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Slow arbuscular mycorrhizal colonisation of field-grown cotton caused by environmental conditions in the soil

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Abstract Slow arbuscular mycorrhizal colonisation is characteristic of a growth disorder of cotton occurring in crops in northern New South Wales, Australia. To determine whether or not slow colonisation is caused by poor survival of mycorrhizal fungi between crops, we examined colonisation of cotton in field crops and in a series of pot bioassays. Cotton roots were sampled at sites with or without severe symptoms of the growth disorder in each of three fields in 1991 and two fields in 1993. The bioassays were at intervals over the winter fallow prior to the crops in both years. In each bioassay, soil was collected from the field sites and sown with cotton in pots in a controlled environment cabinet. Colonisation was assessed at 14, 28 and 42 days after sowing. In the bioassay series, colonisation at 14 days, which was representative of primary infections of roots and hence propagule density in soil, tended to decline over the winter fallow. In contrast, colonisation at 42 days, which included secondary spread of infection, first declined and then returned to its original level or higher. In the field, plants affected by the growth disorder were colonised slowly, while healthy plants were colonised rapidly. In the bioassays, however, colonisation in the soil from sites with the growth disorder equalled or surpassed that in soil from sites with healthier cotton. Thus, the slow colonisation and growth of field-grown cotton did not result from a lack of mycorrhizal inoculum and was most likely caused by soil factors.

Key words Bioassay · *Gossypium hirsutum* · Infectivity · Vertisol

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Introduction

Arbuscular mycorrhizal (AM) fungi are ubiquitous in plant ecosystems and have the capacity to increase the fitness of their plant hosts, especially by supplying extra phosphorus (P) to the plant (Abbott and Robson 1991; Allen 1996). When AM colonisation develops poorly, crop growth and nutrient uptake may be reduced accordingly (Thompson 1987, 1994). Slow AM colonisation and reduced nutrient uptake have been observed in cotton (*Gossypium hirsutum* L.) affected by a growth disorder (Nehl et al. 1996). The disorder occurs in cracking grey clays (Vertisols) in northern New South Wales (NSW), Australia. Affected plants are stunted early in the growing season and gradients in the severity of symptoms occur within individual cotton fields. In these fields, AM colonisation, shoot growth and P uptake are correlated positively (Nehl et al. 1996). Cotton is a mycotrophic plant in which growth and nutrient uptake is usually increased by AM colonisation (Rich and Bird 1974; Smith and Roncadori 1986). However, it is uncertain whether slow AM colonisation in the cotton growth disorder is a symptom or the cause of reduced plant growth (Nehl et al. 1996). In either case, an explanation for the slow colonisation will aid our understanding of the disorder.

AM colonisation commences with primary infection by propagules in soil. These colonies expand within the root and extraradical 'runner' hyphae initiate secondary colonies elsewhere in the root system (Wilson and Tommerup 1992). Initiation of primary colonies continues along with secondary spread (Walker and Smith 1984). The proportion of colonised root increases exponentially at first and then reaches a plateau (Wilson and Tommerup 1992; Pattinson and McGee 1997). If mycorrhizal inoculum is sparse, then roots exploring the soil will encounter few propagules and primary infections will be sparse. Unless secondary spread of infection can compensate, colonisation will not keep pace with the expanding root system and the plateau level of colonisation will also be low.

The density of AM inoculum in soil is a function of both production and survival of propagules, including spores, infected root fragments and extraradical hyphae. Spores and hyphae appear to be important for survival of AM fungi in soils used to grow cotton in Australia (McGee et al. 1997; Pattinson and McGee 1997). Sporulation by AM fungi varies according to crop species, soil properties and seasonal conditions (Dodd et al. 1990; Abbott and Robson 1991) and can be decreased by high input agriculture, soil salinity and P fertiliser (Abbott and Robson 1991; Douds et al. 1993; Douds 1994; Mårtensson and Carlgren 1994).

The viability of AM propagules in soil is affected by a range of biological and non-biological factors. Soil-borne fungi can parasitise AM spores (Lee and Koske 1994) and hyphae (Rousseau et al. 1996), arthropods graze on hyphae (Allen 1996) and soil disturbance reduces the viability of inoculum, particularly hyphae (Abbott and Robson 1991; McGee et al. 1997). Since AM fungi cannot grow saprophytically, propagules become senescent with time if deprived of a living plant host. In agricultural soils, a decline in numbers of viable AM propagules can occur during long weed–free fallows or during rotation with non-mycotrophic plants (Johnson and Pfleger 1992). Long fallows are associated with reduced colonisation and growth of sunflower (*Helianthus annuus* L.), linseed (*Linum usitatissimum* L.) and cotton (Thompson 1987; Brown et al. 1990; Thompson 1994). Hence, time is an important factor affecting the survival of fungi during fallows between cotton crops (McGee et al. 1997). Furthermore periodic wetting and drying may decrease survival during fallows, especially in combination with soil disturbance (Pattinson and McGee 1997).

The cotton growth disorder in northern NSW is dissimilar to long fallow disorders because the symptoms are perennial, despite annual cropping (Nehl et al. 1996). Moreover, the symptoms occur in localised areas within fields in which cropping practices are applied relatively equally. Hence, the severity of the growth disorder is associated with soil characteristics. Stunting of cotton is most severe in the soils with heavier texture and higher P, Mn and Na contents than elsewhere. Waterlogging, soil compaction, soil sodicity, soil pH, Mn toxicity and nutrient deficiencies are unlikely to be direct causes of stunting (Nehl et al. 1996). However, these or other soil factors may inhibit the production or survival of inoculum. By comparing colonisation of cotton in field crops to that in a series of pot bioassays, we confirmed that slow colonisation of cotton in the field is caused by environmental factors in the soil and not a lack of arbuscular mycorrhizal inoculum.

Materials and methods

In 1991 there were four bioassays using soil collected on 30 April, 5 July, 14 August, and 24 September (Julian days: 120, 186, 226, 267, respectively) from the Narrabri farm of Auscott Ltd, in northern NSW. Cotton in this region is normally sown in early October and harvested from late March onward. The four bioassays spanned the period from the end of one crop to the start of the next (Fig. 1). Each bioassay included soil from a site where cotton crops are stunted early in the season and yield is poor (hereafter stunted-crop soil), in each of three fields (field numbers 8, 18 and 20). For comparison, each bioassay also included soil from a site where cotton grows normally (hereafter normal-crop soil). Stunted-crop and normal–crop soils correspond to soil groups (group A and group C, respectively) identified by Nehl et al. (1996). In each bioassay in the present study, plants were harvested at 14, 28 and 42 days after sowing. Thus, each of the four bioassays included a stunted-crop soil and a normal-crop soil from each of three fields, with three harvests and six replicates.

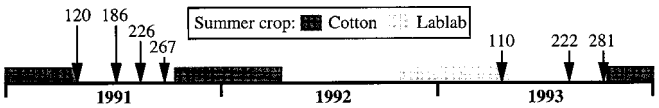

Fig. 1 Time line for crops and bioassay sampling dates (*arrows* show Julian date) in three fields. Crop durations are approximate since plants did not die immediately after harvest

Soil was collected from a depth of 0–20 cm and passed through a 12.5-mm sieve, except that a sieve with an aperture of 6 mm was used for the first bioassay (Julian day 120). Soil was stored in polythene bags at 4–5 °C in the dark for up to 17 days before potting in the bioassays. Soil was used undiluted in the bioassays to avoid diluent effects (Adelman and Morton 1986). Physical and chemical properties of the soil at each site (Table 1) were determined by methods described previously (Nehl et al. 1996). A few days after soil was collected for the third bioassay, N fertiliser was applied to fields 8, 18 and 20 as ammonia gas (83–111 kg N ha^{-1}) and as urea (30 kg N ha^{-1}).

Since the mycorrhizosphere is greatly influenced by the host plant genotype (Linderman 1992) and cotton alone appears to be affected by the growth disorder (Nehl et al. 1996), cotton was used as the host plant in the bioassays. Acid delinted seeds (cv. Deltapine Acala 90) weighing 0.09–0.11 g were surface sterilised for 2 min in a 1% solution of sodium hypochlorite and 0.5% ethanol, then rinsed four times in distilled water. Pots (1000 ml) without drain holes (to enable watering by weight) were filled with 800 g of soil (oven-dry equivalent) and sown with five seeds 20 mm beneath the soil surface.

Pots were placed in a controlled environment cabinet with a 14-h photoperiod (516 ± 6 μE m^{-2} s^{-1}) under fluorescent lights beginning at 6.00 a.m. and temperature and humidity regimes that simulated diurnal fluctuation. Temperature was set at 20 °C (4.00 a.m. to 6.00 a.m.) and 32 °C (10.00 a.m. to 2.00 p.m.), with incremental adjustment between these periods. Humidity was set at 80% (4.00 a.m. to 6.00 a.m.) and 60% (10.00 a.m. to 2.00 p.m.), with incremental adjustment between these periods. Pots were completely randomised among seven wire trays, which were moved to a new position in the controlled environment cabinet after each watering. Pots were watered (deionised rainwater, electrical conductivity <2 μS cm^{-2}) to soil water holding capacity at 0, 2, and 3 days after sowing, then every 2 days until 21 days after sowing, and daily thereafter. Nitrogen fertiliser was added to the soil at 7, 15, 23 and 31 days by addition of 10 ml aliquots of NH_4NO_3 solution (6.7 g l^{-1} of the fertiliser Nitram); in total, this was equivalent to 99 kg N ha^{-1}.

After emergence, seedlings were thinned to two plants in each pot. Harvested shoots and roots were dried in an oven (80 °C) for 48 h before weighing. AM colonisation was assessed in a 0.3–0.5 g subsample of fresh roots. The dry mass of this subsample was estimated using the fresh to dry weight ratio of the remaining roots. Roots were stained using the method of Koske and Gemma (1989) modified by the use of 10% KOH solution to clear the roots and 2% HCl solution to acidify the roots prior to staining for 20 min. The gridline intersect method of Giovannetti and Mosse (1980) was used to assess the percentage root length with arbuscules.

Table 1 Chemical and physical properties of soils used in pot bioassays. The growth of cotton in crops was greater at normal sites than at stunted sites. The analyses were: bicarbonate extractable P; DTPA extractable Zn and Mn; EC and pH in 1:5 suspension of soil in water; cations after equilibrium of the soil in 1.0 M NH_4Cl solution (pH 7.0) (*CEC* cation exchange capacity, *EC* electrical conductivity, *ESP* exchangeable sodium percentage, *WHC* water holding capacity)

Property	Site					
	Field 18		Field 20		Field 8	
	Normal	Stunted	Normal	Stunted	Normal	Stunted
P (mg kg^{-1})	16	62	17	96	30	44
Zn (mg kg^{-1})	0.9	3.4	4.5	4.5	2.3	0.6
Mn (mg kg^{-1})	18	26	29	65	7	99
$Ca^{+}_{0.5}$ (cmol kg^{-1})	28	30	24	22	24	23
$Mg^{+}_{0.5}$ (cmol kg^{-1})	10	12	14	13.5	13.6	15
K^{+} (cmol kg^{-1})	1.5	1.5	1.6	1.5	1.8	2.3
Na^{+} (cmol kg^{-1})	1.3	1.5	1.3	2.1	0.8	1.4
CEC (cmol kg^{-1})	40.5	45	41	39	41.6	42
ESP	2.6	3.4	3.3	5.2	1.9	3.4
Clay (%)	51	59	60	66	60	70
Silt (%)	18	21	15	18	14	17
Fine sand (%)	18	18	12	13	16	12
Coarse sand (%)	11	2	13	3	10	2
WHC (%)	40	46	41	52	43	47
pH (1:5)	8.6	8.3	8.6	8.2	8.1	7.6
EC (1:5, μS cm^{-1})	94	73	82	132	112	173

In 1991, commercial cotton crops were sown in fields 8, 20 and 18 on 7, 11 and 15 October, respectively. At the sites in these fields where soil was collected for the bioassays, AM colonisation was measured in composite root samples from the field crops at weekly intervals from seedling emergence up to 42 days after sowing, using methods previously described (Nehl et al. 1996). At each site, the height of 20 plants was monitored non-destructively. Prior to clearing and staining, root browning was assessed by scoring for the presence or absence of brown discolouration using the gridline intersect method. Shoot mass and shoot nutrient content of cotton were measured at the sites in field 8 by methods previously described (Nehl et al. 1996).

In 1993, there were three bioassays using stunted–crop soil and normal–crop soil from fields 18 and 20 (Fig. 1). This soil was collected on 20 April, 10 August and 8 October (Julian days: 110, 222, 281, respectively) from the same sites as in 1991. Soil was passed through the 12.5-mm sieve and stored in plastic bags at 4–5 °C for up to 8 days before potting. The first application of Nitram solution to bioassay pots was increased from 10 ml to 25 ml. Hence, total N applied to pots was equivalent to 136 kg N ha^{-1}. Nitrogen fertiliser was applied to fields 18 and 20 by aerial application of urea (30 kg N ha^{-1}), 1–2 weeks after soil was collected for the third bioassay in 1993. In all other respects, the conditions and design of the 1993 bioassays matched those in the 1991 bioassays.

The program Systat© Version 5.2 (Systat Inc., Evanston, Ill., USA) was used for all statistical analyses. Before analysis of variance, data were screened for normality using probability plots and transformed if appropriate. Bartlett's c^2 test was used to check for homogeneity of variance before analysis of variance and comparison of means by Scheffé's test. Where normality and homogeneity of variance could not be achieved by data transformation, the Kruskal-Wallis test was used to determine significance.

Results

Field crops

At the sites where soil was collected for bioassays in 1991, slow growth in cotton crops was matched by slow AM colonisation of roots (Fig. 2). In field 8, root browning (Nehl et al. 1996) developed more rapidly in cotton at the stunted-crop site (71% at 21 days) than at

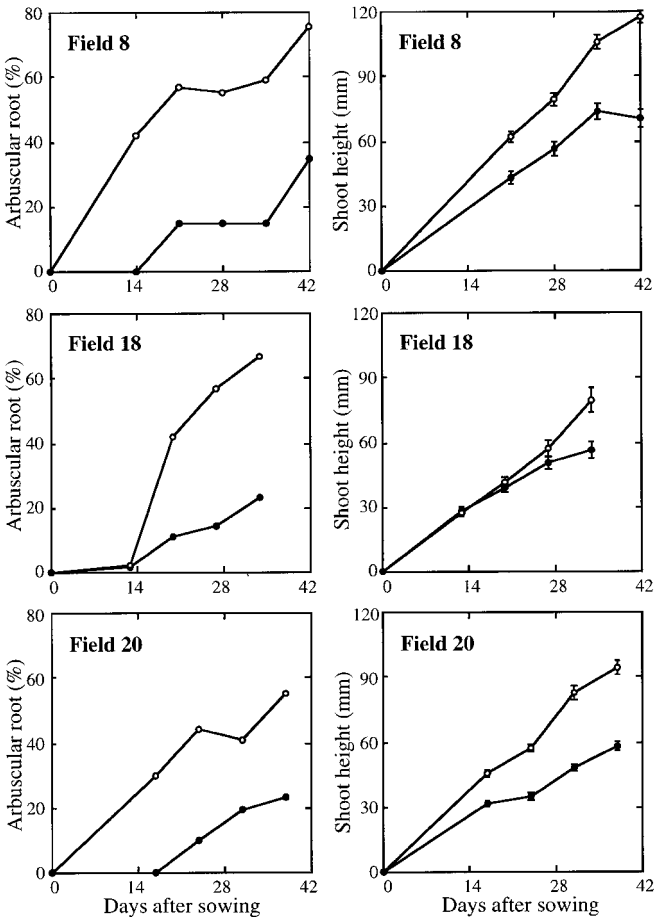

Fig. 2 Early season growth and arbuscular mycorrhizal colonisation of cotton roots, in crops in 1991, at sites where cotton crops are stunted and yield poorly (●) and sites where crops grow normally (○), within the same field. Shoot heights are the means and standard errors for 20 plants. Values for mycorrhizal colonisation are from composite root samples from adjacent plants

the normal-crop site (18%). At 42 days in field 8, shoot mass (1.06 g plant^{-1}) and shoot P content (5.6 g kg^{-1}) of cotton at the normal–crop site were considerably higher than at the stunted-crop site (0.44 g plant^{-1} and 3.0 g kg^{-1}, respectively). Symptoms of the growth disorder in field crops in 1993 in fields 18 and 20 have been previously published as mean values for several stunted-crop and normal-crop sites (Nehl et al. 1996). Hence, data for the field crops at the individual bioassay sites in 1993 are not presented.

Bioassays in 1991

AM colonisation in the bioassays increased most rapidly between 14 and 28 days and then slowed, consistent with a pattern of logistic growth (Pattinson and McGee 1997). Fitted curves (not presented) indicated that colonisation approached the plateau level by 42 days in most of the soils. The growth and AM development of cotton in the bioassays contrasted strongly with that of cotton in the field. In the bioassays, colonisation of cotton in stunted-crop soil mostly equalled or surpassed that of cotton in normal-crop soil from the same field (Table 2). In the bioassay conducted at the time crops were sown in 1991 (Julian day 267, Fig. 1), AM colonisation of cotton in normal-crop soils (Table 2) was consistently lower than in cotton crops at corresponding sites in the field (Fig. 2). Conversely, AM colonisation of cotton in stunted-crop soils (Julian day 267, Table 2) was consistently greater than in the field (Fig. 2). In contrast to the pattern in field crops (Fig. 2; Nehl et al. 1996), the growth of cotton in stunted-crop soils in the bioassays generally equalled or surpassed that in normal-crop soils (Tables 3 and 4).

Over the winter fallow, there was no net change ($P>0.05$) in levels of AM colonisation of cotton at 14 days in any of the soils, although colonisation in soil from field 8 was higher in the second bioassay (Table 2). However, assuming that the 6-mm sieve used in the first bioassay in 1991 disrupted inoculum (McGee et al. 1997; Pattinson and McGee 1997), then the remaining three bioassays in 1991 indicated declining inoculum, in four of the six soils in 1991 (Table 2). Colonisation of cotton roots at 42 days first declined and then increased over the winter fallow in soil from all six sites (Table 2), showing significant curvilinear trends. In all soils, except that from the stunted-crop site in field 18, the colonisation at 42 days in the fourth bioassay was equal to or higher than in the first bioassay (Table 2).

In most soils, cotton root mass at 42 days declined sharply between the first and second bioassays in 1991 and then either levelled off or increased slightly without returning to the original level (Table 3). The potential for shoot growth also declined over the winter fallow. Although the shoot mass did increase slightly in a

Table 2 Arbuscular mycorrhizal colonisation of cotton roots in bioassays, over the winter fallow in 1991, using potted soil collected from sites where cotton crops are stunted and from sites where crops grow normally. Values followed by the same letter within rows were not significantly different at the stated probability level for pairwise comparison by the Scheffé test (*NS* Not significant) Values in brackets are means of $\log_e(x)$ transformed data

Bioassay date (Julian day)	Site						
	Field 8		Field 18		Field 20		
	Normal	Stunted	Normal	Stunted	Normal	Stunted	($n=6$)
	Arbuscular colonisation (%) 14 days						
120	10b	9b	12ab	7b	7b	18a	$P\leq0.007$
186	17bc	28a	11cd	11cd	7d	19b	$P\leq0.029$
226	9bc	16a	11ab	6cd	3d	10bc	$P\leq0.022$
267	9	8	13	8	6	19	
	(2.1)abc	(2.1)abc	(2.6)ab	(2.0)bc	(1.7)c	(2.8)a	$P\leq0.044$
	Arbuscular colonisation (%) 28 days						
120	36	38	26	39	24	38	NS
186	40ab	47a	36bc	28cd	21d	36bc	$P\leq0.045$
226	30ab	36a	28ab	21b	10c	37a	$P\leq0.022$
267	27b	49a	39ab	36ab	28b	45a	$P\leq0.013$
	Arbuscular colonisation (%) 42 days						
120	58a	60a	53ab	59a	48b	58a	$P\leq0.011$
186	38b	52a	39b	42ab	40ab	43ab	$P\leq0.024$
226	39	45	43	41	44	38	NS[a]
267	54	63	53	50	54	54	NS
Quadratic regression	0.844	0.489	0.453	0.752	0.494	0.666	r^2
($n=24$)	<0.001	0.001	0.002	<0.001	0.001	<0.001	P

[a] Kruskal Wallis test used because parametric tests were inappropriate

Table 3 Root growth of cotton in bioassays, over the winter fallow in 1991, using potted soil collected from sites where cotton crops are stunted and from sites where crops grow normally. Values followed by the same letter within rows were not significantly different at the stated probability level for pairwise comparison by the Scheffé test (*NS* Not significant)

Bioassay date (Julian day)	Site						
	Field 8		Field 18		Field 20		
	Normal	Stunted	Normal	Stunted	Normal	Stunted	($n=6$)
	Root dry matter (g plant^{-1}) 14 days						
120	0.081a	0.083a	0.073ab	0.081a	0.076ab	0.069b	$P \leq 0.025$
186	0.085ab	0.070c	0.097a	0.079bc	0.076bc	0.080bc	$P \leq 0.036$
226	0.074ab	0.069b	0.083a	0.076ab	0.072ab	0.069b	$P \leq 0.010$
267	0.081a	0.057ab	0.066ab	0.044b	0.047b	0.064ab	$P \leq 0.025$
	Root dry matter (g plant^{-1}) 28 days						
120	0.20a	0.19ab	0.16b	0.18ab	0.17ab	0.19a	$P \leq 0.043$
186	0.19ab	0.18b	0.19b	0.18ab	0.18ab	0.20a	$P \leq 0.019$
226	0.15	0.16	0.17	0.17	0.14	0.16	NS
267	0.14abc	0.15ab	0.14abc	0.10c	0.10bc	0.16a	$P \leq 0.039$
	Root dry matter (g plant^{-1}) 42 days						
120	0.52a	0.46a	0.36b	0.47a	0.45a	0.51a	$P \leq 0.017$
186	0.30bc	0.25d	0.33b	0.34b	0.26cd	0.41a	$P \leq 0.008$
226	0.28b	0.23b	0.25b	0.29ab	0.23b	0.36a[a]	$P \leq 0.014$
267	0.24ab	0.25ab	0.20c	0.21bc	0.20c	0.27a	$P \leq 0.034$
Linear regression	0.837	0.671	0.855	0.936	0.865	0.767	r^2
($n=24$)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	P

[a] Mean with a high variance but overall data normally distributed

Table 4 Shoot growth of cotton in bioassays, over the winter fallow in 1991, using potted soil collected from sites where cotton crops are stunted and from sites where crops grow normally. Values followed by the same letter within rows were not significantly different at the stated probability level for pairwise comparison by the Scheffé test (*NS* Not significant) Values in brackets are means of square root transformed data

Bioassay date (Julian day)	Site						
	Field 8		Field 18		Field 20		
	Normal	Stunted	Normal	Stunted	Normal	Stunted	($n=6$)
	Shoot dry matter (g plant^{-1}) 14 days						
120	0.19ab	0.20ab	0.22a	0.19ab	0.18b	0.20ab	$P \leq 0.001$
186	0.18a	0.15b	0.19a	0.15b	0.15b	0.19a	$P \leq 0.024$
226	0.18a	0.15b	0.19a	0.17ab	0.15b	0.18a	$P \leq 0.025$
267	0.19a	0.14ab	0.18a	0.11b	0.11b	0.18a	$P \leq 0.048$
	Shoot dry matter (g plant^{-1}) 28 days						
120	0.42ab	0.51ab	0.44ab	0.47ab	0.42b	0.52a[a]	$P \leq 0.048$
186	0.47 (0.68)b	0.37 (0.61)c	0.47 (0.68)b	0.38 (0.62)c	0.36 (0.60)c	0.56 (0.75)a	$P < 0.001$
226	0.44a[a]	0.35b	0.45a	0.39ab[a]	0.35b	0.39ab	$P \leq 0.036$
267	0.41ab	0.37bc	0.39bc	0.27d	0.31cd	0.48a	$P \leq 0.047$
	Shoot dry matter (g plant^{-1}) 42 days						
120	0.95ab	0.84bc	0.67d	0.81c	0.76cd	1.06a	$P \leq 0.018$
186	0.90b	0.74c	0.83bc	0.75cd	0.65d	1.08a	$P \leq 0.004$
226	0.87a	0.72bc	0.76abc	0.67cd	0.59d	0.82ab[a]	$P \leq 0.022$
267	0.70bc	0.76b	0.57cd	0.57cd	0.48d	1.02a	$P \leq 0.021$
Linear regression	0.597	0.208	0.086	0.712	0.754	0.143	r^2
($n=24$)	<0.001	0.025	NS	<0.001	<0.001	NS	P

[a] Mean with a high variance but overall data normally distributed

few soils, toward the end of the winter fallow, all plants in the final bioassay were smaller than in the first (Table 4). Thus the seasonal patterns of change in arbuscular colonisation at 42 days were generally dissimilar to those of shoot and root growth.

Arbuscular colonisation at 42 days was compared to that at 14 days by linear regressions using (i) the means for soils as cases, separately for each bioassay, ($n=6$) and (ii) the means from all soils in all bioassays as cases ($n=24$). None of these regressions were significant. Shoot mass and root mass at 14 and 42 days were each compared to levels of arbuscular colonisation at 14, 28 and 42 days by linear regression using the means from all the soils in all four bioassays as cases ($n=24$). Only one of these comparisons was significant ($P=0.014$), showing a weak relationship between shoot mass at 42 days and arbuscular colonisation at 14 days ($r^2=0.243$).

Arbuscular colonisation, shoot mass and root mass at 42 days in 1991 were compared to soil P content by linear regression using (i) the means for soils as cases, separately for each bioassay, ($n=6$) and (ii) the means from all soils in all bioassays as cases ($n=24$). In contrast to the field crops, there was a weak positive relationship ($r^2=0.201$, $P=0.028$) between shoot mass and soil P content when data from all four bioassays was pooled. None of the other regressions over soil P were significant.

Bioassays in 1993

AM colonisation of cotton in bioassays in 1993 contrasted strongly with observations of colonisation in field-grown cotton, as in the 1991 bioassays. AM colonisation at 14 days in soil from the stunted-crop site in field 20 was approximately twice as high as in soil from the normal-crop site in field 20 (Table 5). Although arbuscular colonisation of cotton in the stunted-crop soil from field 18 was initially lower than in the normal-crop soil, these differences were equalised or reversed by 28 to 42 days (Table 5). Arbuscular colonisation of roots at 42 days in the stunted-crop soils was generally equal to, or greater than in the normal-crop soils from the corresponding field (Table 5).

Linear regressions showed a significant decline over the winter fallow in arbuscular colonisation at 14 days in each soil (Table 5). In the first bioassay in 1993, arbuscular colonisation at 14 days was approximately double that of the first bioassay in 1991. However, by the end of the winter fallow, arbuscular colonisation at 14 days was similar in both years in each soil.

AM colonisation at 42 days in the 1993 bioassays followed a curvilinear pattern over the winter fallow (Table 5) which closely matched that of the 1991 bioassays (Table 2). In 1993, linear regression using the means from all the soils in all three bioassays as cases ($n=12$) showed no significant correlation between arbuscular colonisation at 42 days and that at 14 days, as in the 1991 bioassays.

The patterns of cotton growth in the bioassays in 1993 were similar to the 1991 bioassays (data not presented). Shoot mass and root mass were compared to arbuscular colonisation by linear regression using the means from all the soils in all three bioassays as cases ($n=12$). There was a positive relationship between shoot mass at 14 days and arbuscular colonisation at 14 days ($r^2=0.542$, $P=0.006$). There were no other significant relationships among cotton growth parameters and colonisation in the 1993 bioassays.

Table 5 Arbuscular mycorrhizal colonisation of cotton roots in bioassays, over the winter fallow in 1993, using potted soil collected from sites where cotton crops are stunted and from sites where crops grow normally. Values followed by the same letter within rows were not significantly different at the stated probability level for pairwise comparison by the Scheffé test (*NS* Not significant

Bioassay date (Julian day)	Site				
	Field 8		Field 18		
	Normal	Stunted	Normal	Stunted	($n=6$)
	Arbuscular colonisation (%) 14 days				
110	28a	16b	17b	32a	$P\leq0.005$
222	20a	10b	11b	19a	$P\leq0.041$
281	14a	5b	6b	13a	$P\leq0.016$
Linear regression	0.638	0.697	0.794	0.733	r^2
($n=18$)	<0.001	<0.001	<0.001	<0.001	P
	Arbuscular colonisation (%) 28 days				
110	40b	52a	53a	54a	$P\leq0.007$
222	58a	53ab	47b	47b	$P\leq0.007$
281	53a	35c	37bc	48ab	$P\leq0.027$
	Arbuscular colonisation (%) 42 days				
110	57	49	59	54	NS
222	50a	46ab	41b	43ab	$P\leq0.026$
281	60bc	67a	56c	64ab	$P\leq0.044$
Quadratic regression	0.471	0.697	0.794	0.733	r^2
($n=18$)	0.008	<0.001	<0.001	<0.001	P

Discussion

In the bioassays, AM infection at 14 days occurred as small primary infections and, therefore, reflected the density of propagules in the soil (Walker and Smith 1984). The decline in primary infection of roots over the winter fallow, in the 1993 bioassays, is consistent with reports of declining viability of AM inoculum over time (Thompson 1987, 1994; McGee et al. 1997). Dormancy (Gazey et al. 1993) and changes in the soil microflora (Wilson et al. 1989; Linderman 1992) or soil chemistry (Sylvia and Williams 1992; Miranda and Harris 1994) may influence the infectivity of AM propagules but senescence was the most likely cause of the decline (McGee et al. 1997). Nevertheless, at the end of the winter fallow, AM inoculum was abundant enough in all six soils to establish substantial colonisation of roots in the bioassays.

Pattinson and McGee (1997) found that primary colonisation of cotton roots was linearly proportional to the subsequent maximum level of colonisation. In our bioassays colonisation at 42 days was close to plateau level but was not proportional to primary colonisation and varied according to the origin of the soil sample in time and space. Primary infections may continue to occur as roots explore the soil. However, over the winter period, the pattern of colonisation at 42 days did not match root growth. Hence, factors other than inoculum density affected the spread of colonisation in cotton roots.

Seasonal variation in AM colonisation of plants in the field can be influenced by climatic conditions, soil moisture and nutrient pulses, the application of fungicides and host genotype and growth stage (Abbott and Robson 1991; Cade-Menun et al. 1991; Sanders and Fitter 1992; Eissenstat et al. 1993; Sanders 1993). The standardised conditions in the bioassays eliminated the mitigating effects of the weather and the host genotype and growth stage. No fungicides were used in the bioassays or applied to fields. The recurrent pattern in AM colonisation at 42 days during the winter fallows suggests regulation of colonisation by the host plant (Koide and Schreiner 1992; Douds 1994), perhaps in response to fluctuation in the availability of P or other elements in the soil (Jasper et al. 1979). Seasonal fluctuation in the availability of P (Kemp et al. 1985), if it occurred, is an unlikely factor because the pattern of colonisation was similar in soils with both high and low P contents. Nitrogen fertiliser sometimes increases AM colonisation (Dhillion and Ampornpan 1992). While N fertiliser was applied to the fields prior to the final bioassays in both years, the rate was much lower in 1993. The similar patterns of AM colonisation in both 1993 and 1991, and the likely masking effect of N fertiliser applied in the bioassays, suggest that N was not a major factor. Whether or not the seasonal oscillation in the development of AM colonisation over the winter period was attributable to host regulation in response to plant nutrition (Jasper et al. 1979), or direct effects of the soil on root physiology (Koide and Li 1990), can only be determined by further experimentation.

The soil properties and symptoms of the growth disorder at the stunted-crop site and the normal-crop site in field 8 were consistent with the description of soils and symptoms in fields 18 and 20 (Nehl et al. 1996). In crops in all three fields, growth and AM colonisation in cotton at the stunted-cotton sites was markedly slower than at the normal-cotton sites. This differential pattern of growth and AM colonisation in the crops, was reversed or at least equalised in the pot bioassays. In the bioassays and at pairs of sites within each field, cotton genotypes and climatic conditions were the same, and agricultural practices were essentially the same. Therefore, AM development and plant growth was either constrained or enhanced according to changes in soil conditions, either biological or non-biological, associated with sieving, potting or watering the soil.

In soils from normal-crop sites, the slower rate of AM colonisation of cotton in bioassays conducted at the same time as the crops were sown, possibly resulted from disruption (McGee et al. 1997) or dispersion (Wilson and Tommerup 1992) of inoculum during the sieving and potting of soil. However, it is difficult to conceive a mechanism by which potting could cause an increase in the number of AM propagules in the stunted-crop soils. In the first bioassay in 1991, the densities of spores of *Glomus* spp. in stunted-crop soils were similar to those in normal-crop soils (Nehl unpublished data). Clearly, AM propagules were abundant enough in stunted-crop soils, at the time the crops were sown, to initiate rapid colonisation in the bioassays.

Inhibition of AM colonisation by high levels of soil P (Johnson and Pfleger 1992) is consistent with the strong negative correlation between soil P and AM colonisation early in the cotton crops (Nehl et al. 1996). However, as cotton crops mature, colonisation of roots in the high-P stunted-crop soils reaches the same level as in normal-crop soils, where P is lower (Nehl et al. 1996). Furthermore, in the bioassays, the initiation and spread of AM colonisation were often greater in the high-P stunted-crop soils than in the corresponding normal-crop soils from the same field. Slow AM development in stunted cotton crops is apparently not caused by high levels of soil P.

While the nature of the soil conditions affecting the growth and AM colonisation of cotton remains unresolved, our study has a broader implication for mycorrhizal research in general. It is well known that the perturbations occurring when soil is used in AM pot experiments confound extrapolation to the field (Fitter 1985; Stribley 1987; Merryweather and Fitter 1995). However, the tacit assumption that colonisation in bioassays of different soils occurs in proportion to that occurring in field has not been tested using soils with a wide range of properties (Abbott and Robson 1991). Our observations clearly show that the assumption of proportional colonisation does not hold for differing soil types. Nevertheless, information from pot experi-

ments has relevance if linked to observations in the field (Allen 1996).

In conclusion, poor survival of AM inoculum between crops was not a direct cause of the cotton growth disorder. The agricultural practices in fields affected by the cotton growth disorder (Nehl et al. 1996) appear to maintain adequate densities of AM fungi in the soil. Slow initiation and spread of AM colonisation in cotton crops was caused by environmental factors in the soil and, while the nature of these factors is not yet clear, they were partially or wholly counteracted by pot culture. The seasonal oscillation in AM colonisation in the bioassays appeared to be mediated by host-regulation in response to soil factors, but was not directly related to P and N levels in the soil. Root browning in stunted cotton may indicate the activity of soilborne pathogens, although common fungal pathogens of cotton are not associated with stunting (Nehl et al. 1996). Deleterious rhizosphere bacteria can cause root browning and stunt plant growth without obvious above-ground symptoms (Nehl et al. 1997) and may influence AM colonisation (Linderman 1992). While a better understanding of the interactions between cotton and the biological and non-biological aspects of the soil environment will illuminate the causes of the growth disorder, the slow arbuscular mycorrhizal colonisation observed in stunted cotton crops is clearly a symptom and not a direct cause of the disease.

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