Bacterial stunt of cotton: a balance between beneficial and harmful soil microbes

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Introduction

Farmers have probably always realised that soil is alive. Good soil seems to have certain intangible qualities, over and above its physical structure and chemistry. There is something special about topsoil. What are these intangible qualities? We all know that microorganisms in the soil can cause disease. However, in the last 25 years scientists have started to recognise the importance of some very helpful soil inhabitants, especially the vesicular arbuscular mycorrhizal (VAM) fungi and beneficial bacteria.

Nearly all our crop plants rely on VAM fungi to provide them with nutrients, such as phosphorus, and this includes cotton. To be able to grow in many soils, cotton relies heavily on VAM fungi for uptake of phosphorus and zinc (Nehl and Brown, 1992, Nehl, Allen and Brown, 1994). Unfortunately VAM fungi cannot survive without a living host plant. They cannot grow on trash and organic matter in the soil. Problems can occur when fields are subjected to long bare fallows (usually more than one season) because the VAM fungi die out (Brown, Allen and Constable, 1990; Nehl et al., 1994).

Some of the bacteria that inhabit roots are beneficial to the plant. Beneficial effects on plants can be derived from bacteria that variously produce plant hormones which are absorbed by roots, fix nitrogen (not only in nodules on legumes), solubilise nutrients such as phosphorus, promote mycorrhizal function and regulate ethylene production in roots. Furthermore, it is well established that some rhizobacteria have the capacity to suppress major plant pathogens. Current research in cotton has shown that beneficial bacteria can be used as an effective and environmentally friendly method for control of several soil-borne diseases (Putcha and Allen, 1994).

Unfortunately pathogenic soil microbes can counteract the effects of the beneficial bacteria and fungi. The following account illustrates their combined effects on cotton growth.

Bacterial stunt of cotton

Recently a disease of cotton called 'Galathera syndrome' or early 'season growth disorder' has been investigated. This disease is really 'bacterial stunt' and it occurs in many cotton growing areas, although initial investigations began at Galathera Creek north west of Narrabri. Affected plants are stunted and grow slowly during the first half of the season and yield poorly. Gradients in the severity of stunting are present within individual fields (Nehl and Brown, 1992; Nehl, Allen and Brown, 1996). Growth of severely stunted plants improves in mid-season but too late for substantial recovery of yield. Stunted plants have lower levels of mycorrhizal development than larger plants within the same field but this is not due to long fallows.

The cause of bacterial stunt has been identified by a process of elimination by investigation of the interactions among cotton growth, mycorrhizal fungi, soilborne pathogens and chemical and physical characteristics of the soil.

Interaction between cotton and the soil

Observations of early season cotton growth were made at 100 m intervals along transects in fields 18 and 20 on the Auscott Narrabri farm. Statistical analysis of physical and chemical properties of the soil distinguished three groups of sites (A, B, and C) which corresponded to patterns of yield and early season growth (Nehl et al., 1996). Early season growth and mycorrhizal colonisation of cotton at group A and B sites was much slower than at group C sites. Group B sites showed a recovery of yield later in the season while group A sites did not.

Group A soils had lower pH, finer texture and higher phosphorus, zinc, manganese and exchangeable magnesium, potassium and sodium than group C soils. Thus, paradoxically, the greatest stunting occurred in should what have been the more fertile soils with a more favourable pH for cotton growth. The exchangeable sodium percentage (ESP) of group A soils was higher than that of group C soils but below the critical level of 5 %. While the finer texture and greater ESP of group A soils may make them more prone to structural degradation or waterlogging, symptoms of waterlogging were not observed in cotton during the period of study.

Interaction between cotton and mycorrhizal fungi

On the transects in fields 18 and 20 shoot growth was closely related to mycorrhizal fungal colonisation: the more stunted the plants, the lower the level of

colonisation of their roots (Nehl and Brown, 1992; Nehl et al., 1996). It was feasible that low levels of mycorrhizal colonisation at poor growth sites were due to an absence of viable mycorrhizal fungi at the start of the season. A series of bioassays in pots showed that at the start of the season the colonisation of cotton roots in soil from poor growth sites was equal to or greater than in soil from sites with better growth (Table 1). Hence, the early season growth disorder was not caused by a reduction in the number of mycorrhizal fungi in the soil. Therefore, the low levels of mycorrhizal development in field-grown cotton were due to the environmental conditions in the soil.

Table 1. Mycorrhizal development of cotton (% root colonisation) at 42 days after sowing in potted soil which was collected at the onset of cotton seasons from fields with variable severity of bacterial stunt.

	site						
location	field 18		field 20		field 8		
[1]crop growth	fair	poor	fair	poor	fair	poor	
[2]soil group	C	A	C	A	C	A	(n = 6)
1991	52.8	49.5	54.4	54.4	53.7	63.0	NS
1993	59.7bc	67.3a	56.0c	64.0ab	-	-	[3]$p \leq 0.044$

[1] Based on assessment of early season growth in the field during 1991/92 and 1993/94 seasons.
[2] Soil groups identified by Nehl et al. (1996).
[3] In rows, values followed by the same letter were not significantly different at the stated probability level for pairwise comparison by the Scheffé test.

Interaction between cotton and mycorrhizal fungi

At some sites cotton had a high relative field mycorrhizal dependency (up to 92% at six weeks after sowing). In other words, 92% of the shoot growth of cotton was enabled by the presence of the mycorrhizal fungi (Nehl et al., 1994). This dependency decreased as the level of available phosphorus in the soil increased. In fields 18 and 20 mycorrhizal colonisation in cotton roots declined as phosphorus availability increased (Nehl et al., 1996). Hence the lack of mycorrhizal colonisation in stunted plants probable reflected a lower level of dependency on mycorrhizal fungi (Nehl et al., 1994). In a pot experiment mycorrhizal plants in steam sterilised soil from a site with bacterial stunt of cotton had double the phosphorus content of non-mycorrhizal plants in steam sterilised soil (Table 2). The available phosphorus content of that soil was 88 ppm. Therefore, although the dependency of cotton is lower in the high phosphate soils the mycorrhizal fungi still contribute to uptake of nutrients.

Table 2. Arbuscular mycorrhizal colonisation, growth, shoot nutrient content and root browning of cotton in potted soil from a site with bacterial stunt of cotton.

	treatment[1]				
	+S-I	+S+I	-S-I	-S+I	[2](n = 6)
14 days after sowing					
root browning (%)	0.2b	5b	45a	40a	$p < 0.001$
42 days after sowing					
arbuscular root (%)	0.0	49.6a	39.6b	48.3ab	$p = 0.024$
shoot height (mm)	234a	190b	193b	165b	$p \leq 0.029$
shoot dry mass (g plant^{-1})	2.45a	2.09b	1.58c	1.56c	$p \leq 0.002$
shoot P (g kg^{-1})	2.2b	4.6a	4.4a	4.3a	$p < 0.001$
shoot Zn (mg kg^{-1})	15b	22a	24a	25a	$p \leq 0.001$
root browning (%)	5c	36ab	32b	41a	$p \leq 0.048$

[1] Treatments included steam sterilised (+S) and unsteamed (-S) soil with a layer of either sterilised (-I) or unsterilised (+I) soil with roots of cotton.
[2] In rows, values followed by the same letter were not significantly different at the stated probability level for pairwise comparison by the Scheffé test.

Interaction between cotton and soil pathogens

Sterilisation of soils with stunted cotton consistently increased cotton growth. The concentration of phosphorus and zinc in non-mycorrhizal plants was half that of the mycorrhizal plants and yet the mycorrhizal plants were stunted (Table 2). This suggested that something in unsterilised soil was holding plant growth back, namely soilborne microorganisms that were pathogenic to cotton. Sterilisation of soil in the field also increased cotton seedling growth and these seedlings contained twice as much Mn as stunted seedlings in unsterilised soil. Therefore Mn toxicity was not a cause of stunted seedling growth.

Root browning is a symptom of stunted cotton: the stunted plants have the brownest roots (Nehl et al., 1996). In pot and field experiments root browning developed rapidly in live soil and was eliminated by soil sterilisation (Table 2). The capacity for browning was restored to steam sterilised soil by the addition of live soil and roots (see +S+I treatment in Table 2). Hence, root browning was caused by soil pathogens. Viruses and nematodes were discounted as possible pathogens. Few fungi other than mycorrhizal fungi were observed colonising cotton roots from poor growth sites. Colonisation of roots by *Thielaviopsis basicola* and chytrid fungi increased as shoot growth increased (Nehl et al., 1996). Furthermore, the incidence of verticillium wilt symptoms in mature plants

increased as boll yield increased. It was concluded that pathogenic fungi were not causing the stunting.

Several observations indicated that soilborne bacteria play a causal role in the early season growth disorder. The application of bacterial antibiotics to live soil increased the growth of cotton in pots (Table 3).

Table 3 The influence of streptomycin soil drenches on the growth of cotton in potted soil from a site with bacterial stunt of cotton, at 35 days after sowing.

	streptomycin (mg g^{-1} soil equivalent)			
	0.0	**0.2**	**0.8**	[1](n = 6)
shoot fresh mass (g $plant^{-1}$)	2.30b	3.20a	3.51a	$p \leq 0.001$
shoot dry mass (g $plant^{-1}$)	0.352b	0.521a	0.560a	$p \leq 0.001$
root fresh mass (g $plant^{-1}$)	0.846b	0.962ab	1.152a	$p = 0.017$
root dry mass (g $plant^{-1}$)	0.132b	0.170a	0.168ab	$p = 0.041$

[1] In rows, values followed by the same letter were not significantly different at the stated probability level for pairwise comparison by the Scheffé test.

Bacteria isolated from browned cotton roots were shown to be pathogenic to cotton seedlings, causing root browning and stunted root growth (Figure 1).

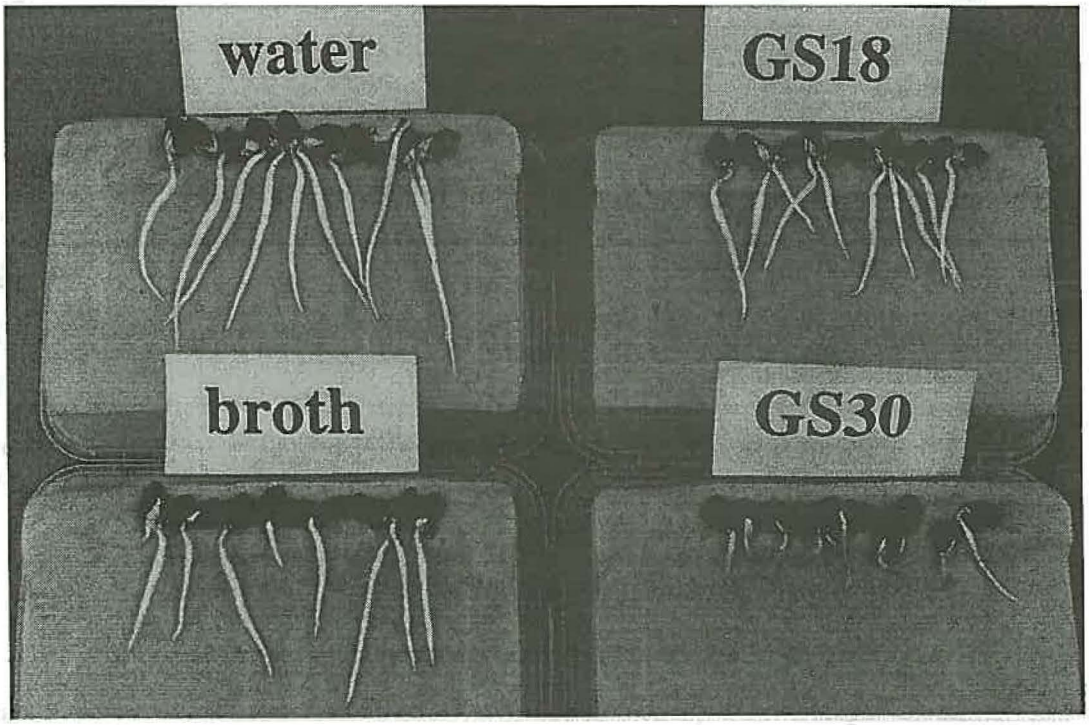

Figure 1 Growth and root browning of cotton in a seedling bioassay at 84 hours after inoculation with non-pathogenic (GS18) and pathogenic (GS30) bacteria isolated from roots of cotton growing in soil with bacterial stunt.

All the isolates of bacteria from cotton roots that were highly pathogenic (Figure 1) belonged to a species of *Pseudomonas*. These pathogenic bacteria are broadly distributed in cotton growing soils. Under the microscope, bacteria were observed inside browned root cells and bacteria streamed out from the cut surfaces of browned roots of field-grown cotton. The severity of their effects on cotton growth appears to be determined by soil type (Nehl et al., 1996).

The balance between beneficial and harmful soil microbes

The observations above have demonstrated that cotton growth is determined by the additive effects of non-biological and biological properties of the soil, including both beneficial and pathogenic microorganisms (Figure 2). The mycorrhizal fungi had the dominant effect on cotton growth in soils which did not show early season stunting (group C soils) and were less important in the more fertile soils in which cotton growth was stunted (group A soils). Conversely soilborne pathogens had the dominant effect on cotton growth in group A soils, and only caused a slight reduction of growth in group C soils (Figure 2).

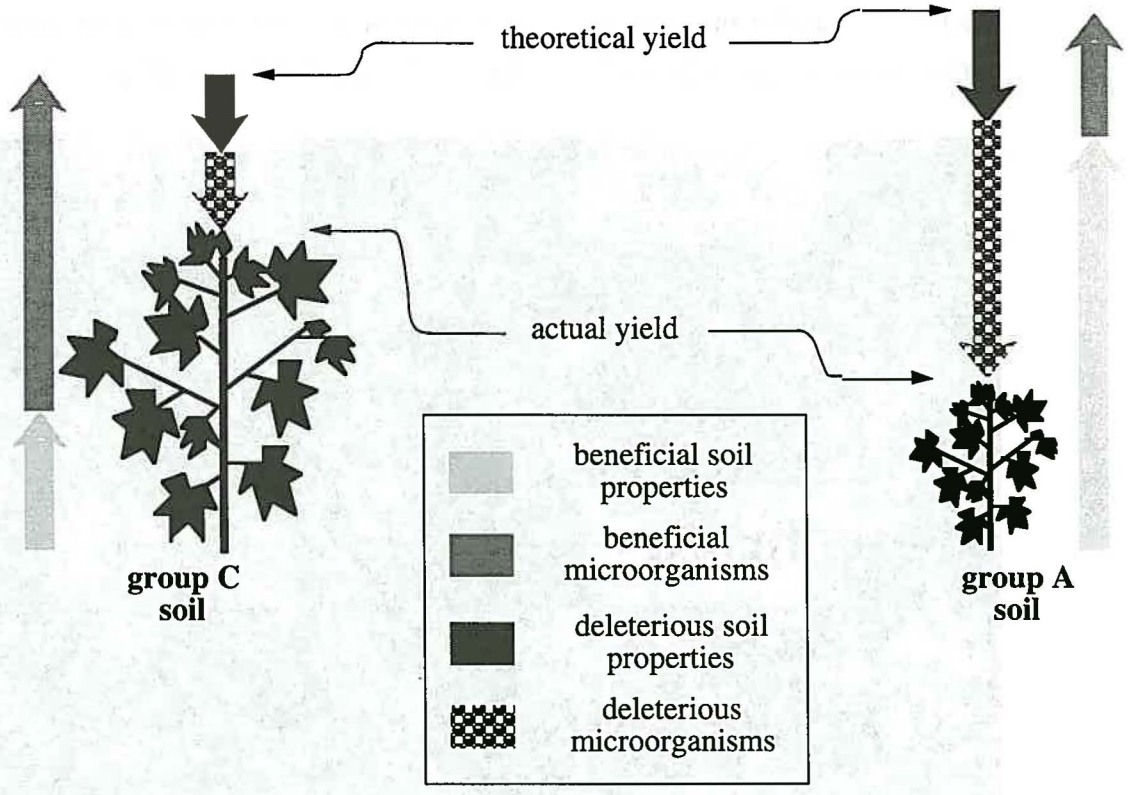

Figure 2. A qualitative depiction of the relative contribution of biological and non-biological soil properties to cotton growth in soils that support slow early season growth (group A) and faster early season growth (group C). Arrows represent changes in growth and/or yield.

This model of the factors affecting cotton growth (Figure 2) depicts an ecosystem which is dynamic. Field experiments showed that the pathogens in group A soils were active throughout the growing season and therefore contribute a constant impediment to cotton growth.

The distribution of bacterial stunt of cotton

Current knowledge of the distribution of bacterial stunt is inadequate. The fact that the disorder was first identified at Galathera Creek is a reflection of the severity of the disorder in that area. At other sites the disorder is less prominent or the severely affected areas are small. A survey of several cotton growing areas was conducted during the 1995/96 season. Soil from 32 sites was collected and tested for cotton growth responses induced by steam sterilisation (as observed in previous experiments, Table 2). Steaming increased the growth of cotton (Siokra1-4) in many of the soils, indicating the presence of pathogens. The greater the growth increase induced by steaming the soil, the smaller the plants in unsteamed soil tended to be (Figure 3). In other words, the growth of cotton in pots was substantially reduced by soilborne pathogens. Plants in some of these

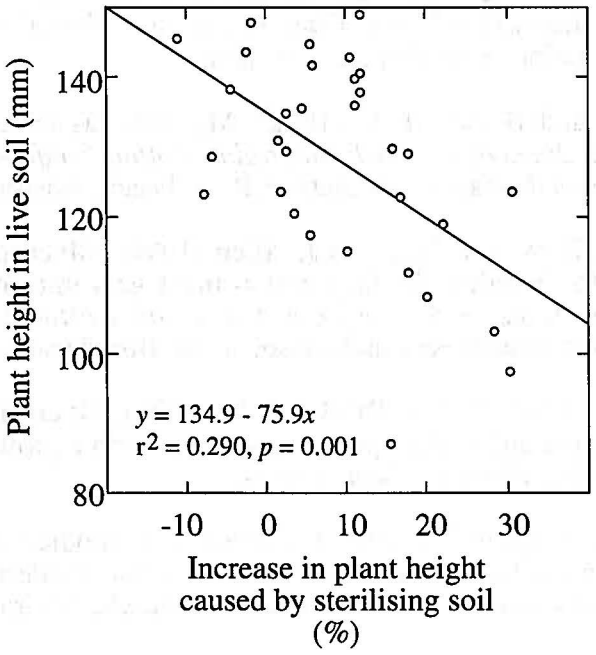

Figure 3. The relationship between the increase in cotton growth induced by steam sterilisation of soils and growth of cotton in unsterilised soil in potted soil from 32 sites, at 21 days after sowing.

were affected by black root rot, a fungal disease. However, preliminary observation of seedlings in live soil suggested that symptoms of fungal disease were not prevalent in several of the soils in which steaming induced a large

growth increase. These soils were from sites in which cotton in the 1995/96 season had symptoms consistent with bacterial stunt. Bacteria have been isolated from the roots of plants in all 32 soils and their pathogenicity on cotton is being evaluated.

In summary, bacterial stunt of cotton is caused by bacteria which inhibit growth and mycorrhizal development of cotton in nutrient rich, heavy clay soils. Cotton appears to be less dependent on mycorrhizal fungi in these nutrient rich soils than elsewhere. While other diseases, such as black root rot, can also cause stunting of cotton, bacterial stunt is a distinct disease. If mild bacterial stunt is widespread then it would go undetected. This is because yield can only be compared to the best fields in the country, which may also be mildly affected. The performance of cotton should be viewed as the net result of a number of positive and negative effects on growth. Current research aims to develop appropriate control strategies for bacterial stunt, based on a better understanding of the soil ecology.

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