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Reduced growth of autumn-sown wheat in a low-P soil is associated with high colonisation by arbuscular mycorrhizal fungi

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Abstract

Autumn-sown wheat (*Triticum aestivum*) was studied over two seasons in south-eastern Australia, on a low-P soil where indigenous arbuscular mycorrhizal fungi (AMF) were known to provide little nutritional benefit to crops. It was hypothesised that AMF would be parasitic under these circumstances. Shoot dry mass and water soluble carbohydrate (WSC) reserves in roots and shoots were measured for wheat grown with or without P-fertiliser, in plots where crop sequences had produced either high or low colonisation by AMF. Application of P-fertiliser greatly increased crop growth and decreased colonisation by AMF. At tillering, colonisation by AMF ranged from 24 to 66% of root length when no P was applied and from 11 to 32% when P was applied. At each P-level, high colonisation correlated with reductions of around 20% in stem and root WSC concentrations (first season) or shoot WSC content and shoot dry mass (much drier second season). Impacts on yield were not significant (first season) or largely masked by water-stress and frost (second season). While the major fungal root diseases of the region were absent, interactions between crop sequence and other unknown biotic constraints could not be discounted. The results are consistent with the parasitic impacts of colonisation by AMF being induced primarily through the winter conditions experienced by the crops until anthesis. It is concluded that wheat in south-eastern Australia may benefit from reduced colonisation by AMF, which could achieved through selected crop sequences or, perhaps, targeted wheat breeding programs.

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that attain all carbon requirements from sugars in host roots (Ho and Trappe, 1973; Pfeffer et al., 1999). AMF colonise the roots of many agricultural plants, including cereals, and are believed to use between 4 and 20% of host photosynthate (Graham, 2000). When benefits to the host, such as enhanced P and Zn uptake, outweigh carbon-costs, growth may be increased by AMF (Smith and Read, 1997). However, if the host receives inadequate return benefits for the carbon supplied to the fungi, growth can be decreased (Johnson et al., 1997). A number of circumstances, that might occur alone or in combination, may cause AMF to decrease host growth, including: P supplied by AMF not required for growth due to high soil P or presence of other growth-limiting factors (Graham and Eissenstat, 1998; Kahiluoto et al., 2001; Khaliq and Sanders, 2000; Olsen et al., 1996); AMF fail to enhance host nutrient uptake; or, the rate of host photosynthesis is low, as may occur under low light conditions (Son and Smith, 1988).

Under field conditions, seasonal fluctuations in climate, along with shifts in the nutritional requirements and physiology of host plants at different growth stages, probably cause the role of AMF to vary between parasitic and beneficial (van der Heijden, 2001).

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The impact of AMF may similarly vary throughout their lifecycle, being greatest at initial establishment when an external network of hyphae is being formed (Lerat et al., 2003; Pearson and Schweiger, 1993). In addition, species of AMF differ in their impact on host plants (Lerat et al., 2003) and there is evidence that under agricultural conditions, particularly under continuous monocultures or in highly P-fertilised soils, populations of AMF may shift toward species less favourable for host growth (Hendrix et al., 1992; Johnson, 1991; Johnson et al., 1991, 1992; Kiers et al., 2002; Modjo and Hendrix, 1986).

Parasitic impacts from AMF are often not considered in agricultural studies, even though there is only limited evidence that AMF make a positive contribution to crop and pasture growth (Ryan and Graham, 2002). In a recent field experiment in south-eastern New South Wales, Australia, low colonisation by AMF of autumn-sown wheat (*Triticum aestivum*) and field pea (*Pisum sativum*) induced via crop sequence did not affect nutrient-acquisition before anthesis, even though crop growth was greatly limited by P (Ryan and Angus, 2003). After anthesis, however, there was a strong positive correlation between colonisation level and Zn-uptake, which resulted in enhanced grain Zn concentrations but not enhanced grain yield. A second set of field experiments in this region also found no deleterious impacts on nutrition and yield of wheat associated with low colonisation by AMF induced by preceding non-mycorrhizal *Brassica* crops (Ryan et al., 2002). In response to these findings, we hypothesised that AMF in south-eastern New South Wales would be parasitic on wheat, at least until anthesis.

To test this hypothesis, wheat shoot dry mass and reserves of water soluble carbohydrates (WSCs) in shoots and roots were examined at the site established by Ryan and Angus (2003). WSC reserves, which in wheat and other cereals consist mainly of fructans (Pollock et al., 1996), are probably the primary source of the sugars utilised by AMF (Pearson and Schweiger, 1993; Pfeffer et al., 1999) and therefore could be more sensitive to colonisation than shoot dry mass (Pearson and Schweiger, 1993). Large variation in the percentage of wheat root length colonised by AMF was induced through use of a bare earth fallow to decrease levels of AM-fungal inoculum and linola or legume-based pasture, which both host AMF, to increase inoculum. In addition, the subsequent wheat crops were grown with and without P-fertiliser. Conditions thought ideal for AMF to show a benefit for crop growth were present: low P soil (Smith and Read, 1997); negligible levels of cereal root pathogens (Ryan et al., 2002), and; a site history that consisted not of wheat, but of untilled, non-fertilised, legume-based pasture (Kiers et al., 2002).

Materials and methods

Site details and experimental design

The experiment was conducted on a commercial farm at Junee, New South Wales, Australia (34°85′ S, 147°57′ E, 280 m elevation), on a red loam soil (Kandosol) (Isbell, 1996). The site had contained non-fertilised subterranean clover (*Trifolium subterraneum*)-annual grass pasture for six years, before being sprayed with paraquat in August 1998 to remove grasses and control cereal root pathogens. After cultivation in April 1999, 2.5 t ha^{-1} of lime were spread. Soil characteristics in the top 100 mm were bicarbonate-extractable P 11 mg kg^{-1} (Colwell, 1963), pH 6.0 (water), pH 5.2 ($CaCl_2$), EC 0.11 dS m^{-1} and total N 1060 mg kg^{-1} (Rayment and Higginson, 1992). The experiment was conducted from 1999 to 2001 (Years 1 to 3). Year 1 plots were progressively divided by additional treatments in Years 2 and 3. Results from a subset of Year 2 and Year 3 wheat crops are presented in this paper (Figure 1).

In Year 1, plots were established of legume-based pasture, linola (*Linum usitatissimum*), canola (*Brassica napus*), bare earth fallow maintained with herbicides, and bare earth fallow maintained with herbicides and tillage. There were 4 replicates of each treatment arranged in a randomised block design with an individual plot size of 12 × 15 m. In Year 2, each Year 1 plot was divided into six 2 × 15 m plots sown on 17 May to wheat (cv. Diamondbird), canola (cv. Oscar) or field pea (cv. Soupa), with or without 20 kg ha^{-1} of P as triple superphosphate (20.2% P, 1.0% S). The herbicide pendimethalin was applied before sowing and the post-sowing, pre-emergence, herbicide S-metolachlor was applied mixed with the insecticide bifenthrin. Post-emergent herbicides were applied on 14 August: clopyralid (wheat, canola); diclofopmethyl (wheat); and haloxyfop-R (canola, field pea). All herbicides were applied at commercially recommended rates. Remaining weeds were removed by hand hoeing.

In Year 3, remaining crop stubble was burnt in April. On 3 May, the site was cultivated once and

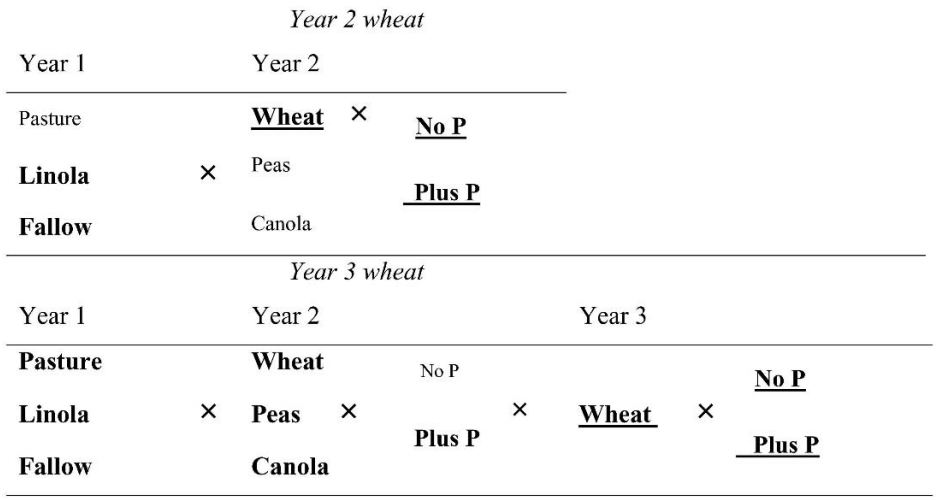

Figure 1. Treatment structure of the experiment. The crop and fertiliser treatments that preceded the subset of Year 2 and Year 3 wheat crops that are the subject of this paper are shown in bold type. These wheat crops were grown with and without P-fertiliser. There were four replicates.

56.5 kg ha^{-1} of N as urea applied at a depth of 65–75 mm. On 18 May, each Year 2 plot was divided lengthwise into three plots all sown to wheat (cv. Sunvale). A 2 × 6 m plot received 16.5 kg ha^{-1} of P as diammonium phosphate (16.5% P, 11% N, 4.5% S) and a 2 × 2 m plot received no fertiliser; a second 2 × 6 m plot is not referred to further in this paper. Seed was treated with fungicides (triticonazole and cypermethrin). The herbicide trisulfuron was applied before sowing and the post-sowing, pre-emergence, herbicide S-metolachlor was applied mixed with the insecticide bifenthrin. The post-emergent herbicides clopyralid and diclofopmethyl were applied on 31 July. All herbicides and fungicides were applied at commercially recommended rates. Remaining weeds were removed by hand hoeing.

Sample collection and analysis

Wheat plots were sampled three times in Year 2 and Year 3 and development rated by the Decimal Code of Zadoks et al. (1974); time 1 (4 September, 2000, DC 30; 24 September, 2001, DC 32), time 2 (9 October, 2000, DC 61; 14 October, 2001, DC 65) and time 3 (8 December, 2000, DC 92; 5 December, 2001, DC 92). These times are referred to as tillering, anthesis and harvest. In each year a subset of treatments was sampled as described below, the aim being to attain the greatest variation in the degree of colonisation by AMF.

In Year 2, wheat sown with and without P-fertiliser was sampled following Year 1 tillage fallow (hereafter referred to as fallow), a treatment expected to decrease AM-fungal inoculum, and linola, a host of AMF. At tillering, shoots and roots (sampled to approximately 100 mm depth with a shovel) were removed from two 0.32 m^2 quadrats in each plot; roots and shoots of an additional 10 plants adjacent to each quadrat were also removed. Samples were quickly transported to the laboratory, washed and stored at 4 °C until processing, which was completed within 48 h. For the material sampled from the quadrat, shoots were oven-dried at 70 °C for 3 days, weighed, finely ground in a puck-mill, pelleted, and analysed for P and Zn using an X-ray fluorescence spectrometer (Philips (PANalytical) 1404, Bankstown, NSW) with dual anode Sc/Mo tube (3kW) (Hutton and Norrish, 1977; Norrish and Hutton, 1977). Root systems from 10 randomly selected plants were stored in 70% ethanol. These roots were later cut into approximately 10 mm lengths, mixed thoroughly, and a sub-sample of approximately 2 m cleared in KOH and stained with aniline blue (Grace and Stribley, 1991). The percentage of root length colonised by AMF was then assessed by the grid line-intersect method at ×100 magnification (Giovannetti and Mosse, 1980). At each intersect between a root and a grid-line it was also noted whether the colonisation, if present, consisted of AMF with coarse (greater than 1.5 μm diameter) or fine (less than 1.5 μm diameter) hyphae; hereafter referred to as coarse endophyte and fine endophyte (Abbott, 1982). The percentage of colonised root length that contained fine and coarse endophyte was then calculated. Note, some points of intersection between a root and a grid line contained

both fine and coarse endophyte. The presence or absence of arbuscules of both fine and coarse endophyte was also recorded at each intersection.

The additional 10 plants were divided into stems plus sheaths, leaves and roots. The samples were frozen, freeze-dried and finely ground in a puck-mill. Concentrations of N were determined using an automatic N and C analyser (ANCA-SL) interfaced to a 20–20 stable isotope mass spectrometer (Europa Scientific, Crewe, UK). Water soluble carbohydrates (WSCs) were analysed following the method of Yemm and Willis (1954). Samples were extracted in ethanol at 80 °C for 1 h, followed by two extractions in water at 60 °C before addition of anthrone solution and heating for 10 min at 100 °C. Absorbance at 630 nm was measured using a spectrophotometer. This procedure was considered to extract mainly fructans and low molecular mass compounds (Borrell et al., 1989).

At anthesis, one 0.32 m^2 quadrat was taken in each plot, while at harvest, two 0.32 m^2 quadrats were taken in each plot. Shoots were oven-dried and total shoot dry mass and grain yield determined. At anthesis, roots to 100 mm depth were removed with a shovel. The percentage of root length colonised by AMF was determined at $\times 30$ magnification.

In Year 3, wheat sown with and without P-fertiliser was sampled following the P-fertilised Year 2 wheat and field peas, both hosts of AMF, and the non-mycorrhizal canola. These Year 2 treatments followed Year 1 tillage fallow or pasture (Figure 1). By sampling only after Year 2 crops that received P-fertiliser, it was hoped to achieve at least one treatment with colonisation close to zero. At each sampling time, shoots were removed from one (tillering) or two (anthesis and harvest) 0.32 m^2 quadrats per plot in the P-fertilised treatments and one 0.16 m^2 quadrat in the non-fertilised treatments; at tillering, roots to 100 mm depth from 10 plants were also removed with a shovel. Samples were quickly transported to the laboratory, washed and stored at 4 °C until processing, which was completed within 48 h. At anthesis, shoots were divided into stems plus sheaths, leaves and spikes. At tillering, the percentage of root length colonised by AMF was determined at $\times 30$ magnification on a subsample of roots.

Remaining roots and shoots were frozen, freeze-dried and shoot dry mass calculated. Nutrient and WSC concentrations were assessed as described above, with only stems being analysed at anthesis. At harvest, shoots were oven-dried, spikes with significant frost damage (defined as greater than 50% of florets without grain) counted, and total shoot dry mass and grain yield determined.

In both Year 2 and Year 3, wheat roots at tillering were visually assessed over a white background for symptoms of fungal root pathogens. In Year 2, roots stained for assessment of AMF were also examined at $\times 100$ magnification for non-mycorrhizal fungal structures. These data are not presented as symptoms were negligible in both years and did not differ between treatments.

Climate data

Rainfall was recorded on site in 1999 and 2000. Rainfall in 2001, long-term rainfall averages and air temperatures in 2000 and 2001 were supplied for the nearby town of Temora by The Bureau of Meteorology, Australia. Sunshine hours were provided for a second nearby town, Junee, by The Bureau of Meteorology, Australia.

Data analysis and presentation

Two sets of data analyses were performed. In the first set, conducted using Genstat (Version 6.1, Lawes Agricultural Trust, Rothamsted UK), a split-split plot ANOVA was used to examine the impact of previous treatment and P-application on colonisation by AMF, shoot dry mass, WSC stores and crop nutrition. In the second set of analyses, the impact in Year 3 of AMF on shoot dry mass and WSC stores in roots and shoots was examined using simple regressions of individual treatment means, which were graphed and an r^2 value calculated using SigmaPlot (Version 8.1, SPSS Inc., Chicago, Illinois).

Results

Climate

Total annual rainfall in Year 1 (1999, 690 mm) and Year 2 (2000, 507 mm) was above or similar to the long-term mean of 537 mm and was characteristically variable from month to month (Figure 2a). Between 13 September and 12 October 2000, there were only 12 mm of rainfall and all crops showed signs of drought stress at anthesis. In Year 3 (2001), only 372 mm of rainfall were recorded. Dry conditions prevailed at the start of the year and while moisture was adequate for germination following sowing on 18 May, further significant rainfall did not occur until 8 June (20 mm).

Table 1. Impact of Year 1 and Year 2 treatment and P-fertiliser application on colonisation by AMF, and growth and nutrition of Year 2 and Year 3 wheat at tillering; estimated means and LSD at $P = 0.05$ for significant effects (ns= not significant). In Year 2, there was a significant interaction between Year 1 treatment and P-application for the proportion of root length colonised by AMF that contained coarse endophyte.

		AMF (% root length)	Fine endo. (% AMF)	Coarse endo. (% AMF)		Root WSC (mg g^{-1})	Shoot WSC (mg g^{-1})	Stem WSC (mg g^{-1})	Leaf WSC (mg g^{-1})	Shoot dry mass (t ha^{-1})	Shoot WSC content (t ha^{-1})	Shoot N (g kg^{-1})	Shoot P (g kg^{-1})	Shoot Zn (mg kg^{-1})
Year 2 Tillering (4 September)				No P	Plus P									
Year 1	Linola	45	75	51	42	54	230	290	150	1.5	0.32	32	2.8	16
	Fallow	29	79	17	54	74	270	360	180	1.5	0.40	29	2.7	14
	LSD	**13**	**ns**	**15**		**18**	**ns**	**40**	**ns**	**ns**	**ns**	**0.3**	**ns**	**1**
P-fert.	No P	56	86			65	260	350	190	0.9	0.24	31	2.2	16
	Plus P	19	68			64	240	310	140	2.0	0.48	31	3.4	14
	LSD	**13**	**13**			**ns**	**ns**	**40**	**40**	**0.6**	**0.10**	**ns**	**0.6**	**1**
Year 3 Tillering (24 September)														
Year 2	Wheat	42	–	–		33	210	–	–	2.7	0.59	25	2.0	13
	Peas	24	–	–		36	230	–	–	3.5	0.82	23	1.9	13
	Canola	28	–	–		36	200	–	–	3.4	0.70	25	2.1	14
	LSD	**8**	**–**	**–**		**ns**	**20**	**–**	**–**	**0.4**	**0.14**	**ns**	**ns**	**ns**
Year 1	Pasture	34	–	–		33	210	–	–	3.1	0.66	25	2.1	14
	Fallow	29	–	–		37	220	–	–	3.3	0.77	24	1.9	12
	LSD	**ns**	**–**	**–**		**3**	**ns**	**–**	**–**	**ns**	**ns**	**ns**	**ns**	**1**
P-fert.	No P	44	–	–		36	210	–	–	2.3	0.50	26	2.0	14
	Plus P	19	–	–		33	220	–	–	4.1	0.91	22	2.0	12
	LSD	**6**	**–**	**–**		**ns**	**ns**	**–**	**–**	**0.4**	**11**	**2**	**ns**	**1**

Table 2. Impact of Year 1 and Year 2 treatment and P-fertiliser application on colonisation by AMF, and growth of Year 2 and Year 3 wheat at anthesis and harvest; estimated means and LSD at $P = 0.05$ for significant effects (ns=not significant). In Year 3 at anthesis, there was a significant interaction between Year 2 treatment and P-application for shoot dry mass accumulation after tillering and shoot dry mass.

		AMF (% root length)	Stem WSC (mg g^{-1})	Shoot dry mass accumulation after tillering (t ha^{-1})		Shoot dry mass (t ha^{-1})		Shoot dry mass (t ha^{-1})	Grain yield (t ha^{-1})	Harvest index	Frost damage (% spikes)
Year 2	**Anthesis**	**(9 October)**						**Harvest (December 8)**			
Year 1	Linola	64	–	3.7		5.1		9.1	3.9	0.43	–
	Fallow	30	–	4.0		5.5		9.9	4.1	0.41	–
	LSD	**18**	**–**	**ns**		**ns**		**ns**	**ns**	**ns**	**–**
P-fert.	No P	50	–	2.6		3.4		7.6	3.1	0.41	–
	Plus P	43	–	5.0		7.0		11	4.9	0.43	–
	LSD	**ns**	**–**	**1.5**		**1.9**		**2.0**	**0.7**	**ns**	**–**
Year 3	**Anthesis**	**(14 October)**		No P	Plus P	No P	Plus P	**Harvest (December 5)**			
Year 2	Wheat	–	470	2.3	3.0	4.1	6.6	7.1	2.7	0.38	20
	Peas	–	480	2.9	2.3	5.6	6.5	7.3	2.6	0.35	30
	Canola	–	415	2.8	2.6	5.1	7.2	8.3	3.2	0.39	19
	LSD	**–**	**30**	**0.7**		**0.7**		**0.7**	**0.3**	**0.02**	**5**
Year 1	Pasture	–	440	2.6		5.7		7.3	2.7	0.37	26
	Fallow	–	470	2.7		6.0		7.8	3.0	0.38	20
	LSD	**–**	**20**	**ns**		**ns**		**ns**	**0.2**	**ns**	**4**
P-fert.	No P	–	470					6.7	2.6	0.39	22
	Plus P	–	440					8.4	3.0	0.36	25
	LSD	**–**	**22**					**0.6**	**0.2**	**0.01**	**ns**

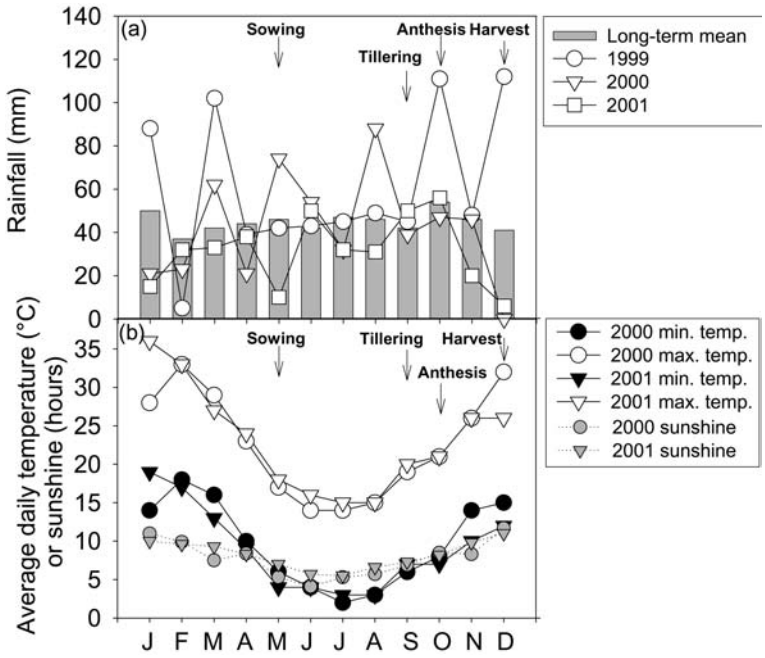

Figure 2. Climate data: (a) total rainfall per month in 1999 (Year 1), 2000 (Year 2) and 2001 (Year 3), and the long-term (66 year) average, and; (b) average daily maximum and minimum temperatures and average daily sunshine hours per month in 2000 and 2001.

Conditions then remained relatively dry throughout crop growth. Only 38 mm of rainfall fell between anthesis and harvest in Year 3 (14 October to 5 December), compared with 99 mm between the same dates in Year 2. In Year 3 a heavy frost occurred on 13 October, the night preceding the anthesis sampling. Anecdotal reports suggested this frost caused yield losses for nearby commercial crops ranging from 20 to 80%. Mean daily minimum and maximum air temperatures and sunshine hours were similar in Year 2 and Year 3, and were all relatively low between May, when the crops were sown, and October, when the crops reached anthesis (Figure 2b).

Colonisation by AMF

The percentage of wheat root length colonised by AMF varied greatly in both years in response to preceding treatment (Tables 1 and 2). In Year 2, colonisation was higher following linola than fallow and in Year 3 colonisation was higher following wheat than peas or canola. Year 1 treatments had no impact on colonisation in Year 3. Application of P-fertiliser substantially lowered colonisation at tillering (Table 1), but not anthesis (Table 2).

In the more detailed assessment of colonisation at tillering in Year 2, Year 1 treatment had no impact on the proportion of colonised root that contained fine endophyte or, when P-fertiliser was applied, coarse endophyte (Table 1). However, when P-fertiliser was not applied, coarse endophyte was more than twice as common following linola than fallow. For both fine and coarse endophyte, the proportion of colonised root length that contained arbuscules was greater than 95% (data not shown).

WSC concentration

In Year 2 at tillering, concentrations of WSCs in roots and stems were higher following fallow than linola (Table 1). In Year 3 at tillering, concentrations of WSCs in shoots were higher following peas than canola or wheat and, in roots only, slightly higher following Year 1 fallow than pasture (Table 1). Thus, while high colonisation by AMF corresponded with lower WSC concentrations in Year 2 at tillering (Table 1), in Year 3 such a relationship was only present in shoots when P-fertiliser was not applied (Figure 3a, b). In Year 3 at anthesis, WSC concentrations in stems were lower following canola than wheat or peas, and lower following Year 1 pasture than fallow. Application of P-fertiliser lowered WSC concentrations in stems and leaves in Year 2 at tillering and in stems in Year 3 at anthesis (Tables 1 and 2).

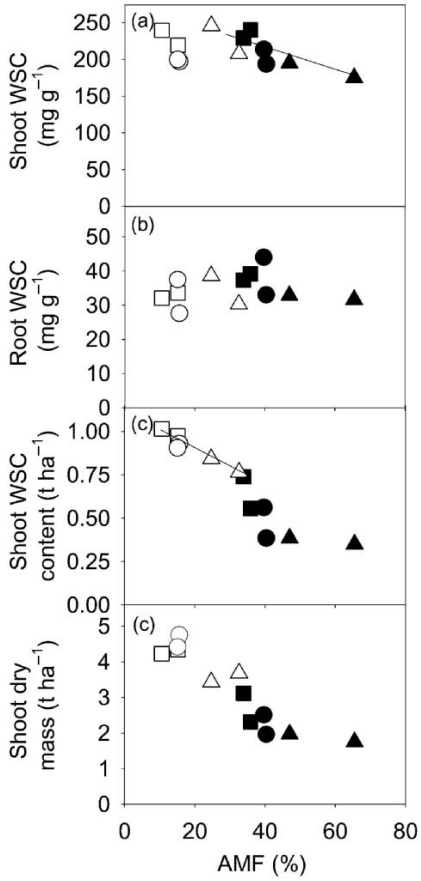

Figure 3. The relationship between the level of colonisation of wheat by AMF at tillering in Year 3 and: (a) WSC concentrations in shoots; (b) WSC concentrations in roots; (c) shoot WSC content (shoot WSC concentration × shoot dry mass); and, (d) shoot dry mass, for wheat grown with 0 (filled symbols) or 16.5 kg ha^{-1} (clear symbols) of P after wheat (triangles), peas (squares) and canola (circles), each of which followed pasture or fallow (not distinguished). Each point is the mean of four replicates; significant correlations are marked (shoot WSC concentration, not P-fertilised, $r^2 = 0.72$, $P < 0.03$; shoot WSC content, P-fertilised, $r^2 = 0.92$, $P < 0.002$).

Shoot dry mass, shoot WSC content and grain yield

In Year 2, shoot dry mass, shoot WSC content (WSC concentration × shoot dry mass) and grain yield did not differ with Year 1 treatment (Tables 1 and 2). In Year 3 at tillering, shoot dry mass and shoot WSC content were lower following wheat than following peas or canola and, hence, there tended to be a negative correlation with the level of colonisation by AMF (Figure 3c, d). In Year 3 between tillering and anthesis, accumulation of shoot dry mass was lower following

wheat than peas and canola when P-fertiliser was not applied, but higher following wheat when P-fertiliser was applied (Table 2). Thus, at anthesis, shoot dry mass was particularly low following wheat when P-fertiliser was not applied and differed little between treatments when P-fertiliser was applied. At harvest, shoot dry mass and grain yield were highest following canola. Frost damage occurred most frequently following peas and these crops also had the lowest harvest index. Application of P-fertiliser consistently increased shoot dry mass, shoot WSC content and grain yield; the impact being greatest earlier in the season, and greater in Year 2 than Year 3.

Crop nutrition

In Year 2 and Year 3, concentrations of N, P and Zn in shoots showed either no, or a quite small, degree of variation in response to treatment at both tillering (Table 1) and anthesis (results not shown). Application of P-fertiliser resulted, at tillering, in higher shoot P concentrations in Year 2, a slight lowering in Zn concentrations in both years, and a slight decrease in shoot N concentration in Year 3.

Discussion

AMF are generally regarded as beneficial symbionts (Smith and Read, 1997). However, their complete reliance on host sugars (Ho and Trappe, 1973; Pfeffer et al., 1999) confers the potential for parasitism if no benefits accrue for the host plant. As discussed below, our results are consistent with the indigenous AMF, in the absence of a nutritional benefit for the host plant, reducing WSC reserves and/or growth of autumn-sown wheat. However, the use of crop sequences to manipulate the degree of colonisation by AMF means interpretation of our data cannot be made without caution. The following sections explore our findings more closely and discuss their broader implications.

Distinguishing impacts of AMF from other influences of crop sequence

There has been relatively little investigation of the influence of AMF on crop growth under field conditions (Ryan and Graham, 2002). In part, this reflects the difficulties with establishing control plots with no or reduced colonisation by AMF. All methods commonly used to reduce AM-fungal colonisation and/or functioning such as soil fumigation or fungicides (Kahiluoto et al., 2001), growing non-host crops such as brassicas (Ryan et al., 2002), long bare fallows (Thompson, 1987), or tillage (McGonigle et al., 1999) have the potential to alter the balance between plant pathogenic and beneficial micro-organisms (Daniels Hetrick et al., 1988; Ryan et al., 2002). The treatments also have the potential to affect soil physical properties, soil water, and availability of soil nutrients (e.g. Kirkegaard et al., 1994). Such side-effects result in uncertainty around the true contribution of AMF to crop growth and nutrition, and limit the conclusions that can be drawn.

The present study was designed to minimise the impact of the crop sequences on factors other than AMF (see Ryan and Angus, 2003). Pasture grasses were removed nine months before the experiment was sown and, as a result, only extremely low levels of known cereal root fungal pathogens were recorded. Six years of legume-based pasture ensured a high level of soil mineral N was initially present and greater than 200 kg ha^{-1} of N to 1.8 m depth was measured before sowing in Year 2 (Ryan and Angus, 2003). Nitrogen was then applied pre-sowing in Year 3. Shoot N concentrations also indicated that N was unlikely to be the cause of differences in growth between treatments (Table 1). Heavy rainfall at the start of Year 2 ensured equal soil water across the site (Ryan and Angus, 2003). However, in Year 3, the dry conditions probably influenced the differences between treatments at the end of the season through the vigorous growth before tillering of the P-fertilised wheat following peas and canola reducing soil water reserves (Fischer and Kohn, 1966). Thus, in the low rainfall conditions experienced after tillering, growth of the P-fertilised crops was greatest for wheat following wheat (Table 2). We also cannot rule out the possibility that the low-P soil at the site may have interacted with crop sequence in Year 3. In particular, the impact of AMF on wheat following canola in the absence of P-fertiliser was probably masked by low soil available P as removals of P in grain of Year 2 crops were 9.3 kg ha^{-1} for wheat and 10.8 kg ha^{-1} for peas, but 18.0 kg ha^{-1} for canola (Ryan and Angus, 2003) (see Figure 3 – dark circles). The differential impact of frost in Year 3 also eliminates the possibility of assessing the influence of AMF on yield.

Finally, in Year 3, the low colonisation by AMF of wheat following peas was unexpected, as the peas were highly colonised (Ryan and Angus, 2003). This low colonisation was unfortunate as the only highly

colonised treatment was, therefore, wheat following wheat. Reductions in growth or changes in root morphology have been reported when a crop is grown continuously and have been attributed to a buildup of soil organisms other than AMF (Olsson and Alström, 1996; Rovira et al., 1990). For instance, deleterious pseudomonad bacteria may increase in abundance in wheat rhizospheres as the frequency of wheat in the rotation increases (Rovira et al., 1990). We cannot discount the possibility that deleterious organisms, other than the known pathogens that were assessed, contributed toward the poor growth of wheat following wheat.

AMF acting parasitically?

In the absence of a nutritional benefit from high colonisation by AMF before anthesis, our results are consistent with a negative impact from AMF on wheat growth and WSC reserves. The clearest evidence is the lower stem and root WSC concentrations in Year 2 following linola compared with fallow (Table 1) and the negative relationship between colonisation level and shoot WSC content and shoot dry mass in Year 3 (Table 1, Figure 3c, d). Application of P-fertiliser also strongly affected colonisation by AMF, but the large increase in shoot dry mass in response to P-fertiliser meant that associated changes in WSCs could not be ascribed to AMF. Whilst the percentage of root length colonised is undoubtedly a crude measure of AM-fungal activity, all mycorrhizal structures are constructed using host sugars and the prolific presence of the relatively short-lived arbuscules (Alexander et al., 1988) indicates the fungi were physiologically active. A strong negative correlation between the percentage of root length colonised by AMF and shoot dry mass has also been reported for pot-grown tobacco (Modjo and Hendrix, 1986), while in a 27-month field-study of *Citrus*, a decrease in the percentage of root segments which contained AMF (induced by benomyl application) correlated with an increase in tree stem diameter (Graham and Eissenstat, 1998). Lerat et al. (2003) reported a strong positive linear relationship between the percentage of barley (*Hordeum vulgare*) root length colonised by AMF and the carbon-sink strength of the roots for two out of three species of AMF tested.

In the present study, the negative impact on crop growth from high colonisation by AMF was greater in Year 3 than Year 2. This may reflect drier conditions (Figure 2a). Cereals store excess WSCs that are later remobilised to aid grain filling (Evans and Wardlaw, 1996). WSCs play a particularly important role in yield when conditions after anthesis restrict photosynthesis (van Herwaarden et al., 1998). In Year 3, water deficit before tillering may have restricted photosynthesis leading to lower WSC concentrations than Year 2. Redistribution of an already small reserve of WSCs to the AMF, away from the host, would have exacerbated the impact of water deficit on photosynthesis, leading to reductions in crop growth. Shoot dry mass may have been sacrificed to maintain a minimal level of WSC reserves. Thus the impact of AMF was manifested primarily in WSC concentrations in Year 2 and in shoot WSC content and dry mass, particularly for the P-fertilised crops, in Year 3.

The full cost to the wheat of colonisation by AMF cannot be calculated for our experiment, as no crops were completely free of colonisation. However, our data do suggest that at each P-level at tillering, an additional 15% of total root length colonised by AMF imposed an approximate penalty of 20% for either WSC concentrations (Year 2) or shoot WSC content or dry mass (Year 3). The latter figure indicates a much higher total carbon contribution to AMF than the 4 to 20% often suggested (Graham, 2000). However, it is possible that other soil organisms also contributed to this carbon cost.

Why a parasitic impact?

As stated in the introduction to this paper, parasitic impacts from AMF could be induced by a number of circumstances that might occur alone or in combination, including: P supplied by AMF not required for host growth due to high soil P or presence of other growth-limiting factors (Graham and Eissenstat, 1998; Kahiluoto et al., 2001; Khaliq and Sanders, 2000; Olsen et al., 1996); AMF fail to enhance host nutrient uptake; or, the rate of host photosynthesis is low, as may occur under low light conditions (Son and Smith, 1988). High soil P can be discounted in the present study. Instead, it appears AMF played little role in crop nutrition before anthesis. While differences in AMF colonisation at tillering were of a magnitude reported to correlate with variation in crop P and Zn nutrition in north-eastern Australia (Thompson, 1987), uptake of P and Zn before anthesis in our experiment was not enhanced by increases in colonisation. Such an inability to enhance crop nutrient uptake could reflect formation of an ineffective symbiosis due to the presence of parasitic species of AMF, or environmental or management constraints on the ability of AMF

to either absorb nutrients from the soil or provide nutrients to the host plant.

It has been hypothesised that agricultural practices, especially frequent addition of P-fertiliser and continuous monocultures, may favour build-up of populations of parasitic AMF (Kiers et al., 2002). For instance in Kentucky, USA, *Glomus macrocarpum* is a known pathogen on tobacco (*Nicotiana tabacum*) and rotation with crops that decrease the occurrence of *G. macrocarpum* enhances yields (Hendrix et al., 1992; Modjo and Hendrix, 1986). While in Minnesota, USA, Johnson et al. (1991) found the impact of crop sequence on AM-fungal spore numbers differed between species of AMF, with species that proliferated under a continuous monoculture often detrimental to yield of that crop, but beneficial to yield of other crops (Johnson et al., 1992). While the current experiment was conducted under circumstances dissimilar to those hypothesized by Kiers et al. (2002) to favour evolution of parasitic AMF, it is still possible that species of AMF intrinsically parasitic to wheat were present. Indeed, in a glasshouse experiment, Graham and Abbott (2000) found species of AMF isolated from Australian low-P natural ecosystems could aggressively colonise and reduce the growth of wheat.

The contribution of coarse endophyte to overall colonisation in Year 2 shows that the abundance of AMF species was being altered in response to treatment and, therefore, may have greatly differed between Year 3 treatments. However, the relationships between colonisation level and post-anthesis Zn-uptake (Ryan & Angus, 2003), WSC concentration (Table 1, Figure 3a) and WSC content (Figure 3c), suggest colonisation level largely over-rode any functional differences between species of AMF.

The positive impact of AMF on Zn-uptake in spring, post-anthesis, in 2000 (Ryan and Angus, 2003) is consistent with the lifting of an environmental constraint at this time; the exceptionally dry spring in 2001 may have inhibited such an effect. Ryan and Angus (2003) hypothesised that until anthesis, soil temperatures below 10 °C reduced the ability of AM-fungal hyphae to transport nutrients (see Cooper and Tinker, 1981). Until anthesis, crops were also experiencing short winter day lengths and low light levels (Figure 2b). In addition, colonisation by AMF was being established between sowing and tillering, with carbon demands probably at a peak (Lerat et al., 2003; Pearson and Schweiger, 1993). This combination of factors may have lead to the wheat being unable to compensate through photosynthesis for sugars used by the fungi (Son and Smith, 1988). It is also noteworthy that the colonisation by AMF consisted primarily of arbuscules, in contrast to a recent survey of spring-sown wheat in southern Idaho, USA, where colonisation consisted primarily of hyphae without arbuscules (McGonigle et al., 2004). The abundant arbuscules may have enhanced the parasitic impact through high carbon requirements for construction and provision of a large surface area for the AMF to access host sugars.

Alternatively, the wheat crops received management typical of commercial crops in south-eastern Australia, including seed fungicides (Year 3 only) and herbicides (Years 2 and 3). While Ryan et al. (1994) found these inputs to have no impact on the level of AM-fungal colonisation in wheat, their impact on nutrient uptake mediated by AMF is unknown.

Implications for management of AMF in cropping systems in south-eastern Australia

The benefits for wheat of preceding non-cereal break crops have long been appreciated in south-eastern Australia and have been considered to largely reflect reduced occurrence of fungal root pathogens (Gardner et al., 1998). However, two of the most popular break crops are narrow-leaved lupins (*Lupinus angustifolius*) and canola. Narrow-leaved lupins are only very weakly colonised by AMF (Trinick, 1977) and canola is non-mycorrhizal. We suggest a proportion of the yield enhancement of wheat following break crops may be due to reduced colonisation by AMF (see also Harris et al., 2002; Ryan et al., 2002).

If this is confirmed to be the case, farmers could choose to capture yield benefits from lowering colonisation by AMF by including non-mycorrhizal crops in rotations or applying a rate of P-fertiliser sufficient to reduce colonisation to negligible levels (Olsen et al., 1996). Alternatively, AMF could be manipulated through crop breeding. Wheat cultivars vary greatly in degree of colonisation by AMF (e.g. 0–38% Azcón and Ocampo, 1981) and response to inoculation by AMF (Azcón and Ocampo, 1981; Hetrick et al., 1993). In *Citrus*, mycorrhizal dependency also varies with host genotype (Graham et al., 1997) and high dependency appears linked to a relatively loose regulation of carbon expenditure on AMF (Jifon et al., 2002). Genotypes with a high dependency greatly benefit from AMF at a low P supply, but when P is plentiful are more likely to suffer a growth depression (Jifon et al., 2002). Perhaps such dependent genotypes would also be more susceptible to growth depressions

under P-limiting conditions when AMF colonisation is present, but not aiding host P-uptake. Thus, for south-eastern Australia, we recommend investigation of whether selection for a low degree of colonisation by AMF and poor mycorrhizal dependency, could enhance growth and yield of autumn-sown wheat.

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