



## ***Part 3 – Final Report Guide (due 31 October 2008)***

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### ***Background***

At commencement of this project, Fusarium wilt had been reported on at 73 farms in NSW and was spreading exponentially. If that rate of spread were to continue, Fusarium wilt would be present on 90% of cotton farms in NSW by 2010 (Figure 1). When Fusarium wilt progresses unchecked, fields and even whole farms can be rendered unsuitable for cotton production. Fusarium wilt has now reached the cooler growing areas in NSW where the climatic conditions may enhance infection and disease progress to a greater extent than in Queensland and northern NSW. It is essential that the spread of this disease be minimised, that disease progress be monitored in the newly infested areas of NSW and that research on control measures continue.

Black root rot increased exponentially during the 1990's and continues to do so. The distribution of black root rot continues to expand. The disease was observed for the first time in the Murrumbidgee Valley in November 2003. In the Macintyre, Gwydir, Namoi and Macquarie Valleys, the disease has now been recorded on 100% of the farms inspected regularly since 1984. Among these farms black root rot was observed in 78% of the fields and in 40% of the plants inspected in November 2003. Yield losses of up to 40% can occur when the disease is severe, especially in the cooler production areas.

Verticillium wilt was once the most widespread and important disease of cotton in Australia. The widespread adoption of varieties with resistance to Verticillium wilt during the 1990s has effectively reduced the incidence and importance of the disease (around 5% of the crop surveyed in NSW in 2004). However, prior to commencement of this project, the incidence of Verticillium wilt was increasing again in some areas (e.g. 12% of the crop in the Namoi valley was infected in the 2001/02 and 2002/03 seasons). During the 1990's, seedling mortality for the whole of NSW declined from an average of 40% down to 20%. Seeding mortality has increased and was greater than 30% in the last four seasons leading up to commencement of this project, and up to 60% in cooler regions. New formulations of seed treatment fungicides have become available to the cotton industry and it is important that they have independent evaluation.

A small outbreak of what appeared to be root knot nematode was observed in cotton at Emerald in the 2002-03 season (J.K. Kochman, personal communication) and monitoring of further outbreaks of this pathogen is required. In NSW root lesion nematodes were observed in unusually high numbers in the soil in association with severe symptoms of Verticillium wilt (defoliating and dying cotton plants). Root lesion nematodes are known to increase the severity of Verticillium wilt in other crops and may be having an impact in Australian cotton. The distribution of nematodes appears to be very limited and their potential impact under Australian conditions is not certain.

The research proposed in this application was designed to enable: further development and confirmation of disease management strategies; transfer of this information to growers for the more effective control of cotton diseases; collaborative development of control measures in other projects; and monitoring of the distribution and potential importance of diseases of cotton.

### *Objectives*

#### **Objective 1. To monitor the distribution and importance of all diseases in cotton and identify environmental and cultural factors influencing the emergence or re-emergence of disease threats**

- Disease surveys were conducted in November and March in each year of the project, including all production areas in NSW. Survey data was added to the existing database to analyse changing trends in the severity and/or distribution of seedling disease, black root rot, Alternaria leaf spot, Verticillium wilt, Fusarium wilt, boll rots and cotton bunchy top.
- The incidence of Fusarium wilt across transects of infested fields was assessed in each year of the project and trends were investigated.

#### **Objective 2. To continue to develop and/or evaluate control strategies for black root rot, seedling disease and Fusarium wilt (e.g. seed treatment fungicides, induced resistance, rotation crops, biofumigation crops, cover crops, late sowing with Bollgard™)**

- Seed treatment experiments were completed at Narrabri, Hillston, or Warren in each year of the project, including evaluation of the potential of fungicides and some 'biological' products and novel products claimed to induce systemic acquired resistance. Experiments on the effect of seed treatment/planting date on seedling mortality and cotton yield were completed in each year of the project.
- Experiments on the timing of sowing, cover crops, in-furrow fungicides, in-furrow biological control agents, crop rotation, biofumigation and systemic acquired resistance were completed, variously for the control of seedling disease, black root rot, Fusarium wilt and Verticillium wilt.

#### **Objective 3. To conduct investigations of host-pathogen-soil interactions (including herbicides) contributing to the severity of black root rot of cotton and identify features that might be exploited for disease control.**

- Several studies of the impact of very long fallows at Bourke on mycorrhizal development in cotton were completed in 2004-05 and 2005-06
- Observations of the progress of symptoms of black root rot were confirmed in field experiments, indicating that symptoms are most severe during the period from three to five weeks after sowing. Delaying sowing minimises this period of exposure to severe symptoms and slow the build up of the pathogen.
- Commercial products claimed to enhance mycorrhizal colonisation or contain inoculum of mycorrhizal fungi were evaluated

#### **Objective 4. Continue long-term field experiments on the role of black root rot, mycorrhizal fungi (VAM) and other soil organisms in the soil ecosystem (soil 'health')**

- The long-term experiment on the impact of continuous bare fallow on survival of mycorrhizal fungi, commenced in 1997-08 at ACRI, was completed in 2006-07
- Long-term biofumigation experiment at ACRI was continued in the first two years of the project and then transferred to CRDC project DAN188C in the 2006-07 season

#### **Objective 5. Facilitate delivery and deployment of cotton disease management strategies that utilise available methods in a cost effective, integrated manner with the least impact on the environment**

- Previous recommendations for modifications to the Integrated Disease Management guidelines for control of seedling disease, black root rot and Fusarium wilt were strengthened and confirmed.
- Results were extended to the cotton industry by way of presentations and extension publications, hands-on-research workshops at the Cotton Conference, and publications in scientific literature.

## Methods

### 3.1 Disease surveys

The distribution and incidence of diseases of cotton in NSW was determined in disease surveys of 86, 93 and 62 commercial crops in November and March in the 2004-05, 2005-06 and 2006-07 seasons respectively. The fields surveyed were drawn from the same set of 42 farms, although fewer fields were surveyed in the third year of the project, due to the drought and subsequent lack of planting. As far as practicable, the cotton crops were arbitrarily selected at random and, to avoid bias, fields or areas with severe disease problems were not necessarily inspected according to the suggestions of farmers.

Field history, ground preparation, cotton variety, planting date and seed rate were recorded for each of the fields surveyed. In each field, the incidence of disease was assessed along two transects (each 220 m long), commencing approximately 50 m into the crop from the tail drain (to avoid bias due to waterlogging). Each transect was commenced at an arbitrarily random position along the tail drain, mostly stepping diagonally to the right in a ‘//’ configuration and the starting position was recorded as a GPS reference. Disease incidence was assessed at 10 points along each transect, at intervals determined by pacing 10m at right angles to the rows of cotton and then pacing 20 m along the rows towards the head ditch. At each of the 10 points along transects, assessment was commenced at the point in the cotton row nearest to one’s toe on the 20th pace (i.e. the ‘step-point’). To avoid muddy conditions at the tail drain transects were occasionally commenced at a point 20 m into the crop elsewhere on the perimeter of the field.

In the November surveys, 10 seedlings were dug from the soil at each step-point on the transects using an asparagus knife and the number of plants with characteristic symptoms of black root rot was recorded. Some or all of the seedlings were examined under a dissecting microscope to confirm the presence of chlamydospores of the pathogen, *T. basicola*, in association with the symptoms. Plant stand was recorded in 1.0 m of cotton row at each step-point on the ‘left hand’ transect, thus giving a stand assessment totalling 10 × 10 m of cotton row. Seedling mortality (% death) was calculated by comparing the grower’s records or estimates of the number of seeds planted per metre, with the number of plants per metre. 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).

In March, 10 plants were inspected at each step-point along the same transects that were assessed in November. The GPS coordinates were used to locate the November starting position. If the gap in the stand from the November assessment was located, plants closer to the head ditch were used for the March assessment, skipping two or three plants at the head ditch end of the gap as a buffer. If the gap was not located, the GPS reference alone was used as the starting position. The number of plants with symptoms of Verticillium wilt, Fusarium wilt, sudden wilt, cotton bunchy top and other diseases was recorded. The presence of Fusarium wilt was confirmed by laboratory tests conducted by Queensland DPI, to confirm vegetative compatibility groups. The severity of Alternaria leaf spot was assessed by visual



estimation of the percentage of leaf area with necrotic lesions, averaged over the 10 plants. The incidence of boll rots was estimated by expressing the total number of affected bolls on the 10 plants as a percentage, assuming 10 fruit per plant. If a disease was observed in the field but not in the plants assessed that were assessed quantitatively at the step-points, its presence was recorded in the database with a value of 0.01% incidence, to indicate its presence in the field. In addition to the annual disease surveys, changes in the incidence and severity of Fusarium wilt over time were monitored in successive cotton crops along transects across infested fields.

### 3.2 Field experiments

Field experiments were used to compare disease incidence with farm management practices and environmental factors. Wherever possible, field sites had prior evaluation of the distribution of pathogens in soil, using selective media, and/or the severity of disease. In seedling disease experiments at ACRI, a seedling disease ‘nursery’ was created each year by growing vetch in the winter prior to cotton and incorporating the vetch approximately 10 days prior to sowing cotton (see details below). The vetch provided a substrate for proliferation of *Rhizoctonia* and/or *Pythium*.

Fungicide seed treatments for seedling disease control were evaluated under a range of climatic conditions, including sites in the seedling disease nurseries at ACRI, and in commercial fields in the Macquarie and Lachlan Valleys, and on the Darling Downs, in collaboration with Cotton Seed Distributors Ltd, Deltapine Australia Ltd. and CSIRO Plant Industries’ Cotton Unit. Fungicides and other seed treatments were applied to cotton seed in combination with the standard fungicides (PCNB and metalaxyl) and deletions thereof, and sown in single row-plots using a cone seeder, or in larger plots using box planters.

Farming practices were evaluated in commercial fields, where available, in fully-controlled experiments. Long-term field experiments were established at the Australian Cotton Research Institute, so that non-standard farming practices could be examined without compromise and, ultimately, results could be presented to industry at field days. A long-term field experiment was continued at the Australian Cotton Research Institute to evaluate the potential for biofumigation with vetch and canola to prevent the build-up of black root rot, from initially low levels, in a continuous cotton system. Field experiments to evaluate options for crop rotation, cultural practices and the potential to induce systemic acquired resistance were conducted both at ACRI and at appropriate field sites.

#### *Seedling disease nurseries*

Since the 1995-96 season, woolly pod vetch (*Vicia villosa*) has been grown as a green manure crop, in the plant pathology field at the Australian Cotton Research Institute (ACRI), with the specific aim of enhancing the activity of seedling pathogens in field experiments (i.e. creating seedling disease nurseries). Seedling pathogens, including *Rhizoctonia* and *Pythium*, are able to grow on organic matter on the soil, particularly fresh crop residues. The legume woolly pod vetch (*Vicia villosa*) has potential to increase the inoculum of these pathogens and was previously demonstrated to have little impact on cotton seedling mortality if it is incorporated at least four weeks before sowing cotton (Rothrock et al. 1995; Final Report DAN154C *Diseases of cotton VIII*).

Having established the utility of vetch for increasing seedling disease pressure on cotton, vetch plots were established each winter at ACRI for seedling disease studies. Generally, approximately one third of Field 4 at ACRI was sown with vetch in May to June and irrigated.

Part of the vetch area was allocated to early and late incorporation of vetch in four-row plots

in a completely randomised block design with six replicates. These plots enabled experiments 8 to be duplicated with low and high pressure from seedling pathogens in the *early* and *late* plots, respectively. Dry matter production of the vetch during this project was variable due to rainfall (Table 1).

**Table 1 Vetch treatments used to create low and high seedling disease pressure by incorporation of the woolly pod vetch in August and September, respectively, at the Australian Cotton Research Institute**

Year	Sown	Incorporated	Above-ground dry matter (tonnes/ha)
2004		19 Aug	1.2 ± 0.13
		20 Sep	3.5 ± 0.15
2005	5 May	19 Aug	2.9 ± 0.07
		22 Sep	Not determined
2006		22 Aug	2.7 ±
		21 Sep	6.8 ±

### *Disease assessment*

Seedling mortality was determined by expressing plant stand, 3-6 weeks after sowing, as a percentage of the seed sown. Disease incidence was based on quantification of symptoms, with confirmation of the presence of some pathogens (e.g. the black root rot fungus, using a field microscope), and other pathogens with laboratory methods (e.g. Fusarium wilt). The severity and incidence of black root rot was assessed by estimating the presence of, and length of tap roots with, characteristic black discolouration, scored on a scale of 0-10. Fusarium wilt and Verticillium wilt were assessed as the percentage of plants with vascular symptoms (scored on a scale of 0-4, where by cutting the stems of cotton just above soil level and ranking the amount of vascular discolouration in the cross section of stems on a scale of 0 to 4, where: 0 = no vascular discoloration; 1 = less than 5% discoloration; 2 = between 5% and 20% discoloration ; 3 = between 20% and 40% discoloration; 4 = greater than 40% discoloration) in pre-determined plots in which plant stand is assessed at the beginning of the season. Data from Fusarium wilt experiments was expressed as three parameters, as follows.

- *Seedling survival* = the percentage of the original plant stand, in October, that was still alive at the end of the season.
- *Adult survival* = the percentage of plants at the end of the season that had little or no infection (0's and 1's in the 0-4 stem rating scale)
- *Total survival* = the percentage of the original plant stand, in October, that survived to the end of the season with little or no disease (0 and 1 on the 0-4 stem rating scale)

Colonisation of cotton roots by arbuscular mycorrhizal fungi (VAM) was assessed by clearing and staining of roots and assessment of the presence of fungal structures (arbuscules) under the microscope.

### *Pathogens*

Populations of pathogens in the soil were measured either (i) in the laboratory, by culturing soil in dilution plates containing selective media (TB-CEN agar for the black root rot pathogen, *T. basicola*) or (ii) in the glasshouse with bioassays using cotton plants as the host. Samples suspected to have Fusarium wilt were sent to Queensland DPI for confirmation of vegetative compatibility groups.

### *Design and analysis*

All glasshouse and field experiments used completely randomised block designs. Data was screened for normality and heterogeneity of variance before analysis. Analysis of variance with spatial analysis (ASREML) was applied to field experiments with planned comparisons of treatments. Linear and non linear regression models were fitted to ‘dose response’ experiments and for comparison between symptoms and other parameters.

## **Results**

### **Seedling Mortality**

- Seedling mortality was greatest with cool wet conditions early in the season, reflecting the major impact of seedling disease pathogens (*Rhizoctonia* and *Pythium*) on mortality
- The risk of seedling disease increases with increasing latitude, with the central to southern regions of NSW being particularly prone
- The disease surveys provided overwhelming evidence that seedling mortality is not related to the incidence or severity of black root rot

### **Black Root Rot**

- The Australian cotton production industry is currently experiencing a widespread, chronic epidemic of black root rot although the incidence of the disease has decreased in the past three seasons, most likely as a result of climatic conditions and drought enforced fallows.
- The Namoi and Gwydir Valleys have been the worst affected areas in NSW, although the disease had been spreading rapidly in the Macintyre Valleys in the past decade.
- In contrast to the recent decline in incidence elsewhere in NSW, black root rot has increased rapidly in the Lachlan and Murrumbidgee production areas.
- The severity of black root rot was assessed in annual disease surveys for the first time, in this project, and was shown to be well correlated with the incidence of black root rot.
- Many farms do not have black root rot and farm hygiene should be practiced to minimise further spread

### **Fusarium Wilt**

- The Australian cotton industry continues to experience widespread, chronic epidemic of Fusarium wilt
- The rate of reporting of new cases has declined in recent years, potentially due to farm hygiene, greater deployment of less-susceptible varieties and drier conditions in spring.
- Fusarium wilt has been reported on a total of 81 farms in NSW
- Fusarium wilt has been observed on 41% of the farms surveyed regularly across NSW and, if the pathogen continues to spread at its current rate, then 100% of the farms in the survey set will be affected by 2022
- The annual disease surveys were modified to ensure that transects in November and March were congruent within each season and to enable comparisons between the incidence of early-season and late-season diseases and/or symptoms
- Preliminary comparisons indicate that there was no relationship between the incidence of Fusarium wilt in March and seedling mortality the previous November in the same crops but few fields with Fusarium wilt were surveyed due to drought



- In NSW the adoption of high-F-rank varieties is well ahead of the incidence of disease
- Transects across infested fields indicate that changing to less susceptible varieties does not always lead to a reduction in the incidence of Fusarium wilt in subsequent crops.
- Fusarium wilt has not been reported on the majority of farms in NSW and farm hygiene should be practiced diligently to minimise further spread

### **Ventricillium Wilt**

- Verticillium wilt was controlled in the 1990's using resistant varieties
- In most regions Verticillium wilt occurs at very low levels
- The incidence of Verticillium wilt decreased recently in the Namoi Valley, probably due to greater use of resistant varieties
- Extension needs to emphasise the value of resistant varieties on an ongoing basis to prevent resurgence of this disease
- While Verticillium wilt has not been reported in some areas and occurs at very low levels in others, the disease is still observed in 45 to 60 % of fields surveyed annually across NSW
- Farm hygiene should be practiced to minimise further spread of Verticillium wilt

### **Boll Rots**

- Cotton boll rots were observed at very low levels across NSW in the 2004-05, 2005-06 and 2006-07 seasons
- Boll rot is depended upon rainfall events in January and February and a high incidence of boll rots has not occurred since 2000 and 2001, when some regions experienced heavy rainfall events during boll filling
- From 2002 to 2007, the number of bolls affected was generally less than 1%, whereas from 1993 to 1999, the disease mostly affected 1 to 2% of bolls
- This apparent decline in boll rots over recent years probably reflects both the drought and the expansion of the surveys into southern NSW, where summer rainfall is low.

### **Alternaria Leaf Spot**

- Alternaria leaf spot was consistently observed at low levels in most crops inspected in NSW
- Alternaria leaf spot currently poses no threat to upland cotton (*Gossypium hirsutum*) in Australia, although Pima crops are more susceptible.

### **Other Diseases**

- Cotton bunchy top was rarely observed during the course of this project and the disease poses little threat to cotton production, while ever such low levels persist
- Bacterial blight was not observed in any of the disease surveys, including the small number of Pima crops that were inspected
- Sudden wilt was observed in a few isolated plants each season but poses no threat to cotton production
- Dieback caused by *Sclerotinia* was observed in a single crop where tall plants had lodged and pressed against the soil but *Sclerotinia* is not likely to threat crops with normal growth habit.



## ***Outcomes***

### ***Objective 1. To monitor the distribution and importance of diseases in cotton by regular disease surveys and identify environmental and cultural factors influencing the emergence or re-emergence of disease threats.***

The disease surveys identified the following trends:

#### ***Seedling disease.***

Environmental conditions have an overriding influence on seedling disease. Seedling mortality across NSW was relatively low during most of the 1990's but increased dramatically in 2000, 2001 and 2002 in association with cool wet conditions early in the season. Subsequently seedling mortality declined but not as low as it was in the 1990's. The disease surveys confirmed that seedling mortality increases with increasing latitude, with the southern regions of NSW being particularly at risk. The disease surveys provided overwhelming evidence that seedling mortality is not correlated with the either the incidence or severity of black root rot. *Black root rot*. Black root rot remains widespread in the Australian cotton industry although the incidence of the disease has decreased in the past three seasons, most likely as a result of climatic conditions and drought-enforced fallows. Black root rot has been observed on all of the farms surveyed regularly in NSW. The Namoi and Gwydir Valleys have been the worst affected areas in NSW in recent years, although the disease has been spreading rapidly in the Macintyre Valleys in the past decade. The severity of black root rot was assessed in annual disease surveys for the first time, in this project, and was shown to be well correlated with the incidence of black root rot. Many farms do not have black root rot and farm hygiene should be practiced to minimise further spread.

#### ***Fusarium wilt.***

The Australian cotton continues to experience widespread, chronic epidemic of Fusarium wilt. The rate of reporting of new cases has declined in recent years, potentially due to farm hygiene, greater deployment of less-susceptible varieties and drier conditions in spring. Fusarium wilt has been reported on a total of 81 farms in NSW and has been observed on 41% of the farms surveyed regularly across NSW. If the pathogen continues to spread at its current rate, then 100% of the farms in the survey set will be affected by 2022. The Macintyre Valley has been the most affected by Fusarium wilt, although the disease has been spreading rapidly in the Gwydir and upper Namoi Valleys. The adoption of less susceptible varieties by growers in the Macintyre Valley was reactive, closely following the appearance of the disease on farms. In contrast, the adoption of high-F-rank varieties across NSW is well ahead of the incidence of disease, with 100% of fields surveyed in 2006-07 having an F-rank of 100 or higher. The annual disease surveys were modified to ensure that transects in November and March were congruent within each season and to enable comparisons between the incidence of early-season and late-season diseases and/or symptoms. Preliminary comparisons indicate that there was no relationship between the incidence of Fusarium wilt in March and seedling mortality the previous November in the same crops but few fields with Fusarium wilt were surveyed due to drought. Transects across infested fields indicate that changing to less susceptible varieties does not always lead to an immediate reduction in the incidence of Fusarium wilt in the following crop. Fusarium wilt has not been reported on the majority of farms in NSW, reaffirming the need for ongoing diligence with farm hygiene.

#### ***Verticillium wilt.***

Verticillium wilt was controlled in the 1990s using resistant varieties and now occurs at very low levels in most regions. The incidence of Verticillium wilt decreased recently in the Namoi Valley, probably due to greater use of resistant varieties. Extension should emphasise the value of resistant varieties on an ongoing basis to prevent resurgence of this disease.

While Verticillium wilt has not been reported in some areas and occurs at very low levels in others, the disease is still observed in 45 to 60 % of fields surveyed annually across NSW. Farm hygiene should be practiced to minimise further spread of Verticillium wilt.

**Boll rots**

Cotton boll rots were observed at very low levels across NSW in the 2004-05, 2005-06 and 2006-07 seasons. Boll rot is depended upon rainfall events in January and February and a high incidence of boll rots has not occurred since 2000 and 2001, when some regions experienced heavy rainfall events during boll filling. From 2002 to 2007, the number of bolls affected was generally less than 1%, whereas from 1993 to 1999, the disease mostly affected 1 to 2% of bolls. This apparent decline in boll rots over recent years probably reflects both the drought and the expansion of disease surveys into the Lachlan and Murrumbidgee Valleys, where summer rainfall is low.

**Alternaria leaf spot.**

Alternaria leaf spot was consistently observed at low levels in virtually all crops inspected in NSW. Alternaria leaf spot currently poses little threat to upland cotton (*Gossypium hirsutum*) in Australia, although Pima crops are more susceptible.

**Other diseases.**

Cotton bunchy top was rarely observed during the course of this project and the disease poses little threat to cotton production if such low levels persist. Bacterial blight was not observed in any of the disease surveys, including the small number of Pima crops that were inspected. Sudden wilt was observed in a few isolated plants each season but poses no threat to cotton production. Dieback caused by *Sclerotinia* was observed in a single crop where tall plants had lodged and pressed against the soil but *Sclerotinia* is not likely to threat crops with normal growth habit.

**Objective 2. To continue to develop and/or evaluate control strategies for black root rot, seedling disease and Fusarium wilt (e.g. seed treatment fungicides, induced resistance, rotation crops, biofumigation crops, cover crops, late sowing with Bollgard™)**

A range of potential tools for control of cotton diseases were evaluated in glasshouse and field experiments with results as follows:

Strategy	Focus	Result	Implications	
			Science	Industry
Seed quality	Seed vigour index (SVI) and seed mass	Seedling mortality decreased as SVI increased but SVI was unrelated to growth	The mechanisms by which SVI increase stand establishment are not related to growth or seed size	Growers awareness that high SVI does not equate to better growth
Crop residue management	Evaluation of vetch residue impact on seedling mortality	Late incorporation of vetch increases activity of <i>Rhizoctonia</i> and I causing greater seedling death was confirmed repeatedly	Vetch residues can be used to create a disease nursery for research	Crop residues should be managed to avoid enhancement of seedling disease in cotton
Timing of sowing	Delayed sowing date	Delayed sowing decreases seedling disease	Grater understanding of factors contributing to severity of seedling disease	Delayed sowing recommended as a management tool for seedling disease



Strategy	Focus	Result	Implications	
			Science	Industry
	Seed treatment prior to sowing	Confirmation that seedling pathogens vary in dominance from field to field and year to year	Increased understanding of factors contributing to severity of seedling disease	Realisation that fungicides are only effective if their target pathogen is exerting disease pressure
Fungicides	Seed treatment prior to sowing	New fungicide combinations rarely performed better than the standard treatment	Confirmation that fungicides vary in capacity to control seedling pathogens	Importance of fungicidal seed treatment emphasised
Fungicides	Seed treatment prior to sowing	Non-fungicidal seed treatments, including acibenzolar-S-methyl were ineffective in seedling disease experiments	Confirmation that systemic acquired resistance against seedling disease is probably not feasible	Awareness that non-fungicidal treatments are likely to be ineffective
Fungicides	Seed treatment with Dynasty™	Dynasty™ performed as well as, or better than, the standard fungicides	Confirmation of effectiveness of components of Dynasty™ in seedling disease control	Independent evaluation of effectiveness of Dynasty™
Fungicides	In-furrow application for seedling disease	Azoxystrobin decreased seedling disease substantially at some sites and not others	Confirmation that pressure from different species of seedling pathogens is variable	Future deployment of in-furrow fungicides, pending further research
Fungicides	In-furrow application of azoxystrobin against Fusarium wilt	No effect on Fusarium wilt	Azoxystrobin not effective against Fusarium wilt	Confirmation of existing assertion that in-furrow fungicides do not control Fusarium wilt
Fungicides	Acibenzolar-S-methyl for Fusarium wilt	Severity of Fusarium wilt decreased and seedling mortality decreased in crops with Fusarium wilt.	Pathogen-specific SAR response that persists with dilution through time and in growing tissues.	New tool for control of Fusarium wilt of cotton, possibly against Fov as a seedling pathogen
SAR	Pre-plant and at-planting herbicides	Consistent observations of no effect on seedling disease or black root rot	Exclusivity of mechanisms of herbicide action and pathogen infection	Confidence that herbicides will not enhance black root rot
Biofumigation	Canola rotation	No effect on black root rot	Rotation cropping unlikely to release the biofumes that are released when crop incorporated as fresh green material	Awareness that rotation with canola will not control black root rot

**Objective 3. To conduct investigations of host-pathogen-soil interactions (including herbicides) contributing to the severity of black root rot of cotton and identify features that might be exploited for disease control.**

A range of potential plant-soil-fungal interactions were evaluated in glasshouse and field studies with results as follows:

Strategy	Focus	Result	Implications	
			Science	Industry
Herbicides	Long-term vetch and canola	Poor growth of the biofumigation crops resulted in and increase in black root rot across the trial site	Threshold level of release of biofumes required for effective decrease in pathogen density in soil	Good growth and incorporation of biofumigation crops is essential, even when used regularly
Survival of AMF	Extended bare fallows	Substantial numbers of AMF surviving up to four years of bare, dry fallow	AMF have evolved to cope with climatic vagaries of the Australian landscape, including drought-induced fallows	AMF generally survive in adequate numbers. Commercial claims for replacement with fertiliser questionable
Biotechnology	Effects of transgenic cotton on AMF	Transformation with insect resistance and herbicide tolerance genes had no effect on colonisation of roots by AMF	Bt toxin is not detrimental to AMF	Confidence that transgenic cotton has no impact on AMF
Enhancement of AMF colonisation	Seed treatment with flavonoid	Phytotoxic effect on cotton growth at recommended rate	Flavonoids can be phytotoxic	Careful evaluation of novel seed treatments is required
Enhancement of AMF colonisation	Amendment of soil with commercial AMF inoculant	Inoculant contained no AMF and carrier was potentially detrimental to native AMF in soil	Inoculants need quality control	Careful evaluation of novel soil amendments is required

**Objective 4. Continue long-term field experiments on the role of black root rot, mycorrhizal fungi (VAM) and other soil organisms in the soil ecosystem (soil ‘health’)**

Long-term experiments were continued, as follows:

Strategy	Focus	Result	Implications	
			Science	Industry
Survival and dispersal of arbuscular mycorrhizal fungi (AMF) and <i>Thielaviopsis</i>	Long-term experiment at ACRI with nine-year fallow	AMF fungi survived in adequate numbers for cotton, <i>Thielaviopsis</i> colonised bare soil in the absence of hosts, probably by movement in irrigation water	Both AMF and <i>Thielaviopsis</i> can survive long periods in the absence of a host and/or colonise bare soil in the absence of plants	AMF generally survive in adequate numbers for cotton and both and disperse with irrigation water
Biofumigation	Long-term experiment at ACRI with vetch and canola green manure crops	Vetch and canola growth was poor during most seasons and black root rot spread and increased across the trial site	Threshold levels of biofumigant crop dry matter are required for and effect against pathogens	Good growth of the biofumigant crop required even when used repetitively

### **Objective 5. Facilitate delivery and deployment of cotton disease management strategies that utilise available methods in a cost effective, integrated manner with the least impact on the environment**

Aspects of the management strategy and the results of the research in the project have been communicated by way of presentations and scientific and extension publications, including: the guidelines for integrated disease management; magazine articles and information sheets media releases as listed in Section 9.1, below. Modifications to the existing disease management strategy for seeding disease, black root rot and Fusarium wilt have been devised and confirmed by the research in this project and are presented in Section 8, below. Personal activity at field days and industry meetings included:

- Organised and conducted Hands-on-Research workshop on black root rot at the Australian Cotton Conference, August 2004, including presentations and posters.
- Presentation on biofumigation to Cotton CRC Review, Aug 2005.
- Presentation on effects of rotations on disease at 'Crown rot meeting', Narromine NSW, Dec 2005.
- Four presentations on disease research at the Australian Cotton Research Institute to the Cotton Consultants Association, May 2006
- Presentation on black root rot to FUSCOM meeting, May 2005
- Presentation on seed production protocol to FUSCOM meeting, May 2005
- Presentation on seedling disease and surveys to FUSCOM meeting, May 2005
- Presentation on Verticillium wilt to FUSCOM meeting, May 2005
- Presentation on the effects of climate change on disease, Climate Change Forum, Narrabri March 2007.
- Presentation on arbuscular mycorrhizas in cotton, Cotton Soil Ecology Research Group Meeting, Sep 2006
- Presentation on diseases of cotton, CRDC Disease Research Review, Feb 2007
- Presentation on integrated disease management for black root rot, Decision Maker's Forum, August 2006
- Organised and conducted Hands-on-Research workshop on black root rot at the Australian Cotton Conference, August 2006, including presentations and posters.
- Gave six hours of lectures to the Cotton Production Certificate course each August in 2005, 2006 and 2007 (18 hours total)

## ***Part 4 – Final Report Executive Summary***

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Regular disease surveys have highlighted the changing status of cotton diseases over time and provided valuable insights into the factors affecting their distribution and severity.

- The disease surveys provided overwhelming evidence that seedling mortality is not related to the incidence or severity of black root rot but increases with increasing latitude
- The observed incidence of black root rot has decreased in the past three seasons, most likely as a result of climatic conditions and drought-enforced fallows but has increased rapidly in the Lachlan and Murrumbidgee Valleys.
- The rate of reporting of new cases of Fusarium wilt, now totalling 81 farms in NSW, has declined in recent years, potentially due to farm hygiene, greater deployment of less-susceptible varieties and drier conditions in spring.



- Fusarium wilt has been observed on 41% of the set of farms surveyed annually in NSW and is spreading at a steady rate of increase among those farms
- The incidence of Verticillium wilt decreased recently in the Namoi Valley, probably due to greater use of resistant varieties, although the disease is still observed in 45 to 60 % of fields surveyed annually across NSW
- An apparent decline in boll rots over recent years probably reflects both the drought and the expansion of the surveys into southern NSW, where summer rainfall is low.

Experiments were conducted to develop and/or evaluate control strategies for control of seedling disease, black root rot and Fusarium wilt:

- High seed vigour index, while decreasing seedling mortality, was not related to seedling growth; seedling mortality was not related to seed mass
- The fungicide Dynasty™ consistently performed as well as, and sometimes better than, the standard fungicides
- Seed treatment with fertilisers and other non-fungicidal products, were not effective in controlling seedling disease, nor was Bion® in most cases except in crops with Fusarium wilt, suggesting that Bion® may activate resistance against *F. oxysporum* f.sp. *vasinfectum* as a seedling pathogen.
- Seed treatment with Bion® activated resistance against Fusarium wilt and black root rot of cotton, although its efficacy was variable
- In-furrow application of the fungicide azoxystrobin was very effective in controlling seedling disease in some seasons at some locations.
- Delayed sowing decreased the period of exposure of cotton to conditions that favour black root rot and seedling disease, even when cool and wet conditions coincided with the late sowing.
- Pre-plant and at-planting herbicides had no effect on the severity of black root rot or growth of seedling cotton
- In comparison to continuous cotton, nine years of bare fallow did not eliminate arbuscular mycorrhizal fungi (AMF) in the soil, although re-colonisation by AMF probably occurred, as the black root rot pathogen, *T. basicola*, also colonised the fallowed plots in the absence of plant hosts.
- During bare fallows of up to four years at Bourke, AMF survived in sufficient numbers for normal development of mycorrhizas in cotton
- MF were not affected by transgenic cotton varieties
- Seed treatment with a plant flavonoid reputed to increase mycorrhizal development in the field was phytotoxic at the recommended rate and no conclusion about mycorrhizal effects could be drawn.
- Evaluation of a commercial inoculum, putatively containing mycorrhizal fungi, showed that no mycorrhizal fungi were present in the product.

Results of these experiments and observational studies have been incorporated in strategies for integrated disease management and disseminated to the cotton industry by way of publications, media releases, field days and meetings with growers and consultants.