table 4). For cultivars, populations ranged from 1339/kg soil after sorghum cv. MR 43 to 6860/kg soil after maize cv. DK 764. Populations in the 0–90 cm interval decreased compared with those in the soil sampled 13 months previously, from –54% change after black gram to <–11% after millet, sunflower and sorghum. There was a small increase in populations after panicum (Table 4).

Densities of *P. thornei* at individual soil depth intervals showed a significant soil depth × Phase 2 crops interaction effect ($P \leq 0.05$). At 15–30 cm soil depth, *P. thornei* populations ranged from 2139/kg soil after panicum to 7530/kg soil after mungbean (Fig. 5). Greatest populations for all crops were recorded at this depth, except for panicum, which had greatest populations at 30–45 cm. There were no significant differences between cultivars within each Phase 2 crop ($P > 0.05$) (data not shown).

Populations of *M. brevidens* at 0–90 cm soil depth showed a significant effect of Phase 2 crops ($P \leq 0.05$) and populations ranged from 505/kg soil following panicum to 1073/kg soil following maize at 0–90 cm (data not shown). At individual depths there was a significant soil depth × Phase 2 crops interaction ($P \leq 0.05$); populations ranged from 131 to 830/kg

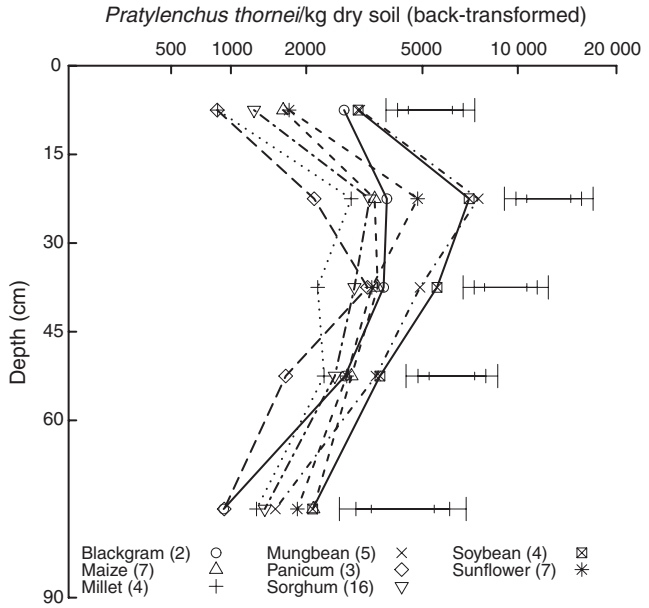

Fig. 5. Populations of *Pratylenchus thornei* per kg soil in the soil profile at 13 months after removal of the Phase 2 summer crops following Phase 1 wheat. Points are plotted on the transformed scale with back-transformed labels indicated on the horizontal axis. Bar maker: l.s.d. ($P = 0.05$) for soil depth × Phase 2 crops. Maximum l.s.d. for comparing black gram and panicum (outer markers); minimum l.s.d. for sorghum (inner markers); mean l.s.d. for all other crops (middle markers). Values in parentheses after crop names are number of cultivars tested.

soil at 0–15 cm, and populations were generally greatest at 30–45 and 45–60 cm (range of 411/kg soil after panicum at 30–45 cm to 1514/kg soil after soybean at 45–60 cm) (data not shown).

Populations of non-parasitic nematodes showed a significant effect of Phase 2 crops ($P \leq 0.05$), and populations ranged from 3268/kg soil after black gram to 5724/kg soil after sunflower at 0–90 cm (data not shown). There was a significant effect of soil depth ($P \leq 0.05$) and populations ranged from 5503/kg soil at 0–15 cm to 3362/kg soil at 60–90 cm (data not shown).

Soil moisture in the respective soil layers to 150 cm, before planting the Phase 3 wheat cv. Strzelecki, was 47.1, 50.7, 51.7, 51.6, 48.7, 45.0 and 45.6% (l.s.d. 0.4, $P = 0.05$). There were no significant interactions of soil depth with Phase 2 crops or Phase 2 cultivars ($P > 0.05$).

Biomass and grain yield of Phase 3 wheat

Biomass of the Phase 3 wheat cv. Strzelecki at 3 months after planting showed a significant Phase 1 crops × Phase 2 crops interaction effect ($P \leq 0.05$). Biomass was least after mungbean, soybean and black gram following Phase 1 wheat (1556–1808 kg/ha) and greatest after sunflower and sorghum following Phase 1 canaryseed (4615–5114 kg/ha) (Fig. 6). There were significant effects on biomass of the Phase 3 wheat for Phase 2 crop cultivars and Phase 1 crops ($P \leq 0.05$), but no significant interaction. Within each crop, there were no significant differences between cultivars ($P > 0.05$). Biomass ranged from 1123 kg/ha after Phase 1 wheat then mungbean cv. Berken to 6399 kg/ha after Phase 1 canaryseed then sorghum cv. Blazer (supplementary fig. S1).

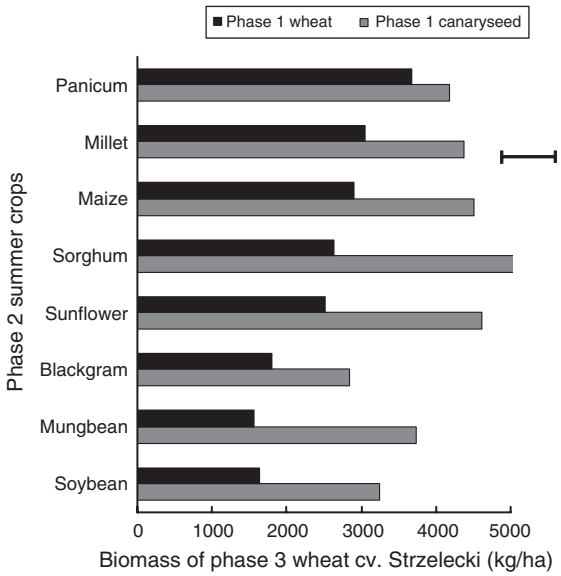

Fig. 6. Biomass (kg/ha) of the Phase 3 wheat cv. Strzelecki at 3 months after planting, following the Phase 2 summer crops and the Phase 1 canaryseed (grey columns) or Phase 1 wheat (black columns). Bar marker: l.s.d. ($P = 0.05$) for Phase 1 crop × Phase 2 summer crops.

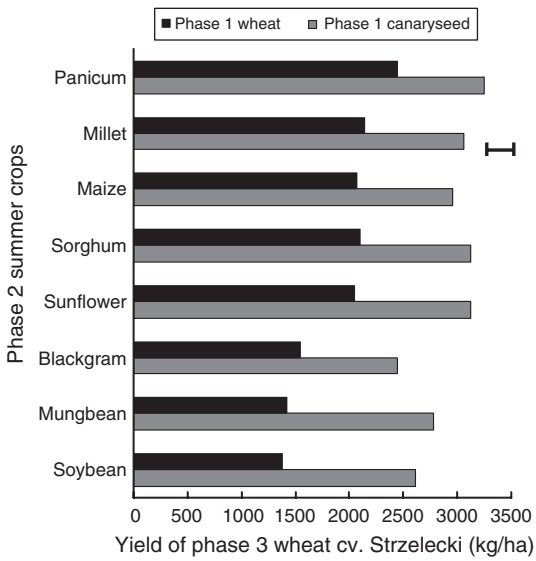

Fig. 7. Grain yield (kg/ha) of the Phase 3 wheat cv. Strzelecki following the Phase 2 summer crops and the Phase 1 canaryseed (grey columns) or Phase 1 wheat (black columns). Bar marker, l.s.d. ($P = 0.05$) for Phase 1 crop × Phase 2 summer crops.

Phase 3 wheat grain yield showed a significant Phase 1 crops × Phase 2 crops interaction effect ($P \leq 0.05$). Grain yield of Phase 2 wheat cv. Strzelecki was least after soybean, mungbean and black gram following Phase 1 wheat (1383, 1424, 1544 kg/ha, respectively). When these crops followed Phase 1 canaryseed, Phase 3 wheat yields increased to 2614, 2779 and 2448 kg/ha, an increase of 47, 49 and 37% for each crop, respectively (Fig. 7). The highest yields after the Phase 1 wheat crop followed panicum,

millets, maize, sorghum and sunflower (2046–2444 kg/ha), and yield increased 25–35% when the Phase 1 canaryseed preceded these crops (2953–3245 kg/ha). Overall, the mean grain yield of Phase 3 wheat cv. Strzelecki following Phase 1 canaryseed was 2996 kg/ha, compared with 1961 kg/ha following Phase 1 wheat (l.s.d. 72, $P = 0.05$), a difference of 35%.

Grain yields of wheat cv. Strzelecki following the Phase 2 crop cultivars were significantly affected by Phase 1 crop or Phase 2 cultivars ($P \leq 0.05$), but there was no significant interaction. Within each crop, there were no significant differences between cultivars ($P > 0.05$). Yields ranged from 1212 kg/ha after mungbean cv. Green Diamond following Phase 1 wheat to 3427 kg/ha after panicum cv. Panorama following Phase 1 canaryseed (supplementary fig. S2), a difference of 65%.

Regression analyses

Biomass

Linear regression of biomass of the Phase 3 wheat *v. P. thornei* population densities from samples collected directly after harvest of the Phase 2 summer crops following Phase 1 wheat and canaryseed was significant for all accumulated depth intervals ($P < 0.001$). There was a strong negative relationship that increased with soil depth; R^2 ranged from 0.61 at 0–15 cm to 0.69 for 0–90 cm ($P < 0.001$, $n = 96$; supplementary table S8). Using the regression equation for *P. thornei* from 0–90 cm, biomass loss of the Phase 3 wheat was 77% at 8099 *P. thornei*/kg soil (Fig. 8*a*).

Regressions of Phase 3 biomass and *P. thornei* following either Phase 1 canaryseed or Phase 1 wheat were significant for all soil depth intervals (all $P < 0.001$, except 0–90 cm after Phase 1 wheat where $P = 0.003$). There was a negative relationship that decreased with soil depth; for Phase 1 canaryseed, R^2 ranged from 0.35 at 0–90 cm to 0.5 at 0–15 cm; for Phase 1 wheat, R^2 ranged from 0.16 at 0–90 cm to 0.30 at 0–15 (supplementary table S8).

In samples that were collected only from the Phase 1 wheat plots after the Phase 2 summer crops followed by a 15-month fallow, there was a significant, although slightly weaker, negative relationship between Phase 3 wheat biomass and *P. thornei* that was strongest at 0–15 cm ($R^2 = 0.25$, $P < 0.001$) and least at 0–90 cm ($R^2 = 0.14$, $P = 0.005$). Using the regression equation for *P. thornei* from 0–15 cm after Phase 1 wheat then summer crop and 15-month fallow, biomass loss of the Phase 3 wheat was 54% (supplementary table S8).

Grain yield

Linear regression of grain yield of Phase 3 wheat *v. P. thornei* population densities from samples collected directly after harvest of the Phase 2 summer crops following Phase 1 wheat and canaryseed was significant for all accumulated depth intervals, and there was a strong negative relationship that increased with soil depth; R^2 ranged from 0.68 at 0–15 cm to 0.82 at 0–90 cm ($P < 0.001$, $n = 96$; supplementary table S9). Using the regression equation for *P. thornei* at 0–90 cm, yield loss of the Phase 3 wheat was 62% at 8099 *P. thornei*/kg soil (Fig. 8*b*).

Regressions of Phase 3 grain yield and *P. thornei* after either Phase 1 canaryseed or Phase 1 wheat were significant for all soil depth intervals ($P < 0.001$), and R^2 decreased with soil depth; for Phase 1 canaryseed, R^2 ranged from 0.45 at 0–90 cm to 0.55 at 0–15 cm; for Phase 1 wheat, R^2 ranged from 0.44 at 0–90 cm to 0.70 at 0–15 and 0–30 cm (supplementary table S9).

In samples that were collected only from the Phase 1 wheat plots after the Phase 2 summer crops followed by a 15-month fallow, there was a significant ($P < 0.001$), although slightly weaker, negative relationship between Phase 3 wheat yield and *P. thornei* that was strongest at 0–15 cm ($R^2 = 0.48$) and least at 0–90 cm ($R^2 = 0.38$). Using the regression equation for *P. thornei* from 0–15 cm after the Phase 1 wheat then summer crop and 15-month fallow, yield loss of the Phase 3 wheat was 34% (supplementary table S9).

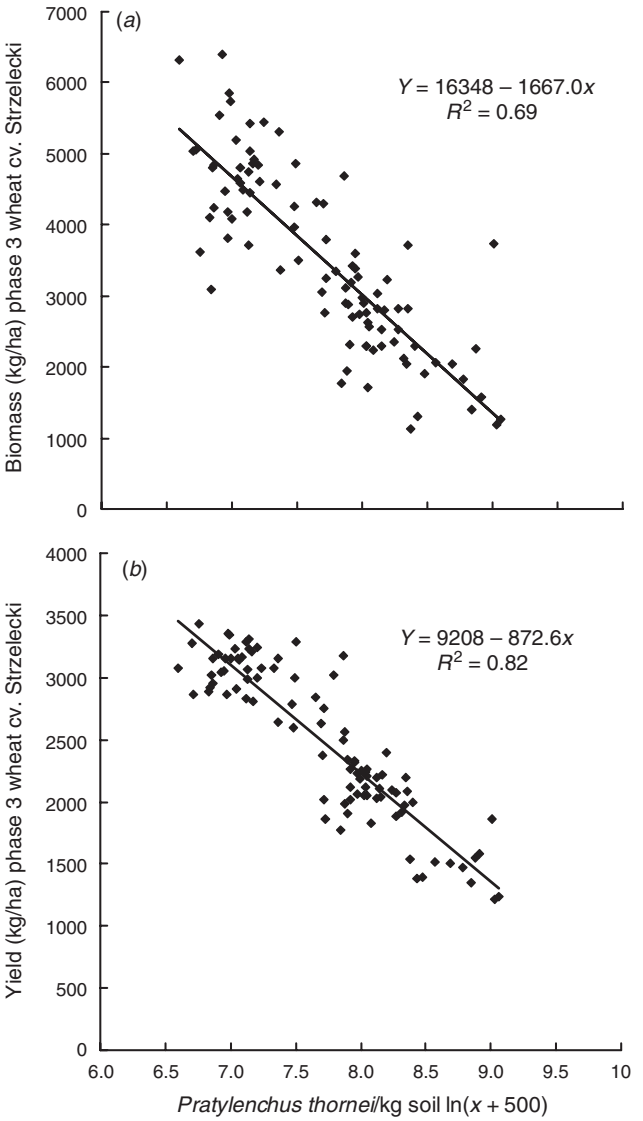

Fig. 8. Relationship between Phase 3 wheat cv. Strzelecki (*a*) biomass (kg/ha) and (*b*) grain yield (kg/ha) and populations of *Pratylenchus thornei* per kg dry soil at 0–90 cm soil depth at 1 month after harvest of the Phase 2 summer crops (15 months before planting the Phase 3 wheat crop) for means of Phase 2 crops cultivars after Phase 1 wheat and canaryseed; $P < 0.001$; $n = 96$.

There was no interaction between Phase 3 wheat biomass or grain yield and *M. brevidens*, non-parasitic nematodes or soil water after the Phase 2 summer crops at either sampling time (data not shown). There was a significant, positive relationship between Phase 3 wheat biomass and Phase 3 grain yield: $y = 994.89 + 0.425x$ where y is grain yield (kg/ha) of the Phase 3 wheat and x is biomass (kg/ha) of the Phase 3 wheat ($R^2 = 0.78$, $n = 96$, $P < 0.001$).

Discussion

This is the first report of a field experiment demonstrating the impact of sequential rotation of partially resistant and susceptible winter crops followed by a range of summer crop cultivars on populations of *P. thornei* and on the yield of a subsequently planted, intolerant wheat cultivar. The greatest gains in wheat grain yield were made when two partially resistant crops were grown in sequence; from the regression equation, wheat yield was reduced by 62% when two susceptible crops were grown in sequence and *P. thornei* populations at 0–90 cm were 8099/kg soil.

Host status and tolerance of summer crops

The study provides new information on the partial resistance of millets (*Echinochloa* spp., *Pennisetum* sp. and *Panicum* sp.) to *P. thornei*. The study also confirms previous reports of the poor host status or partial resistance of sorghum, panicum and sunflower, and the good host status or susceptibility of soybean and mungbean to *P. thornei* (Van Gundy *et al*. 1974; O'Brien 1982, 1983; Thompson 1994; Tobar *et al*. 1995; Di Vito *et al*. 2002).

There is inconsistency in the literature regarding the host status of maize, which has been described as a fair to poor host of *P. thornei* (Van Gundy *et al*. 1974; Thompson 1994). In our study, seven maize cultivars were tested and the responses ranged from partially resistant (RF $\leq$1.0) to susceptible (RF 2.4, 7650 *P. thornei*/kg soil at 0–90 cm). In fact, populations of *P. thornei* following maize cv. DK 764 were similar to those following the susceptible soybean and mungbean cultivars. The RF values reported in this experiment are likely to be conservative estimates because of the detrimental impact of the field environment on nematode multiplication and survival (Trudgill 1991), and they therefore require repetition (De Waele and Elsen 2002). Nonetheless, our results demonstrate the importance of determining the host status of current commercial cultivars, rather than a generalised crop rating.

The summer crops, other than mungbean, appeared tolerant of *P. thornei*, because there were no differences in crop biomass after either Phase 1 crop. This is an important consideration for growers because selection of rotation crops with both resistance and tolerance confers economic benefits (Whitehead 1998). Susceptible crops with tolerance (soybean and black gram) or moderate tolerance (mungbean) did not exhibit obvious symptoms of nematode infection, and large populations of *P. thornei* built up and survived to damage the next intolerant wheat crop in the sequence. The results of our study do not support the observation of O'Brien (1982) of poor growth or intolerance of sorghum associated with *P. thornei* in a field, but this may reflect differences in cultivar response, larger *P. thornei* populations in that study than in the present study, or others causes such as mycorrhizal deficiency. Further research on the impact of *P. thornei* on the grain yield of the summer crops is needed, particularly for mungbean.

Distribution of P. thornei *in the soil profile*

When all treatments were analysed, the strongest relationship between wheat yields in the final year of the experiment were found using *P. thornei* populations at 0–90 cm soil depth rather than from shallower soil samples ($R^2 = 0.82$ at 0–90 cm *v.* 0.68 at 0–15 cm). This concurs with experiments on population densities of the reniform nematode, *Rotylenchulus reniformis*, after deep fumigation or rotation with resistant crops, in which populations at 0–120 cm, rather than 0–30 cm, were better predictors of loss of cotton biomass (Westphal *et al*. 2004). However, whereas Westphal *et al*. (2004) found that populations of *R. reniformis* increased with depth, in our study *P. thornei* populations were greatest at 0–30 cm. The conclusion from both experiments was that the presence of the nematodes throughout the soil profile was a better predictor of the effect on biomass and yield rather than the nematode populations at shallow depths. Ideally, growers should collect deeper samples to understand the distribution of parasitic nematodes in their soil, similar to the current practice of assessment of soil nitrate and soil water.

In the present study, the vertical distribution of *P. thornei* changed with crop species. For example, although *P. thornei* was found after all crops to a depth of 90 cm, the greatest populations at harvest of mungbean were found at 0–45 cm soil depth, of sorghum at 15–45 cm and of soybean at 0–15 cm. Studies of root densities or water-use efficiency of summer crops and wheat grown under rain-fed conditions have shown that roots are active to 1.2 m for mungbean and to 2.0 m soil depth for sunflowers (Garay and Wilhelm 1983; Bremner *et al*. 1986; Newell and Wilhelm 1987; Hochman *et al*. 2001). In the deep Vertosol soils of the northern Australian grain region, mungbean extracted less water than wheat and sorghum below 30 cm (Hochman *et al*. 2001). In a study with soybean, root density was greatest in the top 30 cm of the soil profile until flowering, but as the soil dried, more roots were found at 90–120 cm (Garay and Wilhelm 1983). Root densities differ between crop species, and *P. thornei* populations mirror their distribution, supporting other research that showed distribution of *Pratylenchus* spp. was related to root biomass and crop species (MacGuidwin and Stanger 1991; Pudasaini *et al*. 2006).

In addition to the differences in *P. thornei* distribution between different plant species, there was variation dependent on the previous crops in the sequence. For example, after canaryseed followed by black gram, populations decreased from 6042/kg soil at 0–15 cm to 2794/kg soil at 15–30 cm; in contrast, after wheat followed by black gram, populations were ~10 000/kg soil at both 0–15 and 15–30 cm. *Pratylenchus* are migratory throughout their lifecycle (after hatching), and when plant hosts senesce they can leave the roots and move in soil-water films (Castillo and Vovlas 2007). During weed-free fallow periods, Vertosol soils such as those in the present study can accumulate 200–250 mm of water at 0–1.8 m depth (Dang *et al*. 2006). After fallow periods, soil water content did not differ

between treatments, indicating that rainfall in the present study was sufficient to replenish the soil profile. However, the rate of water use during crop growth and therefore the rate of soil water accumulation during fallow periods varied between crop species until the profile was replenished. Thus, the rate of nematode movement in water films, which is greatest when soil pores are full of water (Wallace 1968), partly reflects water use by the previous crops in the sequence. In addition to needing water films for migration, the rate of migration and consequent infectivity of plant roots by *P. penetrans* was influenced by other factors including temperature, plant host, plant age and nematode developmental stage (Pudasaini *et al*. 2007).

In the present study, populations of *P. thornei* decreased by up to 54% at 0–90 cm after the 15-month, weed-free fallow following harvest of the summer crops. In general, the most marked population decline was at 0–15 cm, with an increase in populations at 60–90 cm. In an experiment at the same site as the current study, populations of *P. thornei* decreased by as much as 95% at 0–15 cm while increasing at 30–60 cm following some winter crop–summer fallow sequences (Owen *et al*. 2010). Similarly, populations of *P. jordanensis* were greater at 16–30 cm than at 0–15 cm in fallow fields, while populations were larger at 0–15 cm in-crop (Mani 1999). Nematode death and downward vertical migration may have contributed to these changes in population distribution; this could occur in response to topsoil drying during the low rainfall between April and September, and topsoil warming during the summer months. In addition *Pratylenchus* spp. have remarkable ability to endure harsh conditions because of their thick cuticle, impervious egg membranes and survival inside plant roots (Egunjobi and Bolaji 1979; Tobar *et al*. 1995; Castillo and Vovlas 2007). In very dry soil, *P. thornei* are capable of anhydrobiosis, and in this state can endure temperatures up to 40°C (Glazer and Orion 1983). Despite the decline in populations of *P. thornei* in our study, weed-free fallow periods were not sufficient to control *P. thornei.*

It is interesting that *P. thornei* populations measured 1 month after harvesting the Phase 2 summer crops were a better predictor of reduction of wheat biomass and grain yield than populations measured 1 month before planting the Phase 3 wheat crop (after a 15-month fallow). This suggests a hysteresis effect or lag, which may be an artefact of the nematode extraction method used and/or an alteration in the behaviour of the nematodes after the 15-month fallow. The Whitehead tray extraction method may underestimate the total number of viable nematodes in the sample compared with a DNA assay, which detects all stages of the nematode's lifecycle, including eggs and recently killed nematodes (Hollaway *et al*. 2003). In our study, a DNA assay on samples collected after a 12-month fallow after the Phase 1 crops detected greater numbers of *P. thornei* than the Whitehead tray method. If a greater proportion of *P. thornei* individuals were present as eggs or anhydrobiotic nematodes after the 15-month fallow, then extraction by the Whitehead tray method for 48 h may not have been sufficient to allow hatching and rehydration before migration of the nematodes to the collection water. In one experiment, *in vitro* egg hatching of *P. thornei* increased from 21% after 3 days incubation to 41% after 9 days (Castillo *et al*. 1996), and in another study, using the Whitehead tray extraction, there was an increase in *Pratylenchus* spp. extracted at 8 days in fallow soil after harvest of maize, presumably due to egg hatching (Egunjobi and Bolaji 1979). Conditions during the Phase 3 wheat crop may have allowed sufficient recovery of nematodes stages so that populations rebounded to levels seen before the 15-month fallow. Other studies have demonstrated recovery rates of up to 85% after reactivation of anhydrobiotic nematodes, with improved survival when the previous crop was a good host (Tobar *et al*. 1995) and increased reproductive rates compared with fresh nematodes (Glazer and Orion 1983).

Modelling the interaction of survival of *P. thornei*, subsequent infection of susceptible crops in relation to soil water and temperature and crop sequencing in the deep Vertosol soils may further assist growers and researchers with decisions about the most efficient sampling depth and time of sampling for predicting yield loss.

Wheat yield loss and crop sequencing

From regression analysis, there was 77% loss of biomass at anthesis and 62% loss of grain yield in a *P. thornei*-intolerant wheat cultivar with 8099 *P. thornei*/kg soil at 0–90 cm soil depth. Similarly, in an experiment at the same site, Thompson *et al*. (2012) reported that wheat biomass loss was 65% at anthesis and yield loss was 76% when wheat was grown after wheat (3500 *P. thornei*/kg soil at planting in the topsoil) compared with wheat after sorghum (no *P. thornei* detected). In contrast, in South Australia, wheat yield loss ranged from 14% to 26% with 45 000 and 6600 *P. thornei*/kg soil, respectively (Nicol *et al*. 1999; Taylor *et al*. 1999). A range of damage thresholds have been reported for wheat and *P. thornei*, from 500 to 2500 nematodes/kg soil (reviewed in Castillo and Vovlas 2007), that are influenced by edaphic, environmental, cultural and varietal conditions. Ideally they should be determined over several years of field experiments in different locations (Ferris 1978).

The benefit of growing two partially resistant crops sequentially before the intolerant wheat cultivar was clearly demonstrated in the present study and agrees with an observation of Mexican fields infested with *P. thornei* and an experiment in Australia in which wheat yields were higher when they followed at least 2 years of non-wheat crops (Van Gundy *et al*. 1974; Thompson *et al*. 2012). In our study, *P. thornei* populations after the Phase 1 wheat crop had a major effect on the next wheat crop 3 years later, which was further compounded by the susceptible summer crops. Accumulating nematode populations are greater under tolerant–susceptible crops (Roberts 2002) such as the summer crops tested. Consequently, growing two or more partially resistant crops in succession is recommended to reduce populations of *P. thornei* throughout the soil profile when a very susceptible crop is grown initially.

Crop rotation is important in balanced, profitable, sustainable farming systems and is associated with increased yield, which is partly explained by disease management of the targeted pathogen, weed control and improved soil properties such as water availability, soil structure and microbial activity (Karlen *et al*. 1994). Legume crops grown in rotation with wheat are particularly beneficial due to increased nitrogen supply and farm profitability through market diversity (Cox *et al*. 2010). However, the susceptibility of the summer legume crops to *P. thornei* offset their benefits, and it is important to increase the resistance levels

over current cultivars grown in the Australian northern grain region. For other important but susceptible crops, such as wheat and chickpea, lines with improved resistance to *P. thornei* have been produced by hybridisation of wild relatives with commercial cultivars (Thompson *et al*. 2011; Sheedy *et al*. 2012). Similarly, soybean lines with immunity to the soybean cyst nematode (*Heterodera glycines*) were produced using wild relatives of soybean (Bauer *et al*. 2007), and wild *Vigna* spp. hybrids may offer some improvement of mungbean lines against insects and diseases (Young *et al*. 1992; Nadarajan and Gupta 2010). In addition, there were differences in susceptibility of the soybean and mungbean cultivars tested in the current study that could be exploited in breeding programs to select more resistant cultivars. Breeding for durable resistance to root-lesion nematodes is a reliable management strategy, but until resistant lines of legume crops can be produced, the full benefits of crop rotation will not be realised.

Merlinius brevidens *and non-parasitic nematodes*

The present study identified new crop hosts of *M. brevidens*, namely millets, soybean and most panicum cultivars; one poor host, panicum cv. Panorama, was identified. *Merlinius brevidens* (syn. *Tylenchorhynchus brevidens*, *Geocenamus brevidens*) has a broad host range (Siddiqi 1972; Tobar *et al*. 1995) and is widely distributed in grain-producing regions in Australia, the USA and Spain, often in association with *P. thornei* (Talavera *et al*. 1998; Smiley *et al*. 2004; Thompson *et al*. 2010). *Merlinius brevidens* was not associated with yield loss of the Phase 3 wheat crop in the present study. Similarly, when *M. brevidens* was found with other nematodes such as *P. thornei*, *P. neglectus* and *Heterodera avenae*, wheat yield loss was related to populations of the more damaging *Pratylenchus* and *Heterodera* nematodes (Smiley *et al*. 2005*a*, 2005*b*; Thompson *et al*. 2012).

Non-parasitic (or free-living) nematodes were found to 90 cm depth in the soil profile, with the greatest populations in the topsoil (0–15 cm) at 1 month after removal of the Phase 2 summer crops. The distribution of fine and structural roots, which in turn alters soil organic matter, may be the major influence of distribution of non-parasitic nematodes in the soil profile (Bell *et al*. 2006; Stirling 2011). Notably, populations after sunflower were markedly greater than other summer crops (7701–18 793/kg soil for sunflower compared with <6000/kg soil for other crops); however, it is unknown whether this crop fostered more diverse trophic groups of non-parasitic nematodes.

Conclusion

Management of *P. thornei* is central to wheat production in many regions, but it is restricted by the intolerance and susceptibility of wheat cultivars, the broad host range of the nematode, and the resilience and accumulation of nematode populations throughout the soil profile. We demonstrated that growing two partially resistant crops in sequence (e.g. canaryseed, followed by millets, panicum, sorghum, sunflower or most maize cultivars) caused populations of *P. thornei* at 0–90 cm soil depth to decrease and counteracted a 62% yield loss of the next wheat crop in sequence, compared with a sequence with two susceptible crops (e.g. wheat followed by mungbean, black gram or soybean). Reducing the impact of *P. thornei* will require development of resistant cultivars of wheat and legume crops together with the provision of nematode diagnostics services and extension of information on the host status of cultivars of crops.

Acknowledgements

We thank Tammy Bull, Ian Dempsey and Ros Reen for their technical assistance and Alex Gwynne and family for their ongoing support and generous use of their farm for our research. Soil analyses were conducted by the former Analytical Chemistry Section, of the Department of Natural Resources and Mines, Toowoomba, Queensland. This work was funded by the Grains Research and Development Corporation.

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