MYCORRHIZAS AND EARLY SEASON GROWTH DISORDER: THE LAZY COTTON PLANT GETS INTO TROUBLE

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The lazy cotton plant

From a humanised viewpoint cotton may be thought of as a lazy plant. It "employs" microscopic fungi to do some of the hard work of collecting nutrients from the soil. This type of relationship, a symbiosis, is known as a mycorrhiza (literally: "fungus root"). Usually both partners in a mycorrhiza benefit from the relationship.

Mycorrhizal fungi take up chemical elements which are relatively immobile in the soil, especially phosphorus, and donate them to the plant. Phosphorus diffuses very slowly through soil. Once the phosphorus in the soil solution that is immediately adjacent to a root becomes depleted it is replaced very slowly. The more soil that a root system comes into contact with, the more phosphorus it has access to. Mycorrhizal fungi colonise the cotton plant's roots and develop a network of filaments (hyphae) which spread through the soil surrounding the roots. The hyphal network contacts a much larger volume of soil than the roots of the cotton plant. In this way the mycorrhizal fungi act as an extension of the plant's root system. In return for their "labours" the mycorrhizal fungi are "paid" with sugars produced as a result of photosynthesis by the plant.

Species of plants which don't form mycorrhizas at all, or rely less upon them, usually have alternative strategies for obtaining nutrients. For example, non-mycorrhizal species may have a finer more extensive root system than mycorrhizal plants, or longer root hairs (Manjunath and Habte, 1991; Hetrick, Wilson and Cox, 1992; Hetrick, Wilson and Todd, 1992). However, for most plant species the cost of maintaining the mycorrhizal fungi in their roots is surpassed by the subsequent gain in nutrients.

The most common type of mycorrhiza is known as vesicular-arbuscular mycorrhiza (VAM) or arbuscular mycorrhiza after the fungal structures which grow inside the roots of the host plant. Arbuscules are highly branched structures which form inside individual root cells. Arbuscules have a large surface area in contact with the contents of the plant cell. Most of the exchange of nutrients and sugars between the plant and the fungus occurs in the arbuscules.

Does cotton need to be mycorrhizal for optimum economic growth?

Species of mycorrhizal plants can vary in the level to which successful growth depends on their fungal partners (Plenchette, Fortin and Furlan, 1983). Many of our crop plants have the ability to form mycorrhizas, cotton being no exception. Mycorrhizal dependency can be tested by relating the growth of mycorrhizal plants to that of non-mycorrhizal plants in the same soil. Mycorrhizal fungi are usually present in soil. To produce non-mycorrhizal plants the soil must be sterilised in some way to eliminate the mycorrhizal fungi. We examined the mycorrhizal dependency of cotton by fumigating soil with methyl bromide at two sites on a commercial farm near the Australian Cotton Research Institute (ACRI) at Narrabri in the 1992/93 season. In both fields soil fumigation eradicated mycorrhizal fungi and no colonisation of cotton roots occurred (Table 1).

Table 1. Growth, mycorrhizal colonisation, nutrient uptake and mycorrhizal dependency of cotton (cv. Deltapine 90) grown in methyl bromide fumigated and unfumigated soil at six weeks after sowing.

site		field 17		field 18	
soil fumigation		YES	NO	YES	NO
mycorrhizal colonisation (% roots with arbuscules)		0	45.4	0	57.7
shoot dry matter (g/plant)		2.19	9.93	0.59	6.97
nutrient concentration (whole shoots)*	P (mg/g)	3.16	3.66	1.30	3.43
	Zn (µg/g)	20.9	25.5	46.0	30.9
total nutrient content (whole shoots)*	P (mg)	5.9	31.5	0.67	20.4
	Zn (µg)	38.8	230.7	16.9	169.2
relative field mycorrhizal dependency (%)	shoot	78		92	
	total P*	81		97	
	total Zn*	83		90	

*These values are exclusive of the first two fully expanded leaves of each plant which were retained for separate analysis (results not presented).

At the site in field 18 the differences between mycorrhizal and non-mycorrhizal plants were dramatic. The mycorrhizal plants in field 18 were almost 12 times larger than non-mycorrhizal plants (Table 1). In the presence of mycorrhizal colonisation shoot phosphorus concentration was increased by about two and one half times and the above ground accumulation of phosphorus was increased by 30 times. There was a higher concentration of zinc in the shoots of non-mycorrhizal plants compared to that of mycorrhizal plants in field 18. The higher zinc concentration was probably due to accumulation of zinc over time, rather than any enhanced root function, because the shoots of these plants had ceased growth at four weeks after sowing. Even so, the above ground accumulation of zinc was increased tenfold in the mycorrhizal plants in field 18 (Table 1).

At the site in field 17 mycorrhizal plants grew larger and took up more nutrients than non-mycorrhizal plants. However, the relative increases were not as conspicuous as those in field 18 (Table 1). The non-mycorrhizal plants were larger in field 17 than in field 18 and did not cease growth as did the non-mycorrhizal plants at the field 18 site. This shows that the mycorrhizal dependency of cotton can vary from site to site. Mycorrhizal dependency can be quantified by expressing the increase in plant dry matter between mycorrhizal and non-mycorrhizal plants as a percentage of the dry matter of the mycorrhizal plants (Plenchette, et al., 1983). Mycorrhizal dependency, based on dry matter, was much higher in field 18 than in field 17 (Table 1). We have extended the application of this calculation of mycorrhizal dependency by using it for above ground nutrient accumulation, as well as for dry matter accumulation (Table 1). In field 18, for example, cotton was 97 % dependant on the mycorrhizal fungi for uptake of phosphorus. In other words the activity of the mycorrhizal fungi was directly and indirectly responsible for the uptake of 97 % of the phosphorus found in the mycorrhizal plants. Some of this extra uptake would have occurred in the fungal hyphae and some would have occurred in the roots of the plant.

The relative field mycorrhizal dependency of cotton grown in field 18 was higher than in field 17, for both shoot growth and nutrient uptake. This difference is consistent with the observed levels of mycorrhizal colonisation (Table 1). The percentage of roots containing arbuscules was significantly lower in field 17 (Table 1). Mycorrhizal colonisation was expressed as the proportion of roots containing arbuscules because the arbuscule is considered to be the site where most of the nutrient exchange between the plant and the mycorrhizal fungus occurs. Hence arbuscular colonisation gives a measure of how symbiotic the mycorrhizal partnership was.

It can be hypothesised that the lower mycorrhizal dependency of cotton grown in field 17 was due to the higher levels of available soil nutrients at that site. The levels of available phosphorus and zinc were 2.75 and 2.33 times greater respectively in the soil at the field 17 site (Table 2). These availabilities were probably related to the clay content of the soil which was higher in field 17 than in field (Table 2).

Table 2. Properties of soil at two sites used to test the mycorrhizal dependency of cotton.

site	field 17	field 18
clay (%)	64.3	52.0
bicarbonate extractable phosphorus (ppm)	44	16
DTPA extractable zinc (ppm)	2.1	0.9

The plants used in the mycorrhizal dependency experiment were not grown to maturity. Nevertheless, it would appear that without mycorrhizal fungi in the soil cotton yields would be reduced substantially, especially at the site in field 18. In summary, it has been shown that the mycorrhizal dependency of cotton can vary from field to field and soil to soil. The results presented here cannot necessarily be extrapolated to other sites or regions. Mycorrhizal dependency has to be assessed on an individual basis. However, a lower mycorrhizal dependency value does not necessarily diminish the importance of the growth increase that the plant gains from its partnership with the fungi. The operative criterion, in deciding how important the mycorrhizal fungi are, should be whether the size of the contribution of the fungi to plant growth is large enough to be relevant to the aims of the farmer. In most soils it probably is.

What affects mycorrhizal development on cotton and can we manage our farms to ensure that the "lazy cotton plant" does not get into difficulties?

Genotypic compatibility. It is likely that the degree of stimulation of cotton growth will vary among different species, and genotypes within species, of mycorrhizal fungi and among cotton species and cultivars. For example, Raju, Clark, Ellis and Maranville (1990) reported that the response of sorghum to mycorrhizal colonisation varied among the species of mycorrhizal fungi present. Also, Hetrick et al (1992) reported variation in mycorrhizal dependency among wheat cultivars. Research on this sort of variation in cotton has been initiated by Dr Peter McGee at Sydney University.

Long fallow disorder. Arbuscular mycorrhizal fungi are unable to grow in the absence of a living host plant. During a long bare fallow the number of propagules of arbuscular mycorrhizal fungi in the soil is likely to diminish and this can contribute to long fallow disorder (Thompson, 1987). If the crop following the long fallow is dependant on mycorrhizal fungi then it's growth may be diminished due to the reduced colonisation by the mycorrhizal fungi. Long fallow disorder has been reported in cotton in the USA (Smith, Hicks, Lloyd, Gannaway and Zak, 1989) and in Australia (Brown, Allen and Constable, 1990).

We investigated the effect of a bare fallow on mycorrhizal colonisation, growth and yield of cotton at four sites on a commercial farm near the ACRI (Table 3). Six plots at each site were kept free of weeds from the end of the 1991/92 cotton season until the start of the 1993/94 cotton season. In the 1992/93 season the four sites were sown with *Lablab purpureus*. Lablab is known to be a mycorrhizal plant (Mahdi and Atabani, 1992) and we had previously observed mycorrhizal colonisation in lablab sown in the area. In the weed free plots the lablab was

removed shortly after germination. In another six plots at each site the lablab was allowed to grow normally through the 1992/93 season. A commercial cotton crop was grown over all 12 plots at each site in the 1993/94 season. Shoot dry matter production and arbuscular mycorrhizal colonisation were measured at nine weeks after sowing. Boll dry matter production was measured at 24 weeks after sowing.

Statistical analysis of shoot growth, mycorrhizal colonisation and boll yield showed that there was no significant difference between means for the bare fallow treatment and the lablab rotation treatment. Thus the prior presence of the rotation crop was of no benefit to the early growth and the yield of cotton in the 1993/94 season. Some of the sites studied were chosen because they are known to he affected by a growth disorder. The level of mycorrhizal colonisation (combined for the fallow and lablab treatments) at the better site in field 18 (Table 3) was comparable to levels that have been previously reported in healthy cotton (Brown, Allen and Constable, 1990; Nehl and Brown, 1992). Colonisation at the poorer sites (Table 3) was no lower than would be expected from our knowledge of the growth disorder (Nehl and Brown, 1992). Thus, in the fields we examined, the potential for development of mycorrhizal colonisation of cotton was not diminished by a bare fallow over at least one season, and not increased by rotation with lablab. However, bare fallowing over a longer period could result in a decrease in colonisation of the subsequent crop. Brown Allen and Constable (1990) reported a case where cotton growth was reduced markedly following two consecutive seasons of bare fallow. Our findings are not necessarily applicable to all cotton growing soils. In different soil types the survival of the arbuscular mycorrhizal fungi might be affected differently by a bare fallow of the same duration as that reported here.

Early season growth disorder. A second growth disorder of cotton that involves reduced levels of mycorrhizal colonisation is an early season growth disorder. This disorder is sometimes called Galathera syndrome after the Galathera Creek north of the ACRI. The early season growth disorder differs from long fallow disorder because it occurs perennially and can be localised within individual fields. It was reported at the 6th Australian Cotton Conference that the early season growth disorder was associated with reduced levels of mycorrhizal colonisation of cotton roots (Nehl and Brown, 1992). Similar observations were made in the lablab/fallow trial reported here. In each field the percentage mycorrhizal colonisation at the "poor" site was about one third lower than that at the better site (Table 3).

Table 3. Growth, mycorrhizal colonisation and root browning (at nine weeks after sowing) and boll yield of cotton (cv. CS189+) in fields affected by the early season growth disorder.

field:	18	18	20	20
*history:	fair	poor	fair	poor
†shoot dry matter (g/m)	34	9	23	13
†mycorrhizal colonisation (% roots with arbuscules)	61a	41b	56a	39b
†root browning (%)	83a	96b	85a	95b
†boll dry matter (g/m)	501a	381b	465ab	471ab

*assessment of early season growth during 1991/92 season.
†Values followed by different letters are significantly different ($p < 0.05$).

Cotton plants were smallest at the "poor" sites in each field (Table 3). Plants at the "fair" site in field 20 were only two thirds the size of those at the "fair" site in field 18 (Table 3). The boll dry matter production at 24 weeks after sowing did not reflect the pattern of early shoot growth exactly. Boll production at both sites in field 20 was lower than, but not significantly different from, boll production at

the "fair" site in field 18. This suggests that the cotton was able to compensate later in the season for the early reduction in growth. The apparent lack of correlation between early season mycorrhizal colonisation and subsequent yield suggests that the problem is not caused solely by reduced early mycorrhizal development.

Another factor associated with the early season growth disorder is root browning. The percentage (by length) of roots showing browning was higher at the poor growth sites (Table 3). Pot and field trials, not reported here, have shown that root browning develops rapidly during the first two weeks of cotton growth in the "poor" soils. Root browning does not develop in plants grown in "poor" soil that has been partially sterilised by steaming or methyl bromide fumigation. Partial sterilisation of "poor" soils consistently had a beneficial effect on cotton growth. This suggested that the root browning and the stunting of cotton growth was caused by one or more soilborne microorganisms. In further experiments, bacterial antibiotics (streptomycin and penicillin) stimulated increased cotton growth when they were applied to "poor" soil. Browned roots were therefore examined for the presence of bacterial pathogens. Thirty eight strains or species of bacteria were isolated either from cotton roots that were surface sterilised in chlorine bleach for five minutes or from rhizosphere soil. These isolates were used in an *in vitro* bioassay to assess their pathogenicity toward cotton. Suspensions of cultures of the isolates were applied to seeds sown on moist paper towelling in sterile plastic containers. Root growth was reduced dramatically by nine of the isolates which have been identified by fatty acid fingerprinting as a species of *Pseudomonas*. Currently investigations of the pathogenicity of these isolates in soil are underway.

Conclusion

It is probably more apt to describe cotton as a "busy" plant rather than a "lazy" plant. Usually the mycorrhizal fungi that cotton "employs" enable it to grow faster and produce more. Mycorrhizal problems associated with fallowing can probably be avoided in most cases by restricting the length of the fallow or by rotation with a mycorrhizal crop plant. The early season growth disorder remains a problem. However, further elucidation of its cause will enable strategies for control to be formulated in future.

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