Journal of Agricultural Science, Cambridge (2001), **137**, 27–36. © 2001 *Cambridge University Press*
DOI: 10.1017/S0021859601001101 *Printed in the United Kingdom*

Effect of genotype of *Trifolium repens* on mycorrhizal symbiosis with *Glomus mosseae*

W. R. EASON[1]*, K. J. WEBB[1], T. P. T. MICHAELSON-YEATES[1], M. T. ABBERTON[1], G. W. GRIFFITH[2], C. M. CULSHAW[2], J. E. HOOKER[3] AND M. S. DHANOA[1]

[1] *Institute of Grassland and Environmental Research, Plas Gogerddan, Aberystwyth SY23 3EB, UK*
[2] *Institute of Biological Sciences, University of Wales, Aberystwyth SY23 3DA, UK*
[3] *School of Applied Sciences, University of Glamorgan, Pontypridd CF37 1DL, UK*

(*Revised MS received 26 March 2001*)

SUMMARY

Forty-three near-isogenic lines (NILs) of white clover (*Trifolium repens*), derived from four parental self-compatible genotypes containing the rare self-fertility allele, were inoculated with the arbuscular mycorrhizal fungus *Glomus mossae*. Plant growth response (shoot and root weight and root length), shoot P uptake and mycorrhizal root infection rates were recorded 12 weeks after inoculation. There was generally a high degree of variation between individual lines in all recorded parameters. The most sensitive indicator of plant response to mycorrhizal infection was root length with almost half of all lines showing significant responses (in most cases a decrease in root length). Shoot weight was significantly different between mycorrhizal and nonmycorrhizal plants in nine lines. Parental genotype significantly affected both plant response to mycorrhiza as well as mycorrhizal infection rates. The results suggest that the NILs will prove useful for further studies to elucidate the molecular genetic control of the symbiosis and inform plant breeding strategies of this agronomically important species.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF) are non-pathogenic, symbiotic fungi which infect the roots of most terrestrial plants. They play a key role in increasing plant access to supplies of soil immobile nutrients such as phosphorus, particularly in conditions of low soil P. In exchange for improved plant nutrient supply the fungus benefits from a supply of plant derived carbon (Sanders *et al.* 1975, Smith & Read 1997). AMF may also increase uptake of nitrogen from the soil either directly or through improvements in the plant P supply (Ibijbijen *et al.* 1996*a*, *b*). AMF-infected plants exhibit improved reproductive capacity (Lu & Koide 1994), plant adaptation to water stress (Subramanian & Charest 1999) and health through antagonistic and/or competitive effects on pests and pathogens (Gange & West 1994, Bodker *et al.* 1998). In low-input or organic farming systems AMF may play a critical role because of their role in linking plant and soil processes (Bethlenfalvay & Linderman 1992). It is in these conditions that AMF are likely to have the most significant impact on plant growth and health.

AMF have a low level of host specificity and a single AMF species may infect a large range of plant hosts (Smith & Read 1997). However, the ability of AMF to infect and colonize root systems of different plants may not always result in the same functional relationship between fungus and plant host. The mycorrhizal dependency or growth response of different plant hosts can vary when colonized by the same AMF (Khalil *et al.* 1999). The functional effectivity of AMF-plant interactions can be defined in a number of ways including effects on plant growth and nutrient uptake and/or altered resistance to root pathogens or adaptation to abiotic stresses.

AMF infection and plant response to it can be modified by environmental factors such as soil P availability which under nonlimiting levels may reduce both plant response and infection levels (Hall *et al.* 1977). The symbiosis may even reduce plant growth under certain conditions, for example under low light levels, when carbon supply to maintain the fungus becomes limiting and begins to affect plant growth (Graham & Eissenstat 1998).

It is also clear that the functioning of the symbiosis can be modified by the plant and fungal partners.

* To whom all correspondence should be addressed. Email: william.eason@bbsrc.ac.uk

Scullion *et al.* (1998) demonstrated that AMF inoculum from conventionally managed agricultural soils was less effective (in terms of the growth response of *Trifolium repens*) compared to inoculum from organically managed soils.

There have been a number of studies demonstrating the effect of plant genotype on the arbuscular mycorrhizal (AM) symbiosis (see Smith *et al.* 1992 for a review). Manske (1990) showed that high yielding cultivars of wheat were much less responsive than landraces to AMF infection under low fertility conditions. Hetrick *et al.* (1992*a*, *b*) demonstrated a strong genetic basis for differences in mycorrhizal dependence and root infection among wheat cultivars, also showing that there was a trend for a greater reliance on the symbiosis in older cultivated varieties. Some modern varieties either showed no response or else growth was reduced following infection (Hetrick *et al.* 1992*b*). This may reflect the fact that plant breeding programmes have not selected for plants based on their ability to form effective AM associations. Indeed, it is more likely that plants have been evaluated under conditions such as high soil nutrient availability, which could have indirectly selected plants with a poor ability to form good AM symbioses in low-input systems.

White clover (*Trifolium repens* L.) is an important component of low input pasture systems and can form effective associations with both nitrogen-fixing rhizobium and AMF. This study used near-isogenic lines (NILs) of *T. repens* to elucidate the importance of plant genetic control of the AM symbiosis. NILs are genetically identical with the exception of a small proportion of loci. *T. repens* is normally an outbreeding species and production of NILs was only possible by using the dominant *Sf* allele, which confers true self-compatibility. Plants of four parental genotypes carrying the *Sf* allele were selfed and their progenies selected and selfed, over many generations so that the lines were near-isogenic (Michaelson-Yeates *et al.* 1997). Traits of agronomic importance, including their functional relationship with AMF, are fixed in NILs. This genetic uniformity and stability, coupled with variation in the trait of interest (e.g. AMF infection rates or plant response to AMF), make the NILs ideal for studies on the plant genetic control of the symbiosis.

In this study, the objective was to identify closely related clover lines differing in both the colonization and the plant's response to infection by AMF. This study will provide the basis for future work examining the molecular-genetic control of the symbiosis.

MATERIALS AND METHODS

Plant materials and cultural conditions

Forty-three near-isogenic lines (NILs) of *T. repens* were used in this study. Self-compatible plants of *T. repens* were derived from lines originally developed by E. G. Williams, University of Melbourne, Australia. From 15 self-compatible plants detected in these accessions, four lines (coded H, J, R and S) were maintained through seven generations of selfing (Michaelson-Yeates *et al.* 1997). From four parental genotypes a total of 43 lines were produced, lines 1–11 derived from parental genotype H, lines 12–21 from J, lines 22–34 from R and lines 35–43 from S.

Plants were grown in 0·4 litre pots (Plantpak Ltd, UK) containing sterile, washed, Terragreen (Agsorb, grade 8/16; Oil-Dri, Wisbech, UK). Terragreen is an inert attapulgite clay and has been used in previous AMF studies, as it contains low levels of available P which encourages AMF formation (Tisserant *et al.* 1998, Boddington & Dodd 1999). Fifty ml of a modified nutrient solution, containing no P, was supplied to each pot every week (final concentration in g/mg per dm^3; KNO_3 0·51 g, $Ca(NO_3)_2$ 0·82 g, $MgSO_4.7H_2O$ 0·49 g, KCl 74·6 mg, FeEDTA 13·1 mg, $MnCl_2.4H_2O$ 1·81 mg, $CuSO_4.5H_2O$ 0·08 mg, $ZnSO_4.7H_2O$ 0·22 mg and $Na_2MoO_4.2H_2O$ 0·13 mg).

Seeds were scarified and sown directly into the pots of moist Terragreen at the rate of three seeds per pot. Following establishment of plants plant numbers were reduced to one per pot leaving similar size plants across treatments. Ten ml of a rhizobium inoculant was supplied to all plants which consisted of a mix of five strains of *R. leguminosium* biovar. *trifolii* (D. Allen, personal communication). The original sugar-based stock culture solution was centrifuged for 1 min at 1000 ***g*** to pellet the rhizobium which was re-suspended in water immediately prior to application to pots.

The experiment was carried out in a glasshouse with supplementary lighting and heating over the period August–October. Temperature was maintained within the range 15–20 °C. Photoperiod was maintained at 16 h by use of artificial lighting (Phillips SON-T AGRO 400W), providing a minimum 200 μmol/m^2 per second (mean levels when dark outside, A. Mizen, personal communication). Pot positions were randomized every week and plants were watered daily to field capacity.

AMF inoculum

AMF inoculum was obtained commercially (Biorize Ltd, France) as a granular preparation of *Glomus mosseae* (La Banque Europeene des Glomales, Isolate 12; http://www.bio.ukc.ac.uk/beg/). This consists of a Terragreen base containing infected plant roots, spores and hyphae. The efficacy of this inoculum is routinely tested using *Allium ameloprasum* L. (leek) where we routinely obtain infection of over 90% of the root length (in similar environmental and soil conditions to this experiment). A layer of this

inoculum (equivalent to 5 cm^3) was placed approximately half-way down in filled pots, which were then filled with Terragreen. In order to ensure that plants in uninoculated pots would have similar background microbial populations apart from AMF, washings of the inoculum were filtered through Whatman No. 42 paper (which did not allow transmission of AMF propagules) and 10 ml of the filtrate was added to each pot. Each treatment (+AMF inoculum and –AMF) was replicated six times.

Plant assessments

Non-destructive assessments of plant shoot growth were taken ten times between 19 and 61 days after sowing. Leaf number assessment followed the method of Carlson (1966). The purpose of these assessments was to provide an indication of when a mycorrhizal growth effect had become established.

Forty-one days after sowing, root cores (approx. 1 cm diameter and 3 cm length) were taken from three randomly selected lines (2, 4 and 34) of both control and AMF-inoculated treatments to assess the mycorrhizal status of the roots. Three randomly selected replicates were taken from each treatment. Other pots in these treatments were also cored (but not assessed) to counter any effect of the coring process on plant growth. Washed Terragreen was used to fill holes left by coring. Per cent of root length infected with AMF was determined on roots washed from the cores (Phillips & Hayman 1970, Giovannetti & Mosse 1980). The purpose of this assessment was to check that AMF root infection had occurred and that controls remained uninfected. AMF assessment of all treatments was carried out at the final harvest, at 84 days.

Eighty-four days after sowing, the plants were harvested and the root and shoot systems were separated. The shoot system was oven-dried to constant weight at 80 °C and its dry weight determined. Chemical analysis of phosphorus (P) was determined on ground subsamples of the shoot system (MAFF 1986). Terragreen was washed from the root system, which was then stored in 50% ethanol for further analyses. Root length was determined using a grid intersect method (Böhm 1979) and % AMF of the root length was determined. Root dry weights (dried to constant weight at 80 °C) were determined from subsamples taken from and based on total fresh weight of well-blotted root systems. Root:shoot dry weight ratios were calculated.

Statistical analysis

Cluster analysis was carried out on combined plant growth data using Genstat 5 (Lawes Agriculture Trust 1997). This is a technique which provides a visual overview of the data structure as indicated by emerging clusters (i.e. groups of units with similarities). Analysis of variance was performed using Genstat to examine the effects of mycorrhizal status, parental family genotype and individual lines and all interactions. Root infection data (%) were transformed (angular) prior to analysis in Genstat (actual percentage values shown as well as transformed data). Least significant difference values for individual line comparisons were calculated at $P \leqslant 0{\cdot}05$, $P \leqslant 0{\cdot}01$ and $P \leqslant 0{\cdot}001$ to account for the increased number of significances as a result of the large number of treatment means. Ratios of growth (comparing mycorrhizal and nonmycorrhizal plants) were determined for selected treatments. These ratios were calculated from treatment means (Davies 1967).

Additional correlation analyses (not shown) were performed using Microsoft Excel 97 (Microsoft Corporation 1997).

RESULTS

Plant growth: non-destructive timecourse assessments

The main purpose of these data (together with assessments of AMF root infection during the experiment) was to guide the timing of the final harvest for the experiment. Examples of lines that differed in their response to AMF inoculation are shown in Fig. 1*a*, *b*. Differences between mycorrhizal and nonmycorrhizal plants were apparent between 30 and 40 days after sowing. Assessments of randomly selected lines (not those shown in Fig. 1) indicated that by day 45, AMF infection had become established with all control plants remaining uninfected (see below). The pairs of lines derived from single parental genotypes showed contrasting responses to AMF inoculation. Line 40 grew better but line 41 worse when inoculated (Fig. 1*a*). Lines 28 and 30 exhibited far less divergence in growth (Fig. 1*b*). Most lines (as the final harvest data also indicate) did not show a shoot growth response to AMF inoculation (these data not shown). Since it was impractical to continue non-destructive assessments beyond day 61 and because plants in some lines were becoming pot bound all plants were harvested 84 days after sowing.

Plant growth: final harvest

Cluster analyses of the final harvest plant data (root and shoot weight and root length) (Table 1) indicate strong groupings as a result of parental family origin. Plants in cluster 1, for example, account for 8 out of 11 control plants from parental genotype H, and 7 out of 12 from parental genotype R, indicating a strong similarity between these two parental genotypes. Plant lines from parental genotypes J and S show different and more diverse patterns of clustering.

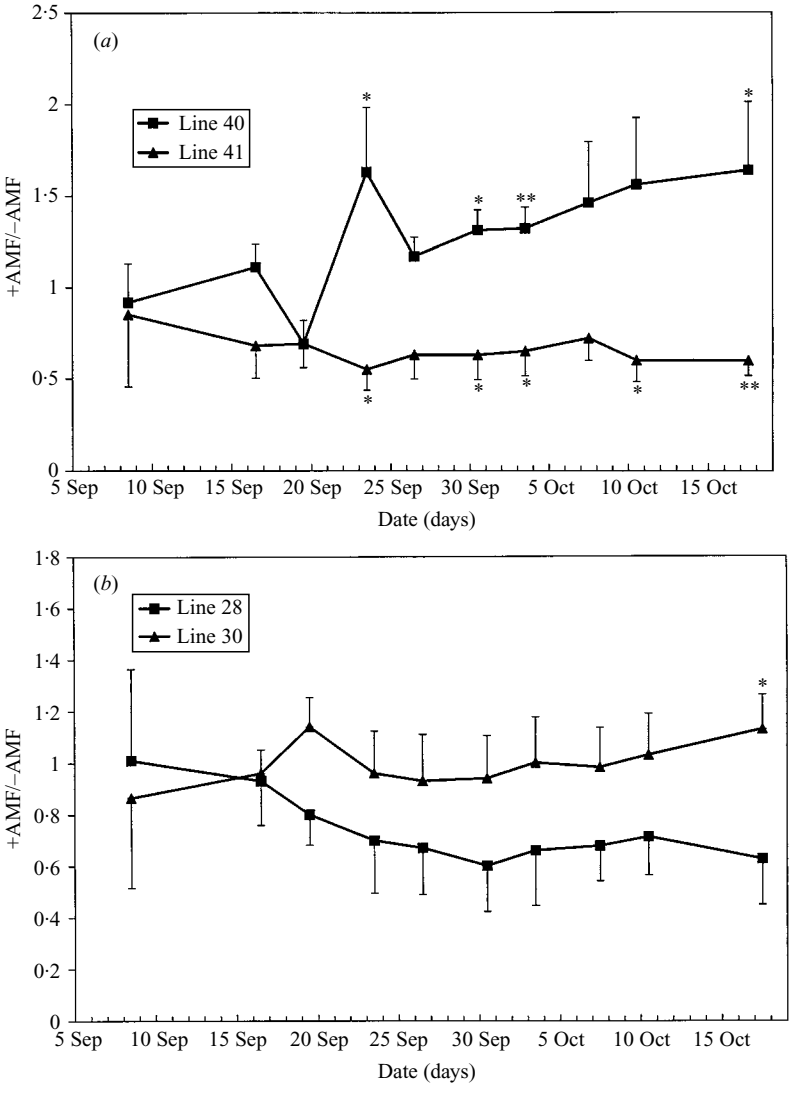

Fig. 1. (*a*, *b*). Shoot growth of near-isogenic lines of *Trifolium repens* following inoculation with the AMF *Glomus mossae*. Each graph shows two lines derived from the same parental genotype which exhibit contrasting growth responses to AMF infection, Fig. 1*a* (lines 40 and 41 from parental genotype S) and Fig. 1*b* (lines 28 and 30 from parental genotype R). Between 19 and 61 days after sowing leaf number was measured. Data are presented as the ratio of growth in AMF compared to control (non AMF) plants (this based on ratios of mean growth data). Bars are standard errors of these ratios. Where there is a significant difference between +AMF and –AMF plants (based on actual growth data, not on ratios) this is indicated (*, $P \leqslant 0.05$; **, $P \leqslant 0.01$).

The cluster analysis also indicates differences between mycorrhizal and nonmycorrhizal control plants for each individual line. Plants from parental genotype S show the highest response to AMF inoculation with 8 out of 9 lines in different clusters for mycorrhizal and nonmycorrhizal treatments. By contrast plants from genotype R have mycorrhizal and nonmycorrhizal plants in the same clusters for 10 out of 12 lines.

There were significant interaction effects of parental genotype (Table 2) on all measured parameters. Mycorrhizal interactions were significant for all parameters except shoot weight. Within parental genotype × mycorrhizal interactions were significant for all parameters except total shoot P. Parental genotype × line and parental genotype × line × mycorrhizal interactions were significant for all parameters.

Although there were no mycorrhizal effects on

Table 1. *Cluster analysis of combined plant growth data* (*root and shoot weight and root length*) *for 43 near isogenic lines of* Trifolium repens *inoculated with the AMF* Glomus mosseae (*m1–m43*) *compared to uninoculated control plants* (*c1–c43*). *At a probability level of 90% six clusters* (*C1–C6*) *are present*, *each significantly different from each other. Data are also grouped into lines from each of the original parent genotypes* (*parent genotype H = lines 1–11*; *genotype J = lines 12–21*; *genotype R = lines 22–34 and genotype S = lines 35–43*). *Where differences occur between mycorrhizal and control* (*m and c*) *lines these are highlighted in bold text*

	Cluster	C1	C2	C3	C4	C5	C6	Cluster	C1	C2	C3	C4	C5	C6	
	Line							Line							
H	m1	m						m22	m						R
	c1	c						c22	c						
	m2					**m**		m23	m						
	c2	**c**						c23	c						
	m3		**m**					m24	m						
	c3	**c**						c24	c						
	m4	m						m25	m						
	c4	c						c25	c						
	m5		**m**					m26	m						
	c5	**c**						c26	c						
	m6	m						m27	m						
	c6	c						c27	c						
	m7					**m**		**m28**		**m**					
	c7	**c**						**c28**	**c**						
	m8					m		m29		m					
	c8					c		c29		c					
	m9	m						**m30**	**m**						
	c9	c						**c30**			**c**				
	m10	**m**						m32			m				
	c10					**c**		c32			c				
	m11					m		m33			m				
	c11					c		c33			c				
								m34						**m**	
J	**m12**				**m**			c34						**c**	
	c12					**c**									
	m13				**m**			**m35**				**m**			S
	c13					**c**		**c35**	**c**						
	m14						**m**	**m36**				**m**			
	c14	**c**						**c36**	**c**						
	m15		m					**m37**		**m**					
	c15		c					**c37**			**c**				
	m16						**m**	**m38**			**m**				
	c16		**c**					**c38**	**c**						
	m17		**m**					m39	m						
	c17	**c**						c39	c						
	m18		m					**m40**	**m**						
	c18		c					**c40**		**c**					
	m19		m					**m41**						**m**	
	c19		c					**c41**		**c**					
	m20		**m**					**m42**						**m**	
	c20	**c**						**c42**		**c**					
	m21		**m**					**m43**			m				
	c21	**c**						**c43**		**c**					

combined shoot weight data (Table 2), if individual lines are examined (Table 3) nine showed significant ($P < 0{\cdot}05$) shoot weight responses to AMF infection, with five having lower shoot weight than non-AMF plants and four having higher shoot weight. However if the significance level is increased to 0·01 (to account for the increased chance of significance associated with the large number of means in the analysis presented in Table 2) this reduces to five lines (two lower and three higher when mycorrhizal).

Root weight was also greater in plants from parental genotype H although with reduced significance. Root weight was significantly different between mycorrhizal and nonmycorrhizal plants for parental genotype J

Table 2. *Effect of inoculation of* T. repens *with the AMF* Glomus mosseae (*Myc*) *on mean shoot and root weight* (*mg*); *root : shoot ratio*; *root length* (*cm*); *shoot* % *P and total P* (*mg*) *and* % *root length infected with AMF compared to uninfected controls* (Con). *Plants are separated into parental genotypes* (*H*, *J*, *R and S*). *An analysis of variance examined* Parental genotype *and individual* Line *interactions with mycorrhizal* (Myc) *treatment. Significant interactions are indicated as follows* (***, $P \leqslant 0{\cdot}001$; **, $P \leqslant 0{\cdot}01$; *, $P \leqslant 0{\cdot}05$; *NS, not significant*). *Replicate numbers and standard deviation* (S.D.) *of analysis of variance also shown. Associated degrees of freedom* (D.F.) *are shown in brackets below* S.D. *values.*

Parental genotype Replicates	H 66	H 66	J 60	J 60	R 78	R 78	S 54	S 54	S.D.† (D.F.)	Parental genotype	Myc	Within parental genotype × Myc	Within parental genotype × Line	Within parental genotype × Line × Myc
	Myc	Con	Myc	Con	Myc	Con	Myc	Con						
Shoot weight	2760	2644	1929	2336	1948	1867	1912	1847	639·5 (384)	***	NS	**	***	**
Root weight	508·3	447·1	224·7	360	349·8	348·7	329·6	328·1	102·8 (383)	***	***	***	***	***
Root:Shoot	0·199	0·171	0·116	0·155	0·185	0·194	0·167	0·182	0·044 (382)	***	***	*	***	***
Root length	11401	9352	5297	9562	5998	6996	4295	9497	2346·1 (382)	***	***	***	***	***
% P	0·422	0·534	0·312	0·369	0·392	0·429	0·369	0·488	0·094 (377)	***	***	***	***	***
Total P	1159	1413	845	631	775	867	703	876	322·0 (377)	***	***	NS	***	***
% AMF	10·58 (5·1)	0 (0)	18·22 (15·84)	0 (0)	9·07 (4·7)	0 (0)	19·47 (14·4)	0 (0)	5·71‡ (382)	***	***	***	***	***

† S.D. are shown as replicate numbers vary (S.E.M. = S.D./$\sqrt{}$(Rep number). Actual mean values shown for % AMF infection rates in brackets (analyses performed on angular transformed data shown). ‡ This S.D. value applies to the transformed data. For individual line results see Table 3.

Table 3. *Individual line significant differences between mycorrhizal and nonmycorrhizal treatments. Individual lines from parental genotypes H, J, R and S are identified by code numbers 1 to 43. Numbers followed by (−) indicate a significant reduction in mycorrhizal plants compared to nonmycorrhizal for the associated parameter (shoot weight; root weight; root : shoot ratio; root length; total shoot P and shoot % P). Numbers followed by (+) indicate a significant increase in the recorded parameter. Significance at three different levels of probability is shown ($P \leqslant 0{\cdot}05$, 0·01 and 0·001). Differences at the 0.05 level are calculated from the least significant difference (LSD) values determined in the analyses shown in Table 2. LSD values for the higher levels of significance were calculated to provide a more rigorous testing of the data, given the high number of treatments in the original analysis of variance. Total numbers of lines showing significant responses (and a breakdown of those showing positive and negative responses) is also shown*

	Significance (%)	Parental genotype H (Lines 1–11)	Parental genotype J (Lines 12–21)	Parental genotype R (Lines 22–34)	Parental genotype S (Lines 35–43)	Totals
Shoot weight	5	2+ 10−	14− 16−	23+ 28− 34+	40+ 41−	9 (4+5−)
	1	2+	14− 16−	28−	40+	5 (2+3−)
	0·1	2+	16−			2 (1+1−)
Root weight	5	3− 10−	13− 14− 16− 20− 21−	28− 34−	40+	10 (8−2+)
	1		13− 14− 16− 20− 21−	28− 34−		7 (6−1+)
	0·1		13− 14−	28−		3 (3−)
Root : Shoot	5	2− 9−	13− 20− 21−	34+	37− 39− 43+	9 (7−2+)
	1	2−	13− 21−		37− 43+	5 (4−1+)
	0·1	2−	21−		37− 43+	4 (3−1+)
Root length	5	2+ 3− 4− 5− 8− 9− 10−	12− 13− 14− 17− 20− 21−	28− 32−	35− 36− 41− 42−	19 (18−1+)
	1	3− 4− 8− 10−	12− 13− 14− 20−	28−	41− 42−	11 (11−)
	0·1	10−	13− 14− 20−			4 (4−)
Total P	5	3− 9− 10−	13− 14− 16− 20−	28−	38− 40+ 41− 42− 43−	13 (12−1+)
	1	10−	16−	28−	38− 40+ 42− 43−	7 (6−1+)
	0·1	10−		28−	38−	3 (3−)
% P	5	2− 3− 6− 7− 8− 9− 10−	14−		36+ 37− 38− 39− 42− 43−	14 (13−1+)
	1	6− 8− 9− 10−			37− 38− 42− 43−	8 (8−)
	0·1	9−			37− 38− 42−	4 (4−)

where there was a reduction in mycorrhizal plant root weight (Table 2). When individual lines are examined (Table 3) most lines which significantly respond to AMF show decreases in root weight compared to non-AMF controls.

Root length appeared to be a more sensitive indicator to AMF infection with a significant reduction in mycorrhizal plants for parental genotypes J and S (Table 2). A total of 19 individual lines exhibited significant responses to AMF infection with most of these (18) showing a decrease in root length compared to non-AMF controls. Although parental genotypes J and S showed the highest mean levels of infection and lowest root lengths there was no correlation between the decrease in root length and the percentage of root length infected when analysed on an individual line basis (analyses not shown).

These findings were also reflected in root:shoot ratios which were significantly smaller in parental genotypes J, R and S when plants were mycorrhizal, although root:shoot ratio was greater in mycorrhizal plants from parental genotype H (Table 2). Seven out of nine individual lines showed significant decreases in root:shoot ratio when plants were mycorrhizal (Table 3).

Shoot nutrient status

Phosphorus (either percentage concentration or total shoot levels) was in most cases less when plants were mycorrhizal (Tables 2 and 3). This effect was most pronounced in parental genotypes H and S where the reduction in concentration (%) was significant.

Mycorrhizal infection

Assessment of randomly selected lines at day 45 showed that infection had occurred by this stage in the three lines selected (Line 2, mean percentage infected root length 10·3% (S.E. 4·70); Line 4, 3·0% (1·53) and Line 34, 18·7% (13·13)). All control treatments for these lines remained uninfected. These assessments indicate that the plants were infected with AMF at the same time as the observed growth effects between inoculated and control plants (Fig. 1).

Mean infection at the final harvest ranged from 5–16% for plants inoculated with AMF (Table 2), with individual lines having from 0 to 37·2% of the root length infected (data not shown). Infection levels were generally consistent within lines but varied significantly between them (data not shown). All control plants remained uninfected. There was a significant effect of parental family origin on infection rates, with plants from parental genotypes J and S having higher levels of root colonization than plants from parental genotypes H and R. There was no correlation between final percentage of root length infected with AMF and plant response (analyses not shown).

DISCUSSION

It is clear from this study that when challenged with the same AMF there is a high degree of variation in both root colonization and plant response between plant genotypes (based on a comparison between both individual lines and parental genotypes), further supporting the theory that there is a functional specificity between AMF and their plant hosts (Khalil *et al.* 1999). Probably the clearest evidence for this comes from a comparison between plants from each of the four parental genotypes, which exhibited contrasting growth responses to infection, as well as differences in root colonization rates. The cluster analysis indicated a strong grouping of response within and between parental genotypes.

The effect of AMF inoculation on root length was strongly linked to parental genotype. Parental genotypes J and S exhibited around a 50% reduction in root length when mycorrhizal. These genotypes also had significantly higher levels of root colonization than the other two parental lines although there was no correlation between the decrease in root length and the amount of infection when examined on an individual line basis. The reduction in root length associated with AMF infection reported here, supports most previous findings that AMF decrease the root:shoot ratio of infected plants (although in this study there was only a significant reduction in root:shoot ratio for parental genotype J). Hetrick *et al.* (1992*b*) showed that plants that depend more heavily on AMF also tend to have a more plastic root morphology, maintaining a less fibrous root system in response to AMF colonization. Kothari *et al.* (1990) found significant changes in root morphology of maize following inoculation with *G. mosseae*, with a 31% reduction in root length and even larger decreases in root hair density and length. Torrisi *et al.* (1999) however reported that there was a localized elongation of roots of cotton following inoculation with *G. mosseae*, although overall root mass in relation to shoot mass declined.

Root infection rates were also strongly affected by parental genotype with parental lines J and S having significantly higher levels of infection than parental genotypes H and R. Root colonization rates were lower than in previous studies which used conventional outbreeding varieties of *T. repens* (Scullion *et al.* 1998, Eason *et al.* 1999). This is the first time however that AMF infection in these inbred *T. repens* has been reported and such levels of infection appear to be characteristic of these lines. However the levels of infection are not exceptional for *T. repens*. Hall *et al.* (1977) reported root length infection rates ranging from less than 1% up to 30%, for two cultivars of *T. repens*, comparable to the range reported in this study. Jongen *et al.* (1996) reported colonization rates of 29–35% for *T. repens* in their study, which is in line

with some of the individual lines in this study (data not shown). For example in this study lines 41, 36, and 35 had root infection rates of 27·3% (S.E. 8·53%), 30·3% (5·81%) and 26·7% (3·4%) respectively.

Infection rates can vary with time and they may not be directly related to effects on plant growth. In this study lines 40 and 41 resulted in contrasting growth responses (Fig. 1) with line 40 showing a positive growth response despite much lower infection levels (at final harvest this was 6·4% (S.E. 3·24%) compared to line 41 at 27·3% (8·53%)). Marschner & Crowley (1996) reported that mycorrhizal infection of *Glomus deserticola* on *Capsicum annuum* decreased from 20% to 6% during the course of their experiment, but that there was still a significant reduction in root length and shoot weight, compared to nonmycorrhizal control plants. Where plants were infected with *G. intraradices*, 48% of the root length was infected, although the reduction in root length and increase in shoot weight was not proportionally higher. Mitchell & Osborne (1996) reported that mycorrhizal barley plants had higher rates of photosynthesis and greater P-use and N-use efficiencies, despite the fact that root infection was only around 3%.

Environmental factors will affect the development and function of plant-AMF associations, for example, root colonization rates may be affected by P availability, light levels and inoculum potential. For example at high soil P levels root colonization is generally reduced (Hall *et al*. 1977). The foliar P concentrations in this study however were within the range expected for *T. repens*, when grown in a low P soil and inoculated with AMF (Scullion *et al*. 1998). That final harvest P levels were lower in most cases when plants were mycorrhizal might indicate that effects on plant P uptake may also have been transitory occurring prior to the final shoot harvest. Indeed this was the case in earlier studies with *T. repens* (Scullion *et al*. 1998). Variation in light levels may also affect plant carbon levels and affect the overall benefit to the host plant so modifying the plant response to infection. At low light levels the plants may become carbon limited and the cost of maintaining the AM symbiosis may result in a decrease in plant growth compared to nonmycorrhizal plants (Graham & Eissenstat 1998).

Environmental factors might then explain the generally poor response of shoot growth to AMF infection in this study (although individual lines within this study did exhibit significant shoot growth increases in response to AMF). However Hetrick *et al*. (1992*a*, *b*) also found a generally poor (or negative growth) shoot growth response to AMF infection in modern wheat varieties compared to older cultivated varieties. Modern plant breeding strategies employed in developing the clover lines in this study are unlikely to have provided conditions which might favour the selection of an efficiently functioning AM symbiosis. For example the standard soil used in the breeding programmes at IGER use steam sterilized soil with added P (135 mg/l of soil) which may have reduced both inoculum and infection levels.

In a study comparing 43 plant host genotypes, a single harvest date selects plants (and their associated mycorrhizas) at different developmental stages. The nondestructive growth data indicate that the timing of AMF response varied between lines. Rates of AMF colonization may vary between lines and some lines may have shown growth effects had the experiment run beyond the final harvest date. Under such circumstances a single harvest is a realistic compromise, given the number of plants involved in this study. Although experimental conditions may have therefore made it impossible to identify all responsive genotypes this does not invalidate the positive responses observed and does provide a relative index of plant response to AMF infection.

In conclusion this study has shown that there is a significant plant host genotype influence on both AMF root colonization and plant growth response. Future work (focusing on lines which exhibit contrasting infection rates and growth responses) will minimize environmental variables still further, in order that a reproducible phenotype response to AMF, can then be related to plant gene expression. This work will focus on plant lines that are closely related (i.e. derived from the same parental line) but which responded differently to AMF infection in this study (e.g. lines 40 and 41). These differences may be due to relatively small differences in the genome between individual lines and as such they may provide the raw material for molecular genetic studies which aim to elucidate the key plant genes involved in the functioning of the AM symbiosis. This will then inform future plant breeding strategies.

This work is supported by the Biotechnology and Biological Sciences Research Council, UK. I would like to thank John Toler for his help with the care and maintenance of this experiment. Thanks also to James Devlin for his help with some of the assessments and Debbie Allen for supplying the rhizobium inoculant.

REFERENCES

Bethlenfalvay, G. J. & Linderman, R. G. (1992). *Mycorrhizae in Sustainable Agriculture*. American Scientific Association, Special Publication Number 54.

Boddington, C. L. & Dodd, J. C. (1999). Evidence that differences in phosphate metabolism in mycorrhizas formed by species of *Glomus* and *Gigaspora* might be

related to their life-cycle strategies. *New Phytologist* **142**, 531–538.

Bodker, L., Kjoller, R. & Rosendahl, S. (1998). Effect of phosphate and the arbuscular mycorrhiza fungus *Glomus intraradices* on disease severity of root rot of peas (*Pisum sativum*) caused by *Aphanomyces euteiches*. *Mycorrhiza* **8**, 169–174.

Böhm, W. (1979). *Methods of Studying Root Systems*. Berlin: Springer-Verlag.

Carlson, G. E. (1966). Growth of clover leaves – developmental morphology and parameters at ten stages. *Crop Science* **6**, 293–294.

Davies, O. L. (1967). *Statistical Methods in Research and Production*. London: Oliver and Boyd.

Eason, W. R, Scullion, J. & Scott, E. P. (1999). Soil parameters and plant responses associated with arbuscular mycorrhizas from contrasting grassland management regimes. *Agriculture, Ecosystems and Environment* **73**, 245–255.

Gange, A. C. & West, H. M. (1994). Interactions between arbuscular mycorrhizal fungi and foliar feeding insects in *Plantago lanceolata* L. *New Phytologist* **128**, 79–87.

Giovannetti, M. & Mosse, B. (1980). An evaluation of techniques for measuring mycorrhizal infection in roots. *New Phytologist* **84**, 489–500.

Graham, J. H. & Eissenstat, D. M. (1998). Field evidence for the carbon cost of citrus mycorrhizas. *New Phytologist* **140**, 103–110.

Hall, I. R, Scott, R. S. & Johnstone, P. D. (1977). Effect of vesicular-arbuscular mycorrhizas on response of 'Grasslands Huia' and 'Tamar' white clovers to phosphorus. *New Zealand Journal of Agricultural Research* **20**, 349–355.

Hetrick, B. A. D., Wilson, G. W. T. & Cox, T. S. (1992*a*). Mycorrhizal dependence of modern wheat varieties, landraces, and ancestors. *Canadian Journal of Botany* **70**, 2032–2040.

Hetrick, B. A. D., Wilson, G. W. T. & Cox, T. S. (1992*b*). Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. *Canadian Journal of Botany* **71**, 512–518.

Ibijbijen, J., Urquaiga, S., Ismali, M., Alves, J. R. & Boddey, R. M. (1996*a*). Effect of arbuscular mycorrhizas on uptake of nitrogen by *Bracharia arrecta* and *Sorghum vulgare* from soils labelled for several years with ^{15}N. *New Phytologist* **133**, 487–494.

Ibijbijen, J., Urquaiga, S., Ismali, M., Alves, J. R. & Boddey, R. M. (1996*b*). Effect of arbuscular mycorrhizal fungi on growth, mineral nutrition and nitrogen fixation of three varieties of common bean (*Phaseolus vulgaris*). *New Phytologist* **134**, 353–360.

Jongen, M., Fay, P. & Jones, M. B. (1996). Effects of elevated carbon dioxide and arbuscular mycorrhizal infection on *Trifolium repens*. *New Phytologist* **132**, 413–423.

Khalil, S., Loynachan, T. E. & Tabatabai, M. A. (1999). Plant determinants of mycorrhizal dependency in soybean. *Agronomy Journal* **91**, 135–141.

Kothari, S. K., Marschner, H. & George, E. (1990). Effect of VA mycorrhizal fungi and rhizosphere microorganisms on root and shoot morphology, growth and water relations in maize. *New Phytologist* **116**, 303–311.

Lawes Agriculture Trust (1997). *Genstat 5 Release 4.1, Genstat 5 Committee*. Rothamsted Experimental Station, Lawes Agricultural Trust.

Lu, X. & Koide, R. T. (1994). The effects of mycorrhizal infection on components of plant growth and reproduction. *New Phytologist* **128**, 211–218.

Manske, G. G. B. (1990). Genetical analysis of the efficiency of vesicular-arbuscular mycorrhiza in spring wheat. *Agriculture, Ecosystems and Environment* **29**, 273–280.

Marschner, P. & Crowley, D. E. (1996). Root colonisation of mycorrhizal and non-mycorrhizal pepper (*Capsicum annuum*) by *Pseudomonas fluorescencs* 2-79RL. *New Phytologist* **134**, 115–122.

Michaelson-Yeates, T. P. T., Marshall, A., Abberton, M. T. & Rhodes, I. (1997). Self-compatability and heterosis in white clover (*Trifolium repens* L.). *Euphytica* **94**, 341–348.

Ministry For Agriculture Food and Fisheries (1986). *The Analysis of Agricultural Materials*. Technical Bulletin 27. London: HMSO.

Mitchell, F. D. T. & Osborne, B. A. (1996). Photosynthesis and nutrient-use efficiency of barley in response to low arbuscular mycorrhizal colonisation and addition of phosphorus. *New Phytologist* **132**, 425–433.

Phillips, I. M. & Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and mycorrhizal fungi for rapid assessment of infection. *Transactions of British Mycological Society* **55**, 158–161.

Sanders, F. E, Mosse, B. & Tinker, P. B. (1975). *Endomycorrhizas*. New York: Academic Press.

Scullion, J., Eason, W. R. & Scott, E. P. (1998). The effectivity of arbuscular mycorrhizal fungi from high input conventional and organic grass-arable rotations. *Plant and Soil* **204**, 243–254.

Smith, S. E. & Read, D. (1997). *Mycorrhizal Symbioses*. New York: Academic Press.

Smith, S. E, Robson, A. D. & Abbott, L. K. (1992). The involvement of mycorrhizas in assessment of genetically dependent efficiency of nutrient uptake and use. *Plant and Soil* **146**, 169–179.

Subramanian, K. S. & Charest, C. (1999). Acquisition of N by external hyphae of an arbuscular mycorrhizal fungus and its impact on physiological responses in maize under drought-stressed and well-watered conditions. *Mycorrhiza* **9**, 69–75.

Tisserant, B., Brenac, V., Requena, N., Jeffries, P. & Dodd, J. C. (1998). The detection of *Glomus* spp. (arbscular mycorrhizal fungi) forming mycorrhizas in three plants, at different stages of seedling development, using mycorrhiza-specific isozymes. *New Phytologist* **138**, 225–239.

Torrisi, V., Pattinson, G. S. & McGee, P. A. (1999). Localised elongation of roots of cotton follows establishment of arbuscular mycorrhizas. *New Phytologist* **142**, 103–112.