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Pre-cropping with canola decreased *Pratylenchus thornei* populations, arbuscular mycorrhizal fungi, and yield of wheat

K. J. Owen[A,B], *T. G. Clewett*[A], *and J. P. Thompson*[A]

[A]Agri-Science Queensland, Department of Employment, Economic Development and Innovation, Leslie Research Centre, PO Box 2282, Toowoomba, Qld 4350, Australia.
[B]Corresponding author. Email: Kirsty.Owen@deedi.qld.gov.au

Abstract. Root-lesion nematode (*Pratylenchus thornei*) significantly reduces wheat yields in the northern Australian grain region. Canola is thought to have a 'biofumigation' potential to control nematodes; therefore, a field experiment was designed to compare canola with other winter crops or clean-fallow for reducing *P. thornei* population densities and improving growth of *P. thornei*-intolerant wheat (cv. Batavia) in the following year. Immediately after harvest of the first-year crops, populations of *P. thornei* were lowest following various canola cultivars or clean-fallow (1957–5200 *P. thornei*/kg dry soil) and were highest following susceptible wheat cultivars (31 033–41 294/kg dry soil). Unexpectedly, at planting of the second-year wheat crop, nematode populations were at more uniform lower levels (<5000/kg dry soil), irrespective of the previous season's treatment, and remained that way during the growing season, which was quite dry. Growth and grain yield of the second-year wheat crop were poorest on plots previously planted with canola or left fallow due to poor colonisation with arbuscular mycorrhizal (AM) fungi, with the exception of canola cv. Karoo, which had high AM fungal colonisation and low wheat yields. There were significant regressions between growth and yield parameters of the second-year wheat and levels of AMF following the pre-crop treatments. Thus, canola appears to be a good crop for reducing *P. thornei* populations, but AM fungal-dependence of subsequent crops should be considered, particularly in the northern Australian grain region.

Additional keywords: brassicas, long-fallow disorder.

Introduction

Root-lesion nematode (*Pratylenchus thornei*) is widely distributed in the Australian wheatbelt and causes substantial damage in the northern grain region (southern Queensland and northern New South Wales). The estimated present value of wheat production lost due to *P. thornei* is $38 million per year in the northern grain region (Murray and Brennan 2009). Root-lesion nematodes are migratory, endoparasitic, and feed and reproduce in the cortex of plant roots (Fortuner 1977). High populations of *P. thornei* result in root damage and loss of root function, which causes symptoms of nutrient deficiencies and water stress in the plant tops (Whitehead 1997). An estimated economic threshold for economic loss in intolerant wheat in the northern grain region is 2000 *P. thornei*/kg dry soil (Thompson *et al*. 2010). The best methods of managing *P. thornei* in Australian dryland cropping systems are the use of tolerant and resistant cultivars, crop rotation with poor host crops, and farm hygiene (Thompson *et al*. 1999, 2008).

If rotation crops are to be used in the northern grain region, it is prudent to consider their status as mycorrhizal hosts. Arbuscular mycorrhizal fungi (AMF) are symbiotic fungi that colonise plant roots and enhance uptake of poorly mobile nutrients such as phosphorus and zinc (Marschner 1986). A decline in the level of AM fungal inoculum causes poor growth of AM fungal-dependent field crops after long periods of clean-fallow (greater than 12 months) and is referred to as 'long fallow disorder' (Thompson 1987). The severity of the disorder depends on (*i*) the degree of reduction of AM fungal inoculum caused by fallow or cropping with a non-mycorrhizal host-crop, (*ii*) the level of P and Zn in the soil, and (*iii*) the mycorrhizal dependency of the crop species (Thompson 1991).

Brassicas have been shown to reduce levels of numerous soil-borne pathogens including fungi, bacteria, and nematodes (Matthiessen and Kirkegaard 2006). Brassicas have a 'biofumigation' ability, which results from the enzymatic degradation of glucosinolates in root residues to produce a variety of products including anti-microbial isothiocyanates, the level of which varies with cultivar (Kirkegaard and Sarwar 1999). Canola (*Brassica napus* ssp. *olifera* var. *annua*) has been promoted as a rotational crop for the suppression of soil-borne diseases of wheat in Australia, such as take-all, caused by *Gaeumannomyces graminis* var. *tritici* (Angus *et al*. 1994; Kirkegaard *et al*. 2000). Potter *et al*. (1998) found that addition of *Brassica* spp. tissue to soil suppressed *Pratylenchus neglectus* populations more than wheat tissue in an incubation experiment. This effect was correlated with the level of 2-phenylethyl glucosinolate (PEG) in the tissues but not other forms of glucosinolates or the total glucosinolate content.

A potential disadvantage of using canola is that it also reduces populations of beneficial soil organisms, such as AMF, because it is a non mycorrhizal-host crop (Thompson 1987; Ryan *et al*. 2002). On the Darling Downs of Queensland, wheat (*Triticum aestivum*), which is considered to have low mycorrhizal dependence, grew well after a canola crop because populations

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of *P. thornei* had been reduced, but linseed (*Linum usitatissimum*), which is resistant to *P. thornei* and highly mycorrhizal dependent, grew poorly because AM fungal levels had been reduced (Thompson 1994). In contrast, in south-eastern Australia, low populations of AMF after canola did not affect the growth or nutrition of the following wheat crops, or the mycorrhizal-dependent crops linola and field pea (Ryan *et al*. 2002; Ryan and Angus 2003).

This paper describes a 2-year field experiment that examined the ability of a range of canola cultivars to control *P. thornei* and which showed that canola's negative effects on mycorrhizal populations may be more important in some situations than its positive benefits of reduction of nematode population density.

Materials and methods

Field site

A field experiment was conducted over two crop cycles during 1999 and 2000 at Formartin (27.46401°S, 151.42616°E; 364 m elevation; 70 km west of Toowoomba) on the Darling Downs, Queensland, Australia. The experimental field site was part of a 15-ha site used for testing crop responses to *Pratylenchus thornei*. The entire site was managed on a 4-year crop rotation cycle, namely sorghum followed by a 15-month clean long-fallow, followed by the *P. thornei*-susceptible wheat cv. Cunningham, then by field trials, and finishing with another long-fallow before the sorghum crop. The soil at the site is a haplic, self-mulching, endohypersodic, black Vertosol (Isbell 1996) of the Waco Series (Beckmann and Thompson 1960; Vandersee 1975).

Field experiment

In 1999 and 2000, 120 kg N/ha as urea was drilled into the soil 3 weeks before planting, and 4 kg N/ha, 7 kg P/ha, and 0.9 kg Zn/ha were applied as Starter Z (Incitec Pivot) at the rate of 35 kg/ha drilled into the soil at planting (following regional agronomic practices). Weeds were controlled during fallow periods by the application of Glyphosate; during crop growth, weeds were removed manually using a chipping-hoe as required.

On 23 June 1999, 14 cultivars representing 8 crop species were planted in 3 randomised blocks of plots each 10 × 1.75 m wide with 7 rows. A clean-fallow treatment was also included in the experimental design. Canola cultivars were chosen for differing levels of PEG in the taproots. Crop cultivars grown, their susceptibility to *P. thornei*, AM fungal host-status, and the PEG levels of the canola cultivars are listed in Table 1. The plots were machine-harvested on 22 November 1999, except for the canaryseed plots, which were harvested by hand to limit dispersal of seeds. Crop stubble was retained in all plots, following regional practices.

In the subsequent winter season, all plots were planted with the *P. thornei*-susceptible and intolerant wheat, cv. Batavia, on 6 June 2000. Plant tops were cut from 1 m lengths of a row in two positions per plot after ear emergence (growth stage 60, Zadoks *et al*. 1974) in September to assess plant biomass and head density. Plant samples, collected from every plot in September 2000 (as described), and grain subsamples, collected at harvest from all plots, were analysed for Kjeldahl N, total P, and total Zn (in diacid digests). The plots were machine-harvested on 7 November 2000.

Soil sampling

The experimental field site was surveyed for pre-plant nematode populations from 16 cores taken across the field in a grid pattern in May 1999. The cores were collected to a depth of 1.5 m with a hydraulically operated soil corer and push tube of 5.0-cm diameter. The cores were cut and bulked in 0.15-m intervals to 0.6-m depth and then in 0.3-m intervals to 1.5-m depth. Two days

Table 1. Crop cultivars planted in the 1999 experiment, their responses to *Pratylenchus thornei* and arbuscular mycorrhizal fungi (AMF), and 2-phenylethyl glucosinolate (PEG) levels in taproots of canola cultivars

Crop	Root length with AMF (%)[A]	Cultivar tested	Response to *P. thornei*[B]	PEG level (μmol/g)[C]	1999 Grain yield (kg/ha)
Wheat (*Triticum aestivum*)	60.5	GS50a	Moderately resistant		3014
		Cunningham	Susceptible		3139
		Janz	Susceptible		2518
Durum wheat (*Triticum durum*)		Yallaroi	Moderately resistant		4098
Triticale (× *Triticosecale* spp.)	54.7	Abacus	Moderately resistant		3929
Barley (*Hordeum vulgare*)	45.6	Tallon	Moderately resistant		3884
Canola (*Brassica napus* ssp. *olifera* var. *annuna*)	0	Karoo	Moderately resistant	25	1168
		Rainbow	Moderately resistant	20	542
		Narrendra	Unknown	20	517
		Monty	Moderately resistant	5–6	698
		Hyola 42	Moderately resistant	5–6	1396
Chickpea (*Cicer arietinum*)	59.3	Amethyst	Susceptible		2102
Faba bean (*Vicia faba*)	Good AM fungal host[D]	Cairo	Susceptible		1043
Canaryseed (*Phalaris canariensis*)	47.2	Morocco	Moderately resistant		NH[E]
l.s.d. ($P = 0.05$)					591

[A]Thompson and Wildermuth (1989); using a similar soil type to that in the present experiment, with a starting population of 10 AM fungal spores g/soil.
[B]Thompson (1994) for all crops other than canola; Hollaway *et al*. (2000) for canola; Sheedy *et al*. (2009) for faba bean.
[C]Kirkegaard and Sarwar (1999).
[D]Thompson *et al*. (1997).
[E]NH, Not harvested; canaryseed was removed by hand.

after harvest of the first-year crops, post-harvest soil samples were collected at random from the inter-row spaces of each plot by taking 4 cores as described.

In the subsequent winter season, all plots were soil-sampled on 6 May 2000 (4 weeks before planting) and again on 15 October 2000 (at anthesis) for assessment of nematode populations (as described for post-harvest soil sampling by taking 4 cores on the middle row).

Soil samples (collected before planting, at anthesis, and harvest of the second-year crop as described above) were analysed for nitrate in 1 N KCl extracts, bicarbonate extractable-P (Colwell 1963), pH (1 : 5 water), and DTPA-extractable Zn (Lindsay and Norvell 1978).

Nematode extraction and enumeration

The soil samples were broken manually to <5 mm aggregates, composited, and a 150-g field-moist subsample, along with the accompanying roots, was processed for nematode extraction by the Whitehead tray method (Whitehead and Hemming 1965) for 48 h at 22°C. The extraction efficiency for our method is ~70% (Reen and Thompson 2009); results presented are not adjusted for extraction efficiency. *Pratylenchus thornei* and *Merlinius 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-plant parasitic or free-living nematodes (distinguished by the absence of a stylet) were counted as a composite. 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.

DNA analysis of nematode samples

Duplicate soil samples (500 g each) from each plot in the second-year wheat crop collected at anthesis (as described previously) were submitted for DNA analysis by PreDicta B (SARDI, South Australia) to detect root-lesion nematodes (Ophel-Keller *et al.* 2008).

Assessment of AMF

AM fungal-spores and sporocarps were collected from second-year pre-plant soil samples (50-g subsample stored for 6 months at 3°C) from the 0–0.15 m depth interval. A 50-g subsample of field-moist soil was added to 800 mL deionised water with 5 g sodium pyrophosphate (to disperse the clay soil) in a jar that was mechanically agitated end-on-end for 1 h. The sample was then transferred to a 38-µm 20-cm-diameter sieve and washed with tap water until all clay was removed and clear water ran through the sieve. The sample was transferred to a conical flask with 1 L of water and inverted 6 times and then rested for 10 s before decanting through a series of 250, 106, 63, and 38 µm mesh sieves and collected in a specimen tube. Spores and sporocarps were counted in a 1-mL subsample using a Hawksley slide under a compound microscope at ×40 and ×100 magnification.

For assessment of AM fungal-colonisation of wheat roots in September in the second year of the experiment, soil was excavated to 0.2-m depth using a garden fork from two 0.3-m lengths of a row (where plants had been cut for biomass as described previously). Soil (500 g) was placed in a 10-L bucket and then 5 L of water plus 5 g sodium pyrophosphate added and stirred. The contents of the bucket were then decanted through a 425-µm mesh sieve. This process was repeated 5–6 times until all of the roots and any attached AM fungal sporocarps were recovered from the soil. The roots were then blotted dry. A 0.6-g subsample of roots was cleared and stained with trypan blue (Phillips and Hayman 1970) and quantitatively assessed for AM fungal colonisation by a microscope grid intersect method to determine percent root length colonised by AMF (Giovannetti and Mosse 1980).

Statistical analysis

All data were analysed by ANOVA in Genstat 10th edition (Payne *et al.* 2007). Nematode data were transformed by $\ln(x+c)$ where x is nematodes/kg dry soil and root and c is a constant. The value of c was optimised using chi-square principles to minimise the residuals in the ANOVA (Proctor and Marks 1974; Berry 1987). AM fungal colonisation (%) data were transformed by arcsin before ANOVA. Correlation matrix analyses were done in Statistix version 1.0 (1996 Windows Analytical Software, Tallahasse, USA) and regression analyses in Genstat 10th edition.

Results

Rainfall

Table 2 lists the monthly rainfalls for 1999 and 2000, the average rainfall from farm records (1976–2000) and the average long-term rainfall records from the nearest Bureau of Meteorology site at Oakey, Queensland. In 1999 there were 33 mm of rain between soil sampling and planting (May–June), which allowed seed germination and crop establishment. This was followed by 189 mm of in-crop rainfall. There were 158 mm of rain during the fallow period between harvest of the first-year crops and planting of the second-year crop. During the 2000 experiment there were 59 mm of rain between May and June, allowing plant establishment, but only 73 mm of in-crop rainfall.

Table 2. Monthly rainfall data for the field site at Formartin, Queensland, in 1999 and 2000, the average rainfall data from farm records (1976–2000), and long-term rainfall data from the nearest Bureau of Meteorology (BOM) site at Oakey, Queensland

Rainfall (mm)	Month											
	J	F	M	A	M	J	J	A	S	O	N	D
1999	28	88	60	0	13	23	62	16	10	35	78	109
2000	53	60	8	6	23	30	8	4	0	31	48	50
Farm Records	69	72	42	41	51	27	35	19	31	48	67	70
Oakey, BOM	80	86	44	36	48	27	35	26	33	57	79	93

Yields of pre-crops

The yields of the 1999 pre-crops (other than canaryseed) were highest for the cereal crops (range of 4098 kg/ha for durum wheat cv. Yallaroi to 2518 kg/ha for wheat cv. Janz). Chickpea cv. Amethyst and faba bean cv. Cairo were 2101 and 1043 kg/ha, respectively, and the canola yields ranged from 517 to 1396 kg/ha for Narrendra and Hyola 42, respectively (Table 1).

Pratylenchus thornei

The distribution of *P. thornei*, *M. brevidens*, and non-plant-parasitic nematodes in the soil profile to 1.5 m from pre-plant soil samples taken in May 1999 is shown in Table 3. *P. thornei* populations were greatest in the top 0.6 m of the soil profile, with greatest numbers of 3800 *P. thornei*/kg dry soil at 0.45–0.6 m and 3700 at 0.15–0.3 m. Populations of *M. brevidens* and non-plant-parasitic nematodes ranged from 40–1081 to 31–2467/kg dry soil, respectively, and followed a similar distribution to *P. thornei*, with peak populations at or below 0.45 m soil depth.

Table 4 shows the distribution of *P. thornei* in the soil profile to 0.6 m from soil samples taken in November 1999 after harvest of the pre-crops. *P. thornei* was found throughout the soil profile to a depth of 1.2 m, although numbers were low in the deepest layers and are not shown beyond 0.6-m depth. *P. thornei* populations were greatest in the 0–0.15 m soil depth for all treatments except the clean-fallow, barley, and 5 canola cultivars, in which peak populations were at 0.15–0.3 m. The susceptible wheat cvv. Cunningham and Janz had peak populations of 41 294 and 31 033 *P. thornei*/kg dry soil at 0–0.15 m, respectively. At 0.15–0.3 m soil depth, *P. thornei* populations from the canola cultivars (2710–5255 *P. thornei*/kg dry soil) did not differ significantly from the population found in the clean-fallow treatment (2008 *P. thornei*/kg dry soil).

The order of the crops from most to least susceptible (based on accumulated nematode counts for 0–0.3 m) was wheat cv. Cunningham, wheat cv. Janz, faba bean cv. Cairo, barley cv. Tallon, chickpea cv. Amethyst, canola cv. Narrendra, canola cv. Monty, canola cv. Rainbow, canola cv. Karoo, canola cv. Hyola 42, triticale cv. Abacus, canaryseed cv. Morocco, durum wheat cv. Yallaroi, wheat cv. GS50a, and fallow.

When the soil was sampled 6 months later in May 2000 following a 6-month clean-fallow period over summer, *P. thornei* populations had decreased considerably (<5000/kg dry soil) in most treatments (Table 5). Generally, the highest *P. thornei* populations still occurred following the susceptible crops, such as wheat cvv. Janz and Cunningham (4500 and 3600/kg dry soil, respectively), faba bean, and chickpea (3641 and 2821/kg dry soil, respectively) at 0.15–0.30 m, and lowest populations were found after growing the moderately resistant wheat cv. GS50a, the triticale cv. Abacus, and the fallow treatment (417, 1160, 1132/kg dry soil at 0.15–0.3 m, respectively). The effects of the canola cultivars were less distinct, e.g. cv. Monty had the lowest population of all the treatments (208/kg dry soil) but cv. Rainbow had one of the highest populations (2086/kg dry soil) of all the treatments at

Table 3. Population densities of *Pratylenchus thornei*, *Merlinius brevidens*, and non-plant-parasitic nematodes in the soil profile to 1.5-m depth in May 1999, 1 month before planting the winter crops at Formartin, Queensland

Values are transformed $\ln(x+c)$ means from ANOVA with back-transformed means in parentheses where $c = 78$, 350, and 25 for *P. thornei*, *M. brevidens*, and non-plant-parasitic nematodes (NPPN), respectively

Soil depth (m)	Nematodes/kg dry soil		
	P. thornei	*M. brevidens*	NPPN
0–0.15	8.0 (2818)	7.2 (1027)	7.2 (1267)
0.15–0.3	8.2 (3681)	7.0 (750)	7.0 (1089)
0.3–0.45	7.8 (2329)	6.8 (566)	7.2 (1331)
0.45–0.6	8.3 (3835)	7.2 (939)	7.8 (2467)
0.6–0.9	7.2 (1231)	7.3 (1081)	6.7 (792)
0.9–1.2	4.8 (39)	6.5 (306)	5.4 (199)
1.2–1.5	4.4 (1)	6.0 (40)	4.0 (31)
l.s.d. ($P = 0.05$)	0.4	0.4	0.7

Table 4. Population density of *Pratylenchus thornei* in the top 0.6 m of the soil profile after harvest of winter crops in November 1999 in the first year of the experiment at Formartin, Queensland

Values are transformed $[\ln(x+1170)]$ means from ANOVA with back-transformed means in parentheses

1999 crop treatment	*P. thornei*/kg dry soil			
	0–0.15 m	0.15–0.3 m	0.3–0.45 m	0.45–0.6 m
Fallow	7.7 (1023)	8.1 (2008)	7.5 (644)	7.6 (790)
Canola cv. Hyola 42	7.8 (1258)	8.3 (2849)	8.1 (2180)	8.1 (2257)
Canola cv. Karoo	7.8 (1279)	8.3 (2710)	8.1 (2050)	8.2 (2389)
Canola cv. Narrendra	8.1 (2047)	8.8 (5255)	8.5 (3540)	8.3 (3077)
Canola cv. Rainbow	8.1 (2210)	8.3 (2967)	8.2 (2345)	8.5 (3809)
Canola cv. Monty	8.4 (3358)	8.6 (4499)	8.3 (2737)	8.1 (2207)
Canaryseed cv. Morocco	8.2 (2462)	8.1 (1937)	7.9 (1472)	7.4 (518)
Durum cv. Yallaroi	8.6 (4421)	7.7 (1046)	7.5 (554)	7.5 (710)
Triticale cv. Abacus	8.6 (4496)	8.2 (2501)	7.5 (633)	7.5 (737)
Chickpea cv. Amethyst	8.8 (5471)	8.3 (2855)	7.9 (1605)	8.3 (2849)
Faba bean cv. Cairo	9.6 (14 546)	9.3 (9503)	8.3 (2948)	8.4 (3279)
Barley cv. Tallon	8.8 (5608)	8.8 (5655)	8.1 (1959)	7.9 (1480)
Wheat cv. GS50a	8.5 (3540)	8.0 (1684)	7.5 (588)	7.5 (724)
Wheat cv. Janz	10.4 (31 033)	9.9 (19 738)	9.5 (12 184)	8.3 (2956)
Wheat cv. Cunningham	10.7 (41 294)	10.4 (32 776)	9.6 (14 294)	8.5 (3557)

l.s.d. ($P = 0.05$); crop 0.5; soil depth 0.2; crop*depth 0.7

Table 5. Population density of *Pratylenchus thornei* in the top 0.6 m of the soil profile following a 6-month clean-fallow period and before planting wheat cv. Batavia in the second year of the experiment in June 2000 at Formartin, Queensland

Values are transformed [ln(x + 128)] means from ANOVA with back-transformed means in parentheses

1999 Crop treatment	*P. thornei*/kg dry soil			
	0–0.15 m	0.15–0.3 m	0.3–0.45 m	0.45–0.6 m
Fallow	6.4 (450)	7.1 (1132)	7.6 (1875)	7.0 (949)
Canola cv. Hyola 42	6.4 (481)	7.6 (1884)	6.9 (917)	7.7 (2257)
Canola cv. Karoo	7.1 (1017)	7.2 (1195)	7.4 (1483)	7.6 (1791)
Canola cv. Narrendra	7.3 (1421)	7.7 (2174)	7.9 (2764)	7.4 (1457)
Canola cv. Rainbow	7.7 (2086)	8.7 (5700)	7.2 (1228)	8.1 (3015)
Canola cv. Monty	5.8 (208)	7.5 (1783)	7.8 (2242)	7.2 (1253)
Canaryseed cv. Morocco	6.8 (811)	7.0 (994)	6.8 (761)	7.6 (1928)
Durum cv. Yallaroi	6.8 (821)	7.5 (1632)	7.2 (1246)	7.0 (91)
Triticale cv. Abacus	6.8 (789)	7.2 (1160)	7.0 (922)	6.6 (1421)
Chickpea cv. Amethyst	7.1 (1050)	8.0 (2821)	7.2 (1257)	6.7 (671)
Faba bean cv. Cairo	7.8 (2325)	8.2 (3641)	8.0 (2785)	7.9 (2438)
Barley cv. Tallon	7.7 (2195)	7.7 (2132)	7.9 (2543)	7.2 (2432)
Wheat cv. GS50a	6.3 (435)	6.3 (417)	7.1 (1064)	5.5 (111)
Wheat cv. Janz	7.5 (1701)	8.4 (4515)	8.2 (3637)	7.8 (2362)
Wheat cv. Cunningham	7.9 (2625)	8.3 (3682)	8.4 (4509)	8.1 (2943)

l.s.d. (P = 0.05); crop 0.8; soil depth 0.3; crop*depth 1.2

0–0.15 m. The effects of the crop treatments on *P. thornei* became even less clear at the lower depths, with several treatments showing an increase in populations when compared with the populations found 6 months previously. For example, after the barley cv. Tallon, populations increased 1.3 and 1.6 times at 0.3–0.45 and 0.45–0.6 m soil depth.

When the plots were over-sown with wheat cv. Batavia and soil-sampled at anthesis in October 2000, *P. thornei* populations remained at uniform but moderate levels (<7000 *P. thornei*/kg dry soil at 0.15–0.3 m) and there were no significant differences ($P < 0.05$) between the treatments (data not shown). Additionally, there was no significant regression relationship between the pre-plant *P. thornei* populations and grain yield of the Batavia wheat (data not shown).

There were no significant differences in numbers of non-plant-parasitic nematodes between treatments at the 3 sampling times during the experiment. Population densities of *M. brevidens* were significantly different between crop treatments ($P < 0.05$) only at the November 1999 sampling, with highest populations at 0.15–0.3 m soil depth (range of 124–3659/kg dry soil for fallow and canola cv. Monty, respectively); at 0.15–0.3 m soil depth the fallow, barley, and canola cvv. Narrendra, Rainbow, and Hyola 42 were significantly lower than the other treatments (data not shown).

DNA analysis

DNA analysis of the samples taken at anthesis of the wheat crop in the second year confirmed that *P. thornei* was the only root-lesion nematode present in the trial site. There was a significant regression relationship between manual counts from the Whitehead tray extraction combined with morphological identification methods and the DNA assay ($y = 0.5909x + 2.4816$, $R^2 = 0.726$; $P < 0.001$; $n = 126$; where y is manual counts and x is the DNA assay). The DNA assay detected higher levels of *P. thornei*, which were not significantly different between treatments ($P < 0.05$).

AM fungal colonisation v. *wheat growth and yield*

The biomass of wheat cv. Batavia produced after an 18-month clean-fallow or after the canola cvv. Narrendra and Hyola 42 was significantly lower than after the chickpea, durum wheat, and canaryseed first-year crops (Table 6). Because of this pattern of response the effect of pre-crop treatments on AM fungal colonisation of the wheat was assessed. Observations of the percent AM fungal colonisation of the wheat cv. Batavia roots revealed that the pre-treatments of canola (excluding cv. Karoo) or fallow resulted in poorer AM fungal colonisation compared with pre-cropping with durum wheat, canaryseed, or triticale (range of 13% AM fungal colonisation of the root length for canola cv. Narrendra to 80% for triticale cv. Abacus) (Table 6). The percent AM fungal root colonisation was also correlated with the number of AM fungal spores collected in the top 0.15 m of soil at the pre-plant sampling in the second year of the experiment (Fig. 1). As with percent AM fungal colonisation, the number of AM fungal spores collected was lowest after fallow and the canola treatments and greatest after the other crop species (e.g. up to 4 times greater at 0–0.15 m under the wheat cultivars than the canola cultivars) (Fig. 2). There were no significant differences between treatments for sporocarps collected on the 63, 106, and 250 µm sieves (data not shown), with the grand mean of 0.7 total sporocarps/g soil. Although AM fungal colonisation of roots following canola cv. Karoo was as high as barley cv. Tallon, it did not reflect on yield. The wheat grain yield after canola cv. Karoo was very low (938 kg/ha) compared with after barley cv. Tallon (1445 kg/ha).

The highest grain yields of wheat cv. Batavia were obtained after pre-treatments with durum cv. Yallaroi (1924 kg/ha) and

Table 6. Biomass, arbuscular mycorrhizal fungal (AMF) colonisation of roots (%), plant dry weight and number of plant heads at anthesis, and grain yield of wheat cv. Batavia grown in 2000 following the 1999 first-year winter crop treatments at Formartin, Queensland

Values for AM fungal colonisation are transformed (arcsin) means from ANOVA with back-transformed means in parentheses

1999 Pre-treatment	2000 Wheat cv. Batavia			
	% AM fungal colonisation	Dry weight at anthesis (kg/ha)	No. plant heads/m^2 at anthesis	Grain yield (kg/ha)
Fallow	28 (22)	1365	122	1310
Canola cv. Hyola 42	23 (15)	1194	117	1064
Canola cv. Karoo	55 (67)	1975	161	938
Canola cv. Narrendra	21 (13)	1078	99	860
Canola cv. Rainbow	39 (39)	2271	169	1211
Canola cv. Monty	39 (40)	2444	190	1304
Canaryseed cv. Morocco	63 (80)	3407	238	1935
Durum cv. Yallaroi	60 (75)	3284	235	1924
Triticale cv. Abacus	64 (80)	3243	225	1619
Chickpea cv. Amethyst	56 (68)	3183	240	1441
Faba bean cv. Cairo	59 (74)	2477	178	1127
Barley cv. Tallon	56 (70)	2505	165	1445
Wheat cv. GS50a	52 (62)	2582	189	1444
Wheat cv. Janz	43 (47)	2179	167	1388
Wheat cv. Cunningham	59 (74)	2265	197	1447
l.s.d. ($P = 0.05$)	21	1331	87.3	619

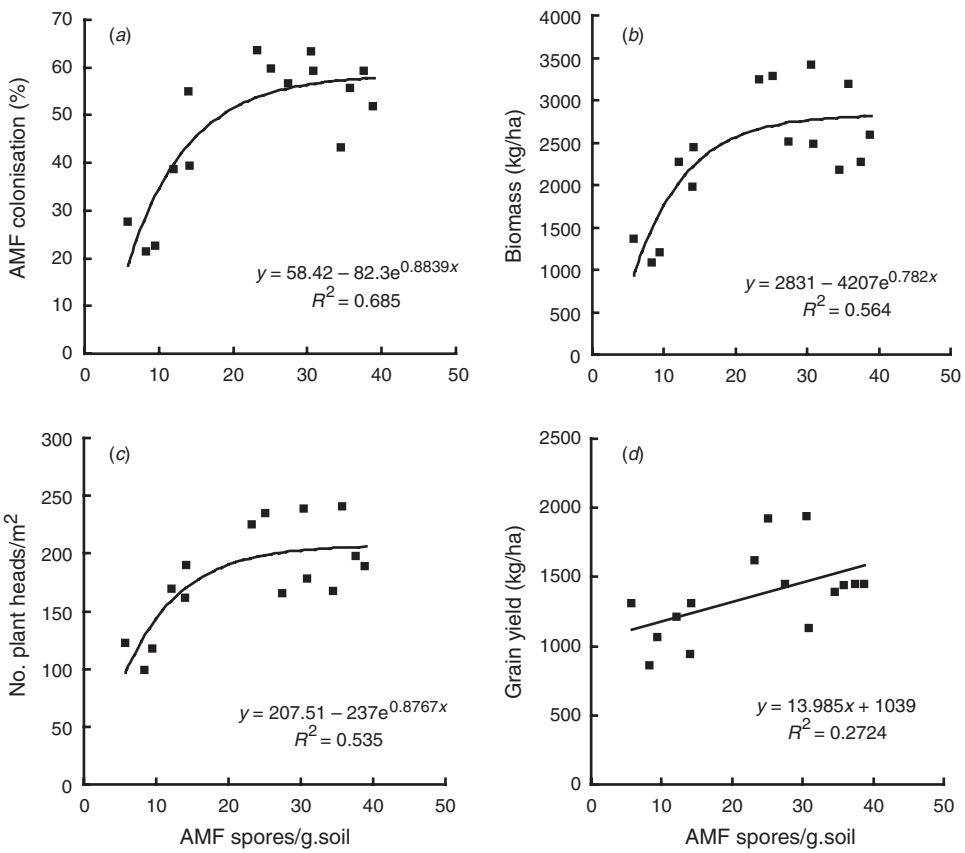

Fig. 1. The relationship between total arbuscular mycorrhizal fungal (AMF) spores/g soil at 0–0.15 m from pre-plant soil samples collected in the second year of the experiment and (*a*) AM fungal colonisation of the roots ($P < 0.001$), (*b*) plant biomass ($P < 0.003$), (*c*) number of plant heads/m^2 at anthesis ($P < 0.004$), and (*d*) grain yield ($P < 0.05$) of the second-year crop, wheat cv. Batavia, on plots planted with various winter crops in the preceding season ($n = 15$). Values are treatment means from ANOVA.

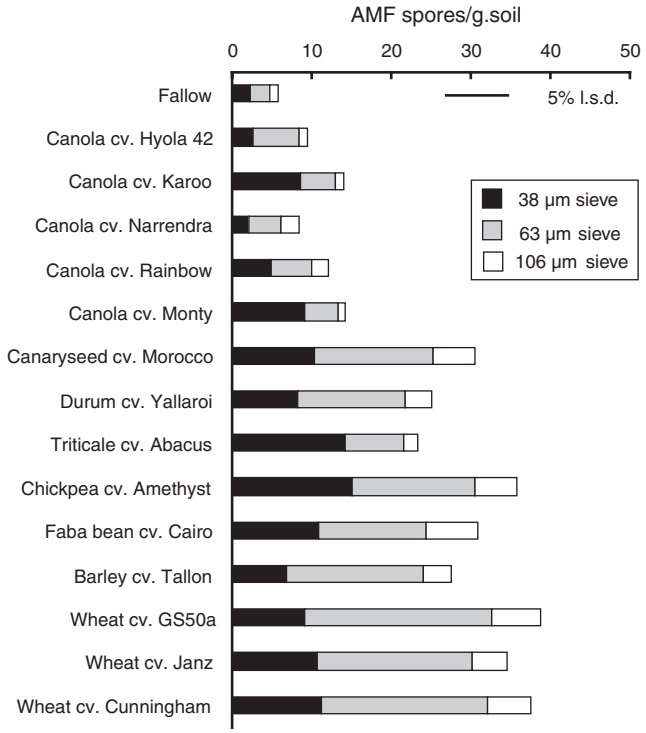

Fig. 2. Total arbuscular mycorrhizal fungal (AMF) spores/g soil at 0–0.15 m collected from three sieve sizes (38, 63, and 106 µm) from pre-plant soil samples in the second year of the experiment following the winter crops from the previous year at Formartin, Queensland; l.s.d. is for total spores from 38, 63, and 106 µm sieves $P = 0.01$ (for 38 µm sieve $P = 0.09$; 63 µm sieve $P = 0.03$, l.s.d. 6; 106 µm sieve $P = 0.04$, l.s.d. 2); l.s.d. values are $P = 0.05$.

canaryseed cv. Morocco (1935 kg/ha) while the lowest yield (860 kg/ha) was obtained after pre-treatment with canola cv. Narrendra (Table 6). Plant dry weight and number of plant heads/m^2 at anthesis are shown in Table 6. Grain yield was correlated with plant dry weight ($R^2 = 0.64$) and the number of heads/m^2 ($R^2 = 0.66$) at anthesis (data not shown).

There were significant positive regression relationships (Fig. 1) between total AM fungal spores found in the soil from 0–0.15 m in the pre-plant samples of the second year of the experiment and AM fungal colonisation of the second-year wheat crop cv. Batavia at anthesis, plant biomass, number of plant heads, and grain yield. There were also significant regression relationships (Fig. 3) between the mycorrhizal colonisation level of the wheat cv. Batavia roots and the biomass of the top growth, plant-head density, and grain yield.

There were significant correlations (Table 7) ($P < 0.05$) between the N, P, and Zn concentrations and biomass and number of plant heads of the wheat plants at anthesis and the presence of AMF (as spores in the pre-plant soil or as colonisation of the wheat roots at anthesis). There were no significant correlations between AM fungal spores at pre-plant sampling or AM fungal colonisation of the wheat roots for the ratios of N, P, and Zn in the wheat plants at anthesis, with one exception being a significant, negative interaction between the AM fungal spores and the N : P ratio.

There were no significant correlations between the levels of AMF (as spores in the pre-plant soil or colonisation of wheat roots at anthesis) and the concentrations of N, P, and Zn of the grain (data not shown).

Chemical analysis of soil

Chemical analysis of soil samples (0–0.15 m) taken before planting the second-year wheat cv. Batavia crop showed that mean soil pH was 8.8, P concentration was 7.1 mg/kg (Colwell P), and Zn was 0.68 mg/kg (DTPA-extractable Zn). Soil water concentrations at 0–0.30 m were 41% at the 1999 pre-plant sampling, 46.3% immediately after harvest in 1999, 42.3% at the pre-plant sampling in 2000, and 27.5% at the anthesis sampling in 2000. There were no significant differences in soil water content between crop treatments at the pre-plant sampling or at anthesis in the second-year wheat crop. There was no significant relationship between pre-plant soil water content and number of plant heads and biomass at anthesis or with grain yield data. Additionally there were no significant differences between treatments for N (range of 49.4 at 0–0.15 m to 17.1 at 1.2–1.5 m mg/kg NO_3), P (7.1 and 2.9 P mg/kg Cowell P at 0–0.15 m and 0.15–0.3 m, respectively), or Zn (0.68 and 0.36 mg/kg DTPA-Zn at 0–0.15 and 0.15–0.3 m, respectively) content of soil before planting the Batavia wheat crop.

Correlation coefficients demonstrated that there were no significant relationships between the second-year pre-plant soil levels of extractable P, Zn, N, and pH (at both 0–0.15 and 0.15–0.3 m) and number of plant heads, fresh and dry weights of the plants, and grain yield of the second-year wheat crop (data not shown).

Discussion

The results presented raise uncertainties about the use of canola as a rotation crop in the northern grain region of Australia, particularly preceding dry seasonal conditions and when soil phosphorus is limiting. Canola's resistance to *P. thornei* caused marked reductions in its populations; however, depletion of AMF after canola, as with clean-fallow, was associated with significant yield reduction in plant growth and grain yield in the following wheat crop.

Arbuscular mycorrhizal fungi

The results of the present study are supported by those of Thompson (1987), which demonstrated the importance of AMF for crop growth in the northern grain region of Australia. Although wheat is generally considered to have low mycorrhizal dependence because it has a fibrous root system (Marschner 1986; Thompson 1987), there are several environmental conditions in the present study that caused AMF to impart benefits to the growth of the second-year wheat crop. These included a dry season (only 73 mm of in-season rainfall), low soil concentration of P (7.1 mg/kg Colwell P), moderate concentration of Zn (0.68 mg/kg DTPA-Zn), and high pH (8.8). The very low soil P in the present study is typical of the soils used for wheat production in the northern Australian grain region (Colwell and Esdaile 1968; D. Lester, pers. comm.) and P fertiliser rates

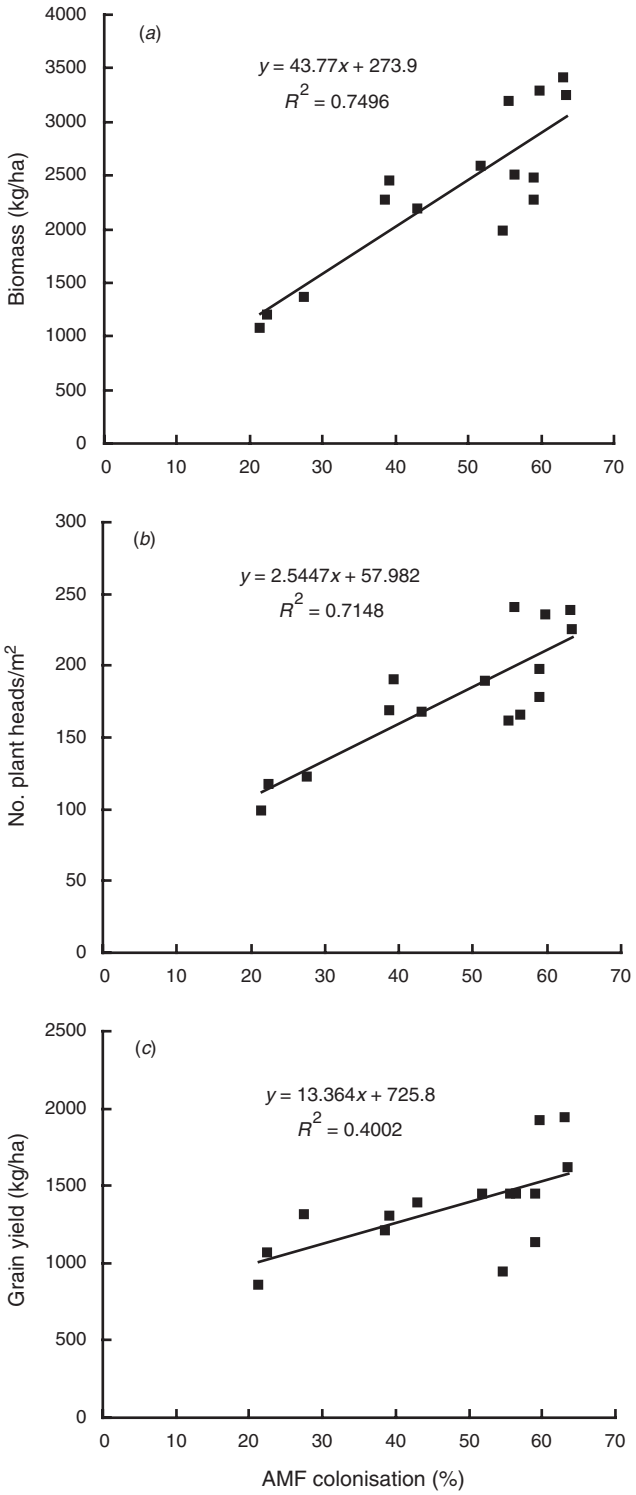

Fig. 3. The relationship between arbuscular mycorrhizal fungal (AMF) colonisation of the roots (%) of the second-year wheat cv. Batavia at anthesis and (*a*) plant biomass ($P < 0.001$), (*b*) number of plant heads/m^2 ($P < 0.001$), and (*c*) grain yield ($P < 0.01$) on plots planted with various winter crops in the preceding season ($n = 15$). Values are treatment means from ANOVA and AM fungal colonisation values are transformed means.

in the region, like those used in the study, are generally very low (5–10 kg P/ha) (National Land and Water Resources Audit 2001).

Wheat grown in the presence of AMF in conditions of water stress has drought tolerance (partly due to increased root depth and root weight) leading to increased leaf area, stem and leaf weight, and tillering (Ellis *et al*. 1985). Although the biomass and grain yield of AM fungal-colonised wheat are higher when water is sufficient, the response of the plants is greater, proportionally, in water-stressed plants (Al-Karaki *et al*. 2004). In extremely dry conditions, however, the benefits to wheat are negligible because AM fungal colonisation is inhibited (Ryan and Ash 1996). In the present study, rainfall in the second year was sufficient at planting and allowed good germination and establishment of the wheat crop, which in turn contributed to colonisation of the wheat roots, leading to improved tolerance of the dry conditions from July to September.

Improved wheat growth and yield in the presence of AMF are likely when soil P concentration is less than 50 mg/kg (Goh *et al*. 1997) and the importance of low soil P is further enhanced by low soil moisture which retards plant P uptake (Strong and Barry 1980). The relationship between AMF and plant P uptake is supported by the results in the second year of the current study. The poorer (although significant) correlation between AMF and grain yield, and lack of significant correlations between AMF and grain P concentration were most likely due to the very dry conditions at the end of the season, which counteracted the gains produced by the wheat plants due to the presence of AMF earlier during the season.

Our results contrast with those reported by Ryan and Angus (2003) and Ryan *et al*. (2002) in which low populations of AMF following canola did not affect wheat yields in the south-eastern Australian wheatbelt. Climatic differences such as higher temperatures and lower rainfall (particularly in the wheat growing season), and differences in farming practices such as the use of summer crops and long fallow in northern Australia, may affect the rate of crop growth leading to different demands in nutrition, and explain regional differences in AMF functioning and community composition (Ryan *et al*. 2002).

Canola

Reductions in *P. thornei* populations immediately following harvest of the canola in the present experiment may not be entirely related to 2-phenylethyl glucosinolate production potential of the preceding canola cultivars. However, maximising the biofumigation potential of canola requires pulverisation and incorporation of the plant tops and irrigation (Matthiessen and Kirkegaard 2006), and this was not performed in the present study. Suppression of take-all and low levels of AM fungal colonisation of brassicas are solely a host effect and are not due to differences in potential for glucosinolate production (Kirkegaard *et al*. 2000; Ryan 2001). In our study the differences between the canola cultivars' effects on residual AM fungal spore populations indicated that there may be some variation between cultivars. However, yield of the second-year wheat crop following canola cv. Karoo was lower than expected based on good levels of AM fungal colonisation of the wheat roots following cv. Karoo. Weeds in the present study were well controlled during fallow and cropping periods, but

Table 7. Correlation coefficients among growth characteristics of the second-year crop, wheat cv. Batavia, arbuscular mycorrhizal (AM) fungal colonisation and nutrient concentrations of the wheat cv. Batavia at anthesis, and AM fungal spores (at 0–0.15 m) from soil collected in June before planting the second-year wheat crop

H, No. plant heads/m^2; FW, fresh weight plants (kg/ha); DW, dry weight plants (kg/ha); PM, plant moisture (%); AMF roots, AM fungal colonisation of second-year wheat crop (%); N, plant nitrogen (%); P, plant phosphorus (%); Zn, plant zinc (%); NU, nitrogen uptake; PU, phosphorus uptake; ZnU, zinc uptake; N : P, nitrogen to phosphorus ratio; N : Zn, nitrogen to zinc ratio; P : Zn, phosphorus to zinc ratio; AMF spores, no. of AM fungal spores at 0–0.15 m/g soil. n.s., Not significant; all other correlation coefficients are significant ($P < 0.05$); $n = 45$

	H	FW	DW	PM	AMF roots	N	P	Zn	NU	PU	ZnU	N : P	N : Zn	P : Zn
H														
FW	0.937													
DW	0.941	0.984												
PM	0.518	0.644	0.525											
AMF roots	0.651	0.719	0.713	0.462										
N	0.395	0.432	0.361	0.491	0.489									
P	0.468	0.526	0.444	0.666	0.423	0.804								
Zn	0.317	0.414	0.377	0.571	0.448	0.433	0.512							
NU	0.935	0.981	0.982	0.563	0.74	0.522	0.563	0.422						
PU	0.908	0.955	0.938	0.604	0.699	0.587	0.705	0.44	0.975					
ZnU	0.911	0.962	0.969	0.576	0.738	0.45	0.517	0.57	0.972	0.938				
N : P	–0.345	–0.405	–0.341	–0.601	–0.231n.s.	–0.392	–0.853	–0.418	–0.391	–0.555	–0.382			
N : Zn	0.136n.s.	0.222n.s.	0.225n.s.	–0.355	–0.208n.s.	0.113n.s.	–0.09n.s.	–0.836	0.179n.s.	0.157n.s.	–0.374	0.227n.s.		
P : Zn	0.229n.s.	0.208n.s.	0.15n.s.	0.222n.s.	0.067n.s.	0.465	0.625	–0.344	0.235n.s.	0.372	0.056n.s.	–0.57	0.657	
AMF spores	0.462	0.469	0.404	0.486	0.364	0.449	0.526	0.43	0.458	0.511	0.482	–0.417	0.199n.s.	0.185n.s.

mycorrhizal-weeds could potentially contribute to unexpected increases in AM fungal inoculum. Further research is required to determine if the yield effect following cv. Karoo was dependent on AMF or other factors.

An important consideration in canola–wheat rotations is the increased nutrient demand of canola, particularly for sulfur, but also nitrogen and phosphorus (Hocking *et al*. 1999). Although soil sulfur levels were not assessed in the present study, there were no significant differences in soil P and N following the first-year crop treatments. Additionally, changes in soil organic matter following different crops can influence mineralisation-immobilisation of available nutrients in soil (Dalal and Chan 2001). However, there were no significant effects between the pre-crop levels of soil water, nitrate, P, or Zn on the performance of the second-year wheat crop in the present study. Overall, the resistance of the canola cultivars to *P. thornei* suggests that it is a useful crop to include in rotations, but only when the effect of loss of AMF is considered, particularly when planting mycorrhizal-dependent crops in the following season, such as cotton, sorghum, maize, chickpea, and faba bean (Thompson *et al*. 1997).

Pratylenchus thornei

Changes in populations of *P. thornei* over the course of the experiment are interesting. In the first year of the experiment, peak populations of *P. thornei* were found closer to the soil surface when a susceptible crop was grown because plant hosts provide stimuli for nematode attraction and a favourable environment for nematode reproduction (Perry 1997). The nematodes were found deeper in the soil profile after the hot summer fallow period. Similarly, the root-lesion nematode, *Pratylenchus jordanensis*, is known to migrate vertically in soil as the topsoil dries (Mani 1999) and there is a positive correlation between survival of *Pratylenchus* spp. and soil moisture (Townshend 1973; Mani 1999).

An unexpected finding was that in the second year of the experiment, populations of *P. thornei* decreased to moderate levels (<5000/kg dry soil) irrespective of the previous season's treatments and largely remained that way despite re-cropping with a susceptible wheat crop. This effect was particularly noticeable following the susceptible wheat cultivars where *P. thornei* decreased up to 95% over the summer clean-fallow period. After harvest of wheat, *P. thornei* survives in an anhydrobiotic state free in soil (Talavera and Valor 2000; Talavera and Vanstone 2001) and in dry wheat roots (Tobar *et al*. 1995). However, survival of *P. thornei* decreases as the frequency of the cycle of anhydrobiosis and rehydration increases (Glazer and Orion 1983) and there is a negative correlation between survival and temperature (Mani 1999).

At anthesis of the second-year wheat crop, there were no longer significant differences in *P. thornei* population densities between the pre-crop treatments. Nematode populations did not increase to very high levels (e.g. 30 000/kg soil as in the previous season following susceptible wheat treatments) despite growing a susceptible wheat crop. This might be due to several factors, including further attrition of anhydrobiotic nematodes by the rehydration/drying cycle and, later in the season, inadequate rainfall to stimulate rehydration (8 mm of rain in July, followed by 4 mm in August and none in October). These dry conditions would, in turn, limit wheat root development, further restricting nematode attraction to the plants, particularly if nematodes had migrated deeper into the soil profile for survival. Mechanical impedance of nematode migration in the very dry conditions during the second year is also expected (soil moisture content fell from 42% at the pre-plant sampling to 28% at anthesis). Under these conditions the water films in soil may be too narrow for nematode movement. In similar soils, when soil moisture content is 45%, the width of undrained pores is only 10 µm (D. Orange, pers. comm.) compared with the average adult *P. thornei* width of 20 µm.

The results from the DNA assay carried out on duplicate subsamples of soil taken at anthesis in the second-year wheat crop were significantly correlated with our methods of Whitehead tray extraction. Anhydrobiotic nematodes tend to be brittle (Hinton 1968) and may be killed during sampling and processing of dry soil by Whitehead tray extraction (Hollaway *et al*. 2003). Although the populations of *P. thornei* detected by the DNA assay were greater because the method may detect recently killed nematodes as well as eggs and anhydrobiotic nematodes, there were no significant differences between the crop and fallow treatments and this supports our results.

A further explanation for the limited population growth of *P. thornei* under the susceptible wheat in the second year of the experiment may be the presence of predatory nematodes and fungi. The experiment began with land that had been previously planted with a susceptible wheat cultivar. Therefore, in some plots a susceptible wheat cultivar was grown consecutively for 3 years, giving opportunity for populations of nematode predators to build-up. It was noted previously (Thompson *et al*. 1980) that on this farm there was an increase in the nematode-trapping fungus, *Arthrobotrys conoides*, with successive wheat cropping (1–3 years). This result is indicative of what might happen in a typical predator–prey cycle where high populations of *P. thornei* might be reduced by the build-up of microbial antagonists.

Merlinius brevidens is widely distributed in the northern grain region (Thompson *et al*. 2010) but its economic impact on crops in the northern grain region of Australia has not been well established. In the present study, population densities of *M. brevidens* increased following several winter crops but this effect was short-lived and did not persist after the summer-fallow period. Further research on this nematode, particularly in more favourable seasonal conditions, is warranted.

Conclusions

Although canola reduced populations of *P. thornei*, this came at the expense of lower wheat yields that were associated with reductions in populations of AMF, which arose because canola is not an AM fungal host. Therefore, planting canola to control *P. thornei* should be carefully considered in the northern grain region of Australia when planting crops in the subsequent season with high mycorrhizal dependency.

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