Australian Government

Cotton Research and
Development Corporation

FINAL REPORT

Managing black root rot of cotton

CRDC Project No. DAN153C

July 2001 to June 2004

DAVID B. NEHL, OM P. JHORAR AND
ANOWAR H. MONDAL

NSW DEPARTMENT OF **PRIMARY INDUSTRIES**

Locked Bag 1000, Narrabri, NSW, 2390, Australia
Tel. +61 2 6799 1500

ISBN 0 7347 1657 5

DAN153C *Managing black root rot of cotton*

CONTENTS

Australian Government
Cotton Research and Development Corporation

Annual, Progress and Final Reports

REPORTS

Part 1 - Summary Details

Please use your TAB key to complete Parts 1 & 2.

CRDC Project Number:		**DAN153C**
Annual Report:	☐	Due 30-September
Progress Report:	☐	Due 31-January
Final Report:	X	Due 30-September (or within 3 months of completion of project)

Project Title: Managing black root rot of cotton

Project Commencement Date: 1 July 2001 **Project Completion Date:** 30 June 2004
Research Program: Diseases and Weeds

Part 2 – Contact Details

Administrator: Mr Graham Denney
Organisation: NSW Agriculture
Postal Address: Lock Bag 21, Orange NSW 2800
Ph: 02 6391 3219 **Fax:** 02 6391 3327 **E-mail:** graham.denney@agric.nsw.gov.au

Principal Researcher: Dr Om Jhorar
Organisation: NSW Agriculture
Postal Address: Locked Bag 1000, Narrabri NSW 2390
Ph: 02 6799 1531 **Fax:** 02 6799 1503 **E-mail:** om.jhorar@agric.nsw.gov.au

Supervisor: Dr Dallas Gibb
Organisation: NSW Agriculture
Postal Address: Locked Bag 1000, Narrabri NSW 2390
Ph: 02 6799 1531 **Fax:** 02 6799 1503 **E-mail:** dallas.gibb@agric.nsw.gov.au

Researcher 2 Dr David B. Nehl.
Organisation: NSW Agriculture
Postal Address: Lockbag 1000, Narrabri NSW 2390
Ph: 02 6799 1528 **Fax:** 02 6799 1503 **E-mail:** david.nehl@agric.nsw.gov.au

Signature of Research Provider Representative: ________________

PLAIN ENGLISH SUMMARY

DAN153C *Managing black root rot of cotton*

Black root rot, caused by a soilborne fungus, was first observed in Australian cotton in 1989. The disease has developed rapidly since then and now occurs in most of the cotton production area in NSW and Queensland. The fungus survives for long periods in the soil as very resilient, dormant spores that are produced abundantly on cotton roots. 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 *T. basicola* virtually impossible to eradicate. Black root rot is favoured by cool temperatures and the infection of roots is, therefore, most severe at the seedling stage, causing stunted growth. No single control measure gives adequate protection against this pathogen and an integrated disease management approach is required.

The objectives of this research project were to evaluate novel and existing control measures for black root rot, conduct ongoing assessment of the impact of farming systems on black root rot and to consolidate and expand upon the existing disease management strategy for black root rot. A range of potential tools for control of black root rot were evaluated in glasshouse and field experiments. Key findings include:

- Confirmation that in-furrow application of the fungicide benomyl was ineffective in controlling black root rot (as previously indicated for other fungicides)
- Treatment of cotton seed with acibenzolar-S-methyl has potential for control of black root rot as part of an integrated disease management strategy
- The effectiveness of biofumigation against black root rot depends upon good growth, incorporation and breakdown of the biofumigation crop
- Delayed sowing has potential to avoid climatic conditions that favour black root rot and seedling disease and should be an effective control; the use of varieties with high fruit retention should enable delayed sowing without loss of yield potential
- Long-term rotation with non-host crops, such as cereals, has potential to decrease the population density of the black root rot pathogen sufficiently to control disease
- The host range of *T. basicola* was extended to include several weed species, indicating that effective control of weeds should help to reduce carryover of the black root rot pathogen

The integrated disease management strategy for black root rot has been modified to include the key findings of this research.

FULL REPORT

1. Background

Black root rot, caused by the fungus *Thielaviopsis basicola*, was first observed in Australian cotton in 1989. The disease has developed rapidly since then and now affects more than 50% of fields surveyed in NSW and occurs in most areas in southern Queensland. The fungus survives for long periods in the soil as very resilient, dormant spores that are produced abundantly on cotton roots (up to 800,000 spores per gram of root in three weeks). 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 *T. basicola* virtually impossible to eradicate. If black root rot continues to spread at its current rate, then 95% of fields in established farms will 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. The major effect of black root rot on cotton is slow early-season growth. In effect, black root rot 'steals' time from the crop, leading to delayed maturity and yield loss. Black root rot contributed to a yield decline of 40% in a farming systems trial in the Macquarie Valley during the 1990's. In the 1999/00 season, a three week delay in maturity and a 26% loss in yield were observed in severely affected areas in a field in the Namoi Valley, while growers elsewhere estimated losses as high as 1.5 bales/acre. Although *T. basicola* does not kill seedlings by itself, severe black root rot may render cotton more susceptible to *Rhizoctonia* and *Pythium*. The cool wet conditions experienced in October 2000 resulted in above average seedling losses and widespread replanting, especially in fields with severe black root rot.

All cultivated cottons are susceptible to black root rot and there are very few effective control measures. Research in CRDC project DAN122C has indicated some potential control measures including summer flooding, induced resistance, biofumigation and rotation for periods greater than one year. Summer flooding can reduce the population of *T. basicola* in soil by up to 98% but its use is constrained by terrain and the availability of water. Soaking cotton seed in acibenzolar-S-methyl (syn. Benzothiadiazole, trade name Boost®) can induce some resistance to black root rot (disease severity decreased by up to 30%) but this remains to be fully tested in the field. Biofumigation with vetch or mustard reduced disease severity by as much as 60% and 70% respectively. Some of these trials will be continued with the same plots in subsequent seasons to determine cumulative effects. An anecdotal study commenced in Project DAN122 C showed a 70% reduction in the severity of black root rot after two years rotation with wheat. Subsequently, a fully replicated experiment that includes rotation with wheat for up to three consecutive years was commenced at Warren. However, a farming systems trial that includes biofumigation, rotations and other control practices for black root rot is warranted. Although fungicides have not given consistent results so far, there is scope for testing new chemistry and application methods. To fully develop effective management practices for black root rot, further research on the control of this intractable disease is required.

The research in this project was designed to enable continued monitoring of the impact of farming systems on black root rot, further evaluation of tools for its management and transfer of this information to growers as part of an integrated disease management strategy.

2. Objectives and extent to which they have been achieved

(i) Evaluate novel and existing methods for control of black root rot

Potential tools for control of black root rot were evaluated in glasshouse and field experiments, including: in-furrow fungicides; activation of systemic acquired resistance using acibenzolar-S-methyl; biofumigation with brassicas and vetch; solarisation in combination with biofumigation; delayed sowing to avoid cool conditions; manipulation of soil temperature with a modified bed shape; cover crops to increase soil temperature and/or reduce wind-chill early in the season.

(ii) Continue to assess the impact of farming systems on black root rot:

The impact of rotation with cereals for three consecutive years was assessed in field experiments at Narromine and Warren. The progress of black root rot with continuous cotton, wheat rotation with and without minimum tillage was monitored in the long-term farming systems experiment at the Australian Cotton Research Institute.

(iii) Consolidate and expand the management strategy developed in the previous project (DAN122C) for control of black root rot

A management strategy for black root rot was developed and incorporated in the CRDC guidelines, *Integrated Disease Management*, published in 2002. The management strategy has now been revised and includes recommendations from the research conducted in this project.

(iv) Facilitate delivery and deployment of the management strategy for black root rot

Aspects of the management strategy and the results of the research in the project have been communicated by way of scientific and extension publications, including the guidelines for integrated disease management, presentations at conferences, meetings and field days, media releases and interviews.

3. Methodology and justification

Disease assessment

Seedling mortality was determined by expressing plant stand as a percentage of the seed sown, with stand counts being conducted two or more metres of row, in any given replicate plot. This comparison gave an estimate of seedling mortality, which included seed viability as well as the impact of seedling disease (*Rhizoctonia* and *Pythium*), grazing insects in the soil (such as wireworms) and physical problems (such as fertiliser burn).

The severity and incidence of black root rot was based on quantification of symptoms, with confirmation of the presence of *T. basicola* using a field microscope). The severity of black root rot was assessed on the basis of discolouration on taproots and rated on a scale of 0-10 where 0 = no blackening, 1 was >0 and ≤ 10%, 2 was >10 and ≤ 20%, 3 was >20 and ≤30%, etc. with 10 being >90 and ≤100% of the tap root blackened. When the severity of disease on taproots in the field experiments was very high among the treatments, the number of relatively healthy lateral roots on each plant was counted. Lateral roots were considered to be relatively healthy when <50% of their length was discoloured or blackened. The lateral root counts provided resolution of the impact of experimental treatments when the severity of symptoms on tap roots was otherwise at a 'saturation' level.

Field experiments

Field experiments were used to evaluate the impact of farm management practices, environmental factors and novel control methods, on the severity and epidemiology of black root rot. Wherever possible, field sites had prior evaluation of the distribution of *T. basicola* in soil, by assessment of the severity and incidence of black root rot in the previous cotton crop or assays of the population density of *T. basicola* using the selective medium TBCEN agar.

In biofumigation experiments at ACRI and on commercial farms, vetch, mustard or canola were grown during the winter prior to cotton and incorporated, where possible, at least four weeks prior to sowing cotton. Fungicides were evaluated were evaluated under a range of climatic conditions, including sites in at ACRI, and in commercial fields in the Macquarie, Lachlan and Barwon Valleys. Acibenzolar-S-methyl (trade name Boost®) was applied as either an in-furrow spray or by soaking seed in an aqueous solution or by incorporating acibenzolar-S-methyl with the conventional seed treatments.

Farming systems practices were evaluated in controlled experiments. Evaluation of the impact of one, two and three consecutive crops of wheat on black root rot, in the long-term field experiment established at Narromine as part of CRDC project DAN122C was continued. Since that trial had only single replicates of each treatment, a fully replicated large-plot trial was established on a commercial farm at Warren. The progress of black root rot was monitored in the long-term farming systems experiment at the Australian Cotton Research Institute. This experiment included three treatments: cotton every summer with normal tillage practices, cotton every summer with minimum tillage, and cotton rotated with wheat every other year and minimum tillage.

Glasshouse/growth room experiments

In experiments with potted soil, naturally infested soil was used where possible. Pots were watered by submerging the base in water for the minimum period required to wet the soil by capillary action, thus simulating subbing-up of cotton beds in the field during furrow irrigation. Pot experiments were generally assessed at between three and five weeks after sowing. In the glasshouse, temperatures were maintained with heating set to prevent temperatures falling below 15°C, cooling set to prevent temperatures exceeding 32°C and natural variation between these limits according to climatic conditions. In the growth room, temperatures were set to a 12 hour night/day cycle of 18/23°C.

Design and analysis

All glasshouse and field experiments used completely randomised block designs where possible. Internal replication was used for growers' trials that used un-replicated plots or a small number of replicates. Blocks were included in analysis of variance using the general linear model in the program SYSTAT version 10 (SPPS Inc.) followed by pairwise comparison of means. Linear and non linear regression models were fitted to 'dose response' experiments and to comparisons between symptoms and other parameters, also using SYSTAT 10.

4. Results and discussion

4.1 Novel and existing methods for control of black root rot

4.1.1 In-furrow fungicides

- In-furrow application of the fungicide benomyl provided a low level of control of black root rot in some fields and had no effect on the disease in other fields
- Shoot growth was not increased in any of the experiments by in-furrow application of benomyl
- Even if repeatable results could be obtained, benomyl would have to be applied at uneconomic rates to achieve a moderate decrease in disease severity

The potential of in-furrow fungicides to control black root rot of cotton was examined in several experiments in CRDC project DAN122C (*Black root rot and slow early season growth of cotton*). The fungicide triadimenol (Baytan®, Bayer Australia Ltd) reduced the severity of black root rot on cotton in some field trials but not in others. Furthermore, this fungicide tended to have phytotoxic effect on cotton, delaying emergence by approximately two days and slowing plant growth. In DAN122C, an experiment using potted soil demonstrated that several concentrations of the fungicide benomyl (Benlate®, Dupont) were able to reduce the severity of black root rot in cotton without a phytotoxic effect. Subsequently, the potential for benomyl, applied as an in-furrow spray over the seed at planting, to control black root rot of cotton in the field was evaluated in this project. Unless stated otherwise, the fungicides were applied using portable injection equipment developed for this purpose.

In a field near Narromine, cotton (V2RR) was sown on 4 October 2001, at rate of 16 kg/ha with Temik and Cotogard. The fungicide benomyl (Benlate®) was applied as an in-furrow spray to 8-row plots at the rate of 500 g/ha, using the grower's injection system, which sprayed the solutions of benomyl into the planting slot immediately behind the seed. In this experiment, benomyl had no effect on the severity of black root rot in cotton (Table 4.1). Shoot growth at 54 days after sowing was significantly lower in the plots treated with benomyl, suggesting that benomyl was phytotoxic. However, benomyl showed no signs of being phytotoxic to cotton in any of the other experiments in the field (Table 4.2) or in potted soil (reported in project DAN122C). An alternative explanation is that the reduction of shoot growth may have been a reflection of the density of spores of *T. basicola* in the soil prior to commencement of the trial, which averaged 300 and 378 cfu/g soil for untreated and treated plots respectively (although these values were not significantly different).

Table 4.1. Lack of effect of the fungicide benomyl, applied as an in-furrow spray, on stand establishment and black root rot of cotton in a field near Narromine

	Untreated	Benomyl	Probability
1 November 2001			
Plant stand (plants/m)	15.3	17.2	Not significant
Disease severity (0-10 scale)	9.0	9.1	Not significant
Healthy lateral roots (No./plant)	5.2	4.9	Not significant
Shoot growth (mg/plant)	113	107	Not significant
27 November 2001			
Shoot growth (mg/plant)	473	353	$p = 0.019$

In a field near Trangie, cotton was sown on 21 September 2001 and the fungicide benomyl was applied as an in-furrow spray to single row plots (15 m long) at three different rates, with water as the control. This experiment was repeated in a field near Warren, that was sown on 4 October 2001 (Sicala 40 at 14.5 seeds/m), in a field near Hillston that was sown on 3 October 2001 and in a field near Walgett that was sown on 1 October 2001. In the experiments at Trangie, Warren and Walgett, there were weak trends of decreasing disease severity of symptoms on tap roots with increasing rates of benomyl (Table 4.2). The reductions in disease severity observed at Trangie, Warren and Hillston were too weak to reflected by significant increases in seedling growth. Benomyl tended to increase stand establishment at Trangie and Warren but the correlations were not very strong (Table 4.2).

Table 4.2. The effect of in-furrow application of the fungicide benomyl (Benlate®) at different rates on stand establishment, black root rot and growth of cotton in the 2001-02 season

Benlate® applied (g/ha)	Plant stand (plants/m)	Disease severity on tap roots (0-10 scale)	Relatively healthy lateral roots (No./plant)	Shoot growth (mg/plant)
Trangie				
0	6.3	9.7	0.3	51
246	9.2	8.3	2.2	52
365	8.8	8.4	3.0	66
718	9.4	7.9	3.0	62
[z]Regression	$r^2 = 0.23$	$r^2 = 0.32$	$r^2 = 0.47$	Not
Probability ($n = 24$)	$p = 0.017$	$p = 0.004$	$p < 0.001$	significant
Warren				
0	9.3	5.8	4.1	74
417	9.6	4.5	5.3	89
835	10.4	4.2	5.5	88
890	10.9	4.8	4.9	79
[z]Regression	$r^2 = 0.24$	$r^2 = 0.30$	Not	Not
Probability ($n = 18$)	$p < 0.037$	$p < 0.001$	significant	significant
Hillston				
0	9.3	9.7	7.3	65
221	9.3	9.5	6.6	63
524	9.2	9.7	7.7	64
793	8.7	9.6	7.6	64
[z]Regression	Not	Not	Not	Not
Probability ($n = 24$)	significant	significant	significant	significant
Walgett				
0	5.2	8.7	-	90
177	4.7	7.6	-	92
386	5.1	7.4	-	93
526	6.0	7.4	-	96
[z]Regression	Not	$r^2 = 0.17$	Not assessed	Not
[y]Probability ($n = 36$)	significant	$p < 0.013$		significant

[z]Regressions compare all observations to the actual amounts of fungicide applied in each plot, as the rate of fungicide application varied from plot to plot during application. Values in columns are means for the desired rates.
[Y]Plots furthest from the tail ditch had little or no disease and, therefore, 12 observations were removed from the analysis.

At Trangie, benomyl increased the number of relatively healthy lateral roots significantly but the increase in lateral roots was apparently not substantial enough to be reflected by a growth increase at the time of assessment (Table 4.2). In that experiment, the number of relatively healthy lateral roots and the severity of black root rot on tap roots were closely correlated (Fig. 4.1a), confirming the value of assessment of lateral root numbers as a measure of disease severity. Although benomyl improved plant stand establishment slightly in the experiment at Trangie (Table 4.2), plant stand and the severity of black root rot on tap roots were not correlated at all (Fig. 4.1b). This confirms previous observations that black root rot and seedling disease caused by *Pythium* and *Rhizoctonia* are generally not linked, even though benomyl had appears to have affected both types of disease.

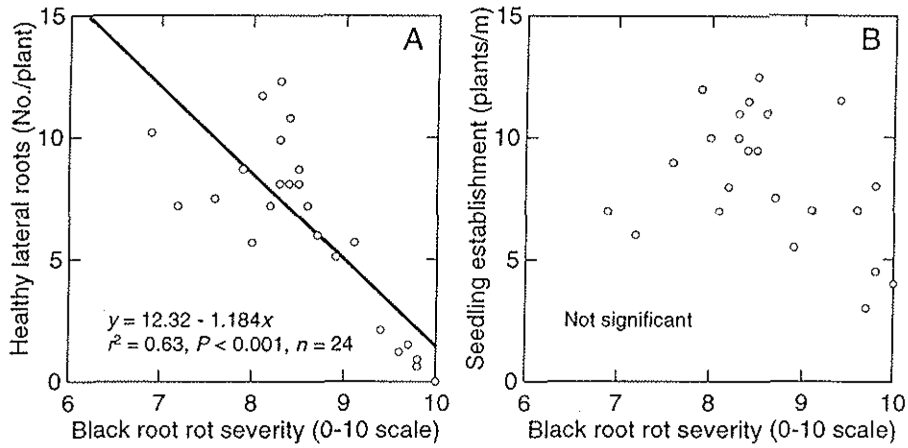

Figure 4.1. When the fungicide benomyl (Benlate®) was applied as an in-furrow spray in a field near Trangie in the 2001-02 season, the severity of black root rot in cotton was not related to plant stand but was closely matched by the number of relatively healthy lateral roots.

While benomyl reduced the severity of black root rot substantially in previous pot experiments, this result could not be repeated in field trials reported here. Climatic conditions that occurred early in the 2001-02 season were cooler than those maintained in the controlled environment used for the pot experiments with benomyl, suggesting that this fungicide may only perform well when conditions are less favourable to the pathogen. Given that a substantial reduction in disease severity on tap roots required rates of 0.5 kg benomyl/ha or more, it seems unlikely that this fungicide would be an economically viable control measure, even if consistent results could be obtained.

4.1.2 Systemic acquired resistance

- The potential for acibenzolar-S-methyl to induce a resistance response in cotton against black root rot was demonstrated in the field by either soaking seed just before sowing or applying an in-furrow spray at sowing.
- Imbibition during soaking causes the seed to swell, resulting in release of fewer seed by mechanical planters.
- Application as an in-furrow spray and, if effective, application with the standard seed dressing would avoid the problem of lower planting rates with the soaking method
- In a dose response experiment, acibenzolar-S-methyl controlled black root rot better at low levels of inoculum, suggesting the potential to slow disease progress in newly-infested fields and decrease further dispersal of the pathogen.

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 a previous project (DAN122C Black root rot and slow early season growth of cotton) a number of experiments were conducted using acibenzolar-S-methyl (also known as benzothiadiazole or BTH) to induce resistance to black root rot in cotton. When acibenzolar-S-methyl was applied to cotton seedlings as a foliar spray a phytotoxic effect was observed and the application was too late to preempt development of disease. Subsequently, acibenzolar-S-methyl was applied to cotton seeds, by soaking prior to planting, and successfully decreased disease severity in pot experiments. In this project, the novel approach of applying acibenzolar-S-methyl directly to the seed was evaluated in cotton crops in the field.

In a commercial cotton field west of Moree, cotton (cv. Sicot 80) was sown on 4 October 2001, in plots (8 rows × 500 m) with two treatments in a randomized block design with three replicates (8 rows buffer between each block). The treatments were water (550 L/ha) and acibenzolar-S-methyl (25 μg acibenzolar-S-methyl /mL, at 550 L/ha) applied as in-furrow sprays (i.e. into the planting furrow) prior to closure by the press wheels. In each plot, the severity of black root rot was assessed in 30 plants at 20 m from the tail drain and, to provide additional replication, in 30 plants at 50 m from the tail-water drain. Analyses of the data by 2-way AOV indicated that the distance from the tail drain did not have significant effects and the data set was treated as if it had six replicates. In an experiment at the Australian Cotton Research Institute, cotton seed (var. NuPearl RR at 15 seeds/m) was soaked in a solution of acibenzolar-S-methyl (25 μg/mL) for three hours, followed by air-drying, immediately prior to sowing, on 9 October 2001. Plots were 8 rows × by the length of the field and the treatments were applied in a completely randomised design with eight replicates. This experiment was repeated in a field near Gunnedah sown with cotton (var. Sicala 40 at 12 seeds/m) on 9 October 2001.

At the ACRI site, soaking seed in acibenzolar-S-methyl decreased the severity of black root rot on tap roots of cotton by one third (Table 4.3). The soaking treatment did not increase the number of relatively healthy lateral roots or shoot growth but it did decrease plant stand substantially. Black root rot was only present at very low levels in a few plants in the experiment near Gunnedah, although plant stand was also decreased in the seed-soaking treatment (Table 4.3)

Table 4.3. Effect of soaking cotton seed for three hours in a solution of acibenzolar-S-methyl, immediately prior to sowing, on the severity of black root rot of cotton in the 2001-02 season

	Unsoaked	Soaked	[z]Probability
Field at ACRI (34 DAS)			
Disease incidence on tap roots (% plants)	93	86	NS
Disease severity on tap roots (0-10 scale)	6.9	4.6	$P = 0.021$
Relatively healthy lateral roots (No./plant)	6.6	5.7	NS
Plant stand (plants/m)	13.0	7.6	$P < 0.001$
Shoot dry mass (mg/plant)	170	187	NS
Field near Gunnedah (28 DAS)			
Disease severity on tap roots (0-10 scale)	0.20	0.19	NS
Plant stand (plants/m)	9.8	7.3	$P < 0.001$
Shoot dry mass (mg/plant)	148	146	NS

[z]Values in rows are not significantly different at the stated probability level (NS = not significant, DAS = days after sowing)

In the field near Moree, application of acibenzolar-S-methyl as an in-furrow spray decreased the severity of disease on tap roots by 24% and increased the number of relatively healthy lateral roots by 350% (Table 4.4). This decrease in disease severity was not reflected by shoot growth at the time of sampling. However, by January it had resulted in a 29% increase in fruit numbers (Table 4.4). This increase in fruit development reflected an advancement of maturity but did not result in a yield increase (Table 4.4), which is consistent with previous observations of the capacity for cotton to compensate for early maturity delays later in the season. Nevertheless, the capacity for acibenzolar-S-methyl to induce a resistance response in cotton against black root rot and to, potentially, advance crop maturity was demonstrated in these field experiments.

Table 4.4. Reduced severity of black root rot of cotton in a field near Moree following seed treatment with acibenzolar-S-methyl applied as and in-furrow spray at sowing in the 2001-02 season

	Acibenzolar-S-methyl (g/ha)[z]		
	0	27.5	[y]Probability
35 DAS			
Disease severity on tap roots (0-10 scale)	9.6	7.3	$P < 0.001$
Relatively healthy lateral roots (No./plant)	1.5	6.8	$P < 0.001$
Plant stand (plants/m)	10.6	11.6	NS
Shoot dry mass (mg/plant)	633	672	
128 DAS			
Fruit development (bolls/m)	21b	27a	$P = 0.004$
193 DAS			
Yield (bales/ha)	8.0	8.3	NS

[z]Acibenzolar-S-methyl was applied as a solution in water (25 µg/mL) at the rate of 550 L/ha.
[y]Values in rows are not significantly different at the stated probability level (NS = not significant, DAS = days after sowing)

Soaking cotton seed in acibenzolar-S-methyl reduced seedling establishment substantially in the field experiments at ACRI and at Gunnedah. The phytotoxic effect of acibenzolar-S-methyl to cotton that was observed previously with foliar application (DAN122C) was not observed in the pot experiments with seed soaking (DAN122C), nor in with application as an in-furrow spray (Table 4.4). This suggests that phytotoxicity was not the reason for lower stand establishment when soaking was used in the field (Table 4.3). During the soaking process, some of the standard fungicide seed coating was removed from the seed and this may have reduced the effectiveness of these fungicides in preventing seedling disease, caused by *Rhizoctonia* or Pythium. However, imbibition during the soaking process increased the size of seeds substantially, which may have affected the planting rate in commercial planters. To test this hypothesis, cotton seed was soaked for three hours in water, air-dried, and run through two boxes on the mechanical planter used at ACRI, with unsoaked seed in the other two boxes. This test showed that the release of seed from the planter was reduced by 20% when seed was soaked. This imbibition effect on the rate of sowing was probably partly responsible for the reduced plant stand in the soaking experiment at ACRI, in which the control treatment was not soaked. In seed-coating experiments (data not presented) and the in-furrow spray experiment near Moree, imbibition did not occur prior to sowing and stands were not decreased by acibenzolar-S-methyl. Application of acibenzolar-S-methyl as an in-furrow spray or as an additive to standard seed treatments of cotton would be preferable for commercial deployment of this product.

The field trials deliberately utilised sites in which the severity of black root rot was high, in order to obtain a relatively even distribution of inoculum across plots. The observed resistance responses were sufficient for a partial control of the disease. The potential for activation of resistance against *T. basicola* at lower levels of inoculum has not previously been evaluated. A dose-response experiment was conducted in a controlled environment room (23/28°C 12 h night/day cycle) using potted soil that was naturally infested with *T. basicola.* To obtain difference inoculum densities, the soil was steamed and amended with various amounts of unsteamed soil. The soil was sown with cotton seed (cv. Sicala V2). The acibenzolar-S-methyl was applied as an in-furrow spray, using a syringe to spray the solution over the seed in a simulated planting slot, with water used as the untreated control.

In the untreated control, the severity of black root rot on cotton tap roots increased as the population density of *T. basicola* increased, reaching a plateau level at around 100 cfu *T. basicola* per gram of soil (Figure 4.2). A substantial level of disease was observed when the soil contained 10 cfu/g, indicating the conditions in the controlled environment room were favourable for disease. Treatment of seed with acibenzolar-S-methyl decreased the rate at which disease severity progressed with increasing inoculum and was, therefore, more effective at controlling disease with the lower levels of inoculum.

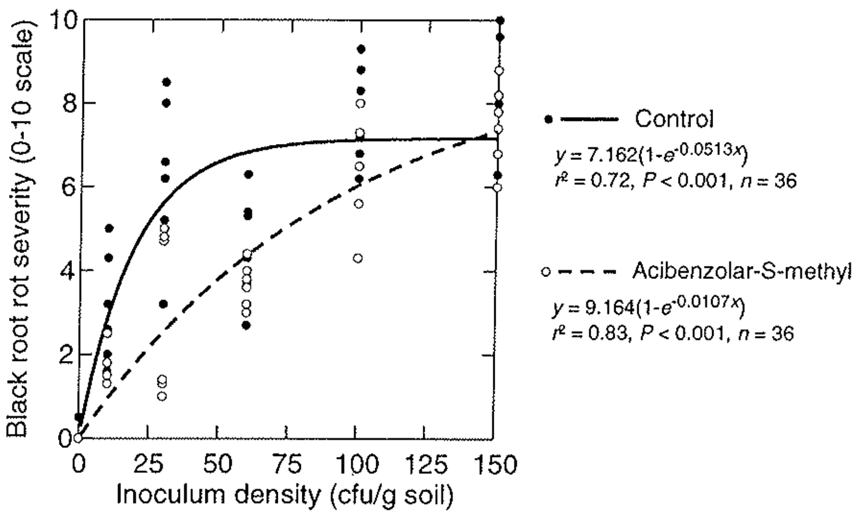

Figure 4.2. Effect of inoculum density of *Thielaviopsis basicola* in soil on black root rot of cotton with and without seed treatment with acibenzolar-S-methyl.

These experiments have clearly demonstrated that acibenzolar-S-methyl has potential as a tool to control black root rot of cotton. Activation of resistance is not likely to provide complete control of the disease in soils that contain a high inoculum density. Nevertheless, acibenzolar-S-methyl did result in substantial decreases in the severity of black root rot in such fields. The observation that activation of resistance was more effective against black root rot at lower inoculum densities, indicates that a prophylactic use of acibenzolar-S-methyl on the whole seed-crop may (i) help prevent the disease becoming severe when fields are infested at low levels and (ii) slow the progress of the black root rot epidemic across NSW and Queensland. Further evaluation of the potential for acibenzolar-S-methyl to control the disease at low levels of inoculum is warranted.

4.1.3 Biofumigation

- In several field experiments biofumigation crops were not effective in controlling black root rot of cotton
- These experiments highlight the need for good growth, good incorporation and good breakdown of biofumigation crops for them to be effective in controlling black root rot of cotton

In a field near Walgett, vetch and wheat were sown in 8 m plots in June 2001 and incorporated in September as a green manure crop, with bare plots as a control. The soil in each plot was sampled on 1 October 2001, along two transects across the plots at distances of 50 m and 400 m from the tail drain. The soil samples were assayed for the population density of *T. basicola* using TBCEN agar and there were no significant differences among treatments in the transect at 50 m (mean 365 cfu/g soil) or the transect at 400 m (mean 199 cfu/g soil). Cotton was sown on 1 October 2001 and disease severity and growth were assessed on 28 October 2001. There were no significant differences in stand establishment, disease severity or cotton growth among the treatments in either transect of the plots. However, there was a slight increase in the number of relatively healthy lateral roots of cotton that followed vetch in the transect at 50 m from the tail drain (Table 4.5). This observation suggests that the vetch had a slight biofumigation effect on the pathogen but not enough to prevent substantial infection of cotton tap roots. At 2.5 tonnes dry mass/ha the vetch crop did not grow as well as in previous experiments, in which a substantial biofumigation effect was been observed. Furthermore, the soil was very dry when the vetch was incorporated prior to sowing the cotton and breakdown of the vetch residues was poor. This experiment suggests that effective biofumigation will require both good growth of vetch, as well as good incorporation and breakdown of its residues.

Table 4.5. Severity of black root rot of cotton in 2001-02 following winter fallow (bare) or vetch as a green manure crop in a field near Walgett that was heavily infested with *T. basicola*

	Bare	Vetch	Probability
50 m from tail drain			
Stand establishment (plants/m)	5.6	5.6	NS
Black root rot severity (0-10 scale)	8.8	8.2	NS
Relatively healthy lateral roots (no/plant)	4.5	5.5	$P = 0.034$
Shoot dry mass (mg/plant)	85	91	NS
400 m from tail drain			
Stand establishment (plants/m)	6.3	6.3	NS
Black root rot severity (0-10 scale)	6.8	6.2	NS
Relatively healthy lateral roots (no/plant)	6.6	8.9	NS
Shoot dry mass (mg/plant)	106	117	NS

In winter of 2000, *Brassica juncea* (cv. Indian mustard 651) was sown in 8-row plots, alternating with 8-row bare plots, across a field near Baan Baa in which severe black root rot had been observed in the 1999-00 season. Stand establishment of the mustard was patchy but reasonably even along a line 30 m from the tail drain of the field. The population density of *T. basicola* in the soil was assayed on 10 October 2001 but was no different between the two treatments (Table 4.6). In general, the population density of *T. basicola* in soil needs to be around 50 cfu/g to cause substantial disease symptoms in cotton. The low level of the pathogen observed in both treatments in this field was reflected by relatively low severity of black root rot symptoms on cotton tap roots (Table 4.6). Given the relatively poor establishment of the mustard crop, this experiment provides further evidence that good growth and incorporation of biofumigation crops is required for effective disease control.

Table 4.6. Effect of a green manure crop of Brassica juncea (Indian mustard) on the population density of *T. basicola* in soil and the severity of black root rot of cotton in a field near Baan Baa in the 2001-02 season

	Bare	Mustard	Probability
T. basicola (cfu/g soil)	19.5	11.5	NS
Stand establishment (plants/m)	8.0	7.9	NS
Black root rot severity (0-10 scale)	3.6	3.2	NS
Shoot dry mass (mg/plant)	99	98	NS

Woolly pod vetch was sown as a green manure crop in a field near Mallawa in winter of 2000. The grower left a single strip of 16 rows that was not sown with vetch. Cool dry conditions were experienced during winter at that site and the vetch grew poorly (. The effect of the vetch on disease in the subsequent cotton crop, sown 4 October 2001, was assessed on 8 November 2001 in three transects crossing from the vetch tot the bare strip to the vetch, at distances of 20, 40 and 60 m from the tail drain. Data from the three transects were pooled for analysis. The vetch resulted in a marginal decrease in the severity of black root rot on cotton tap roots (Table 4.7). The number of relatively healthy lateral roots was not increased by the vetch (Table Kamilaroi) but, given the low probability value for the means belong to the same population (P = 0.062) a significant result may have been possible had the experiment been conducted with a fully replicated design. This evaluation also confirms that successful biofumigation will not be achieved when growth of the biofumigation crop is poor.

Table 4.7. Effect of a green manure crop of woolly pod vetch on the severity of black root rot of cotton in a field near Baan Baa in the 2001-02 season

	Bare	Vetch	Probability
Stand establishment (plants/m)	12.1	12.8	NS
Black root rot severity (0-10 scale)	9.9	9.7	$P = 0.038$
Relatively healthy lateral roots (no/plant)	1.7	2.4	NS
Shoot dry mass (mg/plant)	170	180	NS

In winter of 2001, canola (cv. Karoo) was sown in a field near Trangie and four eight-row strips left unsown (bare) at distances of approximately 200 m apart. Soil from within the bare strips and the next, adjacent eight rows of canola was sampled and assayed for *T. basicola* using TBCEN agar on 5 July 2001. A second field near Narromine was also sown with canola (cv. Rainbow) and five eight-row strips were left unsown, in a randomised design with eight-row strips of canola. Soil samples from the canola and bare plots were also assayed for the presence of *T. basicola* on the 5 July 2001. Both crops were grown through to harvest and the soil at the same positions was assayed again on 27 November 2001. The population density of *T. basicola* was variable among the plots, especially in the field at Narromine, in which half of the trial area had very little pathogen in the soil (Figure 4.3). This variability in the pathogen masked any differences between the treatments with and without canola and there were no significant differences in disease severity in cotton sown when is it was sown in these fields 2002-03 season. However, the mean decrease in population density in the field at Trangie (Figure 4.3) was 53% in the plots with canola, which was significantly greater ($P = 0.011$) than the change in population density in the bare plots (increased by 15%, probably due to sampling variation). A similar decrease was not observed in the field near Narromine but the lack of the pathogen in half the plots was a likely confounding factor.

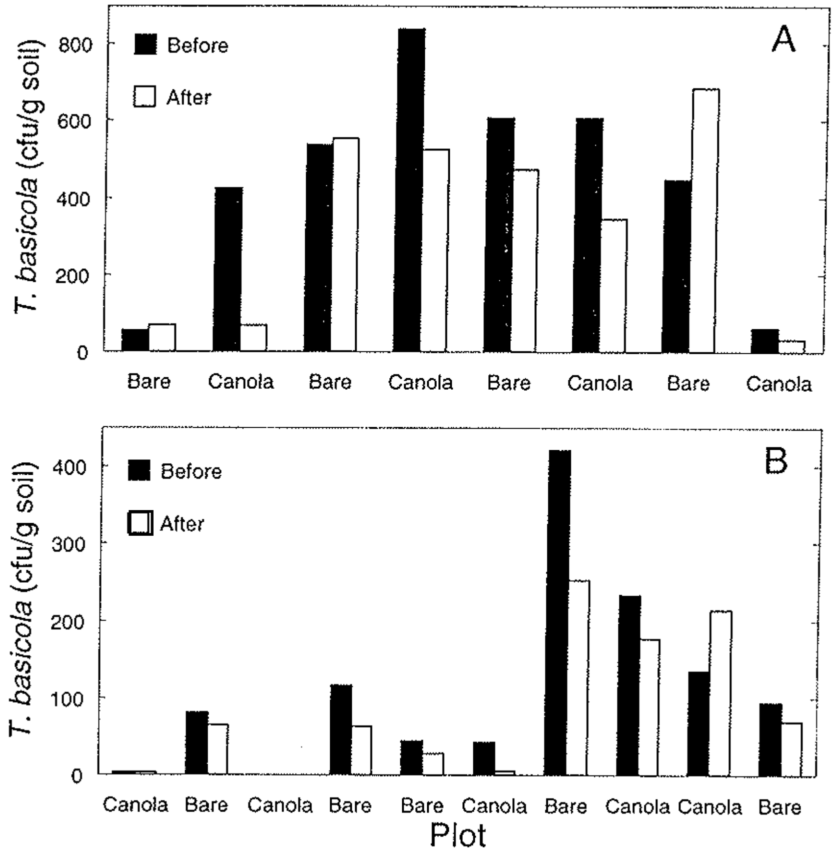

Figure 4.3. Effect of canola cropping in 2001 on the population density of *T. basicola* in soil in fields at Trangie (A) and Narromine (B), assessed just after sowing (before) and after harvest (after).

4.1.4 Delayed sowing

- In a field experiment at the Australian Cotton Research Institute, delaying sowing from the 28 Sep to the 17 Oct decreased the severity of black root rot substantially
- Delayed sowing has potential to control black root rot and seedling disease by decreasing the period of exposure of cotton to climatic conditions that favour black root rot

A replicated experiment with two planting dates was established at the Australian Cotton Research Institute as part of CRDC project DAN122C, *Black root rot and slow early season growth of cotton*. 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, with cotton being sown on 28 September 2000 and 10 October 2000. In that year of the experiment, symptoms of black root rot were absent in plants in plots that were not inoculated. Delaying sowing by 12 days appeared to have no effect on the severity of black root rot, assessed on the tap roots using the 0-10 scale, but this comparison was not based on assessments at after equal periods of time after sowing. The experiment was repeated with a greater differential between sowing dates (19 days) and assessment of black root rot at 28 days after sowing for both planting-date treatments.

Delayed sowing increased plant stand, indicating that the period of exposure to conditions favourable to seedling pathogens (*Rhizoctonia* and *Pythium*) was minimised (Table 4.8). In comparison to the control, inoculation of soil with *T. basicola* decreased plant stand substantially in the 'early' treatment and this effect was completely mitigated in the 'late' treatment. This represents the first clear evidence, in Australia, that seedling mortality can be increased by *T. basicola*. In contrast, the disease surveys conducted annually by NSW Department of Primary Industries (DAN154C *Diseases of cotton VII*) have consistently shown no relationship between seedling mortality and black root rot in commercial cotton crops.

Delayed sowing decreased the severity of black root rot dramatically (Table 4.8). A low level of symptoms was observed on tap roots in both control treatments, which is consistent with the 'background' level of discolouration that is normally observed on cotton seedlings, due to other pathogens or factors in the soil. Inoculation with *T. basicola* caused severe symptoms on tap roots in the early sowing. In the late sowing, symptoms on tap roots in the inoculated treatment were no different to those in the controls. Black root rot decreased the number of relatively healthy lateral roots by 80% in the early sowing and by 40% in the late sowing. Delaying sowing more than doubled the growth rate of cotton in the controls. This effect was less pronounced in the inoculated treatments, where delaying sowing increased growth by around 60%. *T. basicola* clearly caused disease in cotton sown at both dates (Table 4.8). However, by delaying sowing, the window of climatic conditions that were favourable for disease development was shortened sufficiently to gain a substantial decrease in disease severity. The 17th of October is within the recommended planting window for the Namoi Valley and should not incur a yield loss. Furthermore, the new varieties of insect-resistant transgenic cotton offer the potential for sowing to be delayed further without yield loss, due to greater fruit retention. Delayed sowing shows promise as a tool for minimising the impact of black root rot on cotton.

Table 4.8. The severity of black root rot of cotton, at 28 days after sowing, was decreased by delaying sowing in field plots that were either artificially infested (Inoculated) or uninfested (Control) with the pathogen, *Thielaviopsis basicola*, in a field at the Australian Cotton Research Institute

	Sown 28 Sep 2001		Sown 17 Oct 2001		
	Control	Inoculated	Control	Inoculated	[A]Probability
[B]Stand establishment (plants/m)	9.0b	6.6c	11.5a	11a	$P \leq 0.024$
Black root rot severity (0-10 scale)	2.9b	8.2a	1.6b	4b	$P \leq 0.006$
[C]Relatively healthy lateral roots (no/plant)	6.7b	1.4c	10.0a	6b	$P \leq 0.012$
Shoot dry mass (mg/plant)	235bc	186c	501a	305b	$P \leq 0.049$

[A]Values in rows followed by the same letter are not significantly difference by pairwise comparison of means with the Scheffe test at the stated probability level.
[B]Stand was assessed on 26 October, which was 28 and 7 d after the two sowing dates, respectively.
[C]A lateral root was classed as being relatively healthy if the length symptoms of black root rot were visible on less than 50% by length, when dug carefully from the soil.

The experiment in 2001-02 was repeated using the same plots and treatments in the 2003-04 season. In the repeat experiment, delayed sowing increased stand establishment significantly using pairwise comparison of means using Fisher's LSD test ($P \leq 0.003$) but the distinction was less clear using the more-stringent Scheffé test (Table 4.9). In the inoculated plots, delayed sowing decreased the severity of black root rot on tap roots and led to 250% increase in shoot dry mass (Table 4.9), confirming the observations in the previous year. In both experiments, the delay in sowing did not result in a yield penalty (data not presented).

Table 4.9. The severity of black root rot of cotton, at 26 days after sowing, was decreased by delaying sowing in field plots that were either artificially infested (Inoculated) or uninfested (Control) with the pathogen, *Thielaviopsis basicola*, in a field at the Australian Cotton Research Institute

	Sown 3 Oct 2002		Sown 20 Oct 2002		
	Control	Inoculated	Control	Inoculated	[A]Probability
[B]Stand establishment (plants/m)	8.7ab	7.8b	11.0a	10.2ab	$P = 0.029$
Black root rot severity (0-10 scale)	1.6c	6.0a	4.3ab	3.4b	$P \leq 0.032$
Shoot dry mass (mg/plant)	87b	79b	264a	280a	$P < 0.001$

[A]Values in rows followed by the same letter are not significantly difference by pairwise comparison of means with the Scheffé test at the stated probability level.
[B]Stand was assessed on 29 October, which was 26 and 9 d after the two sowing dates, respectively.

4.1.5 Thermal bio-disinfestation

- Solarisation of cotton beds with polythene films may be an effective tool for control of black root rot and other diseases of cotton
- The potential for control of black root rot by sealing the soil with polythene film after incorporation of biofumigation crops is possible but yet to be demonstrated
- The use of polythene films for soil solarisation and thermal bio-disinfestation will depend upon proven cost-effectiveness and strategies to prevent waste film from polluting the environment

Plants that release antifungal substances can potentially be used as biofumigation crops. Most evaluation of biofumigation crops in Australian cotton has included brassicas (mustard and canola) and legumes (e.g. woolly pod vetch). Mustard contain glucosinolates, naturally occurring compounds mainly as potassium and sodium salts. Glucosinolates are hydrolysed by enzymes in the presence of water to yield glucose and unstable aglucone, which spontaneously form an isothiocynate. In the biofumigation process plant based glucosinolates are hydrolysed in field soil to form toxic products, including isothiocyanates. These degradation products may exert control or a suppressive effect on soil borne pathogens, such as *T. basicola*. Woolly pod vetch is used as a green manure crop and releases ammonia when incorporated in soil, providing nitrogen to the following crop. The release of ammonia during decomposition can occur at levels toxic to black root rot fungus. To achieve a high biomass, biofumigation crops should be planted in autumn, soon after harvesting the summer crop. Ideally, mustard should be incorporated after flowering when it contains the maximum level of glucosinolates. Isothiocyanates are released as living cells are ruptured and, therefore, mustard should be incorporated directly as green plants. In contrast, the release of ammonia from vetch occurs as the tissues decay in the soil. Hence, vetch can be slashed and allowed to dry before incorporation without compromising the biofumigation effect. At field day presentations and meetings in 2002/03 in the Macquarie and Namoi Valleys, growers raised concerns that biofumigation crops might increase the severity of Fusarium wilt. Experiments to evaluate further the efficacy of biofumigation crops against black root rot were conducted in the glass house, on research farm and grower fields using various methods of application.

An experiment was conducted using potted soil collected from a field in the Macquarie Valley in which both black root rot and Fusarium wilt of cotton had been observed. The soil was placed in planter boxes in a glasshouse at Trangie. The experiment was a 3×2 factorial design with three biofumigation treatments (mustard, vetch or bare) and two solarisation treatments (polythene and no polythene) following incorporation of the biofumigation plants.

The vetch and mustard decreased the population density of *T. basicola* when incorporated and sealed with polythene but not without the polythene. In contrast to the observations of spore population, the vetch appeared to decrease the severity black root rot by about 25% in the treatments without polythene, with a slight reduction in the treatment with polythene. The mustard appeared to decrease the population density of *T. basicola* by more than half when covered by polythene but not without the polythene (Figure 4.4). In all treatments the population density of *T. basicola* was greater than 100 cfu/g soil, which is sufficient to cause substantial symptoms of black root rot in cotton. Incorporation of vetch and mustard without the polythene cover did not affect seedling mortality in both varieties of cotton that were tested (Figure 4.4). Incorporation of mustard with the polythene cover had no effect on mortality of Sicala V2RR seedlings but appeared to decrease mortality in the experimental line. The polythene cover decreased seedling mortality by approximately half in both varieties, suggesting that the key effect in this experiment was one of solarisation.

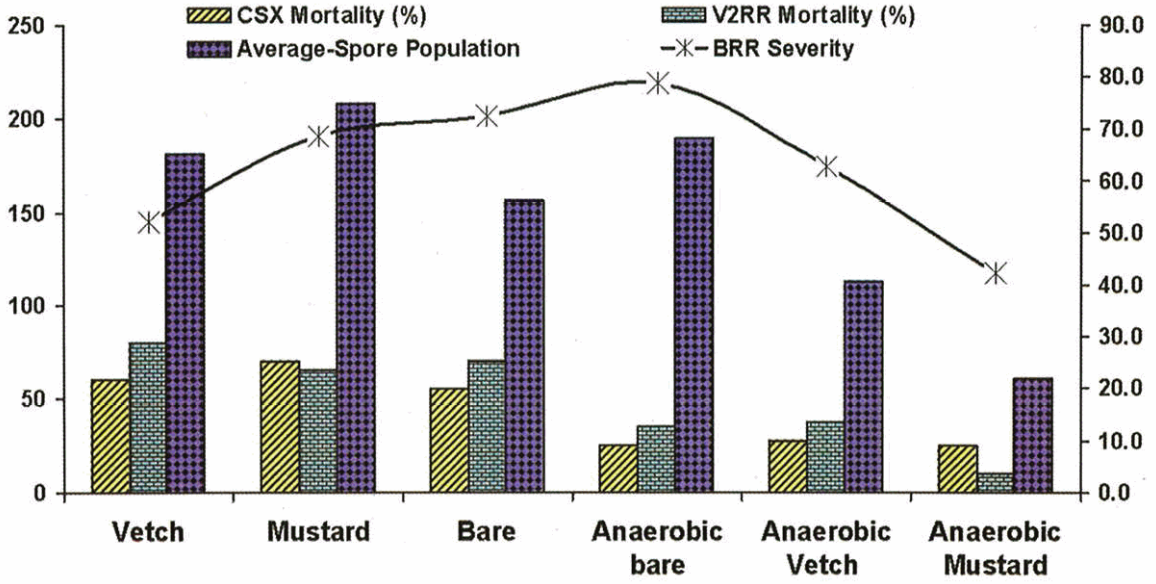

Figure 4.4. Effect of incorporation of vetch and mustard plants on the population density of *Thielaviopsis basicola*, and black root rot severity and seedling mortality of cotton, with and without a polythene cover at incorporation ('anaerobic'), in potted soil collected from a field near Trangie.

Field trials were conducted using polythene film laid over cotton beds on 4 September 2003 at the Australian Cotton Research Institute (Figure 4.5). In comparison to bare soil, the polythene film increased soil temperature by up to 7°C (Figure 4.6). Soil moisture content was approximately 21% under the polythene and 15% in bare plots (Figure 4.7).

Figure 4.5. Covering beds with polythene film after incorporation of a green manure crop of mustard at the Australian Cotton Research Institute on 4 Sep 2003

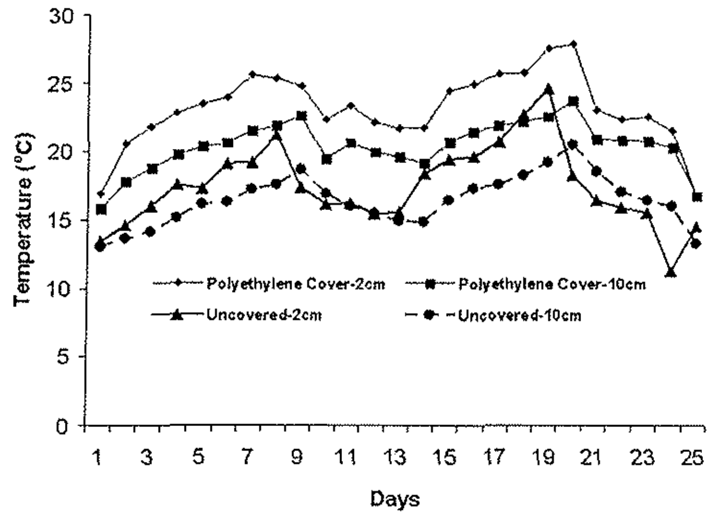

Figure 4.6. Effect of covering beds with polythene sheets on daily average temperature of soil at the Australian Cotton Research Institute, commencing 4 September 2003

Seed cotton yield appeared to be increased when in the treatments that had polyethylene film was applied after incorporation (Figure 3). Since the severity of black root rot on cotton was relatively low at that site (mean of 2.4 on 0-10 scale, on 24 Oct 2003) and not significantly different between treatments (data not presented), control of disease was not a contributing factor to the apparent differences in yield. It is unlikely that covering beds with polythene will be a cost effective method for controlling black root rot unless substantial reductions in disease severity can be produced consistently in the field. Biofumigation crops have been effective against black root rot in previous experiments (previously investigated in CRDC project DAN122C *Black root rot and slow early season growth of cotton).*

The reason for the absence of a biofumigation effect in this experiment cannot be ascertained. Previous observations (DAN122C) have indicated that the effectiveness of biofumigation depends upon good growth of the biofumigation crop, with good incorporation and breakdown of residues. Immediately after incorporation of the biofumigation crops in 2003, the soil was relatively dry and hard and some difficulty was experienced applying the polythene film over the beds. The dry state of soil at incorporation may have impacted upon the efficacy of the biofumigation crops.

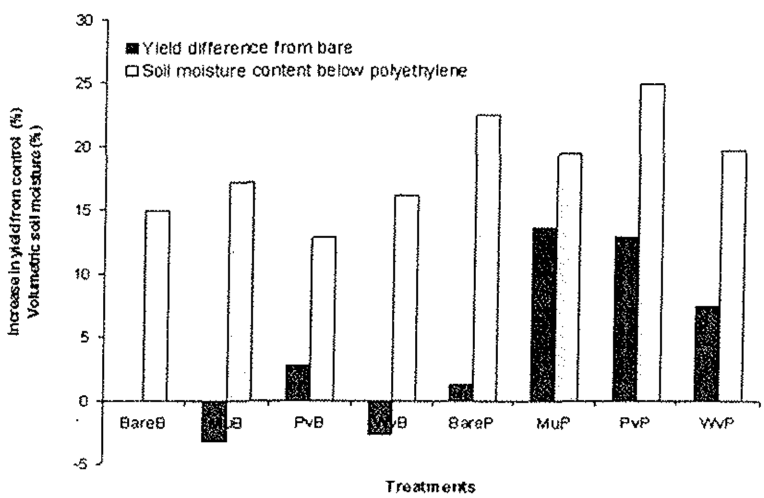

Figure 4.7. Effect of biofumigation crops (Bare = none, Mu = mustard, Pv = pink vetch, Wv = woolly pod vetch) with (P) and without (P) a polythene cover, applied after incorporation, on soil moisture content and cotton yield.

4.1.6 Microenvironment management

- Modified beds may increase soil temperature at the planting line sufficiently to decrease the severity of black root rot but the results in two experiments were inconsistent.
- Cereal cover crops have previously been shown to increase soil temperature
- A consistent effect on the severity of black root rot was not observed in the experiment conducted in this project, with poor stand establishment being a confounding factor

Cool soil temperature (16-21°C) and high moisture (-20 to -10 J/kg) are favourable conditions for black root rot of cotton. Increased soil temperatures or reduced soil moisture levels should be less favourable to the pathogen and reduce the disease severity. The effect of temperature and moisture on black root rot of cotton has been investigated in the USA (Rothrock 1992) but not in Australia. Experiments were conducted a controlled environment room to evaluate the effect of various diurnal temperature regime on the severity of black root rot of cotton. *T. basicola* was able to cause substantial levels of infection of top roots across a wide range of temperatures (Figure 4.8). There were no significant differences in the severity of black root rot among the first four temperature regimes (Figure 4.8). Although, disease severity was lowest in the 23-28°C regime (Figure 4.8) it is likely that active infection by *T. basicola* was greatest during the night period, at 23°C, rather than the day period, at 28°C.

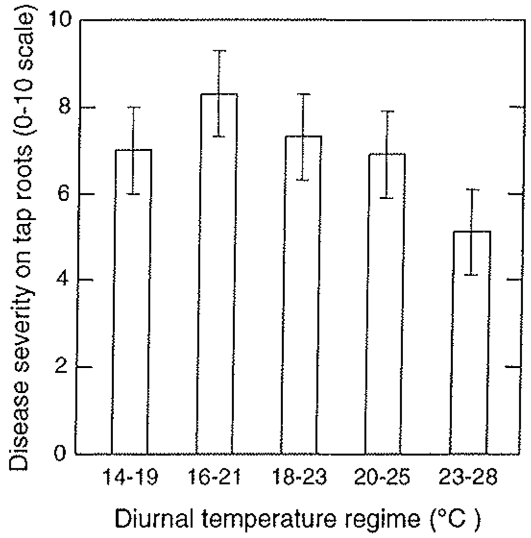

Figure 4.8. The effect of 12/12 h temperature regimes on the severity of black root rot of cotton (bars represent the standard error of the mean of all treatments).

In July 2002, the temperature of raised cotton beds with an east to west orientation was measured at 5 cm below the soil surface, at positions 10 cm either side of the cotton planting line. At that time of year, the path of the sun (ecliptic) was low in the sky and soil temperature on the north side of the bed was approximately 5°C higher than that in the south facing side. In the light of this observation, the potential to increase soil temperature along the cotton planting line by modifying the bed profile, to capture more solar radiation, was investigated. The intention was to increase the surface area of beds, close to the planting line, that received sunlight at incident angles approaching 90° (Figure 4.9).

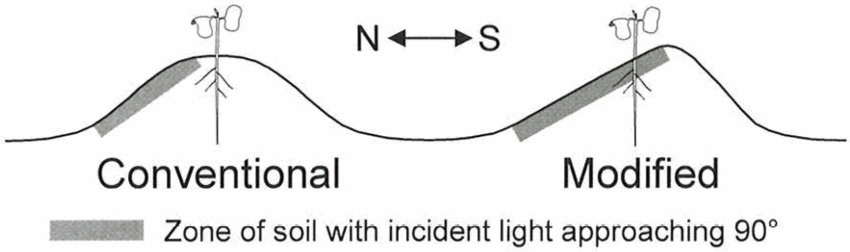

Figure 4.9. Proposed modification of cotton beds to increase the surface area of soil, close to the planting line, that receives sunlight at incident angles approaching 90°, early in the growing season.

Beds were modified by welding a steel plate to one side of a conventional furrow sweep and passing over existing beds in a field at the Australian Cotton Research Institute that was know to be infested with *T. basicola*. The result was initially similar to that proposed (Figure 4.10a), although the bed shape slumped with time (Figure 4.10b). The beds were modified in pairs, alternating with pairs of conventional beds. The severity of black root rot was assessed in five transect lines, perpendicular to the bed direction and at increasing distance from the tail drain of the field. The severity of black root rot decreased with increasing distance from the tail drain (Table 4.10). The modification of beds decreased the severity of black root rot significantly in the two transects furthest from the tail drain but not in the three transects closest to the tail drain (Table 4.10). In contrast to the observations of disease severity, there were no significant differences in cotton growth between conventional and modified beds in any of the transects (Table 4.10). Soil temperatures were not monitored in this experiment and the apparent decrease in disease severity in the two transects furthest from the tail drain was a result of increased soil temperature. The slumping of beds after planting (Figure 4.10b) may have been a confounding factor.

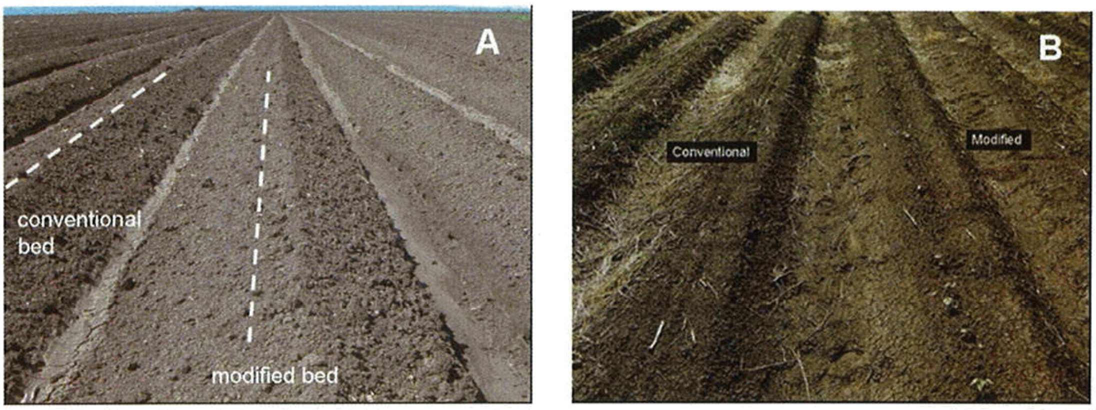

Figure 4.10. Modification of cotton beds prior to sowing cotton (A, dashed line indicates planting line) and after sowing cotton (B)

In 2003 a second experiment to evaluate the potential for modified beds to reduce the severity of black root rot of cotton was conducted at the Australian Cotton Research Institute. This experiment was a 2×3 factorial design with two bed profiles (conventional or modified in shape, as in the previous year), split with three cover-crop treatments (bare soil, a single row of barley on the south side of the planting line, and two rows of barley either side of the planting line). The barley was grown in winter, killed with herbicide and mowed to 20 cm in height prior to sowing cotton. Stand establishment was very low in all plots (Table 4.11). Significant differences in plant stand were observed among the treatments but they are not consistent with either the use of barley, nor the modification of bed shape. Problems were experienced at the time of planting with guidance of the planting machinery, with some seeds being planted into the line of barley stubble, but planting alignment does not explain the very poor seeding establishment in the plots without barley.

Table 4.10. Effect of modifying the shape of cotton beds on the severity of black root rot of cotton growth in a field, with assessment on transects at increasing distance from the tail drain, at the Australian Cotton Research Institute in 2002

	Bed shape		
	Conventional	Modified	Probability
Severity on tap roots (0-10 scale)			
Transect 1	5.5	4.4	Not significant
Transect 2	5.3	3.2	Not significant
Transect 3	6.0	3.9	Not significant
Transect 4	5.2	2.7	$P = 0.016$
Transect 5	4.1	1.3	$P = 0.004$
Shoot dry mass (mg/plant)			
Transect 1	130	125	Not significant
Transect 2	120	116	Not significant
Transect 3	118	100	Not significant
Transect 4	131	129	Not significant
Transect 5	146	123	Not significant

In the plots without barley cover, disease severity was increased by modifying the bed shape, even though the population of spores of *T. basicola* in soil was similar for both treatments (Table 4.11).

Table 4.11. Effect of modifying the shape of cotton beds and growing barley as a cover crops on the severity of black root rot of cotton growth in a field at the Australian Cotton Research Institute in 2003

	Conventional bed shape			Modified bed shape			
	Bare	[A]Barley×1	[B]Barley×2	Bare	[A]Barley×1	[B]Barley×2	Probability
Stand (plants/m)	1.2b	4.2a	1.9b	1.5b	3.6a	1.9b	$P < 0.05$
T. basicola (cfu/g soil)	57	81	93	60	100	57	
Disease severity (0-10 scale)	8.9b	7.9d	9.3ab	9.4a	8.5c	9.0ab	$P < 0.05$
Plant height (mm)	129c	150b	154b	146b	185a	184a	$P < 0.05$
Shoot dry mass (mg/plant)	112c	149b	128bc	151b	185a	125c	$P < 0.05$

[A]One row of barley sown on the south side of the cotton planting line, sprayed with herbicide and mowed to 20 cm in height prior to sowing cotton

[A]Two rows of barley, as for [A], but either side of the cotton planting line

In both the conventional and modified beds, the addition of one row of barely cover decreased the severity of black root rot slightly, whereas two rows did not. The height of cotton plants was increased by both of the barley treatments, in both types of beds (Table 4.11). Since shoot dry mass was not increased in both of the barley treatments, the increase in height was probably due, at least in part, to etiolation of cotton plants. In the modified beds, shoot growth was lower in the plots with two lines of barley stubble then with one line (Table 4.11). This observation was not attributable to shading from the line of barley stubble on the north side of the bed because (i) two lines of barley did not decrease shoot dry mass in the conventional plots, (ii) plant height was not decreased by two lines of barley with either bed shape and (iii) similar problems have not occurred in previous experiments (DAN1222C) in which cotton was sown into a dense cover crops of wheat stubble right across beds. Given the poor stand establishment in the experiment n 2003, it is difficult to draw conclusions about the effects of the treatments on cotton growth.

The experiments evaluating the potential for modified bed shape to increase decrease the severity of black root rot have not given consistent results. In two separate experiments conducted in the 2003-04 season at Moree and Goondiwindi (DAN154C *Diseases of cotton VII*), there was no increase in soil temperature and no effect on seeding disease or Fusarium wilt. It is likely that the use of modified beds to increase soil temperature at the planting line will only have potential at higher latitudes, namely in the Lachlan and Murrumbidgee Valleys, where the spring sun is lower in sky than in northern NSW.

4.2 The impact of farming systems on black root rot

4.2.1 Crop rotation and tillage

- Minimum tillage did not decrease the severity of black root rot consistently in the farming systems trial at ACRI
- Rotation with wheat every second year did not prevent the increase in the severity of black root rot in the farming systems trial at ACRI
- The results confirm the previous observation that the incidence and severity of black root rot increases according to the number of cotton crops growth, irrespective of rotations on a biennial basis.

Black root rot has been monitored, since 1998, in the long-term farming systems experiment conducted by NSW Department of Primary Industries at the Australian Cotton Research Institute. Data from 2004 was available at the time of writing this report and is included for reference. In 1998 and 2004, the incidence of black root rot in was 100 % of plants in all three treatments. The severity of black root rot was similar between the continuous cotton treatments in 1998, 2002 and 2004 but not in 2003 (Table 4.12).

Table 4.12. Effect of tillage system and rotation with wheat on cotton seedling growth and black root rot severity in a farming systems trial at the Australian Cotton Research Institute

	Cotton/cotton Maximum tillage	Cotton/cotton Minimum tillage	Cotton/wheat Minimum tillage	Probability[y]
1998				
Disease severity on tap roots (0-10 scale)	8.7a	7.2a	4.4b	$P \leq 0.006$
Shoot dry matter (mg/plant)	210b	290a	330a	$P \leq 0.026$
1999 (fallow in all plots)				
2000 (not assessed)				
2001 (not assessed)				
2002				
Disease severity on tap roots (0-10 scale)	7.7	6.0	6.0	NS
Shoot dry matter (mg/plant)	-	-	-	
2003				
Disease severity on tap roots (0-10 scale)	7.9a	3.4b	-	$P < 0.001$
Shoot dry matter (mg/plant)	52	48	-	NS
2004				
Disease severity on tap roots (0-10 scale)	6.5a	5.3ab	4.1b	$P = 0.009$
Shoot dry matter (mg/plant)	213	246	307	$P \leq 0.038$

[y] Values followed by the same letter are not significantly different by pairwise comparison of means using the Scheffé test at the stated probability level (NS = not significantly different).

In 1998, the severity of black root rot in the continuous cotton treatments was approximately twice that of the cotton/wheat rotation treatment (Table 4.12). Since the initial onset of black root rot in this field, the wheat rotation treatment included half the number of cotton crops as the continuous cotton treatments. Hence, the lower disease severity in the cotton/wheat rotation in 1998 (Table 4.12) was consistent with previous observations, that the severity of black root rot increases in proportion to the number of cotton crops grown, irrespective or rotation with cereals (Nehl et al. 2004). In 2002, the disease severity was equal among all the treatments (Table 4.12) which was consistent with disease severity continuing to increase in the cotton/wheat plots with each successive cotton crop.

In 2004, disease severity in the cotton wheat plots was equal to that in the minimum tillage continuous cotton plots, although somewhat lower than in the maximum tillage plots (Table 4.12). In 2004, however, disease was assessed on 29 November and the tap roots were beginning to grow rapidly and slough off the damaged outer tissues of the root cortex. Hence, the observed severity on tap roots was lower overall in 2004 than in previous years. The observation that disease severity in the cotton/wheat treatment approached that in the continuous cotton plots over time confirms the conclusions of Nehl et al. (2004) regarding disease increase with repeated cotton cropping.

In 1998, 2002 and 2004, disease severity was equal in both of the continuous cotton treatments (Table 4.12). In 1998 and 2004, disease severity across all treatments was negatively correlated with shoot growth; r^2 = 0.60, P = 0.003 and r^2 = 0.78, P < 0.001, *respectively. In contrast,* disease severity in the minimum tillage plots in 2003 was, apparently, substantially lower than in the maximum tillage plots even though shoot growth was equal between the two treatments and not correlated with disease (r^2 = 0.005, P = 0.865). Since a difference in disease severity between minimum and maximum tillage was only observed in one year, of the four years of assessments, it is concluded that black root rot was not affected greatly by these tillage practices, in a continuous cotton system.

In May 2003, the population density of *T. basicola* was assessed in soil samples taken from the walls of backhoe pits in the four plots in the treatment with continuous cotton and maximum tillage. Soil was collected from 0-10, 10-20, 20-30, 30-40, 40-60, 60-100 and 100-150 cm in depth. Data was plotted against the mean depth of sample collection (Figure 4.11). The greatest density of inoculum of *T. basicola* was located in the top 30 cm of soil. At 50 cm and below the ranged from 0 to 24 cfu/g soil, with 10, 6 and 6 cfu/g being observed at 125 cm in three of the plots. The distribution of inoculum down the profile closely reflects the distribution of cotton roots in the soil profile (Nehl et al. 1997).

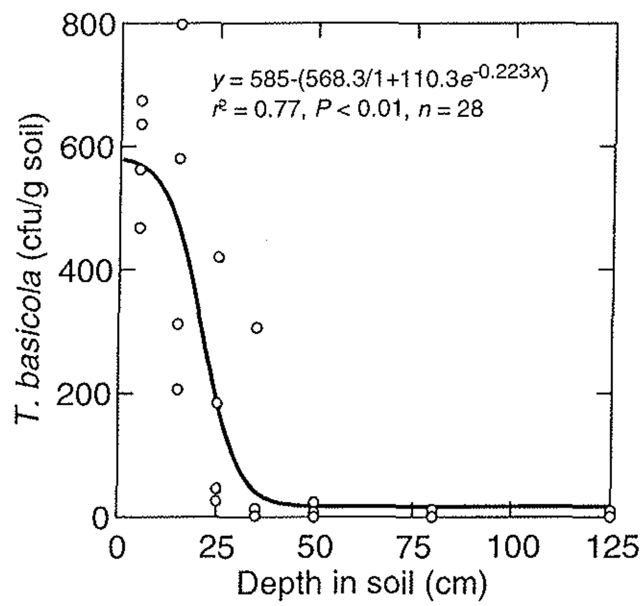

Figure 4.11. Decline in population density of Thielaviopsis basicola down the soil profile in four plots from the continuous cotton-maximum tillage treatment in the farming systems experiment at the Australian Cotton Research Institute.

4.2.2 Long-term crop rotation

- Rotation with three consecutive crops of wheat decreased the severity of black root rot to negligible levels in a field near Narromine
- A fully replicated repeat of this experiment was less successful, when assessed close to the tail drain
- However, seedling growth and cotton yield were increased by one to two bales/ha in the fully-replicated experiment, suggesting that assessment of disease severity close to the tail drain was not representative of the whole field
- Further evaluation of the potential for long-term rotation out of cotton to control black root rot is required before firm recommendations can be made

Farming Systems trials at Warren and the Australian Cotton Research Institute have previously demonstrated that rotation of cotton with cereals for a single season does not reduce the severity of black root rot in cotton, nor prevent its increase over time (reported in CRDC project DAN122C *Black root rot and slow early season growth of cotton*). However, anecdotal reports from a farm near Narromine suggested that the severity of black root rot in cotton may be 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, with single plots (56 rows the length of the field) for each treatment. The severity of black root rot and cotton growth were assessed with internal replication, assessing 10 plants in the 11^{th}, 22^{nd}, 33^{rd} and 44^{th} rows within each 56-row plot, along two transects of the field, 50 m from the head ditch and tail drain respectively.

In October 2001, the severity of black root rot was negligible and the population of *T. basicola* in the soil was very low following rotation with wheat for three consecutive years (CWWW, Table 4.13). After two years of wheat, followed by cotton (CWWC), disease severity in 2001-02 was substantially less than in the cotton-wheat rotation (CWCW) and the wheat-cotton-cotton rotation (CWCC). The observations of disease severity were matched by cotton growth and, to a lesser extent, by yield (Table 4.13, Figure 4.12).

Table 4.13. Decreased severity of black root rot and seedling disease of cotton in the 2001/02 season following long-term rotation with wheat in a field near Narromine

	Previous crops^x				
	CWWW	CWWC	CWCW	CWCC	Probability^y
T. basicola in soil (cfu/g soil)	1b	358a	135ab	313a	$P \leq 0.008$
Disease severity (0-10 scale)	1.2c	6.3b	8.5a	9.0a	$P \leq 0.025$
Healthy lateral roots (no./plant)	22a	10b	8bc	4c	$P \leq 0.008$
Plant stand (plants/m)	9.0ab	8.4b	10.7a	6.3c	$P \leq 0.030$
Fruit development (bolls/m)	21	23	19	17	Not significant
Cotton Yield (bales/ha, hand picked)	9.9a	9.6b	9.8ab	8.1c	$P \leq 0.001$

^{x} Previous crops were wheat/fallow (W) or cotton (C) consecutively from 1997-98 to 2000-01.

^{y} Values followed by the same letter are not significantly different by pairwise comparison of means using the Scheffé test at the stated probability level.

A prominent depression, caused by the press wheels at sowing, was evident along the top of the beds in the plots that followed wheat in 2000-01 but not in the plots that followed cotton, due to the presence of cotton residues. Shortly after disease was assessed, a rainfall event washed herbicide into the soil on beds that had this press-wheel depression, leading to stunting and leaf-burn consistent with symptoms of damage from prometryn. The herbicide damage did not occur in beds that lacked the press-wheel depression. This damage was not present at the time early season disease was assessed (Figure 4.12). The herbicide damage slowed cotton growth and was potentially a confounding factor in the subsequent yield of the crop. Nevertheless, yield was clearly greater in cotton following three years of wheat, than following cotton (Table 4.13). In a separate transect, near the tail drain in the plot that had three years of wheat, the population of *T. basicola* declined from 131 cfu/g soil in 1997 to 3 cfu/g soil in 2001. Rotation with wheat every second year (CWCW) did not reduce disease severity in comparison to continuous cotton (CWCC).

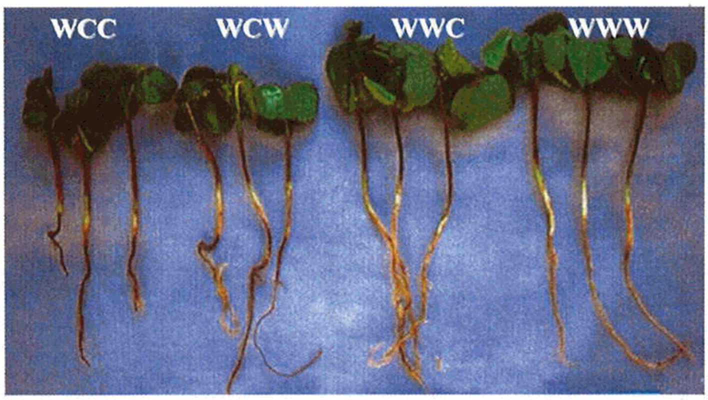

Figure 4.12. Growth of cotton and symptoms of black root rot on tap following long-term rotation with wheat in a field near Narromine (previous crops were wheat/fallow (W) or cotton (C) from 1997-98 to 2000-01, respectively)

When cotton was sown again, in the same field at Narromine in the 2002-03 season, the severity of black root rot was still very low in the plot that had three years of wheat (CWWWC, Table 4.14). The density of spores of *T. basicola* in that plot was higher (Table 4.14) than in the previous year (Table 4.13), suggesting that the pathogen population will recover with continued cotton cropping. Rotation with wheat every second year (CWCW) did not reduce disease severity in comparison to continuous cotton (CWCCC). Rotation with wheat for two consecutive years decreased disease severity and this effect carried forward to the second crop of cotton, in 2002-03 (Table 4.14).

Table 4.14. Decreased severity of black root rot of cotton in the 2002-03 season following long-term rotation with wheat in a field near Narromine

	Previous crops[x]				
	CWWWC	CWWCC	CWCWC	CWCCC	Probability[y]
T. basicola in soil (cfu/g soil, Nov 02)	33b	261a	289a	251a	$P < 0.001$
Disease severity (0-10 scale)	1.4 a	5.4b	7.8c	7.9c	$P \leq 0.001$
Shoot dry matter (mg/plant)	130a	127a	99b	126c	$P \leq 0.044$

[x] Previous crops were wheat/fallow (W) or cotton (C) in 1997-98 to 2001-02, respectively.

[y] Values followed by the same letter are not significantly different by pairwise comparison of means using the Scheffé test at the stated probability level.

The experiment at Narromine provided evidence that rotation with wheat for periods longer than a single season has potential to decrease the population of *T. basicola* in soil dramatically and decrease disease to a negligible level. However, the experiment at Narromine lacked full replication and further evaluation is required before recommendations can be made to growers with certainty. Consequently, a fully replicated experiment with large plots (56 rows × the length of the field) was established on a farm at Warren. The population density of *T. basicola* in the soil, assessed on a transect of the field on 29 November 2000, just after harvest of the first crop of wheat, was relatively even and also very high (Figure 4.13). Rotation with three consecutive crops of wheat did not decrease the severity of black root rot on cotton tap roots, assessed along the transect line 50 m from the tail drain (Table 4.15). However, shoot growth was 24% greater in the plots that followed three years of wheat (CWWW) than in those that alternated with wheat (CWCW). Since the tap roots of cotton reach a saturation level of infection, close to 10 on the 0-10 scale, assessment of the number of relatively healthy lateral roots may have confirmed whether or not the differences in cotton growth were related to black root rot.

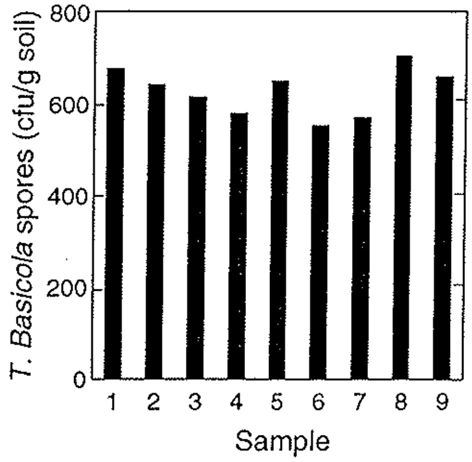

Figure 4.13. Distribution of *Thielaviopsis basicola* in soil along a transect 50 m from the tail drain of a field near Warren on 29 November 2000 (samples were composites)

Table 4.15. Severity of black root rot and growth of cotton at 28 October 2003, following long-term rotation with wheat in a field near Warren

	Previous crops[x]			
	CWWF	CWWC	CWCF	Probability[y]
Disease severity (0-10 scale)	8.6	8.9	9.1	Not significant
Shoot dry matter (mg/plant)	94a	86a	76b	$P \leq 0.038$

[x] Previous crops were wheat/fallow (W), cotton (C) or summer fallow (F), consecutively from 1999-00 to 2002-03.

[y] Values followed by the same letter are not significantly different by pairwise comparison of means using the Scheffé test at the stated probability level.

Yield mapping at harvest showed that the three years of wheat increased cotton yield by one to two bales/ha, in comparison to the other treatments (Figure 4.14). The inconsistent effect of three years of wheat on disease severity, between the experiments at Narromine and Warren, requires discussion on two counts. First, the experiment at Narromine coincided with wet seasons in the winter of 1998 and 1999. In contrast, the experiment at Warren coincided with drought, to the extent that the third crop of wheat could not be sown in winter 2002, and this may have affected the outcome of the experiment. When soil is dry microbial activity comes to a halt. It is quite possible that the potential for *T. basicola* to survive in soil was enhanced by the dry conditions. Alternatively, if the wheat crops were critical factor in the attrition of *T. basicola*, rather than time itself, then the bare fallow over 2002-03 may have favoured greater survival of the pathogen.

Secondly, in the experiment at Warren, the differences in yield among the treatments were less prominent at the position of the transect line 50 m from the tail drain (Figure 4.14), where the severity of black root rot was assessed in 2003. Black root rot is frequently more severe near the tail drain of fields and, the initial population density of *T. basicola* was very high near the tail drain (Figure 4.13). It is possible that, with lower population density of *T. basicola* further into the field, three years of wheat was sufficient to decrease the population density of *T. basicola*, providing enough control the disease to account for the observed yield differences. In the experiment at Narromine, the effect of three years of wheat on black root rot was prominent in the two successive crops of cotton. In the experiment at Warren, assessment of disease in the next crop of cotton, at points further from the tail drain, should indicate whether or not the observed increase in yield was due to disease control.

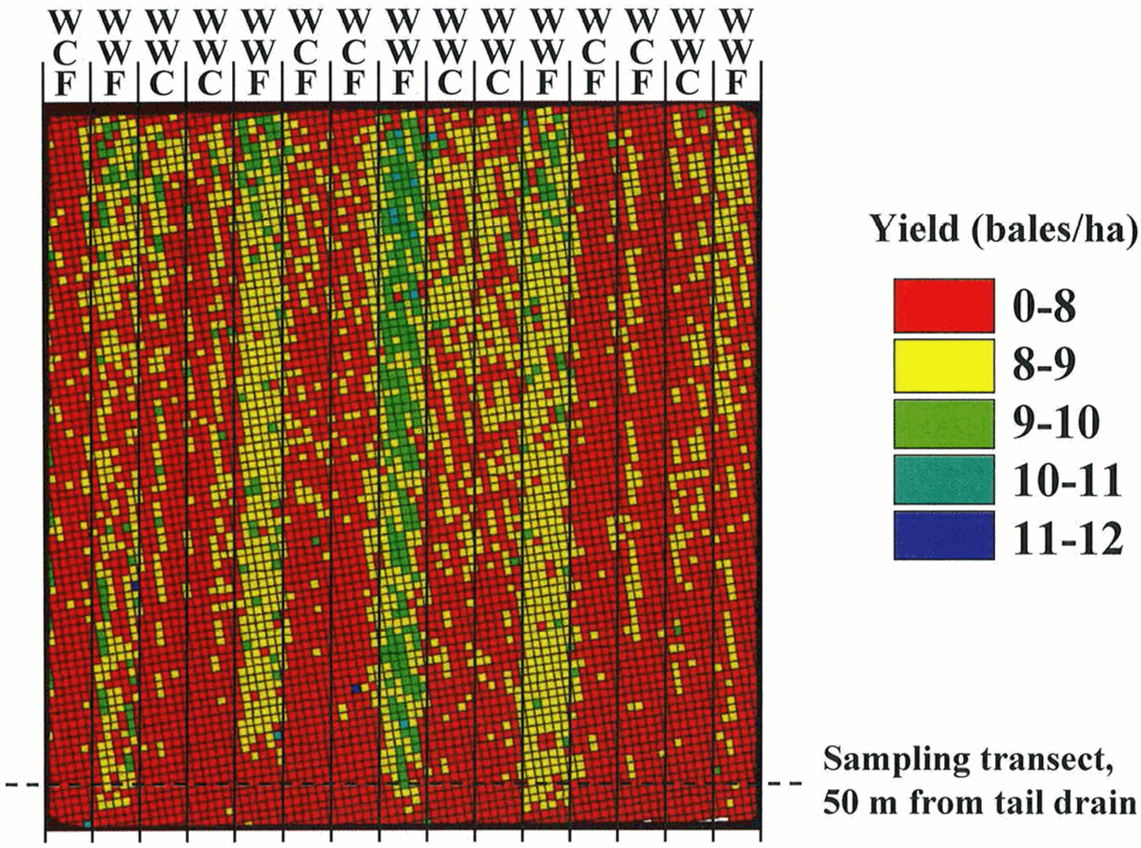

Figure 4.14. Yield of cotton following long-term rotation with wheat in a field near Warren (previous crops were wheat/fallow (W), cotton (C) or summer fallow (F) consecutively from 1999-00 to 2002-03; yield map courtesy of B. Tyrwhitt, Auscott Ltd.)

A crop rotation trial that included two successive years with no cotton was initiated on another farm near Narromine in 2002. The cropping sequence was cotton in 2001-02, canola or bare fallow in 2002-03, wheat or bare fallow in 2003-04 and cotton in 2004-05. The combinations for the two years of rotation were: canola-wheat (CW), canola-bare (CB), bare-bare (BB) and bare-wheat (BW). Additional canola plots were planned for 2003-04 but prevented by drought, thus giving additional replicates of the bare/bare treatment.

Observations of disease severity and growth of cotton at the start of the 2004-05 season were available at the time of collation of this report and are included for reference (Table 4.16). In February 2003, the population of *T. basicola* in soil was not reduced by rotation with canola, in comparison to bare fallow (Table rotations). Due to the dry conditions, dry matter production by the canola was only 0.32 tonnes/ha, suggesting that there was too little growth for a biofumigation effect. During the course of this two-year rotation experiment, the population density of *T. basicola* decreased substantially from the initial measurement of 519 cfu/g soil (five samples in May 2002) but was not significantly different among treatments (Figure 4.15). There was little difference in disease severity among the treatments (Table 4.16). The apparently greater severity of disease in the bare-wheat (four replicates), in comparison to bare-bare (eight replicates), may be an artefact of the differences in replication that occurred due to the drought. This difference in disease severity was not reflected by plant growth (Table 4.16). The apparent magnitude of the decline on population density of *T. basicola* in soil (Figure 4.15) contrasts with the levels of disease that were observed in most treatments in 2004 (Table 4.16). Nevertheless, the assays suggest that the spore population of *T. basicola* did decline over the two years of rotation with non-hosts or bare fallows, which tends to confirm the results of the other rotation experiment at Narromine that had tow and three consecutive years of wheat.

Table 4.16. Effect of rotation crops and bare fallow on black root rot of cotton in the 2004-05 season in a field near Narromine

2002-03	Bare		Canola		
2003-04	Bare	Wheat	Bare	Wheat	[A]Probability
T. basicola in soil (cfu/g soil, Feb 03)	190	140	157	111	NS
Disease severity (0-10 scale)	4.2b	7.5a	4.9ab	6.6ab	$P = 0.018$
Shoot dry matter (mg/plant)	94	90	97	97	NS

[A]Values followed by the same letter are not significantly different by pairwise comparison of means using the Scheffé test at the stated probability level (NS = not significant).

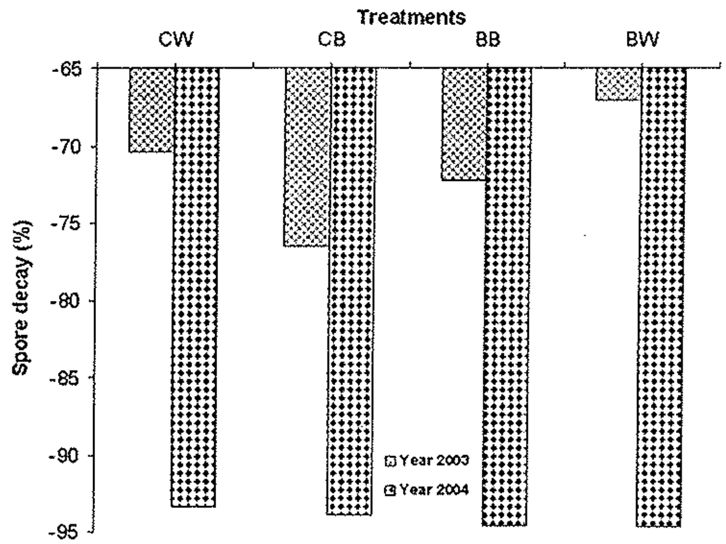

Figure 4.15. Decline in population density of *Thelaviopsis basicola* in soil following two years of bare fallow (BB), bare fallow followed by wheat (BW), canola followed by bare fallow (CB) and canola followed by wheat (CW), in a cotton field near Narromine (initial population density in fives samples across the trial site was 519 cfu/g soil)

In summary, long-term rotation, with three (or, to a lesser extent, two) consecutive crops of wheat, or other non-host crops, has potential for control of black root rot but requires further evaluation before firm recommendations can be given. The observation that long-term rotation in the experiment at Narromine resulted in at least two subsequent cotton crops with negligible disease severity has important implications for deployment of this strategy. Farms that usually rotate cotton with wheat every other year could maintain the same ratio of cropping by alternately growing three crops of cotton and or three crops of wheat, with increased profit margins due to control of black root rot.

4.2.3 Alternative hosts

- Several weed species are good hosts for reproduction of *T. basicola*, including bell vine, wild gooseberry, *Datura* spp. native rosella, velvet leaf and phasey bean
- Pigeon pea is also a good host for *T. basicola*
- Integrated management of black root rot should include diligent control of weeds and careful choice of the location of pigeon-pea refuges to avoid fields infested with *T. basicola*

T. basicola has a broad host range. Alternative hosts have the potential to enhance the carryover of inoculum of *T. basicola* between cotton crops, or even increase it. In this regard, it was deemed important to ascertain the potential for a range of weeds, commonly found in cotton farming systems, to act as alternative hosts for *T. basicola*. In August 2002 a range of weeds were sown in potted soil infested with *T. basicola* to investigate their potential as alternative hosts for the pathogen. Several of these proved to be good hosts for *T. basicola* causing discolouration of the roots in association with production of chlamydospores (Table 4.17). Although all the roots of the cowvine plants were dark brown in colour, no sporulation by *T. basicola* was observed, suggesting that this colour was not the result of infection by *T. basicola*. Control of weeds, especially those species that had a medium and high score for reproduction of *T. basicola* (Table 4.17) should be adopted as routine measure for management of black root rot of cotton.

Table 4.17. Colonisation of *T. basicola* on weeds, commonly found in cotton farming systems, sown in potted soil

Common name	Scientific name	Browning on tap root (0-10 scale)	Density of spores on roots
Bell vine	*Ipomoea plebeia*	6.4	High
Wild gooseberry	*Physalis minima*	2.3	High
Downy thornapple	*Datura inoxia*	3.0	High
Fierce thornapple	*Datura ferox*	2.3	High
Native rosella	*Abelmoschus ficulneus*	5.5	High
Velvetleaf	*Abutilon theophrasti*	3.5	High
Phasey bean	*Macroptyllium lathyroides*	1.4	Medium
Wild melon	*Citrullus lanatus* var. *lanatus*	1.5	Low
Hunter burr	*Xanthium italicum*	2.5	Low
Narrow leaf bladder ketmia	*Hibiscus trionum* var. *trionum*	0.8	Low
Cowvine	*Ipomoea lonchophylla*	10.0	Nil
David's Spurge	*Euphorbia davidii*	0.0	Nil
Dwarf amaranth	*Amaranthus macrocarpus* var. *pallidus*	1.3	Nil
Barnyard grass (two lines)	*Echinochloa* sp.	0.0	Nil
Bathurst Burr	*Xanthium spinosum*	0.0	Nil
Common sow thistle	*Sonchus oleraceus*	0.0	Nil
Black nightshade	*Solanum nigrum*	0.0	Nil
Anoda weed	*Anoda cristata*	0.0	Nil
Narrow leaf bladder ketmia (red flowering type)	*Hibiscus trionum* var. *vesicarius*	0.0	Nil

In addition to the above study, heavy sporulation of *T. basicola* was observed on the roots of pigeon pea used as a trap crop in a commercial field near Narromine. The use of pigeon pea as a trap crop in fields infested with *T. basicola* should be avoided.

In August 2001, hemp (*Cannabis sativa,* variety CHA) was sown (under licence to NSW Department of Primary Industries, at the Trangie Agricultural Research Centre) in potted soil that was collected from a field heavily infested with *T. basicola*. The plants were maintained in a glasshouse without temperature control. Inspection of the roots under the microscope revealed extensive production of chlamydospores of *T. basicola*, suggesting caution in the use of this crop in rotation with cotton, if commercialisation ever proceeds.

5. Conclusions (research outcomes versus objectives)

5.1 Evaluate novel and existing methods for control of black root rot

A range of potential tools for control of black root rot were evaluated in glasshouse and field experiments with results as follows:

			Implications	
Control measure	Focus	Result	Science	Industry
Benomyl fungicide	Application as in-furrow spray at planting	Small but inconsistent decreases in disease severity	Confirms previous observations that fungicides are not a feasible control measure for black root rot, probably due to adsorption by clays	Confirms previous recommendations that fungicides should not be used to control black root rot
Acibenzolar-S-methyl	Activation of resistance by seed treatment and in-furrow spray in field soil	Acibenzolar-S-methyl decreased disease severity effectively in the field	Application of acibenzolar-S-methyl to seed activated a systemic resistance response in cotton against *T. basicola*	Acibenzolar-S-methyl has potential for control of black root rot as part of an integrated disease management strategy
Acibenzolar-S-methyl	Activation of resistance by seed treatment in potted soil	Acibenzolar-S-methyl appears to be more effective at lower levels of inoculum of the pathogen than at high levels	Indicates that the resistance response activated by acibenzolar-S-methyl is less able to cope with high inoculum density	Prophylactic use of acibenzolar-S-methyl as a seed treatment should help slow the development of the disease within fields and slow dispersal of the pathogen
Biofumigation with 'green manure crops'	Field trials with vetch, canola, mustard	Poor control of black root rot	Biofumigation effect confirmed but dependent upon biomass	Effective biofumigation requires good growth, incorporation and breakdown of the green manure crop
Solarisation	Pot experiment and field trial in combination with biofumigation crops	Some control of black root rot in the glasshouse but none in the field	Confirmation that solarisation does not work well in the field during spring conditions	A cost efficient level of control of black root rot by solarisation remains to be demonstrated
Delayed sowing	Field experiments with up to a 19 day delay in sowing,	Good control of black root rot	Delayed sowing has potential to avoid climatic conditions that favour black root rot and seedling disease	Delayed sowing should be an effective control measure, as long as yield potential can be maintained
Manipulation of soil temperature	Modified beds and cover crops	Inconclusive results	Soil temperatures might be increased sufficiently to decrease the severity of black root rot	Cost effective manipulation of bed structure and provision of cover crops may only be effective at higher latitudes

5.2 Continue to assess the impact of farming systems on black root rot:

The impact of crop rotation and minimum tillage on the severity of black root rot was evaluated in glasshouse and field experiments in the Macquarie and Namoi Valleys, with results as follows:

			Implications	
Farming practice	Focus	Result	Science	Industry
Continuous cotton, biennial rotation with wheat and tillage	Farming systems trial at ACRI	Tillage did not reduce disease severity consistently; rotation with wheat every other year did not slow disease progress	Confirmation that population density of *T. basicola* increases with the number of cotton crops grown, irrespective of single-year rotation crops	Confirmation that rotation with non-host crops for a single year will not control the disease
Long-term rotation with cereals	Rotation for one to three years evaluated in the field	Rotation with non-host crops appears to be have potential for control of the pathogen but further evaluation is required	In the absence of a host *T. basicola* may not be able to survive three years in the soil, although drought conditions may favour longer survival	Rotation with non-host crops for three years appears to be an effective control measure; after droughts, infested fields should be returned to cotton last
Weed control	Host status of weeds evaluated in potted soil	Several new weed hosts identified	Ruderal nature of *T. basicola* confirmed	Control of weeds hosts should help to reduce carryover of *T. basicola* between cotton crops

5.3 Consolidate and expand the management strategy developed in the previous project (DAN122C) for control of black root rot

A revision of the existing management strategy for black root rot, previously published in the CRDC guidelines, *Integrated Disease Management* in 2002, has been formulated to include recommendations (highlighted in bold font) arising from the research conducted in this project, as follows:

Control strategy for black root rot of cotton

Planning

(i) Choose varieties that have the capacity to 'catch up' later in the season, **including genetically modified varieties that have high fruit retention**

(ii) **Pending it's registration for use on cotton against black root rot, treat all seed sown with Boost® (a.i. acibenzolar-S-methyl)**

Ground preparation

(iii) Good bed preparation to optimise stand establishment and seedling vigour

(iv) Pre-irrigate in preference to 'watering up'

Early season

(v) **Delay sowing until late October, if possible, to avoid the cool temperatures that favour disease**

(vi) If choosing to sow early, sow when temperatures are warm and rising (a soil temperature of 16°C is OK, 20°C is better), temperature measurements should be taken in the fields where black root rot occurs.

(vii) Replanting decisions should be made on the basis of stand losses, not the size of the seedlings.

(viii) Watch for early onset of water stress (ie. because the root system is weak) and irrigate accordingly, but avoid waterlogging.

Late season

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

After harvest and at all times

(x) 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 adhere to the principal of: COME CLEAN, GO CLEAN

Rotation

(xi) Rotate with non-host crops (eg. cereals, canola) for **up to three seasons,** if possible **(for fields that were on a 1:1 cotton:wheat rotation, consider 3:3 cotton:wheat)**

(xii) **If drought is forcing fallows of two seasons or more, allocate the longest periods of fallow to heavily infested fields**

(xiii) Biofumigation with woolly pod vetch or mustard 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).

(xiv) Avoid rotation with legumes, **including pigeon pea**, excepting woolly pod vetch, and control alternative weed hosts, **especially bell vine, wild gooseberry, *Datura* spp. native rosella, velvet leaf and phasey bean.**

(xv) 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.

5.4 Facilitate delivery and deployment of the management strategy for black root rot

Aspects of the management strategy and the results of the research in the project have been communicated by way of scientific and extension publications, including the guidelines for integrated disease management, presentations at conferences, meetings and field days, media releases and interviews.

6. Cotton R&D Corporation outputs

The research conducted in this project has contributed to the Corporation's economic, environmental and social outputs by modifying the integrated disease management (IDM) strategy for black root rot to provide more effective options for control of black root rot. The IDM strategy aims to increase the profitability and sustainability of cotton production, using methods that have a low impact on the environment, which will have positive flow-on benefits to rural communities. Specifically, this project has contributed to:

- 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.
- Increased awareness of ineffective control measures for black root rot (e.g. in-furrow fungicides)
- 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 (drought has been a limiting factor since then)
- 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 delaying sowing to avoid conditions that favour disease.
- Increased awareness by growers and consultants of the risks of rotation with alternative hosts to *T. basicola*, such as winter legumes, and the need to control weed hosts.
- Decision by Syngenta Crop Protection to proceed with registration of acibenzolar-S-methyl for seed treatment of cotton in Australia

7. Project summary

Black root rot continues to threaten the productivity of cotton production in NSW and Queensland. No single control measure gives adequate protection against this pathogen and an integrated disease management approach is required. A range of potential tools for control of black root rot were evaluated in glasshouse and field experiments, as well as evaluation of the impact of farming systems. Key findings include:

In furrow fungicides

- In-furrow application of the fungicide benomyl provided a low level of control of black root rot in some fields and had no effect on the disease in other fields
- Shoot growth was not increased in any of the experiments by in-furrow application of benomyl
- Even if repeatable results could be obtained, benomyl would have to be applied at uneconomic rates to achieve a moderate decrease in disease severity

Systemic acquired resistance

- The potential for acibenzolar-S-methyl to induce a resistance response in cotton against black root rot was demonstrated in the field by either soaking seed just before sowing or applying an in-furrow spray at sowing.
- Imbibition during soaking causes the seed to swell, resulting in release of fewer seed by mechanical planters.
- Application as an in-furrow spray and, if effective, application with the standard seed dressing would avoid the problem of lower planting rates with the soaking method
- In a dose response experiment, acibenzolar-S-methyl controlled black root rot better at low levels of inoculum, suggesting the potential to slow disease progress in newly-infested fields and decrease further dispersal of the pathogen.

Biofumigation

- In several field experiments biofumigation crops were not effective in controlling black root rot of cotton
- These experiments highlight the need for good growth, good incorporation and good breakdown of biofumigation crops for them to be effective in controlling black root rot of cotton

Delayed sowing

- In a field experiment at the Australian Cotton Research Institute, delaying sowing from the 28 Sep to the 17 Oct decreased the severity of black root rot substantially
- Delayed sowing has potential to control black root rot and seedling disease by decreasing the period of exposure of cotton to climatic conditions that favour black root rot

Microenvironment management

- Modified beds may increase soil temperature at the planting line sufficiently to decrease the severity of black root rot but the results in two experiments were inconsistent.
- Cereal cover crops have previously been shown to increase soil temperature
- A consistent effect on the severity of black root rot was not observed in the experiment conducted in this project, with poor stand establishment being a confounding factor

Crop rotation and tillage

- Minimum tillage did not decrease the severity of black root rot consistently in the farming systems trial at ACRI
- Rotation with wheat every second year did not prevent the increase in the severity of black root rot in the farming systems trial at ACRI
- The results confirm the previous observation that the incidence and severity of black root rot increases according to the number of cotton crops growth, irrespective of rotations on a biennial basis.

Long-term crop rotation

- Rotation with three consecutive crops of wheat decreased the severity of black root rot to negligible levels in a field near Narromine
- A fully replicated repeat of this experiment was less successful, when assessed close to the tail drain
- However, seedling growth and cotton yield were increased by one to two bales/ha in the fully-replicated experiment, suggesting that assessment of disease severity close to the tail drain was not representative of the whole field
- Further evaluation of the potential for long-term rotation out of cotton to control black root rot is required before firm recommendations can be made

Alternative hosts

- Several weed species are good hosts for reproduction of *T. basicola*, including bell vine, wild gooseberry, *Datura* spp. native rosella, velvet leaf and phasey bean
- Pigeon pea is also a good host for *T. basicola*
- Integrated management of black root rot should include diligent control of weeds and careful choice of the location of pigeon-pea refuges to avoid fields infested with *T. basicola*

The key findings have been incorporated into the existing integrated disease management strategy for black root rot, with communication of results by way of scientific and extension publications, including the guidelines for integrated disease management, presentations at conferences, meetings and field days, media releases and interviews.

7.1 Technical advances

The potential for acibenzolar-S-methyl to induce resistance against *T. basicola* when applied to cotton seed, as seed soaking, as an in-furrow spray or in the seed coating was demonstrated in the field and has contributed to Syngenta Crop Protection seeking registration for treatment of cotton seed with this product.

7.2 Other developments

Improved equipment for experimental application of in-furrow sprays was developed in this project.

7.3 Changes to the Intellectual Property Register

The project has contributed to the development of application methods for treatment of cotton seed with acibenzolar-S-methyl and has demonstrated the potential for such treatment to provide a degree of control of black root rot in the field. Acibenzolar-S-methyl is wholly owned by Syngenta Crop Protection under patent. However, changes to the intellectual property register may be required.

8. Further development

8.1 Further development and exploitation of the project technology

A long term experiment to demonstrate the potential for biofumigation to control black root rot has been established at the Australian Cotton Research Institute, as part of CRDC project DAN 177C *Diseases of Cotton VIII*. This experiment will act as a demonstration trial for extension purposes.

A permit from the APVMA has been granted to Dr Stephen Allen, Cotton Seed Distributors, for large-scale field experiments to evaluate the potential for acibenzolar-S-methyl to control black root rot. Dr David Nehl, NSW Department of Primary Industries, is listed under this permit and further experiments with acibenzolar-S-methyl are planned.

Evaluation of the potential for delayed sowing to minimise the period of exposure to early-season conditions that favour black root rot and other soilborne diseases is continuing in CRDC projects DAN 176C, *Severity factors in Fusarium wilt of cotton*, and in DAN177C, *Diseases of cotton VIII*.

8.2 Future presentation and dissemination of project outputs

Extension material, including a black root rot Research Review is in preparation by the Cotton CRC. Preparation of a scientific paper on biofumigation has commenced. The modifications to the IDM strategy for black root rot will be submitted to the Cotton CRC publication approval process and incorporated in the cotton IDM guidelines.

8.3 Future research

The effectiveness of acibenzolar-S-methyl under varying conditions and levels of inoculum in the field needs to be evaluated further to confirm its potential for disease control and develop methods to optimise its effectiveness. There is scope for evaluation of novel biofumigation crops that may be more effective than those crops evaluated so far. The relationship between soil type and the incidence and severity of black root rot requires evaluation to (i) establish the potential for soil suppressiveness and determine its nature and to (ii) enable prediction of risk based on soil types so that control measures can be applied in a targeted and cost-effective manner. Factors influencing the survival of *T. basicola* need to be identified and the length of time for cropping with non-host crops, such as cereals, to reliably reduce inoculum to sub-economic levels needs to be quantified.

9. Communication of results

9.1 Publications

Refereed journal papers

Hulugalle N, Nehl D, Weaver T (2004) Soil properties, and cotton growth, yield and fibre quality in three cotton-based cropping systems. *Soil and Tillage Research* **75**, 131–141.

Nehl DB, Allen SJ, Mondal AH, Lonergan PA (2004) Black root rot: a pandemic in Australian cotton. *Australasian Plant Pathology* **33**, 87-95.

Mondal AH, Nehl DB, Allen SJ (in press) Acibenzolar-S-methyl induces systemic resistance in cotton against black root rot caused by *Thielaviopsis basicola. Australasian Plant Pathology* 00, 000-000.

Conference papers

Allen SJ, Nehl DB, Mondal AH, Jhorar OP (2004) Seed treatments to induce resistance to Fusarium wilt and black root rot of cotton. In *'3rd Australian Soilborne Disease Symposium'*. pp. 45-46. South Australian Research and Development Institute: Rowland Flat **(oral paper also presented)**

Jhorar OP, Nehl DB, Mondal AH (2002) Management of black root rot of cotton: current status and future strategies. In *'Proceedings of the 11th Australian Cotton Conference'*. pp. 689-694. Australian Cotton Growers Research Association: Brisbane

Jhorar OP, Nehl DB, Mondal AH (2004) Modifying seedbed architecture and growing micro-shelterbelts to increase soil temperature and reduce the severity of black root rot of cotton. In *'3rd Australian Soilborne Disease Symposium'* pp. 51-52. South Australian Research and Development Institute: Rowland Flat **(oral paper also presented)**

Jhorar OP, Nehl DB, Mondal AH (2004) Can cultivation be a tool to reduce the population of *Thielaviopsis basicola* in soils used to grow cotton? In *'3rd Australian Soilborne Disease Symposium'*. pp. 153-154. South Australian Research and Development Institute: Rowland Flat

Nehl DB, Mondal AH (2001) Biofumigation to control black root rot in cotton. In *'Conference Handbook, 13th Biennial Conference, Australasian Plant Pathology Society'*. p. 156. Australasian Plant Pathology Society: Cairns **(oral paper also presented)**

Nehl DB, Mondal AH, Allen SJ (2001) Integrated management of black root rot in cotton: a bilateral approach. In *'Conference Handbook, 13th Biennial Conference, Australasian Plant Pathology Society'*. p. 161. Australasian Plant Pathology Society: Cairns **(oral paper also presented)**

Mondal AH, Nehl DB (2001) Benzothiadiazole induces systemic acquired resistance against *Thielaviopsis basicola* in cotton and faba bean. In 'Conference Handbook, 13th Biennial Conference, Australasian Plant Pathology Society'. pp. 358. Australasian Plant Pathology Society: Cairns, Australia **(oral paper also presented)**

Extension articles and material

Hulugalle N, Weaver T, Nehl D (2001) Residual effects of rotation history on cotton growth and soil quality. In *Macquarie Valley Cotton Trial Reports* (Rourke K) pp. 41-43. NSW Agriculture: Warren

Jhorar O, Nehl, D and Mondal, A (2002) Getting to the root of the problem. *Border News* 9th Sep, p. 11.

Jhorar O (2002) Black root rot: reduce the severity. *Cotton Magazine* Sep 2002, p. 12.

Jhorar O (2003) Crop rotations to reduce black root rot severity. *Cotton Magazine* May 2003, p.16.

Jhorar O (2003) Using rotations to reduce black root rot. *The Australian Cotton Grower.* **24**, 47-48

Jhorar O (2003) Black root rot in cotton on increase. *Agriculture Today* Jul, p. 6.

Jhorar O (2003). Fighting fungal disease: biofumigation crops can break the cycle. *Agriculture Today* Aug, p. 6.

Nehl D (2001) Black root rot of cotton. In *Integrated disease management.* Australian Cotton Cooperative Research Centre: Narrabri

Nehl D (2001) Disease research in the Macquarie Valley, 2000/01. In *Macquarie Valley Cotton Trial Reports* (Rourke K) pp. 44-47. NSW Agriculture: Warren

Nehl D (2001) Disease surveys in the Macintyre Valley 2000-2001. In *Border Rivers Cotton Yearbook* (Raymond M) p. 14. Queensland Department of Primary Industries: Goondiwindi

Nehl D, Allen S, Mondal A, Lonergan P (2001) Managing the black root rot menace. *Australian Cottongrower* **22**, 52-55

Rochester I, Roberts G, Peoples M, Kelly D, Nehl D (2001). The benefits of vetch cropping in cotton systems. *Australian Cottongrower* **22**, 22-27.

Media releases and interviews

Cross A, Nehl D. Black root rot concern for cotton, *Media Release*, July 2001

Rochester I, Nehl D. Vetch for control of black root rot, *Media Release*, Aug 2001

Jhorar O. Biofumigation crops for managing black root rot of cotton. *Interview*, ABC Radio, Aug 2003

Grower meetings and field day presentations:

Jhorar O (2003) Black Root Rot Update. *Macintyre Valley End of Season Meeting*. Department of Primary Industries, Queensland Government, Royal Hotel, Goondiwindi, June 2003

Jhorar O (2003) Managing black root rot of cotton. Presented and Published in *Gwydir Valley Field Day Book*. Moree NSW. March 2004.

Jhorar O (2003) Managing cotton seedbed environment to reduce the impact of black root rot of cotton. Presented in *Upper Namoi Area-Wide Management Committee Meeting*. 'Warilea', Narrabri, November 2003

Jhorar O (2004) Improving cotton seedbed environment to reduce the impact of black root rot of cotton. Published in *Upper Namoi Field Day Book*. Baan Baa, March 2004.

Jhorar O (2004) Improving cotton seedbed environment to reduce the impact of black root rot of cotton. Presented and Published in *Upper Namoi Field Day Book*. Breeza, March 2004.

Jhorar O (2003) Improving cotton seedbed environment to reduce the impact of black root rot of cotton. Presented in *Agricultural Science in Action*, a field day at Trangie Agricultural Research Station, Trangie, March 2004.

9.2 On-line resources

The following extension publications have been made available on-line at the Cotton CRC website.

Rourke K, Nehl D (2001) '*Black root rot update, March 2001*' CRC information sheet, Australian Cotton Cooperative Research Centre: Narrabri, http://cotton.crc.org.au/Assets/PDFFiles/Disease/BRRUpd01.pdf

Nehl D (2001) Black root rot of cotton. In *Integrated disease management*. Australian Cotton Cooperative Research Centre: Narrabri, http://www.cotton.crc.org.au/Assets/PDFFiles/Disease/IDMGL02g.pdf

10. Likely impact of research outcomes (cost/benefit)

Long periods of cropping with non-host crops, such as cereals, appear to have potential for substantial control of black root rot. With further confirmation of this method, farms that usually rotate cotton with wheat every other year could maintain the same ratio of cropping by alternately growing three crops of cotton and three crops of wheat. This approach would be cost neutral and would have potential to increase profit margins due to control of black root rot.

If acibenzolar-S-methyl is registered for application to cotton planting seed, it is likely to be a very cost effective measure for partial control of black root rot and other diseases, especially if it proves to be effective at holding back disease progress from low levels of inoculum.

Minimisation of the potential for *T. basicola* to increase on weeds should not incur any costs greater than those dictated by normal weed management practices.

The potential to decrease the severity of black root rot by delayed sowing will require careful crop management to maintain yield potential. Genetically modified cotton varieties that have high fruit retention improve the potential for maintaining yield with delayed sowing.

The cost of biofumigation with woolly pod vetch, is offset by provision of nitrogen to the following cotton crop. A nitrogen benefit will not be forthcoming with non-leguminous biofumigation crops and their deployment will depend on demonstration of very effective control of black root rot.

If growers can utilise several of the above methods in an integrated manner, then cost effective control of black root rot should be possible.

11. Budget

Total funds contributed to DAN153C by the CRDC.

Year	DAN153C
2001-02	144,749
2002-03	161,037
2003-04	160,000
2004-05	10,000
Total	$475786