COTTON RESEARCH AND DEVELOPMENT CORPORATION

FINAL REPORT

Black root rot and slow early season growth of cotton

DAN122C

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BLACK ROOT ROT AND SLOW EARLY SEASON GROWTH OF COTTON

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PLAIN ENGLISH SUMMARY

Black root rot was first observed in Australian cotton in 1989. The disease has spread rapidly since then and now occurs in most areas in NSW and southern Queensland. Black root rot is caused by a soilborne fungus that survives for long periods in the soil as very resilient, dormant spores. Spores are produced abundantly on cotton roots and each cotton crop deposits more spores in the soil. Consequently, the severity of black root rot increases in proportion to the number of cotton crops, irrespective of fallows or rotations. The reproductive capacity and toughness of the spores make this fungus virtually impossible to eradicate. The spores are easily carried in moving water or in mud on vehicles and machinery. If black root rot continues to increase at its current rate, then 95% of fields in the established cotton-growing regions of NSW may have the disease by 2004.

Black root rot is favoured by cool temperatures and the infection of roots is, therefore, most severe at the seedling stage. Although the black root rot fungus does not kill seedlings by itself, severe black root rot renders cotton more susceptible to other seedling pathogens. The major effect of black root rot on cotton is to slow early-season growth. In effect, black root rot 'steals' time from the crop, leading to delayed maturity and consequent yield loss.

Cotton growers are faced with two objectives for control; to either reduce severe infestations to low levels, or to prevent black root rot from becoming severe in the first place. Control methods that have potential to hold black root rot at low levels include: farm hygiene to stop further spread; biofumigation crops ('green manure' crops that kill the fungus); avoiding rotation with crops that are also hosts for the pathogen; cultural methods such as the timing of planting of irrigation.

Biofumigation with woolly pod vetch is proving popular among growers because this plant can provide large quantities of nitrogen to the following cotton crop. Vetch provides an alternative to rotation with other green manure crops that are hosts for the pathogen. Mustard and canola also have potential as biofumigation crops. Other potential control measures include: inducing the natural defences of cotton against black root rot; cereal cover crops, which can increase soil temperatures and improve bed structure and drainage; in-furrow fungicides. These measures require further research before they can be used effectively.

There are currently few options available that can reverse severe infestations of black root rot. All varieties of cotton are susceptible to the disease. Summer flooding is very effective but its feasibility is limited by cost, field topography and water supply. A single season of biofumigation does not reduce the population of the black root rot fungus sufficiently to prevent it from rapidly increasing in the following cotton crop. Longer periods of rotation with non-hosts (eg. cereals for two or three years) may be a practical method. Deployment of current and future control options in an integrated manner will ultimately be the most effective strategy.

FULL REPORT

BACKGROUND

Black root rot is caused by the soilborne fungus *Thielaviopsis basicola*, which causes disease in over 137 species of plants (Honess et al., 1994). *T. basicola* survives for long periods in the soil as resistant resting spores. The wide host range and resistant resting spores make *T. basicola* almost impossible to eradicate from soil. Infection of cotton is favoured by soil temperatures below 20°C. Research in the USA has shown that severe disease symptoms result when the population of the black root rot fungus reaches 100 spores per gram of soil. Populations of 600 to 700 spores per gram of soil are commonly observed in Australian cotton fields.

Black root rot contributes to seedling loss caused by the seedling disease complex. Stand losses of 30% or more have been recorded. Seedlings affected by black root rot are stunted and slow growing and crop maturity is delayed. In California black root rot was regarded as a minor disease 30 years ago but is now considered to be more serious than Verticillium wilt (Note: the defoliating strains of *Verticillium* in the USA are more pathogenic than the strains currently in Australia). Yield reductions of 25 to 50% were attributed to black root rot in California (Garber et al., 1985; Hake et al., 1985) but the potential for yield loss in Australia had not been quantified.

Black root rot was first observed in Australia in 1989. Since then its severity and distribution increased annually. At the commencment of this project, black root rot occurred throughout the Macquarie valley, the Namoi valley and the Darling Downs, and was common in the Macintyre and Gwydir valleys. In some fields 100% of plants were affected. Anecdotal evidence suggested that black root rot may increase the severity of Fusarium wilt (J. Kochman, Pers. Comm.). Permanent bed systems may have increased the severity of black root rot by increasing the population of the pathogen along the planting line.

Prior to this project, the only recommended control measure for black root rot in cotton was to plant late and hence avoid soil temperatures that favour infection. No Australian cultivars were known to have resistance to black root rot, although overseas reports suggest that Pima cottons are more susceptible. The standard fungicides used as seed coatings on Australian cotton seed are not effective against black root rot. Some chemical seed treatments are registered for control of black root rot in the USA but not in Australia. These chemicals had not given consistent results in Australian trials but their efficacy might be improved by 'in-furrow' application. Growers sometimes replant stunted fields but the merits of this practice are uncertain.

The available control measures for black root rot were clearly inadequate. The ongoing increase in the distribution and severity of black root rot in Australia represented a threat to sustainable cotton production in Australia. Much of the research in this project was aimed at gaining a better understanding of the factors contributing the rise in importance of black root rot and providing new options for the integrated management of this disease.

Slow early season growth of cotton can be caused by a lack of mycorrhizal (VAM) development. Mycorrhizal deficiencies occur in fields following very long fallows or land leveling and are associated with reduced cotton growth. Previous research had indicated that 'nurse' crops may have the potential to increase the early season growth and mycorrhizal development of cotton when a lack of mycorrhizal fungi was indicated. A bioassay using linseed to predict mycorrhizal problems prior to cotton cropping required testing in the field.

Another cause of slow early season growth is 'bacterial stunt', in which deleterious bacteria colonise cotton roots. Bacterial stunt occurs in most cotton growing regions, including the upper and lower Namoi, the Gwydir, the Macquarie, Bourke and St George. Bacterial stunt is more severe in certain soils, particularly at sites with heavy clays, where yield losses can be as high as 50%. Field trials for control of bacterial stunt were conducted as part of CRDC Project DAN100C. In these experiments early season cotton growth and subsequent boll load was increased by 40-60 % using mulches and irrigation to maintain moisture near the soil surface. The mulches and extra irrigation dramatically increased root growth in the top 10 cm of the soil, where soil structure was better and nutrient levels were greatest. These control measures for both bacterial stunt and VAM deficiencies required further evaluation to adapt them for use in farming systems.

OBJECTIVES AND EXTENT TO WHICH THEY HAVE BEEN ACHEIVED

(i) **Epidemiology of black root rot** – To investigate the biology of black root rot, including the dispersal and survival of the fungal pathogen (*Thielaviopsis basicola*) and interactions with cropping practices and soils.

The potential for dispersal of the black root rot pathogen to be carried in irrigation water has been demonstrated. The effect of a range of cropping practices on the survival of the pathogen and development of disease was investigated. The relationship between the severity of black root rot and different soils has been quantified, indicating that certain soils are conducive or suppressive to black root rot.

(ii) **Management of black root rot**- To utilise knowledge on the biology of black root rot to develop integrated management practices for its control.

An integrated management strategy for black root rot has been developed, including recommendations regarding rotation crops and novel cropping practices.

(iii) **Interactions with other microbes**- To investigate interactions between black root rot and other soilborne microbes, including interactions with both positive (eg mycorrhizal fungi) and negative (eg. Fusarium) outcomes.

Relationships between the severity of black root rot and mycorrhizal colonisation were quantified. The potential for interaction between *T. basicola* and a rhizosphere bacterium was investigated. Interactions with *Fusarium oxysporum* f.sp. *vasinfectum* were not investigated because of a lack of access to field sites with gradients in severity of the two pathogens were not identified.

(iv) **Management of slow early season growth**- To finalise development of management practices for other causes of slow early season growth, including bacterial stunt and lack of mycorrhiza.

The potential for cereal cover crops to increase the vigour and growth of seedling cotton and reduce the impact of seedling disease by moderating the soil environment (specfically temperature and structure) has been clearly demonstrated in this project. ‘Nurse’ crops prior to cotton may be useful in cases where a lack of mycorrhizal fungi is suspected but will be unnecessary in most situations.

COTTON R&D CORPORATION OBJECTIVES

The research conducted in this project has led to changes in agronomic practices for disease management that have contributed directly to the strategy to improve chemical and non-chemical management of diseases, which is a strategy in Output 1, Sustainability of Natural Resources. These changes in agronomic practices include:

- Widespread adoption of measures to minimise the further spread of soil borne diseases, including black root rot, by all participants in cotton production and research.
- Increasing use of biofumigation crops for control of black root rot, particularly woolly pod vetch. In 2001 individual farms planted as much as 800 ha of vetch and an estimated 5000 ha of vetch was sown in the lower Namoi valley alone.
- Increased awareness by growers and consultants of the need to avoid environmental conditions that favour black root rot by having good bed preparation, sowing cotton when soil temperatures are on a rising trend and pre-irrigating in preference to irrigating after sowing.
- Increased awareness by growers and consultants of the risks of rotation with alternative hosts to *T. basicola*, such as winter legumes.
- Preliminary use by growers of summer flooding and rotation with canola for control of black root rot.

METHODOLOGY AND JUSTIFICATION

Assessment of symptoms

The severity of black root rot in cotton seedlings was assessed as the proportion of the tap root with characteristic blackening (scored on a scale of 0-10) and, in field experiments, the scores from a minimum of 10 plants from each replicate treatment were pooled. This method enabled greater precision than the method used previously, where plants were ranked on a scale of 0-4. In a few cases the percent length of root discoloured by black root rot or the percent length of root with spores of *T. basicola* was assessed quantitatively in the laboratory.

In some experiments the number of relatively healthy lateral roots on seedling plants (where roots with less than 50 % of their length blackened where classed as being relatively healthy) was also assessed. This method enabled resolution of differences between treatments when the tap roots, having been exposed to the fungus for a longer time than lateral roots, were scoring close to 10.

Stand establishment was assessed to give a measure of the interaction between black root rot and other seedling diseases. The dry mass of seedlings was used to measure the effects of black root rot on plant growth. Boll number, boll mass and seed cotton mass were used to assess the effects of black root rot on the maturity and yield of cotton.

Enumeration of *T. basicola* in soil

Soil was sampled as single or composite samples of cores 4 cm in diameter by 15 cm deep. The corer was lubricated with cooking oil spray as necessary and, wherever possible, the samples were taken along the cotton planting line. If soil samples were collected during the early stage of the crop, they were taken from the centre of small gaps in the plant stand to avoid collecting cotton roots and, hence, spores produced during the life of that crop. Soil samples were air-dried and split using a sample divider so that a representative sub-sample could be taken.

The population of *T. basicola* in soil was initially enumerated using the carrot disk technique (Tabachnik et al.,1979). A method using the selective agar medium TB-CEN (Specht and Griffin, 1985) was subsequently adopted. The TB-CEN medium is specific for *T. basicola* and is more accurate than the carrot disk method for quantifying the population of spores in soil.

In the carrot disk method, disks were cut from surface-sterilised carrots and placed in sterile Petri dishes on filter paper soaked in streptomycin solution (0.05%). Soil was diluted 1:100 and in water agar (0.15%) and a 0.1 mL aliquot of the resulting suspension was placed on each carrot disk. The carrot disks were incubated for seven days and then the number of infected carrots was used to calculate the density of *T. basicola* in the original soil sample.

In the TB-CEN method, a 10 g subsample of soil was suspended in 95 mL of sterile water agar (0.1%), shaken vigourously for 15 minutes and then eight replicate aliquots (1 mL) of the resulting soil suspension were dispensed into 9 cm Petri dishes. Then 15 mL of molten (50°C) TB-CEN agar was poured into the Petri dishes, gently mixed with the soil suspensions and incubated at room temperature. The number of colonies of *T. basicola* was determined at one week to 10 days after pouring the plates and the density of colony forming units (cfu) in soil was calculated.

Epidemiology of black root rot

The geographic distribution of black root rot has been assessed throughout the 1990's as part of the annual disease surveys conducted by NSW Agriculture in CRDC project DAN121C and preceding projects. These surveys had been conducted each November, in 70 (±5 s.e.) irrigated fields in NSW since 1984. As far as practicable, the same farms were visited each year and the crops were selected at random, depending on accessibility due rainfall or irrigation. Fields, or sections thereof, that were identified by farmers as having severe disease problems were deliberately avoided.

In each field, 50 to 100 seedlings, in quanta of five to10 respectively, were examined in each of 1-3 randomly selected transects that extended diagonally into each field. Seedlings were selected at points along these transects that were determined by pacing across 5 to 10 rows and then pacing 20 m along the rows. The transects were usually started at a point at least 50 m up the rows from the tail drain of the field, thus avoiding bias due to waterlogging near the tail drain. Occasionally the transects were commenced 20 m into the crop from the irrigation supply channel (head ditch). Seedlings were dug from the soil and individually assessed for the severity of black root rot by estimating the length of the tap root with characteristic blackening on a scale of 0-4, where 4 = 100% of the tap root affected. After 1999, disease severity was assessed using the 0-10 scale described above. The presence of *T. basicola* was confirmed by observation of chlamydospores on the roots using a dissecting microscope. Data from these surveys was analysed using non-linear regression, so that trends could be identified and the relative importance of the disease assessed.

Observational studies and fully replicated experiments were used to examine the importance of a range of factors to the severity and spread of black root rot. These studies included:

- Dispersal of *T. basicola* in soil and water
- Survival of *T. basicola* during the composting of gin trash
- Reproductive capacity of *T. basicola* on different hosts
- Relationship between the severity of black root rot and different soils
- Effects of crop rotation on the incidence and severity of black root rot
- The genetic diversity of populations of *T. basicola*.

Management of black root rot

Fully replicated experiments in the glasshouse and field were used to evaluate a range of control measures for black root rot. These studies included:

- Host-plant resistance in cotton varieties
- Timing of planting
- Summer flooding to eliminate *T. basicola* in soil
- Long term crop rotation with cereals or other crops that may reduce build up of *T. basicola* in soil
- *Biofumigation* by incorporation of 'green manure' crops such as canola, mustard and woolly pod vetch prior to cotton
- The potential of anhydrous ammonia to fumigate soil infested with *T. basicola*
- Chemical agents that may induce systemic acquired resistance
- Application of fungicides to the seed or in-furrow
- Disinfestation of vehicles and equipment using Farmcleanse

Interactions with other microbes

Mycorrhizas (also known as VAM) are an important factor in the growth of cotton and their interaction with black root rot was examined in observational studies and experiments in the field and in the glasshouse.

Some soils can be suppressive to black root rot and this suppression may involve naturally occurring bacteria, such as species of *Pseudomonas* (Sturtz et al., 1986). The potential for suppression of black root rot by the species of *Pseudomonas* associated with bacterial stunt of cotton (CRDC projects DAN7C and DAN17C) was investigated in pot experiments.

The potential for interaction between *T. basicola* and seedling mortality (hence, *Rhizoctonia* and *Pythium*) in the field was assessed in several of the observational studies and experiments that were conducted to develop control measures for black root rot in this project.

Management of other causes of slow early season growth

Slow early season growth of cotton can also be caused by bacterial stunt or a lack of mycorrhizal (VAM) development. Field experiments were used to investigate the potential for practical deployment of methods to enhance early season seedling growth, including cereal cover crops, and bioassays and 'nurse' crops to assess and improve the mycorrhizal status of soil prior to growing cotton.

RESULTS AND DISCUSSION

(i) Epidemiology of black root rot

Assessment of symptoms and effects on yield

The most obvious symptom of black root rot is stunting of cotton plants during the early part of the season. Frequently the disease is first noticed as patches of slow growing cotton. In subsequent seasons the patches may become less obvious as the pathogen spreads throughout the field. The stunting is strongly correlated with the severity of black root rot (Table 1). Diagnosis of black root rot requires examination of the roots. *T. basicola* causes characteristic blackening of the roots due to destruction of the root cortex (Figure 1).

Table 1. Decreased cotton growth and maturity caused by black root rot in a field near Wee Waa.

	Severity of black root rot		
	Less severe	Severe	Probability
10 December 1999			
T. basicola (cfu/g soil)	414	648	$p = 0.012$
Disease severity index (0-10 scale)	0.6	6.1	$p < 0.001$
Healthy lateral roots (No./plant)	9.0	1.0	$p < 0.001$
Plant stand (plants/m)	12.7	11.1	$p = 0.019$
Plant height (mm)	232	72	$p < 0.001$
Nodes per plant	12.1	8.3	$p < 0.001$
Shoot dry matter (g/plant)	5.0	0.6	$p < 0.001$
25 January 2000			
Fruit development (bolls/m)	27	0.5	$p < 0.001$

Despite severe stunting of cotton at the site near Wee Waa, plant stand was only reduced slightly by the presence of black root rot (Table 1). At a site near Pilliga where black root rot was severe and the population of spores in the soil was 1200 to 1400 cfu/g, plant stand was not particularly low. Observations at these sites and others have clearly indicated that *T. basicola* does not generally kill cotton seedlings by itself; seedling death is associated with other seedling pathogens, namely *Rhizoctonia* and *Pythium*. Although some lateral roots may die, *T. basicola* does not usually enter the vascular tissue of cotton and the tap root survives (Figure 1).

As the growing season becomes warmer, affected cotton plants resume growth and the expanding roots slough off the dead cortical tissue (Figure 1). Infection of lateral roots by *T. basicola* will occur throughout the season if conditions are cool. A proportion of older plants may develop an internal stem rot if black root rot is severe (Figure 1). The internal stem rot appears to occur when infection spreads from smaller lateral roots to the centre of the tap root (Figure 1). This internal stem rot contributes further to the survival of *T. basicola* in the field and may enhance the spread of the fungus in floating trash.

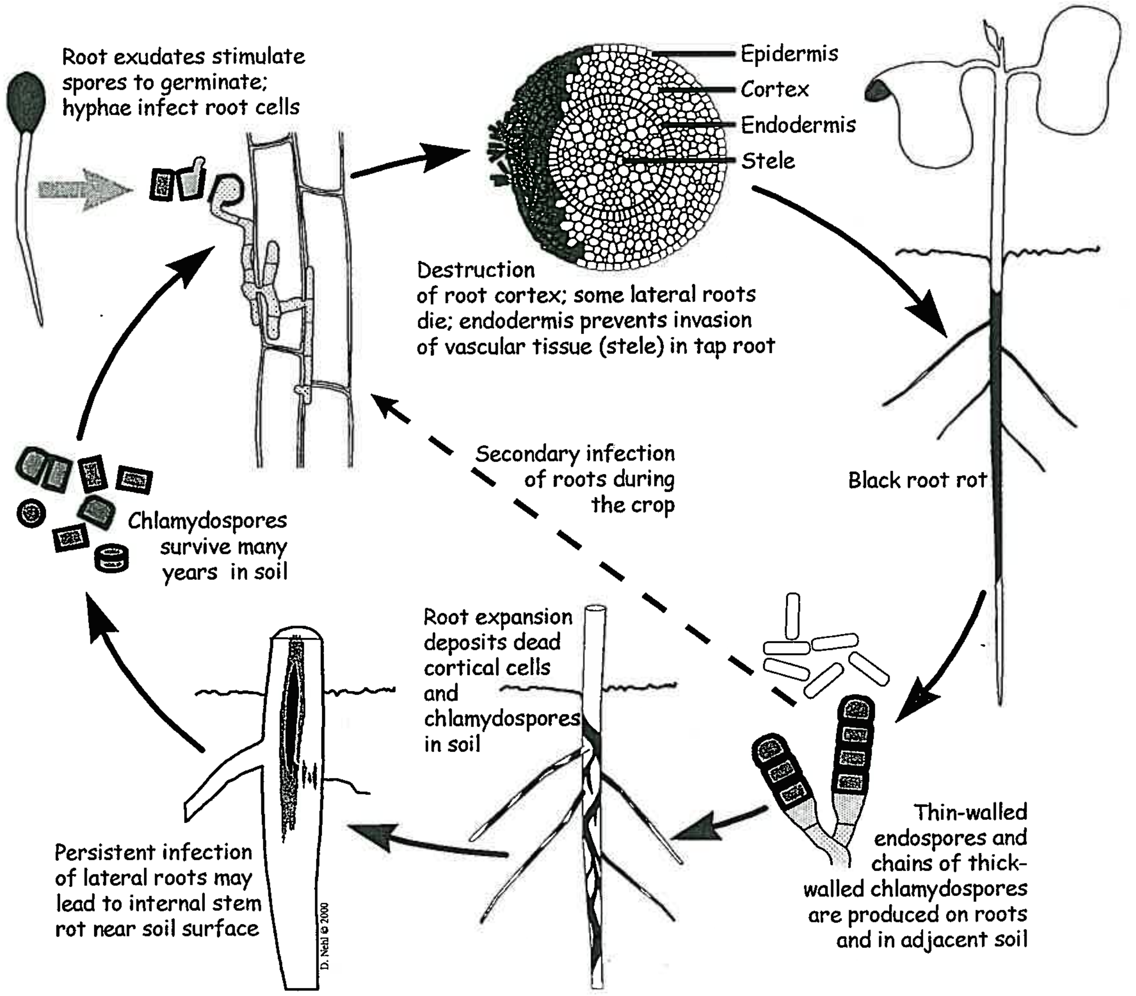

Figure 1. The life cycle of the black root rot fungus, *Thielaviopsis basicola*, in cotton.

Quantification of the effect of black root rot on yield is difficult because replicated plots with and without the pathogen are difficult to generate (few farmers would be willing to have their soil inoculated with *T. basicola*, even for experimental purposes). As an alternative we measured the effects of black root rot on cotton growth, maturity and yield using paired sites, with high and very high populations of *T. basicola* respectively (Table 1). The slight reduction in plant stand in the plots with severe black root rot (Table 1) was not sufficient to affect yield. Black root rot was present throughout the field. The very high population of *T. basicola* and resulting disease were sufficient to delay fruit development and seed cotton maturity by approximately three weeks, compared to areas of the field where the disease was not as severe (Table 1, Figure 2). The result was a 26% loss in yield (Figure 2).

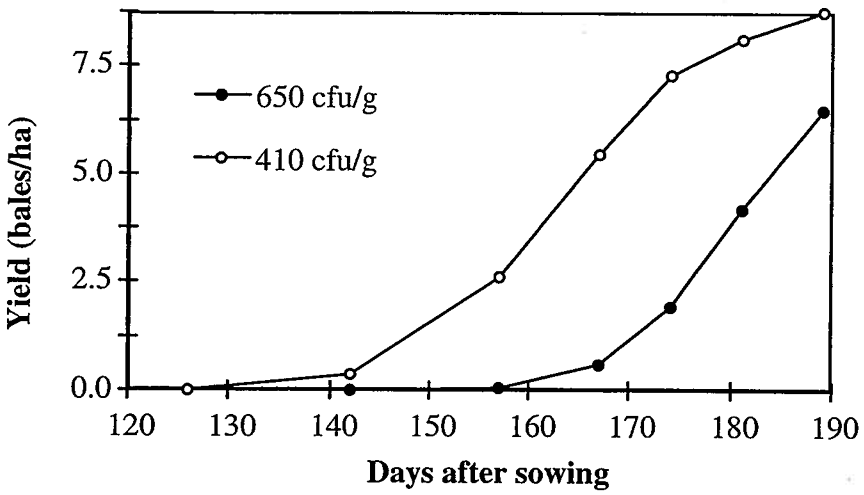

Figure 2. Delayed maturity and reduced yield of cotton caused by black root rot. Data is from replicated sites in a field near Wee Waa (see Table 1) with high (410 cfu/g soil) and very high (650 cfu/g soil) populations of *Thielaviopsis basicola* in the soil. Yield values were calculated from hand-picked seed cotton mass and adjusted to reflect the mechanically harvested yield for the whole field. (cfu =colony forming units)

The yield loss at the Wee Waa site (Figure 2) only occurred in those patches with a very high spore population. However, yield losses are potentially much higher. At the site near Pilliga the grower estimated that black root rot caused a yield loss of 1.5 bales/acre across approximately 50% of each of two fields in the 1999/00 crop. Although the severity of black root rot was not assessed early in the season, the Pilliga soil contained extremely high populations of *T. basicola*; 1200 to 1400 spores/g soil.

In the Cotton CRC farming systems trial at Warren, yield declined in proportion to the number of cotton crops, irrespective of rotation treatments (Figure 4b). This decline mirrored the increase in black root rot severity (Figure 4a). It is possible that other soil factors may have contributed to this yield decline but, so far, nothing other than black root rot severity has been shown to be correlated with the yield decline. Soil compaction was not related to the yield decline because compaction was worse in some of the rotation treatments than in the continuous cotton treatment (N. Hulugalle, personal communication). Similarly, measurements of soil compaction, using paint infiltration of soil cores, at the Wee Waa site were not correlated with the degree of stunting and yield loss (Nehl and Hulugalle, unpublished data).

Geographic distribution of black root rot

Black root rot was first observed in Australian cotton in 1989 (Allen, 1990). Annual disease surveys conducted by NSW Agriculture have shown an exponential increase in the number of fields with black root rot in NSW (Figure 3). Black root rot has now been recorded on 93% of the farms surveyed in the Macintyre, Gwydir, Namoi and Macquarie valleys (Figure 3a). The disease was observed in 65% of fields surveyed in these valleys in November 2000 (Figure

3b). The disease now occurs in all cotton growing regions of NSW except Menindee. Black root rot is also widespread in south west Queensland and the Darling Downs (J. Kochman, personal communication).

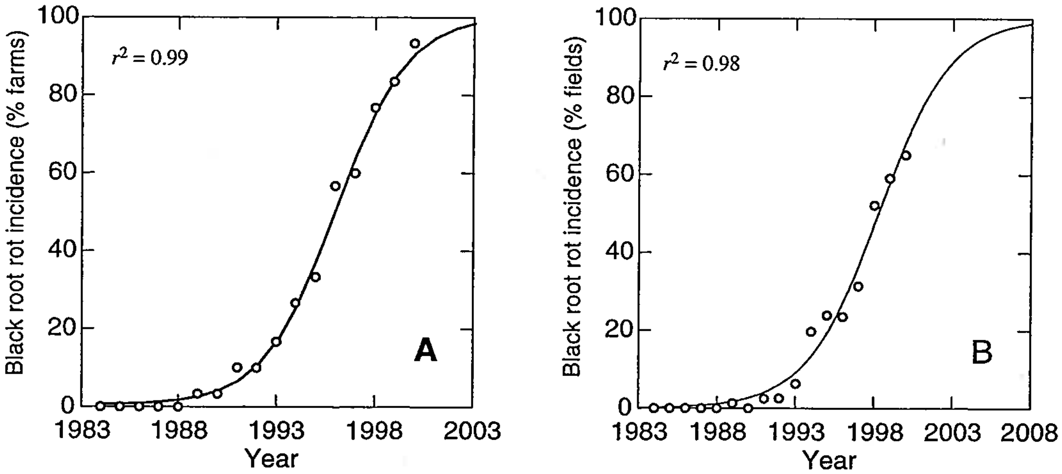

Figure 3. Observed (circles) and predicted (curves) increase in the distribution of black root rot in irrigated cotton in the Macintyre, Gwydir, Namoi and Macquarie valleys.

Local dispersal of *T. basicola* in irrigation water and in soil adhering to vehicles and implements is likely to be a major factor that has contributed to the increasing frequency of black root rot within farms and districts (see section on dispersal below). The factors involved in the spread of *T. basicola* between districts or regions are less conclusive (see section genetic diversity below).

Survival and dispersal of *T. basicola*

Dispersal in irrigation water

In March 2000, samples of irrigation water and floating crop residue were taken from the tail ditch of a field that was heavilly infested with *T. basicola* and from a tail water return channel at various distances from the field. The population of *T. basicola* was enumerated with the TB-CEN agar method by sampling water directly from the tail ditch or by sampling soil that was washed off the floating trash.

Large quantities of spores were leaving the field suspended in the irrigation water (Table 2). The population density of *T. basicola* in mud washed from floating trash was lower on trash collected at the drop box (mostly dead flowers at the time) than on trash collected at the return channel (mostly old stalks). The latter sample was from a main return channel system and included trash from other fields, which may account for the lower population density. Although spores of *T. basicola* suspended in tail water appear to settle with increasing distance from cotton fields (Table 2), soil adhering to floating trash clearly makes *T. basicola* very mobile in both irrigation and flood waters.

Table 2. Density of *Thielaviopsis basicola* in irrigation water and in soil adhering to floating cotton residues on a farm near the Australian Cotton Research Institue, Narrabri (cfu = colony forming units)

	In water		On floating trash	
	Drop box	Return channel	Drop box	Return channel
Million cfu/megalitre	175	46	-	-
Cfu/kg trash	-	-	11750	2671
Distance from field (m)	0	700	0	2000
Water flow	Fast	Medium	Fast	Very slow

Dispersal in soil

Soil samples were collected from the wheel arches of vehicles visiting the Australian Cotton Research Institute on two occasions and assayed for the presence of *T. basicola*. In 1998, three of sixteen vehicles sampled were carrying *T. basicola*. In 2001, three of five vehicles sampled were carrying *T. basicola*. Movement of infested soil adhering to shoes (see Farmcleanse section below), equipment and vehicles is an important avenue for the further spread of *T. basicola* at district, regional, national and international levels.

The expansion of a small patch of black root rot, that was first identified in 1993 in a field near the Australian Cotton Research Institute, was monitored by assessing spatial distribution of black root rot. The diameter of this patch increased successively with each crop of cotton (Table 3). The increase in the size of the patch in the direction of irrigation flow and cultivation can be explained by movement of the pathogen with soil or water. However, the expansion of this patch perpendicular to the rows, during the period when the field was managed with a permanent bed system, indicates that *T. basicola* was spreading by other mechanisms; either by root to root contact, by rain-splash of soil, in soil thrown across beds by cultivation, or a combination thereof.

Table 3. Expansion of single patch of black root rot in a field near the Australian Cotton Research Institute, Narrabri. Following flood damage in 1996/97, the field was redeveloped in 1997/98.

	Width of patch (m)		
	1993	1995	1998
Parallel to rows	41	56	>195
Perpendicular to rows	24	45	>210

The flooding in 1996 and redevelopment of this field in 1997/98, clearly increased the spread of *T. basicola* (Table 3). In the 1999/00 season, black root rot was easily observed in volunteer cotton plants growing along the roadway adjacent to the tail ditch of this field. This indicates that with the practice of grading soil from a tail ditch onto the adjacent roadway, infested soil is then available to be 'picked up' by vehicles or cotton modules, even without entry into the cropped part of the field. The same principle is likely to apply for the spread of inoculum in other diseases, such as Fusarium wilt.

Potential for survival in composted gin trash

In recent years there has been increasing interest in composting gin trash for use as a soil amendment, with cotton farms being a potential end-user. Using TB-CEN, *T. basicola* was not detected in several samples of composted gin trash, sourced from the Wee Waa district. Since *T. basicola* does not generally infect the upper parts of the cotton plant, the risk of it being present in gin trash is less than that for the pathogens causing foliar diseases and vascular wilts. However, *T. basicola* was detected in a sample of composted gin trash that was tested by pathologists at the Queensland Department of Primary Industries (W. O'Neill, pers. Comm.), indicating that dispersal of the pathogen in composted gin trash was possible. Soil adhering to the base of modules is a likely pathway for entry of *T. basicola* into gin trash.

To examine the potential for sterilising composted gin trash using heat, replicated samples of moist, composted gin trash were artificially infested with *T. basicola* and subjected to a range of temperatures for varying periods of time. Saprophytic fungi were completely eliminated by heat treatment at 60°C or more for 10 minutes (Table 4). The results suggest that heat treatment of composted gin trash for at least 10 minutes at 60°C or more will be sufficient to eliminate most fungi. Nevertheless, the effectiveness of heat sterilisation treatments need to be confirmed *in situ* with compost treated under commercial conditions.

Table 4. Survival of fungi in heat-treated composted gin trash.

Total fungal colonies on potato dextrose agar

	Heat treatment duration (min)			
	10	15	20	25
Room temperature	3000	-	-	-
50°C	2270	3530	3380	2880
60°C	0	0	0	0
70°C	0	0	0	0
80°C	0	0	0	0
90°C	0	0	0	0

Farming systems experiments

Cotton CRC Farming systems trial at Warren

This experiment demonstrated that once *T. basicola* became established, the severity of black root rot increased according to the number of cotton crops grown, irrespective of the number of fallows or rotations with cereals. Caution in interpretation of the data is required. If the results from each year are examined individually, then rotation with wheat initially appears to be controlling black root rot, giving lower levels of disease than in continuous cotton in 1994, 1996 and 1998 (Table 5). However, evaluation of the trends over time revealed that the severity of black root rot actually increased at a greater rate in the cotton/wheat rotation than in continuous cotton (Table 5). Plotting this trend using data from all the treatments clearly shows that the severity of black root rot increased in proportion to the number of cotton crops, irrespective of fallows or rotations (Figure 4a). The incidence of black root rot (data not presented) was correlated positively with its severity in 1994, 1995 and 1998 (r^2 = 0.81, 0.76 and 0.75 respectively, $p < 0.001$, $n = 21$). In 2000, 100% of plants had black root rot.

Table 5. The severity of black root rot of cotton after rotation with cereals and legumes in the Cotton CRC Farming Systems Experiment at Warren.

Rotation treatment[w] (1993, 1995, 1997)	Disease severity (0-10 scale) 1994	1996	1998[x]	2000
Cotton	7.9a	8.3a	9.7 (0.31)a	9.5
Fallow	2.0ab	5.9ab	6.2 (1.21)b	7.9
Field pea	1.4b	6.2ab	7.7 (1.00)ab	8.5
Low input wheat	3.3ab	4.7ab	5.6 (1.19)b	7.4
High input wheat	2.4ab	3.4b	5.5 (1.23)b	7.0
Lablab, wheat, cotton	4.2ab	5.1ab	8.3 (0.90)ab	8.2
Faba bean	3.5ab	2.4b	6.4 (1.14)b	8.2
[z]Probability ($n = 3$)	$p = 0.032$	$p \leq 0.023$	$p \leq 0.048$	NS

[w] Wheat was sown across the whole field in 1999.

[z] Means followed by the same letter are not significantly different by pairwise comparison using the Scheffé test. NS = not significant.

[x] Data in brackets were transformed for normality as $\sqrt{\ln(k-x)}$, where k = the highest value + 1.

The increase in disease severity (Figure 4a) was reflected by declining yields (Figure 4b). Regression of the pooled data from 1994, 1996 and 1998 (r^2 = 0.414, p =0.002), indicated that 41% of the variation in yield could be explained by the severity of black root rot. However, the correlation coefficient for this regression may be artificially low because yield was measured for all the plants in each plot (>2 ha) while disease severity was assessed in only 25 plants from each plots. In the 2000/01 season, yields in the experiment ranged from 7.1 ba/ha to 8.6 ba/ha, reflecting the long period of warm weather that occurred after November. It is clear that black root rot contributed substantially to yield losses in this experiment.

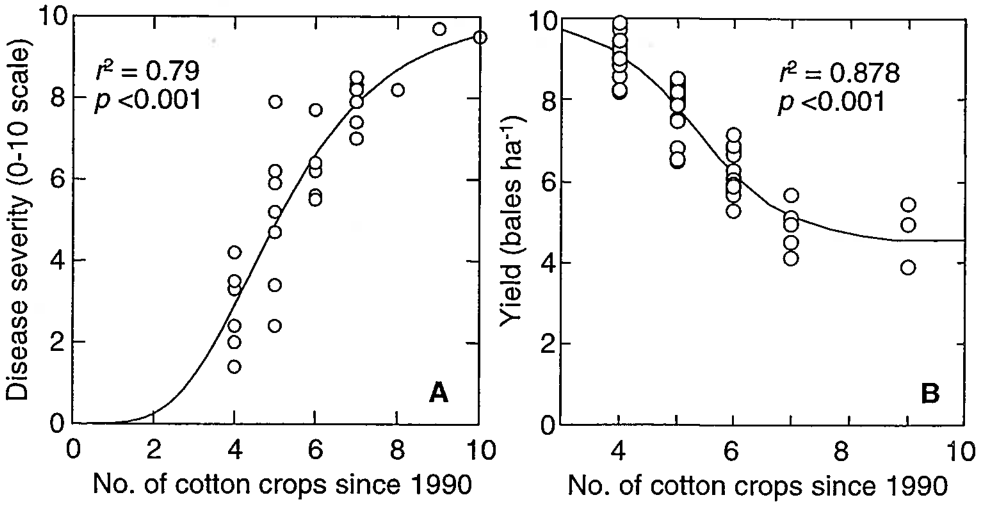

Figure 4. Increase in black root rot severity (a) and decrease in cotton yield (b) with repetitive cotton cropping in the CRC farming systems trial at Warren (treatments are as in Table 5).

Farming systems experiment at ACRI

In the long term farming systems experiment conducted by NSW Agriculture at the Australian Cotton Research Institute since 1985, the incidence of black root rot in 1998 was 100 % in all three treatments. The severity of black root rot was similar between the continuous cotton treatments and approximately double that of the wheat rotation treatment (Table 6). Although seedling growth in the minimum tillage/continuous cotton treatment was similar to that in the wheat rotation treatment, the growth of cotton seedlings was negatively correlated with the severity of black root rot ($r^2 = 0.61$, $p = 0.003$, $n = 12$).

Table 6. Effect of tillage system and rotation with wheat on cotton seedling growth and black root rot severity at six weeks after sowing, and cotton yield.

Rotation sequence	Tillage	Black root rot disease index^z	Shoot dry matter (g/plant)	Lint yield (kg/ha)
Cotton cotton	Maximum	8.7a	0.21b	750c
Cotton cotton	Minimum	7.2a	0.29a	877b
Cotton wheat	Minimum	4.4b	0.33a	1002a
	^{y}Probability	$p <= 0.006$	$p <= 0.026$	$p <= 0.05$

[z] Proportion of blackened tap root, rated on a scale of zero to 10.

[y] Means followed by the same letter are not significantly different by pairwise comparison using the Scheffé test.

Since the initial onset of black root rot in this field, the wheat rotation treatment would have had only half the number of cotton crops as in the other treatments. Hence, the lower disease severity in the wheat rotation treatment (Table 6) is consistent with the observation at Warren, that the severity of black root rot increases in proportion to the number of cotton crops grown (Figure 4), irrespective or rotation with cereals.

Host range

T. basicola, has been recorded as a pathogen in over 137 species of plants (Honess et al., 1994). Non-hosts include all the cereals, sunflower, brassicas such as canola and broccoli, onions and woolly pod vetch. Cereals are not hosts for *T. basicola*. However, most of the legumes that are currently used in rotation with cotton became infected with *T. basicola* when sown in infested soil from cotton fields (Table 7). Although soybean is very susceptible to *T. basicola* in pots under controlled conditions (Table 7), soybean crops are generally sown in December when conditions are not favourable for infection by the fungus. The winter legumes are more likely to increase the population of *T. basicola* in the soil because soil temperatures are likely to be more favourable for disease.

Velvetleaf (*Abutilon threoprastii*), and thornapple (*Datura* sp.) were found to be hosts to Australian strains of *T. basicola* by Prof. C. Rothrock (Appendix II) but little is known about the susceptibility of other weeds.

Table 7. Pathogenicity of *T. basicola* on legumes sown in infested soil from cotton fields, under controlled environment conditions.

Species	Common name	Variety	Host status[z]
Cajanus cajan	Pigeon pea	Quest	+++
Cicer arietinum	Chickpea (Desi)	Amethyst	+
	Chickpea (Kabuli)	?	+++
Glycine max	Soybean	BFC 205	+++
Lablab purpureus	Lablab	Dolichos	+
Phaseolus vulgaris	Green bean	Redlands' pioneer	++
Pisum sativum	Pea	Greenfeast	+
Vicia faba	Faba bean	Barkool	+
		Fiord	+
		Icarus	+
Vicia villosa	Woolly pod vetch	Namoi, Capello	-
Vigna radiata	Mung bean	Berken	+++
Vigna unguiculata	Cowpea	Poona	+++

[z] Severity of infection ranked as high (+++), medium (++), low (+) and nil (-).

Black root rot is a serious disease of tobacco and *Nicotiana debneyi* has a single gene for resistance to *T. basicola*. However, when a range of tobacco varieties with varying levels of resistance to *T. basicola* were sown in soil from a cotton field where black root rot was severe, all were completely resistant (Table 8).

Table 8. Pathogenicity of *T. basicola* on tobacco grown in infested soil from a cotton field, under controlled environment conditions (18-23°C).

Species	Variety	Resistance level	Disease Severity (0-10 scale)
Nicotiana tabacum	Virginia gold	Susceptible	0
	Hicks Q46	Susceptible	0
	Wislica	Tolerant	0
Nicotiana debneyi	TS98	Resistant	0
	L3667	Resistant	0
	L3457	Resistant	0
	W392-2	Resistant	0

The absence of symptoms on tobacco (Table 8) suggests that the strains of *T. basicola* affecting cotton in Australia are adapted for cotton, not tobacco. The implications are twofold. First, any future attempt to transfer the resistance gene from N. debneyi to cotton is likely to meet with failure. Secondly, the host specificity of *T. basicola* will be an important consideration in devising disease management strategies for black root rot (see section on population diversity and below).

The potential for *T. basicola* to reproduce on different hosts will vary according to the host specificity of the strains. In collaboration with this project, the reproductive potential of *T. basicola* on a number of hosts was investigated by Professor Craig Rothrock, while visiting ACRI on sabbatic leave in 1999 (Appendix II). Under favourable environmental conditions, strains of *T. basicola* from Narrabri reproduced in enormous quantities on cotton roots (Appendix II). In most cases reproduction was far greater than on other plants, indicating that the strains of *T. basicola* found in cotton fields are adapted for cotton.

Population diversity and pathogenicity

The pattern of geographic dispersal of black root rot in Australia has been consistent with the introduction of the pathogen to regions of previous absence. However, the pathogenicity of strains of *T. basicola* can be specific for the host from which they are isolated and not others, although this host specificity is not well documented in the scientific literature. Since a variable degree of host specificity evidently exists within the genome of *T. basicola*, it is also possible that continuous cotton monoculture in Australia has selected for virulence in a population that was previously endemic in the Australian ecosystem. If such selection has occurred, then the genes for virulence would need to have been widely dispersed within the population, albeit at low levels. The current epidemic of black root rot in Australian cotton may represent either the introduction and subsequent geographic dispersal of *T. basicola* or the evolution of virulent strains and their subsequent increase within pre-existing populations.

Research undertaken at the University of Queensland may help answer this question. In 1999, replicated samples of soil from cotton fields infested with *T. basicola* at Goondiwindi, Warren and Narrabri were provided to a Postraduate Diploma in Science student. Within the fields at Goondiwindi and Warren the strains were geneticially similar, with similarity coefficients being greater than 95% (Pattermore, 1999). However, there was a low similarity (coefficient of 63%) between strains from the Goondiwindi and Warren sites. Strains from Narrabri were related variously to the populations at Goondiwindi and Warren. Hence, the isolates of *T. basicola* sourced from cotton show a high level of genetic diversity and form distinct groups based on geographic origin within Australia. Isolates collected from lettuce or peat formed a distinct genetic sub-group which lies within the overall grouping of cotton isolates (Pattermore, 1999). On lettuce, genetically identical isolates have been obtained over a large geographic area indicating that inadvertent dispersal by man may be involved (e.g. by machinery or seedlings).

Investigation of the genetic diversity and pathogenicity of *T. basicola* in Australia is continuing in the CRDC funded Postgraduate Scholarship CRC29C.

Suppressive and conducive soils

Anecdotal observations indicate that the severity of black root rot varies with soil type, being more severe on medium clay soils than on very heavy clays or lighter soils. In a field at Goondiwindi the severity of black root rot was significantly correlated with a number of soil properties (Table 9). In a field near Narrabri, infection of cotton by *T. basicola* was also correlated significantly with a range of soil properties (data not presented).

While the association of disease severity with certain soil properties confirms that soils may be conducive or suppressive to *T. basicola*, it does not prove a 'cause and effect'. Although the water holding capacity of soil increases with increasing clay content, the data do not show a strong association with black root rot. Infection of cotton by *T. basicola* at the Narrabri site was suppressed in very heavy clay soils, where the water holding capacity was greatest. The microbial diversity of the soil may be equally as important as its physical and chemical properties.

Table 9. Relationships between the severity of black root rot and soil properties, cotton growth and mycorrhizal development.

	Correlation coefficient	Probability
pH	0.75	<0.001
Electrical conductivity	0.71	0.001
Clay content	0.59	0.010
Organic matter	0.26	Not significant
Bicarbonate extractable P	0.30	Not significant
Exchangeable Ca	0.79	<0.001
Exchangeable K	0.71	0.001
Exchangeable Na percentage	-0.56	0.017
Shoot dry matter	-0.88	<0.001
Mycorrhizal colonisation (VAM)	-0.69	0.002

Further research on the role of soil microbial diversity ('soil health') in soils that are suppressive or conducive to black root rot is the subject of the CRDC postgraduate scholarship CRC18C, with the University of Sydney, NSW Agriculture and the University of New England.

(ii) Management of black root rot

Host resistance

A number of Australian cotton varieties and breeding lines were screened for resistance to black root rot in heavily infested fields near ACRI in 1994 and 1998 (Table 10). All cotton varieties in Australia appear to be equally susceptible to *T. basicola*. No sources of resistance have been identified pathologists in the USA.

Table 10. Field trials screening for resistance to black root rot in commercial varieties and breeding lines of upland and Pima cotton (data for Trial 1 courtesy of S.J. Allen, DAN 69C)

Line/variety	Disease severity (0-10 scale)	Line/variety	Disease severity (0-10 scale)
Trial 1, 1994 (no significant differences)			
SP37	9.8	Siokra 1-4/649	9.5
CAMDE	9.9	Siokra L22	9.9
CABCS	9.8	Siokra L23	9.9
HQ95	9.9	Siokra V15	9.8
CD3H	9.9	Sicala V2	9.7
Acala SJ2	9.7	Sicala 3-2	9.8
Acala Maxxa	9.6	CS 8S	10.0
Sipreme (E15)	9.9	CS 50	9.8
Siokra S324	9.8	CS 189+	9.9
Trial 2, 1998 (no significant differences)			
330	8.4	5690	7.3
2137	8.6	5816	8.2
2190	8.4	Emerald	8.3
3163	8.4	Jewel	8.2
3170	7.9	Opal	8.7
5415	7.9	Pearl	7.4
Trial 3, 1998 (no significant differences)			
56	8.5	436	8.4
224	6.7	441	8.2
225	7.3	442	7.9
226	8.4	444	8.4
228	6.7	449	7.1
338	8.1	459	9.1
339	9.0	465	7.5
340	7.8	470	7.5
341	7.9	471	8.1
342	8.8	472	8.0
343	8.7	649	8.6
389	7.5	Sicala V2	7.8

Transfer of resistance genes from wild species of cotton may be possible but offers no short-term prospects. In the absence of adequate sources of resistance in *Gossypium hirsutum* and *Gossypium barbadense*, an alternative approach would be selecting cultivars that may be managed for performance later in the season (i.e. disease tolerance).

Biofumigation

The concept of biofumigation involves planting a crop that releases compounds that are toxic to pests or pathogens in the soil. Conventional soil fumigation is not a practical option for cotton diseases in Australia. The high clay content in many of the soils used to grow cotton prevents adequate penetration of conventional fumigants. Biofumigation offers a safe, self-generating method of distributing a fumigant throughout the soil profile. Biofumigation involves growing and harvesting the biofumigant plant as either a rotation crop or as a 'sacrificial' crop that is incorporated into the soil prior to planting cotton.

Biofumigation experiments were conducted in several fields over three seasons (Table 11). While the results vary, it must be stressed that the dry matter production in the biofumigant crop and the success of its incorporation will determine the effectiveness against *T. basicola*. Furthermore, the population of *T. basicola* can vary greatly within plots and experiments, adding errors to the spore population data and masking treatment effects. However, biofumigation clearly has potential to reduce the severity of black root rot (Table 11).

Table 11. Summary of results of biofumigation field experiments using Indian mustard and woolly pod vetch for control of black root rot in cotton.

Trial	Crop	Difference from control (%)			
		Disease severity	Spore numbers	Cotton growth	Maturity
Narrabri 98/99	Vetch	-61	-59	NS	ND
Narrabri 98/99	Mustard	-56	ND	NS	ND
Moree 99/00	Mustard	-34	NS	26	26
Narrabri 99/00	Mustard	-31	-65	22	ND
Narrabri 99/00	Vetch	-23 NS	NS	NS	ND
Walgett 99/00	Vetch	ND	-24	ND	ND
Warren 99/00	Mustard	-29	-21	47	26
Warren 99/00	Mustard	-70	-88	19	19
Warren 99/00	Vetch	-38	NS	NS	1NS
Narrabri 00/01	Vetch	-17NS	NS	14	ND
Narrabri 00/01	Mustard	-28	NS	19	ND
Moree 00/01	Vetch	-24	ND	54	ND

NS = Not statistically significant. ND = Not determined

1998/99 biofumigation experiments

A range of plant species were screened for their potential as biofumigation crops for black root rot. In a field experiment in 1998 near Wee Waa, several species from the brassica family (Brassicaceae) were planted in small hand-sown plots (Figure 5). The varieties were:

Karoo	Conventional canola (*B. napus annua*)
Fodder	Fodder rape (*B. napus biennis*)
Ultimo	German fodder radish (*Raphanus sativa*)
Maxi	German white mustard (*Sinapsis alba*)
Indian 651	Indian mustard (*B. juncea*)

Figure 5. Brassicas used for biofumigation to control black root rot in cotton.

Unfortunately winter rains delayed termination of the mustard, radish and canola crops. The plots were sprayed with glyphosate in mid-September and then incorporated (Lillistons) on 29 Sept, one week before planting cotton (Nucotn 37 with QAGP plus Baytan^{TM} seed dressing). Ideally these crops should have been ploughed in before going to seed and at least a month before sowing cotton. Stand establishment was 10 to 11 plants per m in all treatments except Indian 651 and Maxi where it was 8 plants per m. Such stand loss was not observed in other trials with Indian mustard and may reflect the fact that these plants were incorporated one week before sowing. At three weeks after sowing, the severity of black root rot in the Indian mustard 651, Maxi and Ultimo treatments was reduced by half (Figure 6).

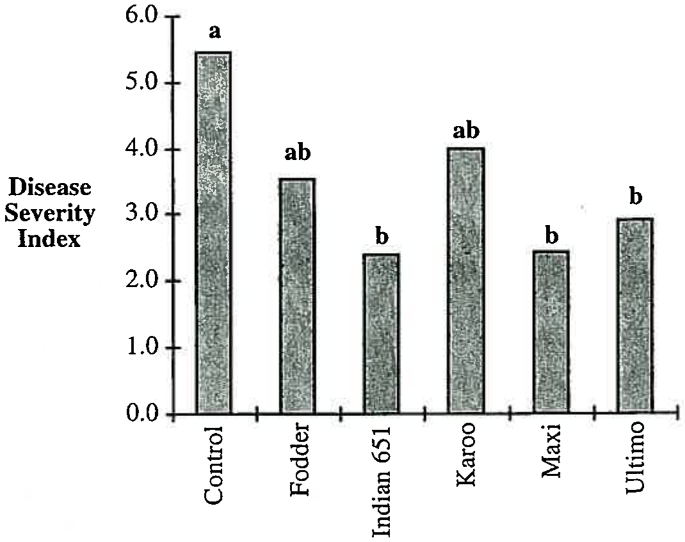

Figure 6. Effect of biofumigation crops on black root rot of cotton at 23 days after sowing. Bars with the same letter are not significantly different ($p <= 0.024$).

Seven days later the 50 % reduction in black root rot due to biofumigation was maintained in the Indian 651 plots (Figure 7). While disease in the other biofumigation plots was lower than in the untreated control, it was not statistically significant.

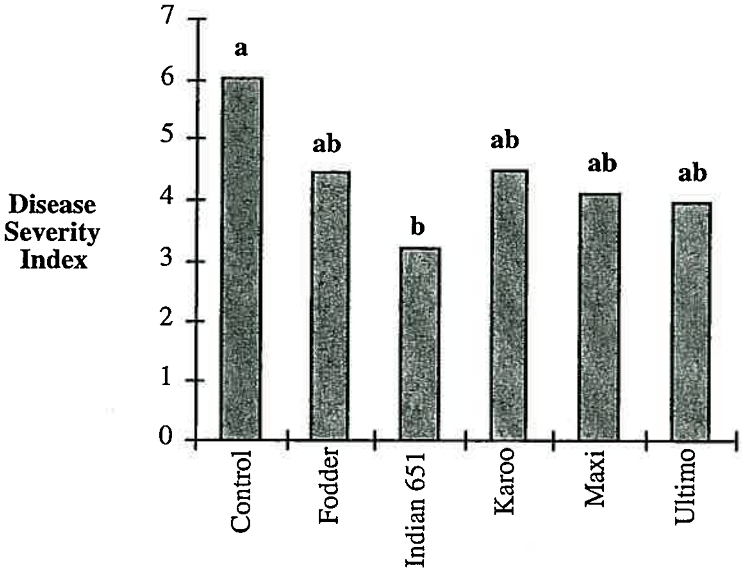

Figure 7. Effect of biofumigation crops on black root rot of cotton at 30 days after sowing. Bars with the same letter are not significantly different ($p = 0.009$).

Despite the reductions in black root rot severity, there were no significant differences in cotton seedling growth between any of the biofumigation crops. The reduced emergence of cotton in the plots with the best control of black root rot (Indian 651 and Maxi) suggests a phytotoxic effect by the mustard that may have masked any benefit to growth arising from less black root rot. The observation of increases in the growth of cotton following biofumigation with Indian mustard in other experiments (Table 11), indicates that mustards will not be phytotoxic to cotton if incorporated early enough (three to four weeks before cotton).

In the same field near Wee Waa, Woolly pod vetch (*Vicia villosa*) was sown in plots 12 rows wide by 40 m long on 30 June 1998. Two rows of vetch were sown along the top of the beds, 10 cm apart, at the rate of 15 seeds per m, resulting in a final seed density of 30 seeds per m. The vetch grew densely with the winter rain, was sprayed with glyphosate in mid-September, and incorporated on 29 September, one week before sowing cotton (Nucotn 37 with QAGP plus Baytan^{TM} seed dressing). Plots with and without vetch were split with application of the fungicide Baytan^{TM} as an in-furrow spray during cotton planting. In a second experiment in the same vetch plots, the fungicide Folicur was applied and an in-furrow spray during cotton planting, and in a third the fungicide Baytan was applied as a cotton seed dressing.

As with some of the Indian mustards, the late incorporation of vetch had a phytotoxic effect on the cotton, reducing stand emergence significantly. This is not surprising since the vetch had a large biomass and was still rotting away just below the soil surface when the cotton was planted into it. This phytotoxic effect has been reported when vetch is used for biofumigation in the USA and can be prevented by ensuring that the vetch is incorporated three to four weeks prior to sowing cotton. However, the phytotoxic effect of vetch on cotton was present in the rows used for the Baytan experiment but *not* in the rows used for the Folicur experiment (Figure 8). Hence, either the depth of planting or the depth of incorporation of vetch led to the phytotoxic effect.

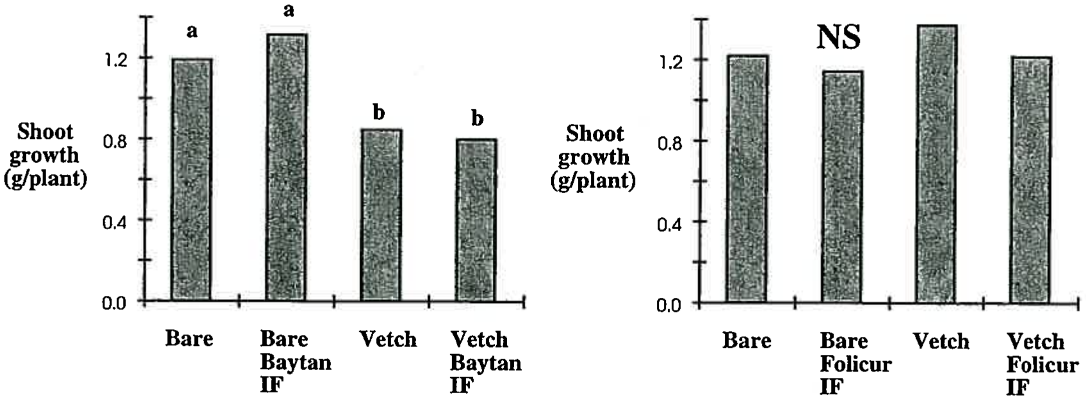

Figure 8. Effect of woolly pod vetch used as a biofumigation crop, and in-furrow fungicides, on growth of cotton at 54 days after sowing. Bars with the same letter are not significantly different. NS = not significant.

Notwithstanding the phytotoxic effect, the vetch treatment led to a large reduction in the severity of black root rot (Figure 9, Figure 10). Baytan also reduced the severity of black root rot when applied as an in-furrow spray. As with the mustards, the phytotoxic effect of the vetch appeared to mask any potential increase in early season cotton growth.

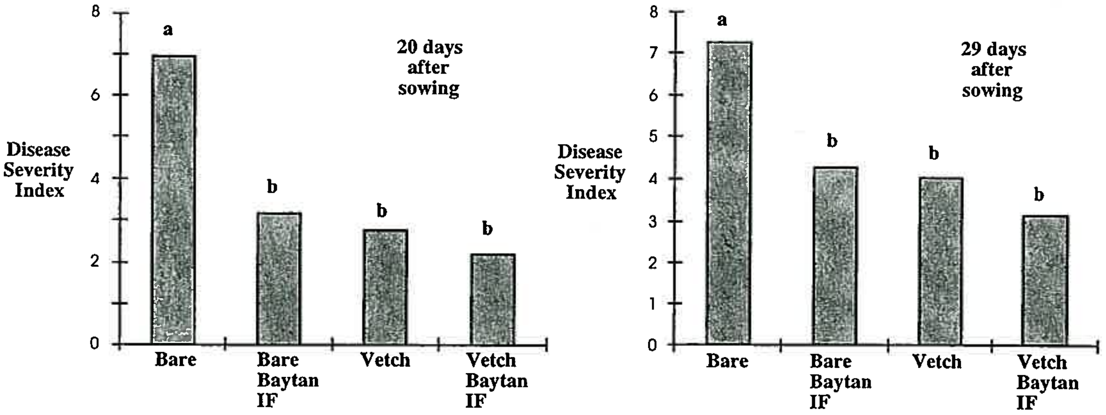

Figure 9. Effect of woolly pod vetch used as a biofumigation crop, and Baytan^{TM} fungicide applied as an in-furrow spray (IF), on black root rot of cotton. Bars with the same letter are not significantly different.

Baytan was not effective against black root rot when applied as a seed dressing (Figure 10).

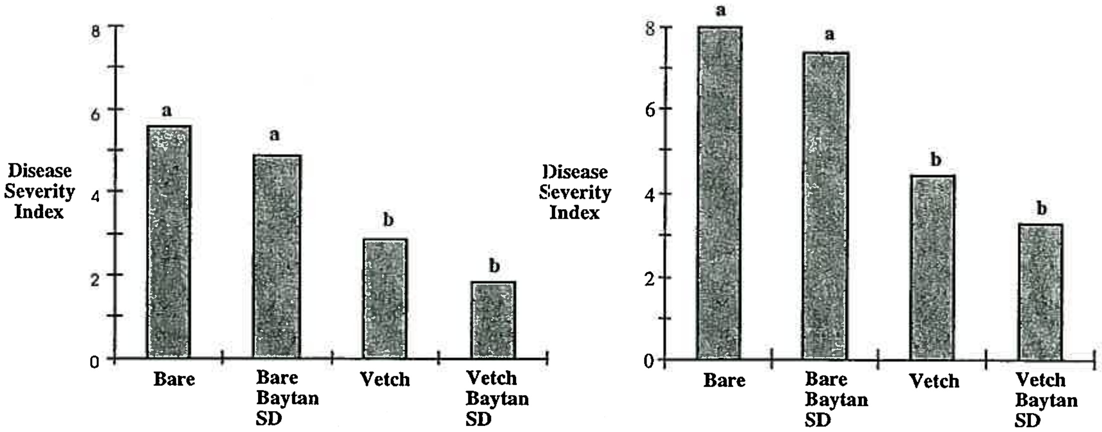

Figure 10. Effect of woolly pod vetch used as a biofumigation crop, and Baytan^{TM} fungicide applied as a seed dressing (SD), on black root rot of cotton at 20 days (LHS) and 29 days (RHS) after sowing. Bars with the same letter are not significantly different.

N.B. Reference to any proprietary names or products here does not represent endorsement of such products by NSW Agriculture.

1999/00 biofumigation experiments

At a farm near Moree in the Gwydir valley, large scale plots (10 × 700 m) were established with or without Indian mustard, during winter of 1999. Shoot dry matter production of the mustard was 1700 kg ha^{-1} and this was incorporated several weeks before sowing cotton. Indian mustard decreased disease severity and this led to an increase in boll production (Table 12). Although the disease severity was assessed on a scale of 1 to 10 for the length of tap root that was blackened, the intensity of root discolouration was less intense than that usually observed with black root rot of cotton, and similar to that observed with bacterial stunt of cotton (caused by deleterious rhizosphere bacteria).

Table 12. Indian mustard 651 as a biofumigant against black root rot of cotton in a field near Moree.

	Bare	Mustard	Probability	% difference
12.11.99				
Disease severity (0-10 scale)	7.2	4.5	*p*<0.001	-38
Healthy lateral roots (No./plant)	7.2	12.6	*p*=0.003	74
Stand (plants/m)	12.1	9.8	NS	-
Shoot dry mass g/plant)	0.19	0.20	NS	-
10.2.00				
Fruit maturity (bolls/m)	25.8	32.4	*p*=0.003	26

At a farm near Trangie, in the Macquarie valley, smaller plots (8 × 50 m) were established with or without Indian mustard, during winter of 1999. Shoot dry matter production of the mustard was not assessed before incorporation, which occurred several weeks before sowing cotton. Indian mustard decreased the severity of black root rot early in the season, leading to a significant increase in shoot growth and fruit maturity (Table 13). This was reflected in the maturation of bolls and total yield but the differences were not significant. The incidence of Verticillium wilt was slightly lower in plots with the mustard treatment but the difference was not significant (Table 13).

Table 13. Indian mustard 651 as a biofumigant against black root rot of cotton in a field near Trangie.

	Bare	**Mustard**	**Probability**	**% difference**
3.11.99				
Disease severity (0-10 scale)	9.5	7.1	*p*=0.02	-25
Healthy lateral roots (No./plant)	1.2	6.2	*p*=0.04	417
Shoot dry mass g/plant)	0.12	0.12	NS	-
16.11.99				
Disease severity (0-10 scale)	8.4	5.9	?	-29
Stand (plants/m)	10.9	10.8	NS	-
Shoot dry mass (g/plant)	0.16	0.20	NS	-
29.11.99				
Shoot dry mass (g/plant)	0.39	0.58	p=0.051	47
21.2.00				
Fruit maturity (bolls/m)	32.8	41.2	*p*=0.037	26
28.3.00				
Seed cotton (kg/ha)	493	651	NS	-
17.5.00				
Total seed cotton (kg/ha)	3242	3528	NS	-
Verticillium wilt (% plants)	5.2	4.2	NS	-

Three experiments were conducted in a field near ACRI, using fully replicated plots with either woolly pod vetch, Indian mustard or a mixture of species. The results of these experiments were confounded by large variation in the population of *T. basicola* in the soil and by difficulties with incorporation. In particular, a 'hot spot' of inoculum occurred across one block in both experiments. The species mixture experiment was located in part of the field with less inoculum of *T. basicola* but where the inoculum was still uneven.

2000/01 biofumigation experiments

Further screening of varieties in 2000 confirmed the biofumigation potential of further species of brassicas, including a mustard (Mustkleen) a canola (Nemfix), as well as pink seeded vetch (Figure 11).

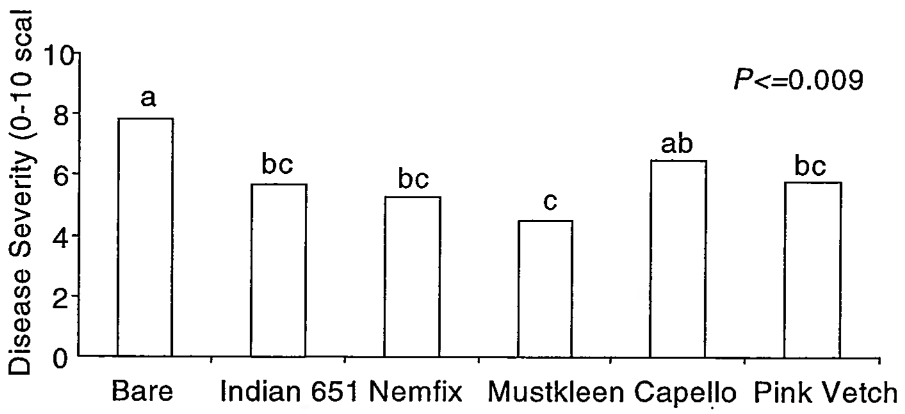

Figure 11. Reduced severity of black root rot in cotton after biofumigation with brassicas and legumes at Narrabri.

Two different varieties of marigold (*Tagetes erecta*) successfully reduced the population of *T. basicola* in potted soil by more than 50% (Table 14). The shoots and roots individually were not sufficient to affect the fungus, indicating that the biofumigation effect did not occur during the life of the marigold plants. The effectiveness of marigold as a biofumigation crop remains to be tested in the field.

Table 14. Reduced density of *T. basicola* in potted soil following incorporation of two varieties of marigold.

Treatment		
Marigold grown in soil	**Material incorporated**	**Density of *T. basicola* (cfu/g soil)^z**
None	None	271a
None	Shoots only "Cupid Mix"	273a
None	Shoots only "Crackerjack"	252a
"Cupid Mix"	Roots only	211ab
"Crackerjack"	Roots only	220a
"Cupid Mix"	Whole plant	133c
"Crackerjack"	Whole plant	141bc

z Values followed by the same letter are not significantly different by pairwise comparison of means using Fisher's LSD test (p<=0,02).

The optimum effectiveness of the biofumigation may require specific methods of termination and incorporation of the biofumigant plant. By incorporating foliage into potted soil, Professor Rothrock found that vetch was more effective if it was burnt down with herbicide before incorporation, whereas Indian mustard 651 was more effective if incorporated as fresh shoots (Appendix II). This result reflects the mechanisms of fumigation. The biofumigation effect of vetch results from the release of ammonia gas as the residue breaks down. Hence, killing the vetch before incorporation should accelerate the effect. In contrast, the biofumigation effect of brassicas involves the release of volatile organic molecules (isothiocyanates) immediately after damage to fresh tissue. Hence, mustards should perform better if incorporated as fresh material.

Although biofumigation does not eradicate *T. basicola* from soil, it does reduce disease severity enough to warrant its use. Woolly pod vetch has been used successfully as a biofumigant for black root rot in cotton in the USA (Rothrock and Kirkpatrick, 1995) and has the added benefit of providing substantial amounts of nitrogen to the following cotton crop (I. Rochester, Pers. Comm.).

Systemic acquired resistance

All plants are equipped with defence systems that enable them to resist infection by most microorganisms. Plants respond differently to pathogens depending on the nature of their defences. Plant pathologists have recently investigated ways to activate these inherent disease resistance mechanisms by applying chemical inducers to the plant, in advance of the pathogen. In this project a number of experiments were conducted using benzothiadiazole (BTH) to induce resistance to black root rot in cotton. In 1998, BTH was applied to cotton seedlings as a foliar spray in fully replicated field experiments in fields infested with *T. basicola* at Narrabri and at Warren. In both experiments there was no reduction in disease severity and cotton seedling growth was depressed, indicating a phytotoxic effect by Bion when applied in this manner.

An alternative method of application was developed by the project's Technical Officer, Mr Anowar Mondal, whereby cotton seed is soaked in a solution of BTH (25 µg mL^{-1}) for three to five hours, allowed to dry, and then sown as soon as possible.

In a number of pot experiments, BTH reduced the severity of black root rot in cotton and legumes that are used in rotation with cotton (Table 15, Table 16 and data not presented). In the second seed soaking experiment, the severity of infection on tap roots was reduced by 16 to 28% across the range of varieties tested (Table 16). Furthermore, the number of relatively healthy lateral roots on each plant was increased by between 40 and 150% (Table 16). If BTH can induce the defences of cotton sufficiently to give similar results in the field then it should be a useful management tool for black root rot.

Table 15. Reduced severity of black root rot of cotton following seed treatment with benzothiadiazole (BTH). Seeds of DP 90, Sicala V2 and Siokra 1-4 were soaked for 18, 5 and 5 hours respectively, sown in soil infested with *T. basicola*, and plants were assessed for black root rot at 21, 18 and 35 days respectively.

	Disease severity (0-10 scale)			Tap root length (cm)
BTH (µg L^{-1})	DP 90	Sicala V2	Siokra 1-4	Siokra 1-4
0.0	3.5a	3.3a	9.7a	5.0b
25	2.9ab	1.9a	8.6a	19a
50	2.4b	1.4a	8.9a	17a
Probability	$p<0.01$	NS	NS	$p<0.01$

Table 16. Reduced severity of black root rot by soaking cotton seeds in benzothiadiazole (BTH) for 3.5 hours before sowing. For each variety, the difference between treated and untreated was significant ($p<=0.05$).

	Disease severity (0-10 scale) BTH (µg L^{-1})		Healthy lateral roots/plant BTH (µg L^{-1})	
Cotton variety	0	25	0	25
Sicala V2	9.3	6.7	17	24
Sicot 189	9.5	7.1	11	28
Delta Sapphire	9.7	8.0	9	20
Siokra 1-4	9.5	8.0	11	25
Sicot 53	9.4	7.3	15	28
Delta Jewel	9.3	6.7	15	32
Delta Emerald	9.2	7.0	19	31

In a different pot experiment, the disease severity of black root rot in cotton was reduced significantly when cotton was grown on a mixture of sterile soil and infested roots of faba bean plants. The faba bean seeds were soaked in BTH prior to sowing in naturally infested soil. The BTH treatment reduced the severity of infection in the faba bean and, hence, the carryover of inoculum to cotton (Table 17). These observations suggest that treatment of susceptible rotation crops could make an important contribution to management of black root rot in cotton farming systems.

Table 17. Reduced severity of black root rot in cotton (cv. Sicala V2) grown in steamed soil inoculated with roots from faba bean (cv. Fiord) plants that were previously treated with bezothiadiazole (BTH) and grown in unsteamed soil from Wee Waa that was infested with *T. basicola*.

	Disease severity (0-10 scale)[a]	
Faba bean seed treatment	Faba bean, prior to cotton	Cotton, after faba bean
Water	4.0a	6.3a
BTH 25 ppm	2.3b	4.8ab
BTH 50 ppm	1.8b	3.9b

[a] Values followed by the same letter in columns are not significantly different ($p<0.05$).

The technique of soaking cotton seed in BTH represents a new development in the use of chemical agents to induce resistance against plant pathogens. BTH has not been previously used to induce resistance against pathogens of plant roots. BTH has potential to reduce both the severity of black root rot and the build-up of *T. basicola* in the soil, and needs to be evaluated further in field trials. The technique has also proved to be effective against other diseases, including Fusarium wilt.

Fungicides

The fungicide triadimenol (Baytan®, Bayer Australia Ltd) is registered as a seed dressing for control of black root rot in California. Experiments prior to this project showed that Baytan® provides a slight degree of control of black root rot when used as a seed dressing on some occasions but not on others. When

Baytan® was used as a seed dressing in the vetch experiment in 1998/99 it had no effect on the severity of black root rot (Figure 10). However, Baytan® appeared to give a degree of control against black root rot when used as an in-furrow spray (Figure 9) in 1998/99.

When used as an in-furrow spray in 1999/00, Baytan® reduced the severity of black root rot by 22% and almost doubled the number of relatively healthy lateral roots on plants (Table 18). Despite this apparent decrease in the severity of infection by *T. basicola*, plants treated with Baytan® did not grow any bigger, even in subsequent measurements of shoot growth at the end of November.

Table 18. Effect of triadimenol (Baytan®) on the severity of black root rot of cotton in a field near Wee Waa on 30th October 1999.

	Disease severity (0-10 scale)	Healthy lateral roots (No./plant)	Shoot dry mass (g/plant)
Untreated	7.5a	10.4b	0.20
Baytan® in-furrow	4.6b	19.7a	0.19
Probability	$p = 0.002$	$p < 0.001$	NS

In the 2000/01 season triadimenol was used in a number of experiments, formulated as either Baytan® or Bayfidan®. Triadimenol did not decrease the severity of black root rot in any experiment (Table 19). Climatic conditions in that season were particularly favourable for seedling disease caused by *Rhizoctonia* and *Pythium*, as well as black root rot. Although triadimenol did not decrease the severity of black root rot at Warren it did increase cotton seedling growth (Table 19). However, seedling disease was very severe in that field, suggesting that the increase in shoot growth was due to the effects of triadimenol on *Rhizoctonia*, not *T. basicola*. In the vetch experiment (Figure 9) triadimenol was effective against seedling disease caused by *Rhizoctonia* and counteracted the seedling disease caused by late incorporation of the vetch (data not presented).

Table 19. Summary of results from replicated field experiments in 2000/01 using triadimenol (Baytan®, Bayfidan®) as an in-furrow (IF) or seed dressing (SD) fungicide for control of black root rot of cotton.

Location	Application	Severity of black root rot	Cotton growth
Hillston	Bayfidan IF × 3 rates	Not significant	Not significant
Warren	Bayfidan IF × 3 rates	Not significant	+50%
Wee Waa	Baytan IF	Not significant	-10%
Wee Waa	Baytan IF Baytan SD Bayfidan IF × 5 rates	Not significant	Not significant
Wee Waa	Baytan IF field 1 field 2	Not significant Not significant	-9.1 -25%

Triadimenol clearly has a phytotoxic effect on cotton that counteracts any benefit that might be derived from reduced infection by *T. basicola*. When Baytan® is used as a seed dressing it consistently delays the emergence of cotton seedlings by approximately two days (A. Hawes, Pers. Comm.) and similar delays were oberved when used as an in-furrow treatment (data not presented). Furthermore, in the experiment at Wee Waa in 1999, triadimenol did not reduce the development of mycorrhizas in cotton (data not presented). Hence, the phytotoxicity does not involve an indirect effect on mycorrhizal colonisation of cotton. Triadimenol is not registered for cotton in Australia. Due to its inconsistent performance, the manufacturer is not seeking registration of triadimenol for use in cotton.

A number of fungicides, including benomyl (Benlate 50WP, 50% a.i.), triticonazole (Real), fluquinconazole (Jockey), TCMTB (Ascend), azoxystrobin (), triadimenol (as a check) were examined for their effectiveness against black root rot in several pot experiments. Cotton seeds were sown in a furrow made across the centre of pots and sprayed with fungicide solution using a syringe; thus simulating in-furrow application in the field. Among the fungicides examined, benomyl was the only one with potential to reduce the severity of black root rot while not having a phytotoxic effect on cotton growth (Figure 12). The other fungicides either had no effect, or reduced severity of infection by *T. basicola* was accompanied by reduced plant growth, indicating phytotoxicity (data not presented).

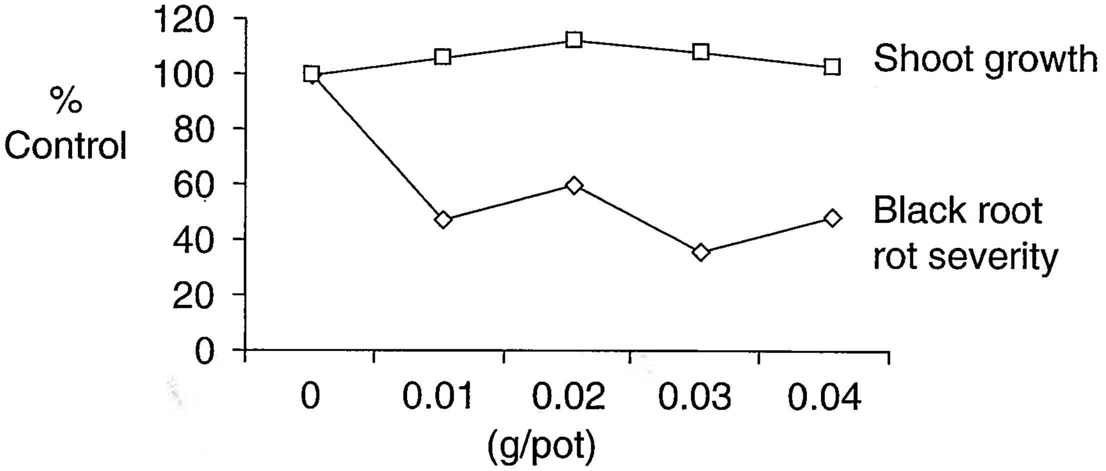

Figure 12. Reduced severity of black root rot of cotton treated with the fungicide benomyl as a simulated in-furrow spray in pots. Data is expressed as a percentage of the untreated control (zero rate).

The combined results of three pot experiments with benomyl suggest that rates as low as 400 g ha^{-1} may be effective and this needs to be evaluated in the field.

Summer flooding

Flooding is used for control of soilborne diseases of cotton in parts of California. Growers there report that the severity of black root rot is reduced substantially in at least the next four cotton crops after flooding. Temperature is an important factor. Flooding is most effective during summer, requiring a minimum of 30 days with maximum air temperature at 30°C or more (O'Niell, 1997). In Australia, the population density of *T. basicola* was decreased dramatically by flooding a field for approximately 57 days, during February and March 2000 (Table 20). The flooding was effective over the range of depths and temperatures across the field.

Table 20 Decline in the population of *Thielaviopsis basicola* after summer flooding of a field at Merah North

	Sampling position	
	50 m from head ditch	50 m from tail ditch
Water depth (cm)	30	55
Soil temperature[A] maximum(°C)	26.2	25.6
Soil temperature[A] minimum(°C)	23.8	24.1
Population before (spores/g soil)	359 ±69	485 ±53
Population after (spores/g soil)	8 ±3	19 ±4
Population reduction	98%	96%

[A] 10 cm below the top of the bed.

Symptoms of black root rot were assessed on tap roots of plants in this field in early November 2000. The mean score for discolouration of tap roots was relatively high (7.0 and 7.4 at the head ditch and tail ditch respectively). However, the intensity of discolouration on the tap roots was low (light brown rather than the typical black caused by *T. basicola*). Microscopic examination showed that *T. basicola* was present up and down the roots but that the damage to roots was mild. Colonisation by mycorrhizal fungi was adequate (28%) considering the age of the seedlings. The climatic conditions experienced during October 2000 were ideal for *T. basicola* and the small number of spores remaining after the flooding would have been able to multiply rapidly on cotton roots. Nevertheless, the cooperating grower reported that early season growth in that field was the best on the farm.

Summer flooding is an effective option for black root rot control but its use in Australia will be restricted by the availability of water and field topography.

Ammonia gas

In 1998/99 a field trial was conducted near Wee Waa to examine the effects of shallow application of anhydrous ammonia gas on black root rot. The gas was applied at approximately 10 cm in depth at the rate of 110 kg of N ha^{-1}. Although the anhydrous ammonia reduced the severity of black root rot by 30% this was not sufficient to affect subsequent shoot growth (Table 21).

Table 21. The effect of shallow (10 cm depth) ammonia gas on black root rot of cotton in a field near Wee Waa.

	Disease severity 4.11.99 (0-10 scale)	Shoot dry mass 23.11.99 (g/plant)
Untreated	8.2a	0.64
Anhydrous ammonia	5.6b	0.69
Probability	$p < 0.001$	NS

This experiment was repeated in the same field in 1999/00 except that the anhydrous ammonia was applied at the commercial rates and depth used on that farm (200 units of N/ha, at 15 cm depth). There were no significant effects of on cotton growth or the severity of black root rot.

The anhydrous ammonia may have been ineffective in the second experiment because, at commercial depths, the gas is not fumigating the soil where it is most needed, in the upper part of the bed. With shallow application, more of the nitrogen will be lost to the atmosphere and the cost is likely to outweigh the benefit.

Long term crop rotation

Farming Systems trials clearly demonstrated that rotation of cotton with cereals does not reduce the severity of black root rot in cotton, nor prevent its increase over time (Figure 4, Table 6). However, anecdotal evidence from a farm between Trangie and Narromine indicated that the severity of black root rot in cotton was reduced dramatically after three consecutive years of wheat. Consequently a rotation trial with one, two and three years of wheat before cotton was established at that farm. The severity of black root rot in the cotton following one year of wheat was assessed in the 1999/00 season. In the 2000/01 season the severity the cotton following two years of wheat will be assessed and results made available.

The density of spores of *Thielaviopsis* was much higher in the plots following cotton than in those after two years of wheat, and this pattern was reflected by disease symptoms (Table 22). Black root rot greatest in the wheat/cotton treatment. Stand establishment was poor in the wheat/cotton treatment, suggesting a substantial impact from seedling disease. However, wet conditions in winter 2000 hampered stalk removal and bed preparation in the wheat/cotton plot and this probably contributed to the poor stand.

Table 22. Effect of cropping history on black root rot and seedling disease in un-replicated plots of cotton (Sicala 40) near Narromine.

	Previous crops		Probability
	Wheat/wheat	Wheat/Cotton	($n = 8$)
Spore population at planting (cfu/g soil)	36	474	$p < 0.001$
Black root rot severity (0-10 scale)	2.2	7.4	$p < 0.001$
No. healthy lateral roots per plant	17.5	9.4	$p < 0.001$
Stand establishment (plants/m)	11	6.9	$p < 0.001$
Boll count (bolls/m 27 Feb)	81	57	$p = 0.001$
Boll mass (g/m 27 Feb)	400	270	$p = 0.002$
Actual yield (ba/ha)	7.9	6.6	N/A

The wheat/cotton plot was replanted after the disease assessment (31 October) and, consequently, a direct comparison of yield cannot be made. However, the differences in fruit maturity and final yield, illustrate the loss caused by having to replant after serious seedling disease. This trial will be finalised in the 2001/02 season. A larger scale fully replicated trial with the same treatments has been established on a farm at Warren.

Timing of planting

A replicated experiment with two planting dates was established at the Australian Cotton Research Institute. A site with no *T. basicola* in the soil was identified and plots with and without the fungus were established by artificial inoculation in April 2000. Cotton was sown on 28 September 2000 and 10 October 2000. Symptoms of black root rot were absent in plants that were not inoculated (Table 23). Planting date had no effect on the severity of black root rot, assessed on the tap roots using the 0-10 scale. Inoculation reduced lateral root development in plants sown on either date. However, in inoculated plots, the early sowing gave an advantage in terms of lateral root development (Table 23). Shoot growth primarily reflected sowing date, with early plants being the largest. The lack of effect of inoculation on shoot growth (Table 23) suggests that disease was not sufficiently severe to stunt cotton growth. When this experiment is sown again in 2001/2 there should be a much higher level of inoculum in the soil. Inoculation and sowing date had no significant effects on stand establishment (data not presented).

Table 23. Effect of planting date and inoculation with *Thielaviopsis basicola* on the severity of black root rot of cotton in a field at the Australian Cotton Research Institute.

Sowing date and treatment	Disease severity (0-10 scale)	Healthy lateral roots (No./plant)	Shoot dry mass (g/plant)
Early	0.8b	21a	3.1ab
Late	0.5b	15b	2.0bc
Early +*T. basicola*	5.1a	9.9b	3.8a
Late + *T. basicola*	5.2a	4.5c	1.8c
Probability	$p < 0.001$	$p <= 0.039$	$p <= 0.001$

Values followed by the same letter in columns are not significantly different at the state probability by pairwise comparison of means using the Scheffe test.

This experiment provides clear evidence that, given reasonable stand establishment, early sowing will enable continued lateral root development in fields with black root rot. Thus, given favourable conditions for stand establishment, early sowing should be advantageous.

Farmcleanse

T. basicola is easily spread in soil adhering to boots, equipment and vehicles. While Farmcleanse was proven to be an effective disinfectant for the Fusarium wilt pathogen, its activity against *T. basicola* was undetermined. The density of *T. basicola* in soil sampled from boots was 325 cfu/g. After the boots were soaked in Farmcleanse (10%) or bleach (1%) for 10 minutes, the density of *T. basicola* was 0 and 63 cfu/g respectively. However, when wet soil was forcibly compacted into the tread of the boots, farmcleanse required 20 minutes soaking to be totally effective. Farmcleanse is a useful disinfectant for *T. basicola* but will not effectively penetrate compacted soil. It should be used as an aid to disinfection after thorough removal of mud or compacted soil.

(iii) Interactions with other microbes

Interaction with mycorrhizal fungi

Observations in several of the experiments described above (Table 9, or data not presented) showed that colonisation of roots by the beneficial fungi that form mycorrhizas (VAM) is clearly reduced by black root rot. In the Cotton CRC Farming Systems Experiment at Warren in 1996, there was a significant negative correlation between mycorrhizal colonisation of cotton roots and the severity of black root rot ($r^2 = 0.57$, $p < 0.001$). This lack of mycorrhizal development is likely to contribute to the negative effect that *T. basicola* has on cotton seedling growth.

Interaction with rhizosphere bacteria

Suppression of black root rot in a soil used to grow tobacco was attributed to naturally occurring rhizosphere bacteria (Sturtz et al., 1986).

Some soils can be suppressive to black root rot and this suppression may involve naturally occurring bacteria, such as species of *Pseudomonas* (Sturtz et al., 1986). It is possible that the reduced incidence of black root rot observed in certain heavy clay soils near ACRI involves rhizosphere bacteria. The potential for suppression of black root rot by the species of *Pseudomonas* associated with bacterial stunt of cotton (CRDC projects DAN7C and DAN17C) was investigated in pot experiments.

Steamed soil was either untreated, inoculated with *T. basicola*, inoculated with the *Pseudomonas* sp., or inoculated with both. Emergence was not affected by individual inoculation with *T. basicola* or bacterium but was reduced substantially by both together (Table 24). *T. basicola* reduced the density of lateral roots (estimated on a scale of 0-4) but the bacterium did not enhance this effect significantly. The pattern of plant growth reflected that of lateral root development (Table 24).

Table 24. Effect of inoculation with *Thielaviopsis basicola* and a species of *Pseudomonas* on emergence and growth of cotton in steamed soil in pots.

Inoculation treatment	Emergence (%)	Lateral root index (0-4 scale)	Shoot dry mass (mg/plant)
Not inoculated	85a	3.8a	79a
T. basicola	80ab	0.7b	47b
Pseudomonas	73ab	3.7a	82a
T. basicola + *Pseudomonas*	68b	0.0b	43b
Probability	$p = 0.026$	$p < 0.001$	$p < 0.001$

Values followed by the same letter in columns are not significantly different at the state probability by pairwise comparison of means using the Scheffe test.

While the bacterium had a deleterious effect on plant emergence, it did not appear to interact significantly with *T. basicola*. The suppressiveness of Australian soils to *T. basicola* may involve other organisms or soil properties.

Interaction with other seedling pathogens

It is clear that in many cases, once cotton has passed the stage of being susceptible to *Rhizoctonia*, black root rot does not cause further seedling death (see discussion with Table 19). Black root rot can be severe without reducing plant stand (Table 1). However, stand loss can sometimes be greater in areas of fields where black root rot is severe. In these situations black root rot contributes to seedling mortality in combination with *Rhizoctonia* and/or *Pythium*. Consequently, some management options are applicable to both diseases (see integrated management strategy below).

(iv)Management of other causes of slow early season growth

Nurse crops and farmer bioassays for mycorrhizas (VAM)

The aim of these experiments was (i) to develop a simple test that farmers could use to assess whether or not a newly developed field, or a fallow field, will have a VAM problem when sown to cotton and (ii) to evaluate the potential for sowing a sacrificial or 'nurse' crop to restore VAM levels in fallow soil prior to sowing cotton.

The concept of a farmer's bioassay involves sowing two short (1 metre) rows of a mycorrhizal plant during the winter prior to cotton and comparing growth with and without the addition of soil from either a nearby crop or from a recently cropped field. The 'inoculum' soil is placed beneath the seeds at sowing and both treatments are given equivalent soil disturbance. Such a bioassay could be repeated at several points within a field or in several different fields. If the bioassay rows are kept to about one metre in length then they can be hand watered to ensure growth.

After six weeks the growth of the plants with and without the addition of VAM inoculum is compared. If there is no difference then adequate VAM fungi is indicated. If the plants in the row without inoculum added are considerably smaller then a deficiency of VAM inoculum is suggested. The intention is for farmers or consultants to be able to make a judgement about VAM levels in the soil based on growth of the bioassay plant.

Linseed was suggested as a good test crop because of its ability to grow in winter and its known dependence on VAM. Linseed bioassays were initially established at a selection of sites at the Australian Cotton Research Institute at Narrabri and at Colly Farms near Collarenebri (Table 25).

Table 25. Initial results of estimating VAM status of soil using a linseed bioassay.

Location	Site history	Mycorrhizal dependency
Colly Farms field 2	no crop for 24 months	81.8%
ACRI, Narrabri - field 4 (edge)	Never cropped to cotton	60.4

If the level of inoculum of VAM fungi is suspected of being low or the results of a linseed bioassay indicate that the level of inoculum of VAM fungi is low, then a 'nurse' crop (Krikun, 1991) could be planted to foster the re-establishment of soil VAM networks and infectivity. Breaking the fallow for a short period with plants that eventually become heavily infected, albeit slowly, would reinstate soil VAM infectivity, with resultant rapid infection of, and nutritional benefit to the subsequent crop. (Hunter et al., 1988). These authors also suggested that controlled short periods of VAM infected weed during the fallow could actually improve subsequent P supply. The closer the weed flush was to subsequent cropping the more likely it would benefit P nutrition.

It proved difficult to locate suitable sites where the concept of farmer's bioassays and nurse crops could be properly evaluated. A trial site was established at ACRI with replicated plots of repeated cotton and repeated bare fallow. It is expected that this site will be ready for field experiments in the 1999/2000 season. Meanwhile a site near Jimbour on the Darling Downs of Queensland became available.

The Jimbour study

Soil samples from three fallowed fields (11A, 11B and 22) on the property Jimbour had been submitted to the Soil Microbiology section of the Queensland Wheat Research Institute for counting of VAM spores. The results (Table 26) had indicated "low to marginal levels of VAM spores", particularly in fields 11A and 11B. Field 11B was therefore identified as an ideal site to evaluate the bioassays and nurse crops.

Table 26. Length of fallow, previous crop, VAM spore counts* and Bicarbonate-P and Zinc# for three fields near Jimbour.

Field	Length of zero-tilled bare fallow and previous crop	Protoplasmic VAM spores /g O.D. soil (0-30,30-60 & 60-90cm)	Other VAM spores /g O.D. soil (0-30,30-60 & 60-90cm)	Bicarb-P (mg/kg)	Zinc (mg/kg)
11A	11 months after wheat	7.5, 0.4, 0.0	14.9, 1.6, 2.8	8	0.3
11B	17 months after sorghum	3.2, 0.2, 0.8	4.1, 5.8, 4.0	8	0.3
22	11 months after barley	4.7, 2.4, 7.2	12.3, 5.0, 10.6	7	0.4

* Counted by the Soil Microbiology section of the Queensland Wheat Research Institute, Queensland Department of Primary Industries. Data courtesy of St John Kent.

Soil Phosphate and Zinc data from Seed and Grain IAMA.

Nurse crops and linseed bioassays were established in field 11B on 5th August, 1997. There were seven nurse crop treatments in each block (replicate) and five replicates arranged in a line with a 20 m buffer between each. Nurse crop treatments included wheat, barley, field pea, vetch, maize, sunflower and the nil crop control. The maize failed to germinate and the vigorous growth of vetch prevented subsequent establishment of cotton. Within each block, plots were separated by 2 m buffers. Each plot (3x1m) was planted by hand and had three rows of plants 0.5 m apart. The rows were sown at 90° to the intended direction for subsequently planting cotton, so that at least two rows of the cotton to be sown in October, (single skip configuration), would pass over the plot at right angles to it. The soil near the surface was dry and all plots were watered with a hand held hose. At the northern end of each block, a linseed bioassay was set up as described previously with soil taken from beneath nearby wheat crops used to provide VAM inoculum. Linseed bioassays were also established in fields 11A and 22. These bioassays were also watered by hand.

The results of the linseed bioassays (Table 27) confirmed the spore count data that had been obtained previously and indicated potential inadequate VAM levels in fields 11A and 11B. The shoot dry mass of VAM inoculated linseed was more than double that of the uninoculated linseed. The results obtained from the bioassays in field 22 were very variable and therefore not significant.

Table 27. VAM colonisation and shoot growth of linseed sown at Jimbour with and without the addition of VAM inoculum. The inoculum consisted of soil taken from adjacent wheat crops in adjacent strips. (Bioassays assessed 48 days after planting)

	-Inoculum	**+Inoculum**	
Strip 11B	3	24	
VAM (%)	(1.6)[z]	(4.9)	$p < 0.001$
Shoot height (mm)	45	67	$p = 0.016$
Shoot dry mass (mg plant^{-1})	19	44	$p = 0.037$
Strip 11A			
VAM (%)	23	30	NS
Shoot dry mass (mg plant^{-1})	28	66	$p = 0.033$
Strip 22			
VAM (%)	30	27	NS
Shoot dry mass (mg plant^{-1})	53	56	NS

[z] Data square root transformed for normality.

VAM colonisation in the uninoculated linseed in field 11B was 3% while the VAM colonisation in the nurse crops at 48 days after sowing was relatively low (mean 5.7%) with no significant differences.

Despite the indications of a potential VAM deficiency, the amount of inoculum in the soil at the start of the cotton season was clearly adequate for development of VAM in cotton (Figure 13). It was comparable to that normally observed in healthy cotton at six weeks after sowing. One explanation for this would be different compatibilities, in terms of the rate of spread of colonisation, between the different hosts and the fungal population at Jimbour. McGee, Torrisi and Pattinson (1998) found that VAM fungi spread rapidly in cotton and that very few propagules were needed for 'normal' levels (50 to 60 %) of colonisation (see also Case study 1 in this report). Hence what was a low number of propagules for linseed may have been adequate for cotton. Alternatively, the population of VAM fungi at Jimbour may have included a mix of VAM species with differential compatibility among hosts. Further study would be required to determine the exact nature of the differential response of cotton and linseed to the VAM fungal population at Jimbour.

The greater growth of cotton in the plots that had wheat in comparison to the fallow (Figure 13), suggests that factors other than VAM fungi were capable of causing growth reductions in cotton in fallowed soil. Hulugalle et al. (1998) found that soil strength and air filled porosity were decreased by fallowing in irrigated cotton fields. Hence the observed benefit to cotton from planting nurse crops in field 11B may have been due to the physical effect of the root system on soil structure.

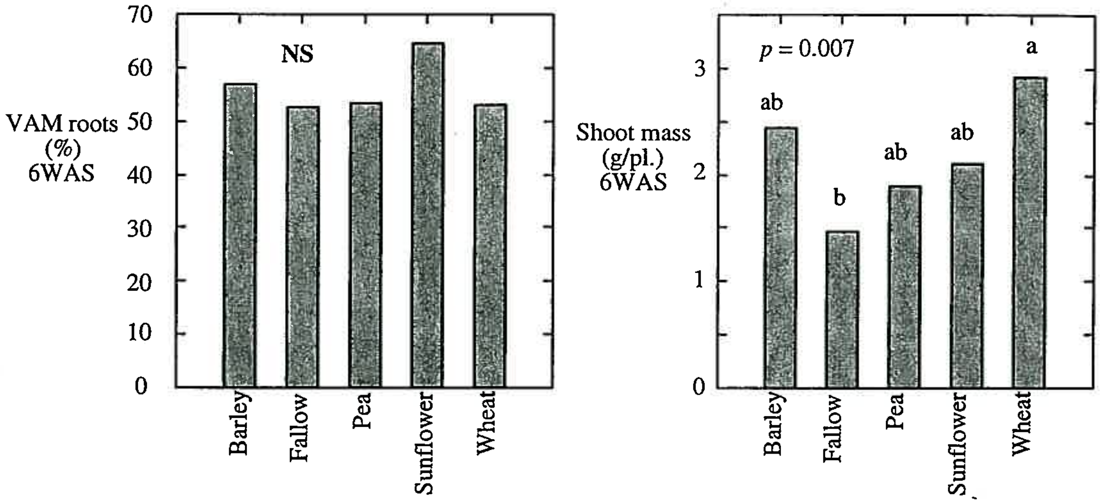

Figure 13. VAM development and shoot growth of cotton following a range of 'nurse crops' at Jimbour. Nurse crops were grown for 48 days during August and September, prior to sowing cotton.

In conclusion, it appears that linseed may not be a suitable plant to indicate potential VAM problems in cotton. A similar differential growth response to that reported here was observed when a linseed bioassay was used in fallowed soil prior to cotton sown at ACRI in 1996 (data not presented). Using wheat as a nurse crop increased cotton growth but this was not a result of enhanced development of VAM. Future research should evaluate alternative indicator plants to linseed and ensure that the length of the fallow is sufficient to result in reduced VAM development in cotton.

Cover crops

Research in CRDC project DAN 100C indicated that mulches had potential to increase early season growth and fruit maturity of cotton, particularly in fields affected by bacterial stunt. In the current project experiments were conducted to put these principles into practice using cereal cover crops.

Wheat (cv. Janse) was sown in a field near ACRI on 3 June 1998. Establishment was poor and the whole crop was sprayed with Roundup on 25 September. On 28 September plots (16 m x 16 m) were marked across the centre of the existing drip irrigation trial. The wheat outside these plots was slashed by the grower. In the 'bare' plots the wheat was cut to ground level using a brushcutter, raked and then the straw was placed on the ground in places where the wheat cover was sparse or absent in the 'wheat cover' plots. This supplementary straw was placed on top of the double beds, not in the furrows. Cotton was sown through all the plots on in October 1998.

In comparison to bare plots, the wheat cover increased early season shoot height, fresh mass and dry mass by 23, 10 and 9.3 % respectively (Table 28).

Cool winds were experienced early in the season and a reduced wind chill may have been a factor contributing to the greater growth of cotton in standing wheat. Drip irrigation had not commenced by the time of this harvest.

Table 28. Effect of wheat cover on cotton growth at 23 November.

	Wheat unslashed	Bare	Wheat slashed	AOV[z] (n= 16)
Shoot height (mm)	174a	142c	153b	$p <= 0.021$
Shoot fresh mass (g plant^{-1})	9.46a	8.57b	8.70b	$p <= 0.022$
Shoot dry mass (g plant^{-1})	1.44a	1.32b	1.31b	$p <= 0.031$

[z] In rows, values followed by the same letter are not significantly different using pairwise comparison of means with Fisher's LSD.

Later in the season cotton growth was greatest with the combination of wheat cover and drip irrigation, and least with surface irrigation of bare soil (Table 29). Shoot fresh mass was 19 % greater with drip irrigation than with surface irrigation (1 way AOV, $p = 0.035$) and 27 % greater with wheat cover than with bare soil (1 way AOV, $p = 0.006$).

Table 29. Effect of wheat cover and drip or surface irrigation on cotton growth and yield of cotton.

	Wheat Drip	Wheat Surface	Bare Drip	Bare Surface	2 way AOV[z] (n= 16)
15 December					
Shoot height (mm)					
Shoot fresh mass (g plant^{-1})	58ab	52ab	48bc	38c	$p <= 0.022$
Shoot dry mass (g plant^{-1})					
21 December					
Flowers (number plot^{-1})	36ab	27b	50a	50a	$p = 0.036$
15 April					
Yield[Y] (bales ha^{-1})	8.1a	7.2ab	6.5bc	5.7c	$p <= 0.004$

[z] In rows, values followed by the same letter are not significantly different using pairwise comparison of means with Fisher's LSD.

[Y] Estimated from seed cotton on the assumption of 38 % GTO and adjusted for row length actually harvested by discounting gaps of more than 0.5 m.

Cotton in the bare plots began flowering earlier than cotton in wheat cover plots (Table 29). Mean flower number in bare soil plots (50 flowers) was 56% greater than in wheat cover plots (1 way AOV, $p = 0.014$).

Despite this difference in flowering, yield was 11.5 % (0.74 bales ha^{-1}) greater with wheat cover than with bare soil (1 way AOV, $p = 0.019$) and 24 % (1.45 bales ha^{-1}) greater with drip irrigation than with surface irrigation (1 way AOV, $p = 0.035$). The interaction between the cover treatments and the irrigation treatments was not significant, indicating that the effect of the wheat cover was the same with and without drip irrigation. Overall, cotton yield was 2.4 bales (42 %) greater in the drip irrigation plots with wheat cover than in plots with surface irrigated bare soil (Table 29). However, the grower indicated that nitrogen manamement may have been the biggest factor influencing the difference between drip and conventional irrigation.

In winter 1999 large plots, with or without wheat cover, were established in a field near ACRI. The field history was cotton in 1997/98, wheat in 1999, followed by a summer fallow. The bed configuration was double beds 2 m wide with minimum tillage. The experiment consisted of a randomised block design with two treatments, 'bare' and 'cover', in four replicate blocks. Wheat plots were sprayed with glyphosate and cotton was sown on 10 October. The bare plots were cultivated on (before sowing) and then the whole field was not cultivated until 14 January 2000.

The bed profiles were measured 27 November at 4 positions in each replicate plot using a template with wooden dowels slotted through holes spaced every 5 cm along a piece of pine timber (75 mm × 40 mm DAR). Sampling positions were selected using a step-point method and beds on 'guess rows' and traffic lanes were avoided. Shoot growth was assessed on 2 December 1999 at the same positions. Cores of soil (90 mm diameter × by 15 cm deep) were taken from beneath each of the sampling positions, combined within each plot, and roots were washed from the soil for assessment of mycorrhizal colonisation and root browning. Stand establishment and the incidence of Verticillium wilt was assessed on 11 May 2000 in the cotton stalks remaining after harvest.

The wheat cover treatment increased early season plant height and dry mass by 19% and 16% respectively (Table 30). There was a trend for slightly lower stand establishment in the wheat plots, although this was not statistically significant. The lower stand in some of the wheat cover plots could have been caused by any of a number of factors including pests, such as wire worm, seedling disease or physical impedance of seedlings. Root browning was relatively low in this field while mycorrhizal (VAM) development was within the usual range observed in cotton, suggesting that bacterial stunt (Nehl et al., 1996) was not an issue in this field. The treatments did not affect mycorrhizal development. Verticillium wilt appeared to be greater with the cover crop treatment and a greater sample size may have shown that this difference was significant (Table 30). The levels of Verticillium wilt were unusually high due to the cool wet conditions during the first half of the season and the moderate susceptibility of the variety. Nevertheless, Verticillium wilt is a factor that needs to be monitored closely in future cover cropping trials.

Table 30. The effect of a wheat cover crop on stand establishment, mycorrhizas (VAM), growth and disease of cotton.

	Bare	**Cover**	
2.12.99			
Shoot height (mm)	128	152	$p = 0.022$
Shoot dry matter (g/plant)^z	4.4	5.1	$p = 0.002$
Root browning (%)	16	14	Not significant
VAM colonisation (%)	44	46	Not significant
1.5.00			
Verticillium wilt (%)	30	47	Not significant
Plant stand (plants/m)	10.9	9.0	Not significant

^{z} Analysed using 'Two-D' spatial analysis software.

The wheat-cover had a profound effect on bed structure (Figure 14). Between sowing and measurement of the bed profile at the end of November there were several rainfall events but no irrigation. This indicates that the beds in the bare plots slumped due to the rainfall while the structure of the beds in the cover plots was maintained. The capacity for wheat stubble to reduce soil loss from cotton fields during irrigation has been demonstrated. This experiment shows that wheat cover crops also have the capacity to maintain bed structure and potentially increase cotton growth, maturity and yield.

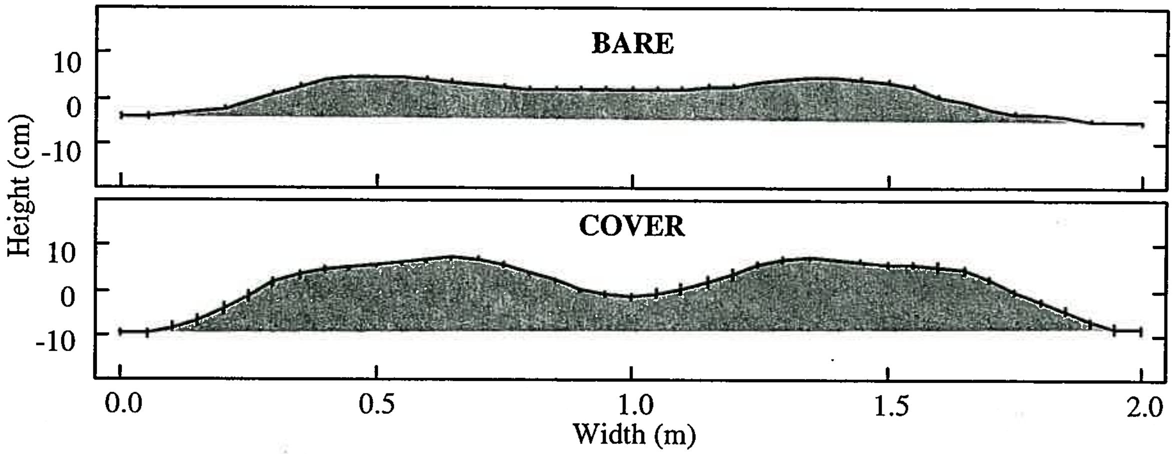

Figure 14. Profile of wide beds in cotton with or without a wheat cover crop. Heights are expressed as the difference from the median. Vertical bars show standard errors. The cotton planting line was at 0.5 and 1.5 m.

A repeat experiment using large plots with or without wheat cover crops was conducted in 2000/01. Poor drainage in parts of the field and wet weather in October caused substantial problems with establishment of the cotton. However, by avoiding these areas and by avoiding certain rows where there were mechanical problems with the planter, replicated observations were undertaken. The wheat increased cotton growth but did not affect black root rot or stand establishment significantly (Table 31). However, these data are pooled from replicated samples taken with within each plot. When data from adjacent pairs of plots were analysed individually, the wheat cover decreased the severity of black root rot by up to 54% ($p = 0.002$).

Table 31. Effect of wheat cover crops on severity of black root rot, stand establishment and growth of cotton in November 2000.

	Disease severity (0-10 scale)	Stand (plants/m)	Shoot dry mass (mg/plant)	Shoot height (mm)
Bare	5.9	9	35	48
Wheat cover	4.2	11	42	83
Probability	NS	NS	$p = 0.034$	$p < 0.001$

The mechanism behind this effect is likely to be temperature. At the onset of irrigation, the temperature plummeted in the bare plots but remained stable in the plots with cover (Figure 15). During the following day the soil temperature maximum was six degrees warmer in plots with wheat cover and this trend continued for the next four to five days (Figure 15). When the field was not wet, the wheat cover tended to moderate soil temperatures, resulting in lower daily maxima and, importantly for seedling pathogens, higher daily minima (Figure 15).

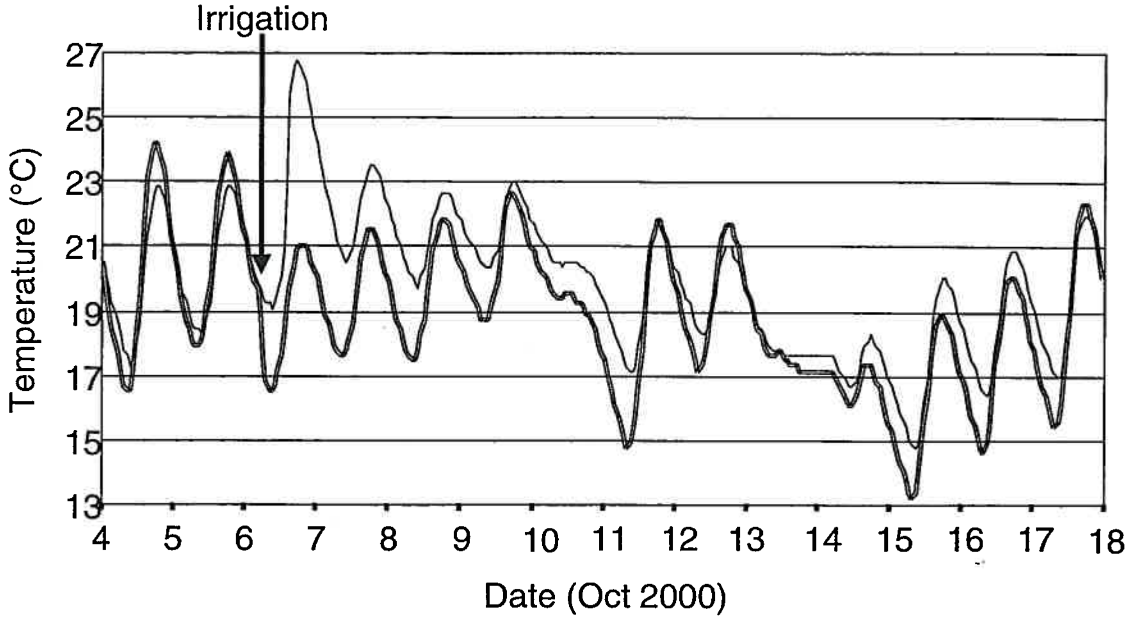

Figure 15. Soil temperature in a cotton field with (single line) and without (double line) a wheat cover crop.

The results from this project and previous studies in DAN100C suggest that the benefits of wheat cover crops to early season growth of cotton are due to a combination of factors; better soil structure, higher soil temperature and moisture content resulting from reduced evaporation and, possibly, reduced wind chill. It is unlikely that the cover crops would reduce the population of *T. basicola* in soil. It is more likely that the greater seedling vigour and higher soil temperature afforded by cover crops would enable the plant to 'grow out' of the condition sooner. Cover crops have potential to improve seedling vigour and reduce the effects of seedling pathogens. In favourable seasons, cover crops may be a useful way to enhance yield of both drip and surface irrigated crops.

CONCLUSIONS (RESEARCH OUTCOMES VERSUS OBJECTIVES)

The research outcomes of this project have met the objectives as follows:

(i) **Epidemiology of black root rot** – To investigate the biology of black root rot, including the dispersal and survival of the fungal pathogen (*Thielaviopsis basicola*) and interactions with cropping practices and soils.

- Black root rot is a chronic disease that delays cotton growth and reduces yield. NSW is currently experiencing a widespread epiphytotic (epidemic) of black root rot.
- Under favourable conditions, the strains of *T. basicola* in cotton-growing soils multiply very rapidly on cotton roots.
- The potential for the black root rot fungus to easily spread from farm to farm in soil and mud adhering to vehicles and machinery has been demonstrated. Soil adhering to floating trash clearly makes *T. basicola* very mobile in both irrigation and flood waters.
- The effect of a range of cropping practices on the survival of the pathogen and development of disease has been investigated. The severity of black root rot increases in proportion to the number of cotton crops grown, irrespective of (single year) rotation crops or fallows.
- Our understanding of the host specificity of Australian strains has been increased. Most legumes used in rotation with cotton are hosts but their relative susceptibility varies. Some weeds are host but the status of others is uncertain.
- The genetic diversity of *T. basicola* in Australian soils varies from region to region. Evaluation of this diversity by the CRDC postgraduate scholarship CRC29C will assist the current management strategies and future development of resistant varieties.
- The relationship between the severity of black root rot and different soils has been quantified, indicating that certain soils are conducive or suppressive to black root rot. Investigation of the role of the soil microflora in the suppressiveness and/or conduciveness of soils, by the CRDC postgraduate scholarship CRC18C will assist in development of management strategies and, possibly, biocontrol agents for black root rot .

(ii) **Management of black root rot**- To utilise knowledge on the biology of black root rot to develop integrated management practices for its control.

- No sources of resistance to *T. basicola* were found in currently available cultivars and breeding lines in Australia or in the USA. In effect, *T. basicola* 'steals time' from the crop and growers should choose varieties that have potential to compensate later in the growing season.

- Biofumigation is an option for reducing the population of *T. basicola* in soils because this fungus is an ecologically obligate pathogen (i.e. it can be cultured in the laboratory but it does not multiply on dead organic matter in the soil). The effectiveness of woolly pod vetch and some lines of mustard and canola as biofumigation crops has been demonstrated. Results will vary according to growth and incorporation of the biofumigation crop. Vetch has proved popular with growers as it has the additional benefit of providing nitrogen for the crop.

- Systemic acquired resistance against black root rot can be induced by soaking cotton seeds in benzothiadiazole (BTH). Further evaluation in the field is required.

- A number of fungicides were evaluated for control of black root rot. Triadimenol is unsuitable for control of black root rot and will not be registered for use on cotton in Australia. Benomyl has potential for control of black root rot but needs to be evaluated in the field.

- Summer flooding reduced the population of *T. basicola* in soil very effectively, without causing undue problems to mycorrhizal fungi. However, the availability of water and the topography of fields will limit the application of this technique.

- Anhydrous ammonia was shown to reduce the severity of black root rot but it was not effective enough to be used as an economic control measure.

- No advantage was gained by delaying planting. In fields infested with *T. basicola*, cotton should be sown early, as long as conditions are favourable for cotton growth.

- Farmcleanse is and effective disinfectant for *T. basicola* but should only be regarded as an aid to the decontamination of equipment and vehicles.

- An integrated management strategy for black root rot has been developed, including recommendations based upon the observations listed above.

(iii) **Interactions with other microbes**- To investigate interactions between black root rot and other soilborne microbes, including interactions with both positive (eg mycorrhizal fungi) and negative (eg. Fusarium) outcomes.

- Mycorrhizal colonisation and, therefore, function is inhibited in plants with black root rot.

- There was no interaction between *T. basicola* and a species of *Pseudomonas* that is associated with bacterial stunt of cotton. The microbial component of suppressive soils may involve other organims.

- *T. basicola* clearly does not kill cotton seedlings by itself, even at very high densities in soil. However, black root rot may contribute to seedling disease, caused by *Rhizoctonia* and *Pythium*, by weakening the root system.

(iv) **Management of slow early season growth-** To finalise development of management practices for other causes of slow early season growth, including bacterial stunt and lack of mycorrhiza.

- The potential for cereal cover crops to increase the vigour and growth of seedling cotton by moderating the soil environment (specfically temperature and structure) has been clearly demonstrated in this project. Cover crops may additionally reduce the impact of seedling diseases.

- The use of 'nurse' crops prior to cotton may be useful in cases where a lack of mycorrhizal fungi is suspected. However, in most situations the level of inoculum of mycorrhizal fungi in the soil will be sufficient for cotton.

- Linseed bioassays were effective at assessing the mycorrhizal status of soil with respect to linseed but had little relevance to cotton.

LIKELY IMPACT OF RESEARCH OUTCOMES (COST-BENEFIT)

Awareness of the risks posed by black root rot to sustainable cotton has been increased during the course of this project. Many growers now take at steps to manage black root rot.

Cotton growers are faced with two different objectives for control; to either reduce severe infestations of *T. basicola* to sub-economic levels, or to prevent population increase in the first place.

There are currently few options available that can reverse severe infestations. Summer flooding is effective but has been used infrequently because of cost and the constraints of topography and water supply. A single season of biofumigation does not reduce the population of *T. basicola* enough to prevent it from rapidly increasing in the following cotton crop. Longer periods of rotation with non-hosts may be a practical method.

Control methods that have potential to hold the population of *T. basicola* at sub-economic levels include biofumigation, avoidance of hosts as rotation crops, systemic acquired resistance and fungicides

Biofumigation with woolly pod vetch is proving popular among growers because it fixes large quantities of nitrogen. A cost-benefit analysis in the USA showed that the nitrogen benefit outweight the cost of sowing and incorporating vetch (C. Rothrock, Pers. Comm). Hence, vetch provides an alternative to rotation with other green manure crops that are hosts for *T. basicola*. Biofumigation with brassicas may not be widely adopted unless growing them as harvestable crops with an economic return has a demonstrable effect on disease.

If systemic acquired resistance against *T. basicola* can be successully induced in the field, then it should be very cost effective control measure.

Cover crops have several advantages apart from those demonstrated in this project; improved infiltration, reduced crusting, reduced soil erosion and benefits to insect management. Cover crops thus provide a package of benefits that warrant their use in many situations.

Some control methods may be deployed within existing farm management structures with little or not cost. These measures include bed preparation, the timing of planting and irrigation, choice of variety and farm hygiene.

Deployment of current and future control options in an integrated manner will ultimately be the most effective strategy.

RECOMMENDATIONS AND APPLICATION TO INDUSTRY

It is recommended that the following disease management strategies be adopted by the industry for the management of black root rot and other causes of slow early season growth.

A control strategy for black root rot of cotton

Planning

(i) Choose 'indeterminate' varieties that have the capacity to 'catch up' later in the season

Ground preparation

(ii) Good bed preparation to optimise stand establishment and seedling vigour
(iii) Pre-irrigate in preference to 'watering up'

Early season

(iv) Time sowing to avoid cool temperatures if possible, but sow early if conditions are warm enough (a soil temperature of 16°C is OK, 20°C is better) and rising. Temperature measurements should be taken in the fields where black root rot occurs.
(v) Replanting decisions should be made on the basis of stand losses, not the size of the seedlings.
(vi) Watch for early onset of water stress (ie. because the root system is weak) and irrigate accordingly, but avoid waterlogging.

Late season

(vii) Anticipate delayed growth and later maturity and manage the crop accordingly (black root rot 'steals' time from your crop).

After harvest and at all times

(viii) Practice good farm hygiene. Farmcleanse (used at 10%) is effective against *T. basicola* and is a useful aid to decontaminate vehicles after mud is removed – COME CLEAN, GO CLEAN

Rotation

(ix) Rotate with non-host crops (eg. cereals, canola) for more than one season if possible.
(x) Biofumigation with woolly pod vetch or mustard (canola?) between consecutive cotton crops or after a wheat fallow. The success of biofumigation depends upon the growth of the biofumigation crop and good incorporation (at least four weeks before cotton).
(xi) Avoid rotation with legumes (except vetch) and control alternative weed hosts (eg. *Datura*).
(xii) Flooding of fields for 30 days during summer reduces the population of *T. basicola* dramatically. This option will be limited by the topography of fields and the availability of water.

A control strategy for bacterial stunt of cotton

Bacterial stunt (also known as *early season growth disorder* or *Galathera syndrome*) occurs in many cotton growing areas and is usually associated with very heavy clay soils. Slow early season growth and severe stunting are often the only above-ground symptoms although the leaves of badly stunted seedlings may have symptoms of zinc deficiency: Root browning develops rapidly in response to pathogenic bacteria that colonise the roots and hinder their function.

Bacterial stunt delays the maturity of the crop and yield can be reduced by as much as 50%; although plants in some areas recover after December and yield well if the season is long enough. Fertilising the soil with zinc may be advantageous. Cover crops (eg. winter cereal, sprayed out before sowing cotton) have increased early season growth in fields where bacterial stunt occurs. The crop should be managed to give maximum time for recovery of growth late in the season.

A control strategy for lack or mycorrhiza (VAM) in cotton

VAM fungi cannot survive without a living host plant and their numbers decline during long, weed free, bare fallows or during rotation with a non-host plant (eg. canola, broccoli). In most cotton growing areas, however, sufficient VAM fungi will survive one season of bare fallow. Problems are more likely after longer periods of fallow. Loss or removal of topsoil can also eliminate VAM fungi and subsequent growth of cotton can be stunted with reduced uptake of phosphorus and zinc. The potential for yield reduction is greatest when the crop is planted late or during short seasons.

If a lack of VAM is suspected then a crop of wheat, which is usually less dependent on VAM fungi, could be grown prior to growing a VAM dependent crop such as cotton. Fertilisation during the crop is unlikely to be of benefit because P and Zn are relatively immobile elements in soil. Foliar fertilisers were ineffective in trials conducted at Narrabri.

COMMERCIAL DEVELOPMENTS

There are no commercially significant developments, patents or licences arising from the research in this project.

TECHNICAL DEVELOPMENTS

The technique of soaking cotton seed in benzothiadiazole (BTH) represents a new development in the use of chemical agents to induce resistance against plant pathogens. Cotton seed is soaked in a solution of BTH (25 µg mL^{-1}) for three to five hours, allowed to dry, and sown as soon as possible. BTH has not been previously used to induce resistance against pathogens of plant roots. This technique was developed by the project's Technical Officer, Mr Anowar Mondal, and has since proved to be effective against other diseases, including Fusarium wilt.

The concept of cover crops is not new. However, the potential for cereal cover crops to increase the vigour and growth of seedling cotton and reduce the impact of seedling disease by moderating the soil environment (specfically temperature and structure) has been clearly demonstrated in this project. This development specifically involved the use of cereal cover crops that were grown during the winter immediately prior to cotton and killed with herbicide before planting cotton.

Similarly, the concept of biofumigation crops has been developed elsewhere, and the potential for woolly pod vetch to control black root rot was demonstrated in the USA several years ago. However, the use of mustards and canola as biofumigation crops for control of black root rot has been demonstrated for the first time in this project. These crops are sown, grown and incorporated during winter. Since the black root rot fungus does not reproduce saprophytically in soil (i.e. it does not grow on dead organic matter) the biofumigation crops can be used in various cropping sequences in combination with cereal rotation crops. The potential for using marigolds as a biofumigation crop for black root rot has been demonstrated for the first time, in a pot experiment.

FURTHER DEVELOPMENT

Technological

The use of cover crops, biofumigation crops, rotation strategies and systemic acquired resistance for control of various cotton diseases, including black root rot, seedling disease, Fusarium wilt and Verticillium wilt, is being developed further as part of the CRDC projects DAN153C (Managing black root rot) and DAN154C (Diseases of cotton VII) with NSW Agriculture.

Further research on the pathogenicity and genetic diversity of Australian populations of *T. basicola* is the subject of the CRDC postgraduate scholarship CRC29C, with the University of Queensland and NSW Agriculture.

Further research on the role of soil microbial diversity ('soil health') in soils that are suppressive or conducive to black root rot is the subject of the CRDC postgraduate scholarship CRC18C, with the University of Sydney, NSW Agriculture and the University of New England.

Outcome deployment

The research outcomes of this project are being included in the Integrated Disease Management (IDM) Manual currently being compiled by the Australian Cotton Cooperative Research Centre. At a recent meeting of the Cotton CRC's Fusarium Wilt Coordination Committee (Fuscom) it was agreed to investigate the feasibility of (i) incorporating the IDM Manual into CottonLOGIC and (ii) developing protocols for growers to use CottonLOGIC to record disease incidence and provide decision support for disease management.

The principles of the disease management strategy for black root rot have already been disseminated to the industry by various means, including: two Cotton CRC information sheets, an article in The Australian Cottongrower, presentations and papers in the proceedings at Australian Cotton Conferences in 1998 and 2000 and at the Cotton CRC Research Conference in 1999, presentations at numerous grower meetings and field days, media releases and radio and television interviews.

COMMUNICATION OF RESULTS

Publications

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Nehl, D. B. (2000). Black root rot - is it on the increase? In *Macquarie Cotton Field Day Notes* p. 26. NSW Agriculture: Warren NSW.

Nehl, D. B. (2000). Black root rot - is it on the increase? In *Lower Namoi Cotton Field Day Notes,* Australian Cotton Research Institute: Narrabri NSW.

Other extension activity

During the course of this project the principal researcher conducted the following additional extension activity:

Media releases	5
Television interviews	4
Radio interviews	9
Press interviews	3
Presentations at grower meetings and field days	29
Lectures	13
Seminars or contributions to research meetings	8
Contributions to cotton newsletters	6
Reports to individual cotton growers	11

APPENDIX I - BUDGET

Total funds contributed to DAN122C by the CRDC.

Year	DAN122C
1998-99	120,229
1999-00	135,025
2000-01	143,098
Total	$398352

APPENDIX II – REPORT BY C. ROTHROCK

REPORT FOR OFF-CAMPUS DUTY ASSIGNMENT (OCDA)

NAME: Craig Rothrock
RANK: Professor
DEPARTMENT: Department of Plant Pathology

PERIOD OF ASSIGNMENT: August 1, 1999- January 31, 2000

ASSIGNMENT:

LOCATION: Australian Cotton Cooperative Research Centre
Australian Cotton Research Institute
Narrabri, Australia
COOPERATORS: Dr. Stephen Allen
Dr. David Nehl

Ecology of *Thielaviopsis basicola* and the Use of Biofumigants for Sustainable Cotton Production

Background:

The modern Australian cotton industry was started in the 1960s with the construction of major dams in northern New South Wales (NSW) and Southern Queensland, and the arrival of two American cotton growers to Wee Waa, NSW. Cotton is currently produced on approximately 350,000 hectares in Australia (Fig. 1). The value of raw cotton tops 1 billion Australian dollars, with meal and oil adding another 100 million dollars. This makes cotton the third largest agricultural export from Australia and Australia the fourth largest cotton exporter in the world. Lint yields are the 2nd highest in the world, 1534 kg/ha compared to 766 kg/ha for the United States. Cotton is generally produced in rotation with wheat. Production is on cracking clays using permanent beds and minimum tillage. Water usage, environmental impact of cotton production and sustainability of current production systems are major concerns for producers and the public-at-large.

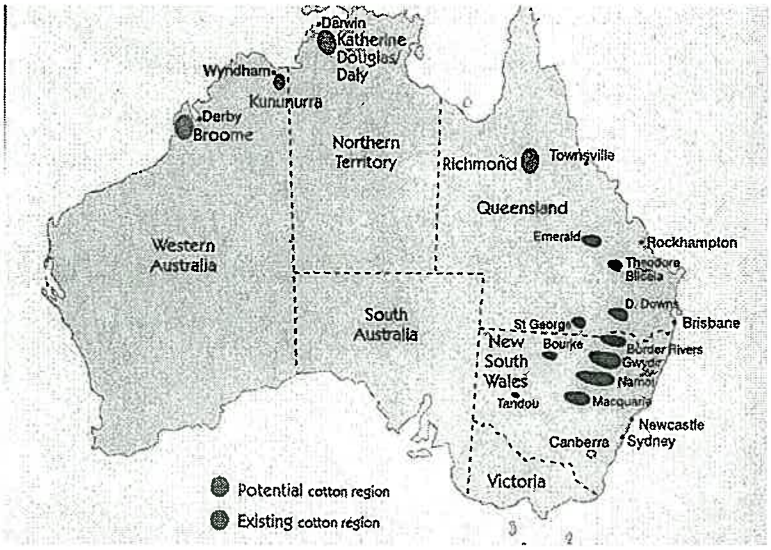

Figure 1. Distribution of Cotton Production in Australia.

Cotton production, as well as cotton research, is highly integrated in Australia. The Australian Cotton Research Institute (ACRI) at Narrabri has staff employed by CSIRO and NSW Agriculture, as well as the cotton industry, and deal with all aspects of production from cultivar development, which are released through Cotton Seed Distributors (CSD), to the environmental impact of cotton production. Three farming systems trials are addressing long-term sustainability of cotton production; including tillage, crop rotation and fertility issues. Information dissemination is strengthened by a National Cotton Extension Team and the Technology Resource Center, located at ACRI. Cotton research has been fostered under the Commonwealth Government's cooperative research center (CRC) program, first as the Sustainable Cotton Production CRC and now the Australian Cotton CRC. The Cotton CRC includes 12 participating groups; CSIRO, NSW Agriculture. Department of Primary Industries Queensland, Agriculture Western Australia, Northern Territory Department of Primary Industry and Fisheries, University of New England, University of Sydney, Cotton Research and Development Corporation, Cotton Seed Distributors, Queensland Cotton, Western Agricultural Industries, and Twynam Cotton. This level of support and integration of researchers and resources make the research responsive to grower and public concerns and allow a level of interaction between researchers and growers that should be emulated by other research efforts.

The United States shares a number of cotton disease problems with the Australian cotton industry. Diseases of major concern are seedling diseases, black root rot, Fusarium wilt and Verticillium wilt. The pathology group at ACRI includes: Dr. Stephen Allen, conducting research on disease resistance and management of cotton diseases; Dr. David Nehl, conducting research on soilborne diseases, including black root rot, and the efficacy of biofumigants; and Dr. Subbu Putcha, conducting research on the biological control of cotton diseases, temporarily located in Canberra.

Research:

Thielaviopsis basicola (syn. *Chalara elegans*) causes black root rot on over 230 plant species in 49 families. The pathogen is most often associated with plants in the Malvaceae, Fabaceae, Solanaceae and Cucurbitaceae. In California, Yarwood detected *T. basicola* in 197 of 402 collections, 46% of virgin soils and 54% of cultivated fields, indicating that the fungus is common in native and cultivated ecosystems. In Arkansas, the pathogen has been detected in over 70% of cotton fields surveyed, with 40% of fields having populations over 100 CFU/g. Symptoms of black root rot are brown to black discolored lesions on the root, which quickly increase until much of the root system takes on a black discoloration. The cortical tissue collapses and the epidermis and cortical tissue may slough off. Black root rot rarely causes plant death, however, early season growth and development are reduced and this delay in development may result in yield reductions.

T. basicola has been characterized recently as a hemibiotroph, an ecological obligate parasite. This characterization suggests a number of areas of research related to the pathogen's survival and ecology that may allow the development of economical management practices. Among these areas of emerging research are the impact of cultural practices on the survival of spores of the fungus, the chlamydospore, and the events from the time of germination of the chlamydospore to infection of the host, preinfection events. Research objectives developed in consultation with researchers in Australia included;

1) Evaluating the potential of green manure crops to suppress *Thielaviopsis basicola*,.
2) Determining the reproductive potential of *Thielaviopsis basicola* on plant species,
3) Examining the nature of chlamydospore survival and germination in the rhizosphere..

Objective 1. Research conducted at the University of Arkansas in the early 1990s as part of a grant from the Sustainable Agriculture Research and Education Program (SARE) demonstrated that the incorporation of a hairy vetch winter cover crop in a cotton production system significantly reduced populations of *T. basicola* through a loss in spore viability and reduced incidence of black root rot on the subsequent cotton crop. Subsequent research in our laboratory indicated that ammonia is evolved into the soil atmosphere at levels in controlled and field studies to account for this loss in viability. Australian researchers are now growing hairy vetch and other cover crops to examine the use of green manure crops as biofumigants for the control of black root rot in cotton. Much of this research is concentrating on lines of canola and Indian mustard selected for elevated levels of glucosinolates, which upon tissue death release high levels of isothiocyanates, volatile sulfur containing compounds that are toxic to many organisms. This approach may have broad application for the control of soilborne fungi and plant parasitic nematodes in cotton and many other field and vegetable crops.

A number of field sites on farms were established in 1999 examining the use of various winter cover crops in cotton production systems by David Nehl. The primary crops were hairy vetch, canola 'Karoo', and Indian mustard '521'. Both of the crucifers had been selected for their high levels of glucosinolates. Tests were located on fields with a history of black root rot. Upon incorporation at Auscott in the Namoi Valley, with the assistance of David Nehl, soil atmosphere sampling equipment was buried to examine the release of ammonia or ITCs and other volatile sulfur compounds from decomposing residue. In addition, the effect of green manure crops on spore germination and viability were examined using a nylon-mesh technique to bury and recover chlamydospores.

As part of these ecological studies, the semi-selective medium TB-CEN for the recovery of *T. basicola* was examined in Australian soils. This medium had shown limited success in Australian soils for quantifying the pathogen, even though it is used routinely in the United States. The medium consists of carrot juice as the source of nutrients. A number of antimicrobial agents are added to the medium to give it selectivity. The medium was divided into the antibacterial components (penicillin G, streptomycin sulfate, and chlortetracycline), the antifungal antibiotic nystatin, and the fungicide Terrazole, active against mucorales and oomycetes. After extensive tests it was determined that nystatin needed to be reduced to ½ or 1/4 the published concentration to be less inhibitory to *T. basicola* and the Terrazole concentration should be doubled to increase activity against Mucorales in these soils. With these changes, populations were evaluated in subsequent research.

Controlled environmental studies were initiated at the time of incorporation of green manures at Auscott. Two studies were conducted using Indian mustard and hairy vetch. In experiment 1, the green manure was either incorporated fresh or desiccated with the herbicide paraquat to evaluate the effect of herbicide burndown treatments on suppression of *T. basicola* by green-manure amendments. Experiment 2 was conducted with frozen plant materials and treatments were rates of the green manures, 1 or 1.5X the rate of the biomass produced in the field study.

From these studies it appears that both green-manure amendments gave some suppression of the pathogen. Herbicide treatment of the plant material lessened the impact of crucifer plant material, while it enhanced the efficacy of hairy vetch (Table 1). These treatment effects or trends should be confirmed by conducting additional controlled and field experiments.

Table 1. Effect of green manure crops on soil populations of *Thielaviopsis basicola*

Amendment	Population(CFU/g soil)
Vetch	122 ab
Vetch+herbicide	73 c
Mustard	87 bc
Mustard+herbicide	108 abc
None	142 a

Objective 2. If *T. basicola* is a hemibiotrophic parasite, the reproduction of the pathogen on the host is critical to the survival of the pathogen. Various plant species, including possible rotation or alternative crops and weeds were examined for the ability of the pathogen to reproduce on the root system of these plants. The bioassay might also detect the potential of root exudates from the plant species to reduce populations of the pathogen through stimulating chlamydospore germination or reducing spore viability.

For these experiments, individual chlamydospores were prepared by lysing the sheath around chlamydospore chains using chitinase by the method of Candole and Rothrock (Phytopathology 97:197-202). Four experiments were conducted to examine reproduction on plants in soil infested with approximately 100 chlamydospores/g of soil. Two experiments were conducted in the greenhouse and two experiments were conducted in a growth chamber to give a range of temperature and light regimes. Three weeks after planting plants were carefully removed, root systems placed in sterilized distilled water and agitated on a wrist-action shaker for 15 minutes, and 1 ml of suspension used with a pour-plate technique to determine colony forming units. Additional data included plant stand, growth stage, dry top and root weight, and disease severity.

Cotton allowed the greatest reproduction of all crops (Table 2). The weed Velvetleaf in the Malvaceae also promoted high levels of reproduction of the pathogen. Reproduction also occurred on alfalfa, soybean and chickpea (Kabuli). Other reported hosts did not support reproduction under the conditions of these studies including; chickpea (Desi), tomato, and cucumber.

Table 2. Reproduction of *Thielaviopsis basicola* on plant species.

	Colony forming units/g of rhizosphere soil		
Crop	Growth chamber 1	Greenhouse 1	Growth Chamber 2
Cotton	25,119 a[z]	48,273 a	7,065 ab
Velvetleaf	--	14,652 a	20,399 a
Chickpea (kabuli)	2,512 b	1,423 b	414 cdefg
Alfalfa	1,000 b	1,535 b	710 cde
Soybean	631 b	56 cde	1,791 bc
Sunflower	79 c	86 cde	73 gh
Cucumber	50 c	27 e	326 cdefg
Chickpea (desi)	40 c	179 cd	104 efgh
Tomato	40 c	95 cde	184 defgh
Hairy vetch	40 c	94 cde	936 cd
Soil	**37**	**84**	**85**
Pea	32 c	69 cde	118 efgh
Bean	32 c	64 cde	95 fgh
Wheat	32 c	61 cde	40 h
Sorghum	25 cd	61 cde	106 efgh
Cowpea	20 cde	59 ef	99 fgh
Faba bean	20 cde	238 c	186 defgh
Onion	16 cde	1 f	37 h
Canola	3 e	67 cde	63 gh
Mustard521	--	85 cde	524 cdef

Objective 3.

Based on the reproduction on the various plant species, host and nonhost crops were selected to examine the role of root exudates from plant species on the germination of chlamydospores. Seeds were planted in sterilized sand and after seven days plants were carefully removed. A moist chamber was designed from a plastic petri plate by placing the plate vertically, lining it with wet filter paper and removing an area in the top of the moist chamber for the stem of the plant. The root system of the plant was placed in the moist chamber with the root tip in a microfuge tube containing a suspension of chlamydospores. The chamber was closed with a cotton collar around the opening where the stem exited the moist chamber. After 24 hours, the root was removed, the spores spun down by centrifugation and most of the supernatant was decanted. Germination of spores was then assessed.

Cotton root exudates allowed the greatest germination of chlamydospores compared to the other crops evaluated (Table 3). This coincides with cotton's reproductive potential and susceptibility. Chickpea, another host, allowed good germination, however, this did not differ from the nonhost wheat, a plant that did not allow reproduction of the pathogen. Soybean, another host crop, stimulated less germination than wheat. The data that indicate some nonhost plant species allow a level of germination as great as host crops need to be confirmed in *in vitro* and *in vivo* systems.

Table 3. Germination of chlamydospores of *Thielaviopsis basicola* in root exudates of plant species.

Crop	Germination (%) Exp.1	Exp. 2	Exp. 3
Cotton	36 a	38 a	16 a
Chickpea (kabuli)	18 cd	20 bc	9 bc
Soybean	1 e	13 c	2 de
Chickpea (desi)	30 ab	26 b	6 cde
Wheat	25 bc	27 b	8 bcd
Canola	8 de	15 c	13 ab
Mustard	--	14 c	6 cde

Chlamydospore germination on TB-CEN after 24 hours was 42%, 42% and 31% for Experiments 1, 2 and 3, respectively.

This research has the potential to address new strategies to control an important soilborne pathogen of numerous crops. For these cultural practices to be implemented, understanding the ecology of *T. basicola* is essential. Other benefits include the characterization of the role of specific exudates in initiation of preinfection events for soilborne pathogens and examining the use of nonchemical control practices for the management of soilborne pathogens.

Other activities:

Fusarium wilt: Field tour of cotton and cotton research in the Goondiwindi area, November 24-25, 1999.

Fusarium wilt, caused by *Fusarium oxysporum* f.sp. *vasinfectum*, is a destructive disease in the southeastern United States and in much of the rest of the world. The first confirmed record of the disease in Australia was in the Darling Downs of Queensland in March of 1993. As of the 1999-2000 growing season, the disease has been observed in over 30 farms in NSW and at least a third of the farms on the Darling Downs. In the lower part of the Darling Downs, it is estimated that up to 30% of farms are no longer suitable for cotton production (D. Nehl, personal communication). Dr. Natalie Moore, a pathologist at DPI, has characterized isolates from affected fields. Research suggest that there are two separate strains of the pathogen in Australia, one from the Darling Downs and a second from the Boggabilla area, based on different DNA fingerprints and vegetative compatibility groups. These data also indicate the pathogen was not introduced from another cotton production area in the world where the disease occurs, but is indigenous.

This disease was devastating in fields observed in the Goondiwindi area, with affected areas of fields having up to 70% plant death within 6 to 8 weeks after planting. Field studies observed examined the influence of residue management on survival of the pathogen and disease development and cultivar susceptibility. In addition to the research effort, there is an active education campaign, "come clean go clean," for growers, as well as researchers, to limit the spread of the pathogen. This program emphasizes the removal of infested soil from vehicles and farm equipment with the product Castrol Farmcleanse. Disease development and distribution in Australia differs from Fusarium wilt in the United States in that it is not associated with the root-knot nematode and thus can occur on finer-textured soils.

Meetings attended:

Australasian Plant Pathology Meeting, Canberra, September 26-October 1, 1999.
Micro-organisms and Cotton Productivity, Narrabri, September 7, 1999.
Open Day for the Australian Cotton Research Institute, Narrabri, December 8, 1999.

Presentations:

Walker, N. R., Rothrock, C. S., and Kirkpatrick, T. L. 1999. *Thielaviopsis basicola* (black root rot) and *Meloidogyne incognita* (the root-knot nematode), an important new interaction on cotton in the United States. Australasian Plant Pathology Meeting, Canberra, September 26-October 1, 1999.

Rothrock, C. S. Diseases of Cotton in the United States. Cotton Production Class, Narrabri, October 2, 1999.

Rothrock, C. S. Managing Cotton Diseases in the United States. Annual Meeting of the Upper Namoi Cotton Growers Association, Gunnedah, September 22, 1999.

Rothrock, C. S. Downunder and Outback in Narrabri: A Plant Pathologist's Odyssey. Australian Cotton Research Institute, Narrabri, December 16, 1999.

Rothrock, C. S. Cotton Pathology Research. Cotton Research & Development Corporation and Cotton Seed Distributors Open House of the New Cotton Breeding and Pathology Wing of the Australian Cotton Research Institute, ACRI, Narrabri, November 4, 1999.

Meetings with additional scientists:

Brisbane; Queensland Department of Plant Industry and University of Queensland, August 5-6, 1999.

Dr. Elizabeth Aitken, University of Queensland, Brisbane, and the Cooperative Research Center for Tropical Plant Pathology. Discussed the host specificity of *Thielaviopsis basicola* and the genetic diversity of the pathogen.

Dr. Natalie Moore, Department of Primary Industries Queensland, Farming Systems Institute at the Indooroopilly Research Center. Discussed Fusarium wilt of cotton and the origin and diversity in the pathogen *Fusarium oxysporum* f. sp. *vasinfectum.*

Dr. R. G. O'Brien, Department of Primary Industries Queensland, Farming Systems Institute at the Indooroopilly Research Center. Discussed host specificity of *Thielaviopsis basicola* and black root rot on lettuce.

Adelaide; Waite Institute, University of Adelaide and CSIRO Land and Water, October 29-29, 1999.

Dr. Steven Neate, CSIRO Land and Water. Discussed population diversity in *Rhizoctonia solani* and farming systems.

Dr. David Roget, CSIRO Land and Water. Discussed the development of predictive models for Take-all of small grains.

Products

Research proposals:

Diversity and pathogenicity of *Thielaviopsis basicola*. PIs Dr. Elizabeth Aitken and Dr. David Nehl,
Collaborators Drs. Craig Rothrock, Stephen Allen and Joe Kochman. Australian Cotton Cooperative Research Center and Cooperative Research Center for Tropical Plant Protection.$115,375.

Factors Influencing Host Specific Responses of the Soilborne Pathogen *Thielaviopsis basicola*; A New Strategy for Disease Management. Craig S. Rothrock. Research Enhancement Grant, Dale Bumper's College of Agricultural, Food & Life Sciences. $20,000 (Pending).

Publications:

Rothrock, C. S. and Nehl, D. B. 2000. Reproductive potential of *Thielaviopsis basicola* on plant species and chlamydospore germination in response to host and nonhost exudates. (Abstr.) Phytopathology (In Press).

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