If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October.
If not, please provide a written report by 30 September.

Part 1 - Summary Details

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

CRDC Project Number:

Project Title:

Project Commencement Date: **Project Completion Date:**

CRDC Program: - Please Select One -

Part 2 – Contact Details

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Signature of Research Provider Representative: ______________________

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

Background

Fusarium wilt of cotton is caused by form species the soilborne fungus *Fusarium oxysporum* f.sp. *vasinfectum* (hereafter ***Fov***). *Fov* causes disease specifically in cotton, including upland varieties and Pima. The disease was first confirmed in cotton in Australia on the Darling Downs in 1993. Two strains have since been distinguished in Australia based on vegetative compatibility grouping (VCG). Strains in VCG 01111 are widespread in NSW and Queensland, whereas strains in VCG 01112 have only been found on a few farms near Boggabilla and, on one occasion, in one field at Moree. It was recognised at an early stage that the strains in Australia were different to those affecting cotton overseas. Recent evidence indicates that the Australian strains of *Fov* are most closely related to a single lineage of *F. oxysporum* occurring on native Australian species of *Gossypium* (Wang et al. 2003).

Repetitive monoculture of cotton probably helped to select for those strains, among the native population of *F. oxysporum*, that happened to be pathogenic (i.e. strains 01111 and 01112). At the commencement of this project, Fusarium wilt of cotton had been reported on 38 farms in NSW; Menindee, the Lachlan and Murrumbidgee Valleys were the only regions where the disease had not been reported. When Fusarium wilt progresses unchecked, fields and even whole farms can be rendered unsuitable for cotton production. Fusarium wilt has now reached some of 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.

Observational studies and experiments on control of Fusarium wilt were established in the CRDC project DAN121C, *Diseases of Cotton VI*, including the effects of rotations with cereals and legumes, biofumigation with mustard and vetch, incorporation and burning of cotton trash in wheat stubble, and induction of systemic resistance. 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.

Verticillium wilt, caused by the soilborne fungus *Verticillium dahliae*, was once the most widespread and important disease of cotton in Australia. The widespread adoption of varieties with resistance to Verticillium wilt during the 1990's effectively reduced the incidence and importance of the disease (around 5% of the crop surveyed in NSW at commencement of this project). However, conditions in the 1999/00 season were ideal for Verticillium wilt and in some fields, including some of the resistant varieties, 40 to 50% of plants were infected.

Active populations of Verticillium are still widespread in cotton growing soils. In California, where there are several different and more virulent strains of the pathogen, the repeated use of resistant cultivars resulted in the selection of more virulent strains and an effective breakdown in the level of cultivar resistance to the disease. An experiment conducted as part of DAN121C indicated that continuous monoculture of resistant varieties in Australia may be selective for strains of Verticillium with greater virulence. For this reason it is of great importance to monitor the incidence of Verticillium wilt with repeated cultivation of resistant varieties.

Seedling disease is caused by *Rhizoctonia solani* and *Pythium* spp. Under favourable conditions, these fungi cause pre- and post-emergent damping off (death) of seedlings. Seedling mortality includes the impact of seedling disease (caused by *Rhizoctonia solani* and

Pythium spp.) as well as seed viability, the activity of soil insects such as wireworms and physical problems such as fertiliser burn. During the 1990's, seedling mortality for the whole of NSW declined from an average of 40% down to 20%. This decline partly reflects improved cultural practices, rather than a declining prevalence of the seedling pathogens *Rhizoctonia* and *Pythium*, because high stand losses still occur when climatic conditions favour disease. For example, with the cool wet conditions in October and November 2000, seedling mortality in the Gwydir, Namoi and Macquarie valleys was 35 to 37% and replanting was common.

Seedling disease is greater in cooler climatic zones and higher seedling losses (eg. 35% in the Lachlan valley) are associated with the southward expansion of cotton production in NSW. Effective control of seedling diseases is important so that the costs and problems associated with replanting can be avoided. Black root rot is caused by a soilborne fungus (*Thielaviopsis basicola*) that infects the roots of cotton early in the season. *T. basicola* produces massive numbers of thick-walled resting spores that are deposited into the soil in greater numbers with successive cotton crop. The fungus is easily spread in infested soil adhering to vehicles, machinery, tools and boots, and in flood and irrigation water. These characteristics of *T. basicola* have contributed to the development of a widespread, chronic epidemic of black root rot in Australian cotton.

Black root rot increased exponentially during the 1990's and, at the commencement of this project, was widespread in all established cotton production areas in NSW and much of Queensland. In November 2000, the disease was observed in 53% of fields and 15% of plants in the set of 30 farms inspected regularly by NSW DPI in the Macintyre, Gwydir, Namoi and Macquarie Valleys. Yield losses of up to 40% can occur when the disease is severe, especially in the cooler production areas. All cultivated cottons are susceptible to black root rot and there are very few options for effective control. This escalation in the incidence of black root rot represents an increasing threat to cotton production.

The pathogen that causes Alternaria leaf spot *(Alternaria macrospora*) survives on crop residues from the previous season. Its survival is favoured by dry winter conditions and the retention of cotton crop residues on the soil surface. Alternaria leaf spot is regarded as one of the major factors affecting Pima cotton production but few of the commercial upland cultivars are very susceptible.

Phytophthora boll rot occurs when soil, containing zoospores, is splashed onto low bolls or when bolls are temporarily submerged either in water in the furrow or when water backs up from the tail drain. A well-established and closed canopy minimises the chance of soil splashing onto low bolls. Other boll rots develop when bolls are exposed to extended damp conditions as a result of wet weather and confinement in a dense or rank canopy. There are no control measures for boll rots. Boll rots are observed each year and can be severe under the right conditions. Continued monitoring of these diseases is required.

Symptoms of cotton bunchy top include small bolls, small leaves, short internodes and a distinctive leaf mottle occurring around the margins of the leaves (the leaf mottle may be masked if infestation by aphids or mites is severe). leaf mottle symptoms may occur unaccompanied by the bunchy growth habit. Cotton bunchy top was apparently widespread in the 1998-99 season but has only occurred at negligible levels since then.

The research in this project was designed to enable continued monitoring of the distribution and potential importance of diseases of cotton, further development and confirmation of disease management strategies, and transfer of this information to growers for the more effective control of cotton diseases.

Objectives

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. Disease surveys were conducted in November and March in each year of the project, including all production areas in NSW except Menindee. 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 identified.

Objective 2.
To continue to develop and/or evaluate control strategies for Verticillium wilt, Fusarium wilt, Alternaria leaf spot and seedling diseases of cotton. Seed treatment experiments were completed at Narrabri, Hillston, Warren or Dalby in each year of the project, including evaluation of the potential of fungicides and some ‘biological’ products. Experiments on the effect of seed treatment/planting date on seedling mortality and cotton yield were completed in two years of the project. Experiments on the timing of sowing, cover crops, seed treatment fungicides, in-furrow fungicides, crop rotation, biofumigation, systemic acquired resistance and transgenic cotton lines were completed, variously for the control of seedling disease, black root rot, Fusarium wilt and Verticillium wilt.

Objective 3.
To initiate investigations of host-pathogen interactions involved in soil-borne diseases of cotton and identify features that might be exploited for disease control. Studies of the progress of disease black root rot were completed confirming that symptoms are most severe during the period from three to five weeks after sowing. Delaying sowing could minimise this period of exposure to severe symptoms and slow the build up of the pathogen.
A study suggesting that the incidence of Fusarium wilt may be influenced by soil types was commenced.

Objective 4.
Facilitate delivery and deployment of cotton disease management strategies. Recommendations for modifications to the Integrated Disease Management guidelines for control of seedling disease, black root rot and Fusarium wilt were devised. Results were extended to the cotton industry by way of presentations and extension publications, and published in scientific literature.

Methods

Disease surveys

The distribution and incidence of diseases of cotton in NSW was determined in disease surveys of 99, 87 and 66 commercial crops in November and March in the 2001-02, 2002-03 and 2003-04 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, trash carryover, 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), and at an arbitrarily random position along the tail drain. 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'). In November, the two transects commenced at a single starting position (at which GPS coordinates were recorded) and ran diagonally to the left and right in a '\/' configuration. In March the two transects commenced at separate, newly-selected starting positions, mostly stepping diagonally to the right in a '/ /' configuration. 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 (or occasionally 5 seedlings, due to the constraints of time or weather) 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). Samples of cotton residues (trash) on the soil surface were collected near each step-point along the 'right hand' transect from within a steel quadrat (30 cm × 30 cm) and dried in an oven. To ensure random sampling of trash, the quadrat was thrown ahead, during pacing of the transect, usually landing < 3 m away from the step-point. Trash samples were not collected from farms known to be infested with the Fusarium wilt pathogen. In March, 10 plants were inspected at each step-point and 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.

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. 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 initiated 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, trash management and the potential to induce systemic acquired resistance were conducted both at ACRI and at appropriate field sites.

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
- In NSW, seedling mortality was relatively low during most of the 1990's but increased dramatically in 2000, 2001 and 2002
- The risk of seedling disease increases with increasing latitude, with the southern regions of NSW being particularly prone
- Growers tend to respond to changes in the level of seedling mortality by altering sowing rates in the following season
- Seedling mortality is not increased by black root rot

Across NSW, seedling mortality declined by approximately 50% during the early 1990s, levelling off at close to 20% in the late 1990s (Figure 1). Since the fungicides used in the standard seed coatings during this period did not change, the large decline in mortality in the early 1990's probably represents an improvement in farming practices, such as improved bed preparation, planting accuracy, and avoidance of herbicide damage and fertiliser burn.

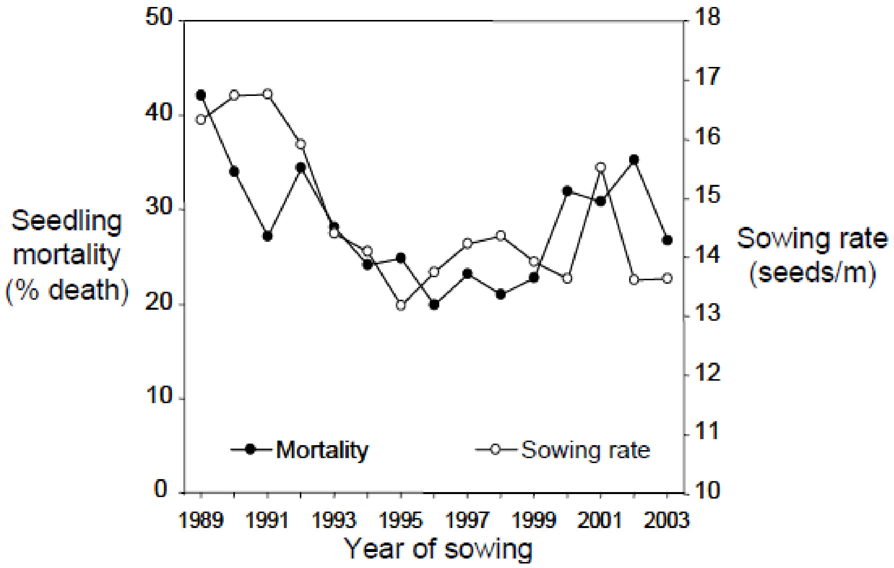


Figure 1. Changes in cotton seedling mortality in fields surveyed in the Macintyre, Gwydir, Namoi, Macquarie and Lachlan valleys in NSW. Farms in the Lachlan Valley were included in the surveys for the first time in the 1990-00 season. Early season-surveys in the Bourke-Walgett region and in the Murrumbidgee Valley commenced in the 2001-02 season.

Seedling mortality increased dramatically in the crops sown in 2000, 2001 and 2002 (Figure 1). The cool wet conditions in October and November in 2000 and 2001 were very favourable to *Rhizoctonia* and *Pythium*, and these pathogens would have had a major impact on seedling mortality in those seasons. Crops planted in mid to late September, 2001 generally established better than crops planted in October, when conditions became wetter and cooler. In 2002, there was very little rainfall during October and early November in most areas. However, hot dry winds resulted in rapid drying of the upper soil profile leading to problems with seedling establishment, which in turn increased seedling mortality (Figure 1, Table 1). In 2003, cool conditions were experienced during October and early November 2003 in most areas but the absence of rain was a mitigating factor that reduced the incidence of seedling disease in comparison to the previous three seasons (Figure 1, Table 1).

Table 1. Cotton seedling mortality by region, on farms surveyed in NSW each November

	Seedling mortality (% death)						
Season	Bourke-Walgett	Macintyre Valley	Gwydir Valley	Namoi Valley	Macquarie Valley	Lachlan Valley	Murrumbidgee Valley
1989-90	-	34	41	42	48	-	-
1990-91	-	21	32	38	40	-	-
1991-92	-	29	25	26	31	-	-
1992-93	-	32	41	32	33	-	-
1993-94	-	22	25	32	33	-	-
1994-95	-	25	20	26	26	-	-
1995-96	-	29	24	19	28	-	-
1996-97	-	23	17	18	24	-	-
1997-98	-	26	21	22	26	-	-
1998-99	-	22	21	18	28	-	-
1999-00	-	24	20	19	25	30	-
2000-01	-	23	37	35	36	26	-
2001-02	30	28	30	27	39	34	41
2002-03	18	40	30	35	36	52	33
2003-04	32	18	21	25	23	48	45
Mean	**26.9**	**26.4**	**27.0**	**27.6**	**31.7**	**38.1**	**39.6**

In any given year, seedling mortality tended to be higher in the Macquarie, Lachlan and Murrumbidgee Valleys (Table 1) reflecting the higher latitude of these regions. There was a direct correlation between mean seedling mortality for each region and mean latitude of the farms surveyed in that region (Figure 2). This relationship to latitude reflects the tendency for climatic conditions to be cooler as latitude increases, thus favouring the seedling pathogens.

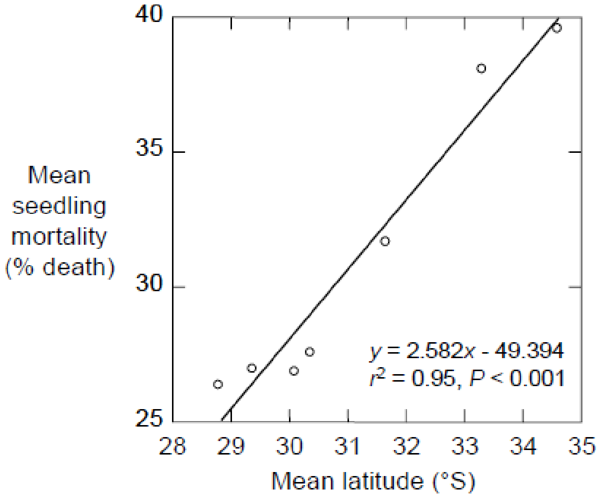


Figure 2. Seedling mortality of cotton increased in direct proportion to the mean latitude of the farms surveyed within each region of NSW. In order of increasing latitude (reading left to right) the valleys/regions were the Macintyre, the Gwydir, Bourke-Walgett, the Namoi, the Macquarie, the Lachlan and the Murrumbidgee.

Throughout the 1990's there was a significant relationship between the choice of sowing rate by growers and seedling mortality (Figure 3). For crops sown between 1990 and 2001, approximately 60% of the variation in sowing rate could be explained by seedling mortality in the previous season. This trend was exemplified during the early 1990's, when sowing rate declined markedly as mortality declined, and following the rapid increase in seedling mortality between 1999 and 2000, which resulted in growers sowing 1.9 more seeds/m in 2001 (15.5 seeds/m) than in 2000 (13.6 seeds/m, Figure 1). Although the mean seedling mortality for NSW was relatively high in 2001-02 (30.9%), growers generally reported less replanting in that season, than in the previous season, probably because of the higher sowing rate. However, in 2002 and 2003 growers returned to sowing 13.6 seeds/m, despite ongoing high levels of seedling mortality in 2001 and 2002 (Figure 1). If the regression analysis (Figure 3) includes the data for the 2002 and 2003 sowings, then the correlation becomes less strong ($r^2 = 0.33$), although still significant ($P = 0.032$). Hence, other factors influenced grower's choice of sowing rate in those two seasons. (Figure 1).

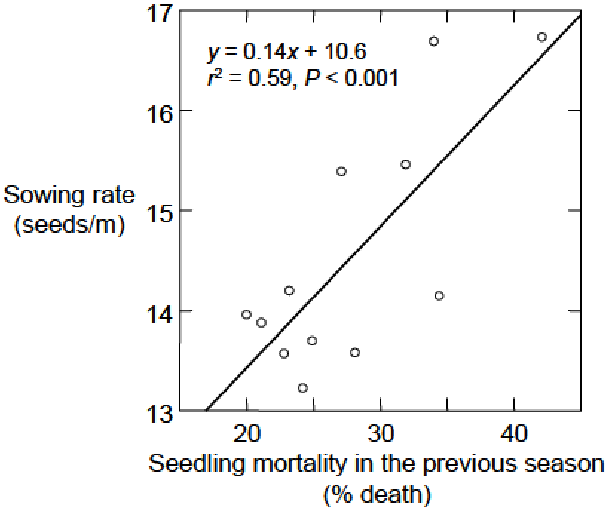


Figure 3. Cotton growers tended to sow more seeds if seedling mortality was higher in the previous cotton crop. Data are for mortality from 1989 to 2000 and for sowing rate from 1990 to 2001, in all regions surveyed across NSW.

Comparison of seedling mortality with the incidence of black root rot along the same transects clearly demonstrated that there was no link between the two, inn all three seasons of the surveys in this project (Figure 4). This suggests that anecdotal reports that seedling mortality is increased by black root rot are unfounded.

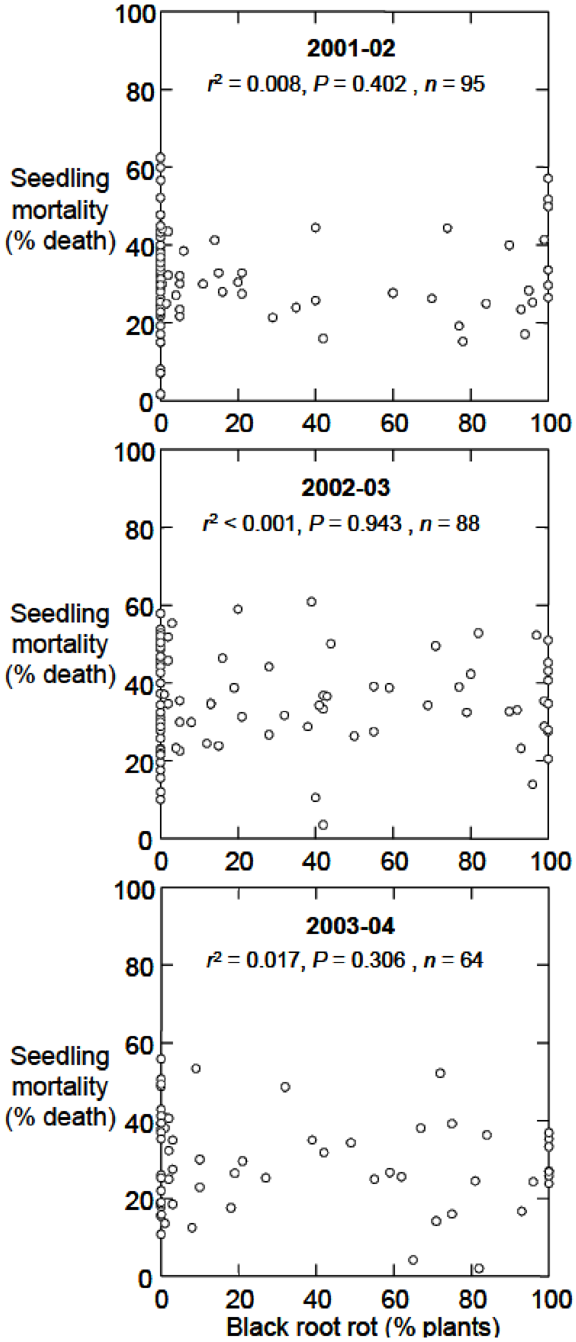


Figure 4. Lack of relationship between black root rot and seedling mortality of cotton across NSW in three seasons of disease surveys.

Black root rot

- The Australian cotton production industry is currently experiencing a widespread, chronic epidemic of black root rot
- Black root rot has been observed on all of the farms surveyed regularly in the Macintyre, Gwydir, Namoi and Macquarie Valleys, and in 78% of fields and 39% of plants within those farms
- If the pathogen continues to increase its distribution within these four valleys at its current rate, then approximately 95% of fields, and 90% of plants within those fields, will be infested by 2011

- 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 Valley
- Black root rot was detected in the Murrumbidgee Valley for the first time in November 2003, as part of the surveys in this project
- *T. basicola* is not distributed evenly within fields and assays of soil do not necessarily detect the pathogen, even when it is located nearby in the field
- Many farms do not have black root rot and farm hygiene should be practiced to minimise further spread

During the three years of this project the distribution of black root rot in NSW, continued to expand, both within and between farms. By the 2003-04 season, black root rot had been reported in all production areas of Queensland and NSW (except Menindee). Black root rot has now been observed in all of the farms surveyed regularly in the Macintyre, Gwydir, Namoi and Macquarie Valleys as part of this project (Figure 5). In these four valleys 78% of the crops inspected in 2003 had the disease, averaging 39% of plants (36% the previous season, Figure 5). If the pathogen continues to increase its distribution within these valleys at its current rate, then approximately 95% of fields, and 90% of plants within those fields, will be infested by 2011 (Figure 5b,c). Across the whole of NSW (i.e. including survey data from the Bourke-Walgett area, and the Lachlan and the Murrumbidgee Valleys), black root rot was observed in an average of 67% of cotton crops and 32% of plants in November 2003 (64 and 31% in the previous season, respectively). In November 2003, black root rot was observed for the first time in one field in the Murrumbidgee Valley (Table 2).

Since the mid-1990s, crops in the Namoi and Macquarie Valleys have been the worst affected, with the disease being observed in 80 to 100% of fields and 50 to 60% of the plants, on the farms surveyed there in recent years (Table 2, Figure 6). The disease has increased rapidly in the Macintyre Valley, affecting 82% of fields and 22% of plants inspected in that valley in 2003. The apparently low incidence of black root rot within crops (i.e. % plants) in 1998 and 1999 (Figure 6) probably reflects delays in the conduct of the surveys in those seasons, rather than a real reduction in disease incidence (see **p. ??** for assessment method appraisal).

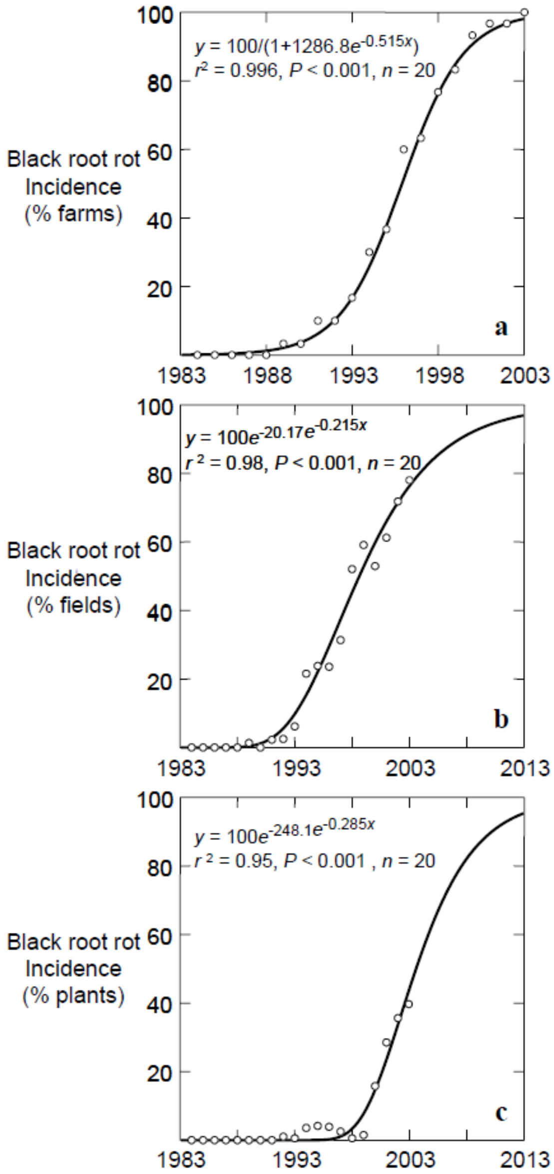
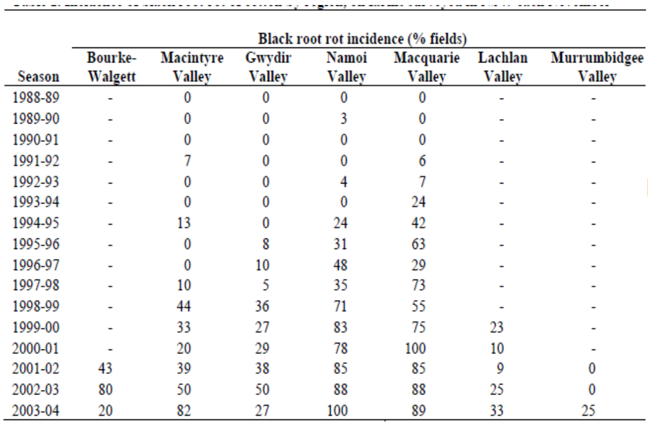


Figure 5. Observed (○) and predicted (▬) increase in the incidence of black root rot of cotton in farms surveyed annually across NSW (data for Macintyre, Gwydir, Namoi and Macquarie Valleys only)

Table 2. Incidence of black root rot of cotton by region, on farms surveyed in NSW each November



Season	Black root rot incidence (% fields) Bourke-Walgett	Macintyre Valley	Gwydir Valley	Namoi Valley	Macquarie Valley	Lachlan Valley	Murrumbidgee Valley
1988-89	-	0	0	0	0	-	-
1989-90	-	0	0	3	0	-	-
1990-91	-	0	0	0	0	-	-
1991-92	-	7	0	0	6	-	-
1992-93	-	0	0	4	7	-	-
1993-94	-	0	0	0	24	-	-
1994-95	-	13	0	24	42	-	-
1995-96	-	0	8	31	63	-	-
1996-97	-	0	10	48	29	-	-
1997-98	-	10	5	35	73	-	-
1998-99	-	44	36	71	55	-	-
1999-00	-	33	27	83	75	23	-
2000-01	-	20	29	78	100	10	-
2001-02	43	39	38	85	85	9	0
2002-03	80	50	50	88	88	25	0
2003-04	20	82	27	100	89	33	25

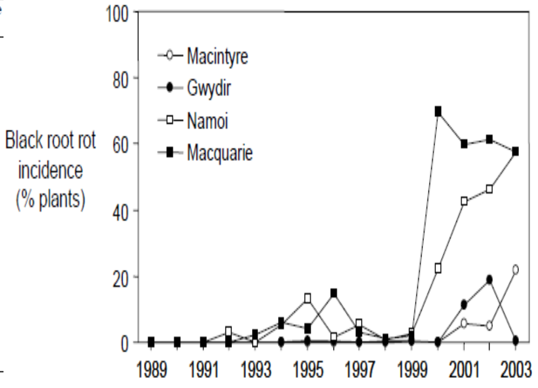


Figure 6. Incidence of black root rot of cotton in the major cotton-producing valleys surveyed in NSW

each November.

In each field in November 2001, samples of soil were taken at the endpoints of the two transects and assayed as a composite sample for the presence of the black root rot pathogen, *T. basicola*, using the selective medium TB-CEN agar. Fields in the Macintyre valley were excluded from the assay to reduce the risk of inadvertently transferring *F. oxysporum* f.sp. *vasinfectum* to the Australian Cotton Research Institute. Symptoms of black root rot were observed in 41 of the 78 fields in which the population of *T. basicola* was assayed in 2001. Variation in the population of *T. basicola* in soil, collected from the endpoints of the transects in the 78 fields, accounted for 85% of the incidence of black root rot within those transects (Figure 7). However, the population of *T. basicola* in the soil samples was not a good predictor for disease incidence because of the angular shape of the curve. Furthermore, the soil assay did not detect the pathogen in nine of the 17 fields in which the incidence of black root rot was less than 30%, reflecting the irregular distribution of this fungus in soil. Conversely, as the population of *T. basicola* increased beyond 100 cfu/g soil there was no relationship with disease incidence (Figure 7).

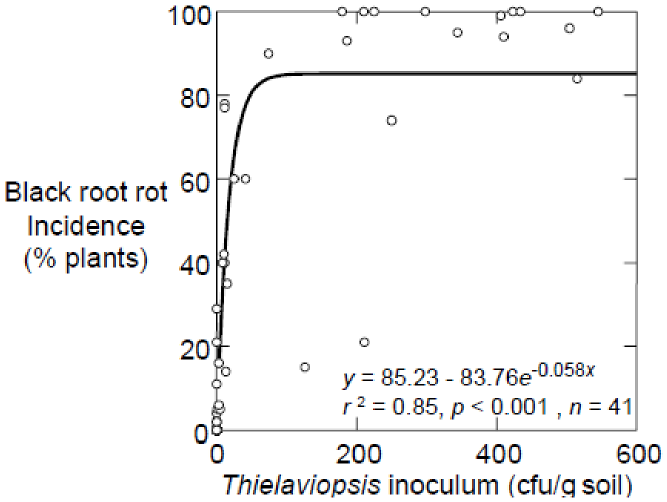


Figure 7. Relationship between the incidence of black root rot over transects of cotton crops and inoculums density of *Thielaviopsis basicola* in soil at the transect endpoints, in fields surveyed across NSW (excluding Macintyre valley) in November 2001 (cfu = colony forming units).

Fusarium wilt

- The Australian cotton industry is currently experiencing a widespread, chronic epidemic of Fusarium wilt
- Fusarium wilt has been reported on a total of 75 farms in NSW
- Fusarium wilt has been observed on 30% of the farms surveyed regularly across NSW
- If the pathogen continues to disperse at its current rate, then approximately 90% of farms in NSW will be affected by 2012
- The annual disease surveys should be modified to assess congruent transects within each season to enable comparisons between the incidence of early-season and late season diseases and/or symptoms
- The Macintyre Valley has been the affected the most 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 closely followed the incidence of the disease among farms
- 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 an immediate reduction in the incidence of Fusarium wilt in the following crop
- Transects of an infested field in the Macquarie Valley suggest that Fusarium wilt may progress much more quickly in cooler cotton growing regions but the absence of crops, due to the drought, has prevented further confirmation of this hypothesis
- Fusarium wilt has not been reported on the majority of farms in NSW and farm hygiene should be practiced diligently to minimise further spread

The incidence of Fusarium wilt in NSW continued to increase during the course of this project (Figure 8). In 2001-02, there were 15 new reports of Fusarium wilt on farms in NSW. This included seven new cases in the Gwydir valley, three in the Macquarie, two in the Macintyre, two at Bourke and one at Brewarrina for the first time. Fusarium wilt was confirmed in another three samples from anonymous locations. There were several newly-affected fields on farms where the disease had previously been reported. The cool wet conditions in October and November appeared to be favourable for Fusarium wilt.

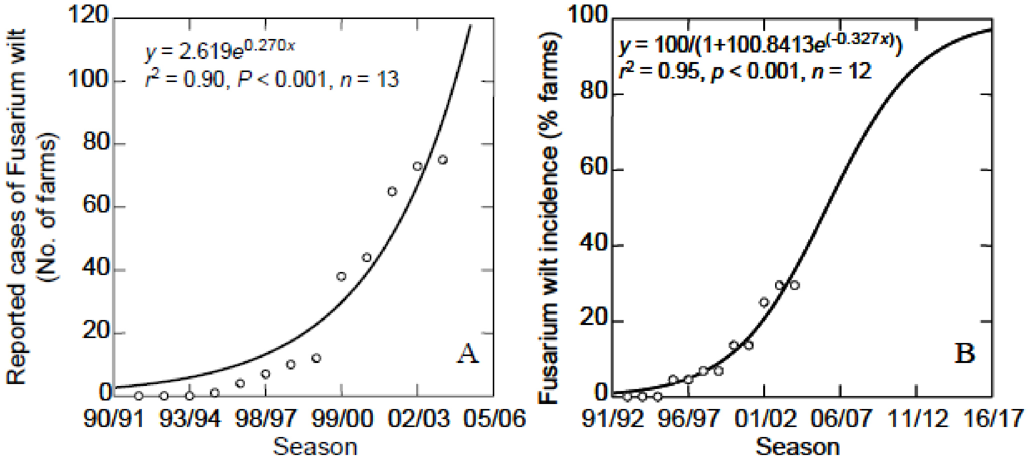


Figure 8. Observed (○) and predicted (▬) increase in Fusarium wilt in NSW: (A) the total number of farms where the disease has been reported; (B) the proportion of farms in which the disease has been observed in annual disease surveys (curves were calculated with substitution of the numbers 1 to *n* for seasons).

In 2002-03, there were seven new reports of Fusarium wilt on farms in NSW. This included three new cases in the Gwydir valley, one in the Macquarie valley, and one in the upper Namoi valley. All these new cases involved strain 01111. Strain 01112 remained confined to a few fields close to Boggabilla, and strain 01111 is now also present in many of those fields. In most areas, there was very little rainfall during October and early November 2002 and the generally hot dry summer appeared to be less favourable to development of symptoms of Fusarium wilt.

In 2003-04, one new case of Fusarium wilt (strain 01111) was reported on a farm in the Macquarie Valley, and one in the upper Macintyre Valley, bringing the total number of cases to 75 farms in NSW (Figure 8). The absence of reports of new outbreaks in that season probably reflected a combination of factors, including the dramatically reduced area sown to cotton due to drought, the impact of farm hygiene measures and seasonal conditions. The lack of rain in October and November 2003, followed by a warm, dry summer were not favourable to expression of the disease, which may have minimised the chance that new outbreaks would be observed.

During much of the 2003-04 season, Fusarium wilt appeared to be less severe than in previous years (Figure 9). Examination of disease progress at Moree and Boggabilla in CRDC Project DAN176C, by C. Anderson (NSW DPI), indicated a rapid increase in the incidence of internal infection mid-season, the incidence of externally visible symptoms of Fusarium wilt lagged considerably. Externally obvious symptoms became more severe toward the end of the season, especially on the Darling Downs where, in several fields, less than 10% of plants (cv. Sicot 189) survived (S.J. Allen, personal communication).

Figure 9. The severity of Fusarium wilt varies with seasonal conditions (these photographs were taken at approximately the same position in a field near Boggabilla, NSW)

Hence, even though disease severity was relatively mild in 2003-04, disease incidence continued to rise. Fusarium wilt has been observed on 30% of the 44 farms surveyed in 2003-04 (Figure 8b). In that season, the disease was observed in 20 and 36% of the fields inspected in the Gwydir and Macintyre Valleys respectively. On the Darling Downs the disease was observed in 75% of fields and 11% of all plants inspected in the 2003-04 season (S.J. Allen, personal communication). Fusarium wilt has not been reported in the lower Namoi, Menindee, Lachlan and Murrumbidgee areas of NSW. However, it must be assumed that the pathogen is more widespread than its reported distribution. An epidemic of Fusarium wilt is clearly developing in NSW. If the pathogen continues to spread at the same rate, 90% of farms in NSW will be affected by 2012 (Figure 8b).

When the incidence of Fusarium wilt was compared to seedling mortality, in farms surveyed annually in NSW, there was no significant relationship (data not presented). However, observations of Fusarium wilt in the surveys in March used different transects to the observations of seedling mortality in November, thus confounding comparison between the two parameters. To be able to identify whether or not interactions between early-season and late-season diseases and/or symptoms are occurring, the disease surveys need to use the same transects within each season.

The commercial deployment of less-susceptible cotton varieties in the Macintyre valley has closely mirrored the incidence of disease among farms. During the late 1990's the rapid increase in the incidence of Fusarium wilt in the Macintyre Valley, was closely followed by adoption of less-susceptible varieties (Figure 9). In recent seasons in NSW, the use of less susceptible varieties increased accelerated in comparison to disease incidence (Fig. 9). This increase in adoption of less-susceptible varieties in NSW may reflect a heightened awareness, on farms where the disease has not been observed, of the need for pre-emptive measures and/or the higher F-ranks (resistance rankings) of recently-released cotton varieties.

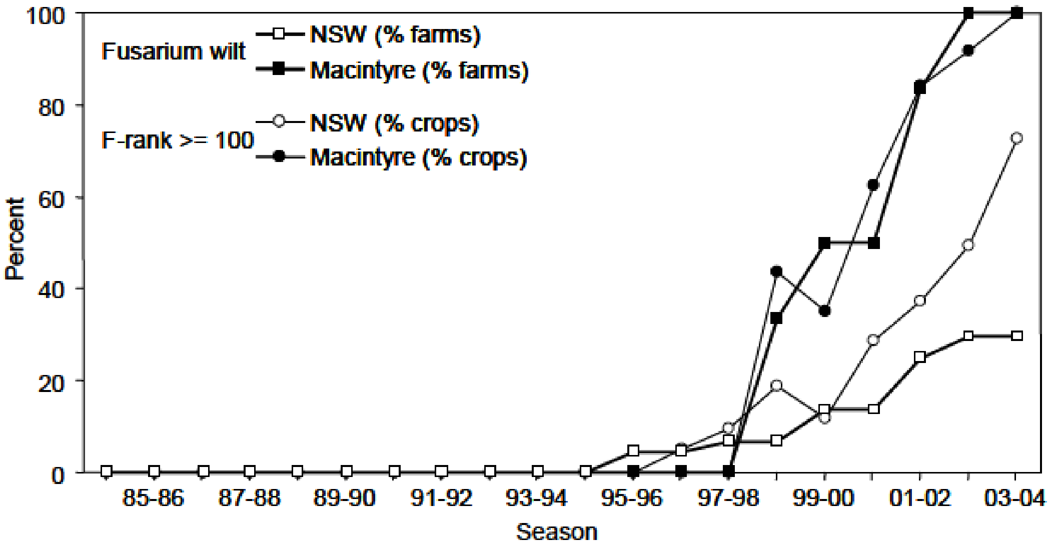


Figure 9. Relationship between the incidence of Fusarium wilt in farms surveyed annually by NSW Department of Primary Industries and the deployment of less-susceptible cotton varieties (F-rank of 100 or more) in the Macintyre Valley and NSW.

To quantify the progress of disease from season to season, the incidence of Fusarium wilt was assessed across transects of successive cotton crops in a number of fields in the Macintyre, Gwydir, Namoi and Macquarie valleys. In each transect, the incidence of Fusarium wilt was assessed in ten plants every 10 rows (occasionally every five rows). One transect, at Boggabilla (field 7), was initiated during the course of CRDC project DAN121C and was subsequently assessed twice during the course of this project (Table 3). A further 13 transects were initiated during the course of this project. Due to the lack of planting due to drought, a repeat assessment was only possible in three of these 13 transects (Table 3).

In field 7 at Boggabilla, the incidence of Fusarium wilt remained relatively stable during the three seasons of observations (Figure 10). The southern half of that field was almost continuously infested with *Fov*. In the transects near Carroll (Table 3), the incidence of Fusarium wilt was 0 and 0.4% of plants in field IW1 and 0.3 and 0.2% of plants in fields, in the first and second assessments respectively. These fields had a few small patches of Fusarium wilt, well away from the tail drain and the transects were deliberately located close to the tail drain to be able to monitor the increase of the pathogen starting from an initially low level of disease.

Table 3. Transects initiated in projects DAN121C and DAN154C to assess the incidence of Fusarium wilt across cotton fields

Valley	Locality	Field	Year of first assessment	Subsequent assessments
Macintyre	Boggabilla	7	2000	2002, 2004
		4	2003	-
		E3	2003	-
Gwydir	Garah	84	2002	-
		49	2002	-
Upper Namoi	Carroll	IW1	2000	2001
		1	2003	2004
Macquarie	Warren	19	2000	2002
		23	2000	-
		8	2002	-
		9	2002	-
		3	2002	-
	Narromine	23	2003	-
		25	2003	-

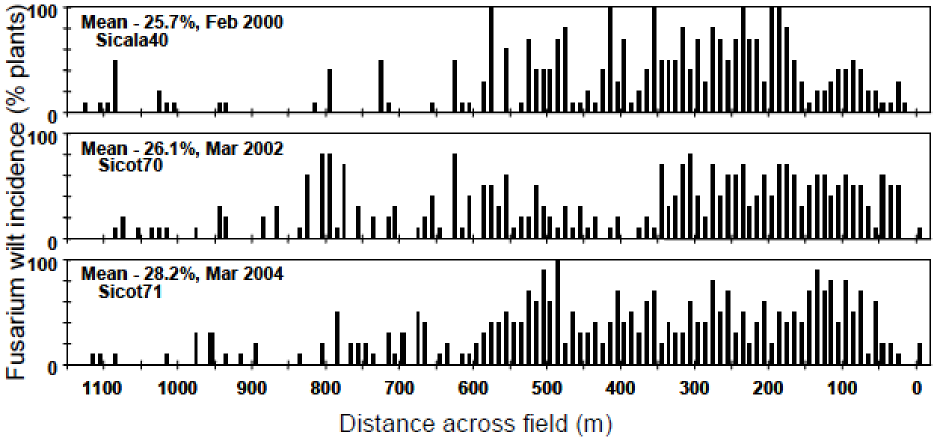


Figure 10. Incidence of Fusarium wilt of cotton 50 m from the tail drain in field 7 near Boggabilla (walking right to left = south to north).

To enable assessment of the spread of Fusarium wilt in the direction of irrigation and/or cultivation, three transects parallel to the trail drain in field 19 at Warren were assessed at 5m intervals in the June 2000 (Figure 11). The field was not cropped again until the 2001-02 season, when a less-susceptible variety was sown. Despite the change in varieties, disease incidence progressed dramatically in the 2001-02 crop (Figure 12). In both crops the incidence of Fusarium wilt was greatest in the transect running 100 m either side of the patches of bare plants and was progressively lower towards the tail drain (Figure 12). This pattern suggests that inoculum moved towards the tail drain with irrigation water, becoming progressively diluted at the same time. In the 2001-02 crop, small numbers of infected plants were observed in rows downstream along the tail drain from the major area of infestation (Figure 12). Since some of these incidences were at 100 and 193 m from the tail drain, infested soil may have been moved up the rows from the tail drain by cultivation and/or other machinery movements.

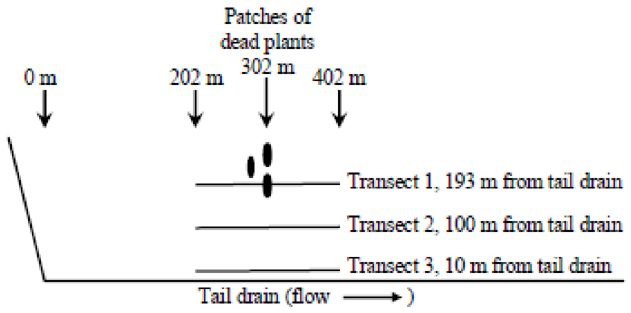


Figure 11. Location of transects assessed for the incidence of Fusarium wilt of cotton in a field near Warren, in which patches of dead plants were observed in June 2000

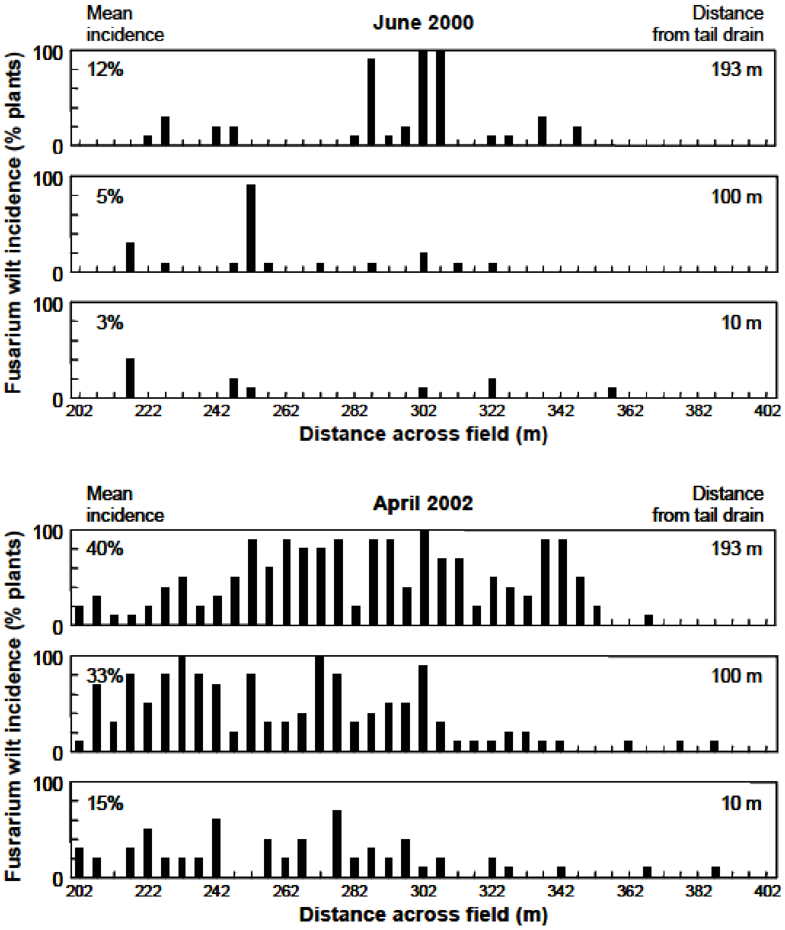


Figure 12. Incidence of Fusarium wilt of cotton in field 19 near Warren in a susceptible variety (Siokra V16) in the 1999-00 season and in a less susceptible variety (Sicot 70) in the 2001-02 season.

Apart from the transects assessed in this project (Table 3), four transects were initiated in the Macintyre Valley by Dr Stephen Allen, as part of DAN95C, *Diseases of Cotton V*. Dr Allen has since continued assessments of these transects (summarised in Figure 13). In field E3, near Boggabilla, and field 17, near Boomi, the first transect was in crops of very susceptible varieties. Less susceptible varieties were used in the following crop and disease incidence was reduced considerably. In field C4, near Boggabilla, a large increase in incidence was observed between the first and second transects, which were both in crops of susceptible varieties, and changing to a less susceptible variety in the third crop also resulted in lower disease incidence. These falls in incidence (Figure 13) reflect the observation that a less susceptible variety requires a 10-fold greater amount of inoculum of Fov in the soil to give the same level of disease as in a susceptible variety (Wang et al. 1999). However, the changeover to less susceptible varieties did not reduce disease incidence in all fields, with rises being recorded in fields seven and 10, near Boggabilla, and field 19, near Warren (Figure 13).

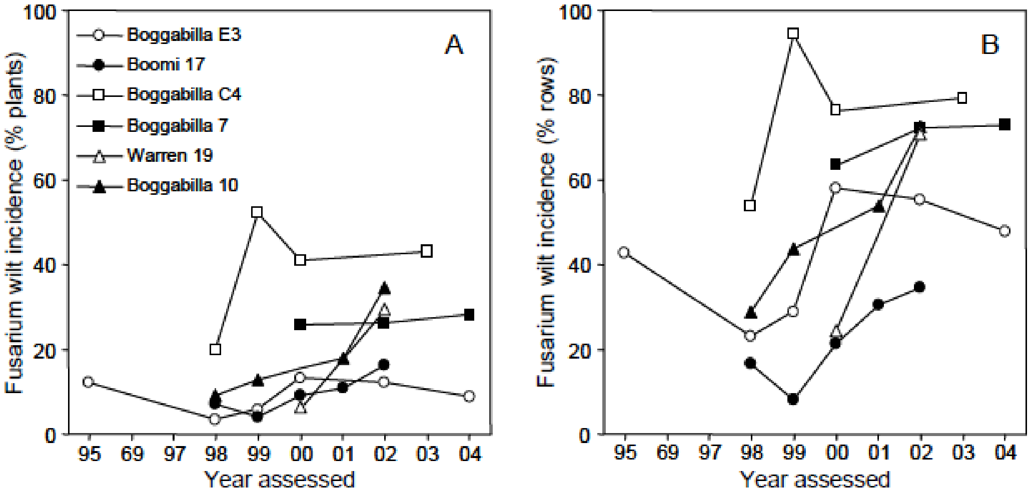


Figure 13. Changing incidence of Fusarium wilt of cotton along transects of fields (data for fields E3, C4 and 10 near Boggabilla, and field 17 near Boomi, courtesy of S.J. Allen).

Even with continued use of less susceptible varieties of cotton, disease incidence continued to rise with each crop, excepting the last two assessments in field E3 near Boggabilla (Figure 13). These observations suggest that inoculum levels in the soil are also increasing with the use of less susceptible varieties. Effective control of Fusarium wilt will ultimately require the development of varieties and/or treatments that result in zero increase in inoculum in the soil. The distribution of Fusarium wilt in all the established transects will continue to be monitored as part of CRDC project DAN177C (*Diseases of cotton VIII*).

Calculation of the incidence of Fusarium wilt as a percentage of the rows examined gives a measure of the area of the infested area of a field, rather than the frequency of plants infested. The changes in the infested area of fields (Figure 13b) were exaggerated in comparison to the concurrent changes in the mean frequency of infected plants (Figure 13b). Hence, while the deployment of less susceptible varieties may result in lower disease incidence at given points in a field, the pathogen may continue to spread to new areas in the field, thus increasing the mean incidence. For example, in field 7, near Boggabilla, the mean incidence of Fusarium wilt only rose slightly over the period of observation (Figure 10) but infestations at the northern end of the field increased (Figure 10).

The dramatic rise in the incidence of Fusarium wilt in field 19, near Warren, between assessment in 2000 and 2002 (Figure 12) was much greater that any other rise between two crops, in any of the transects except for that in field 17, near Boomi, in when two susceptible crops were grown (Figure 13). This suggests that climatic conditions in the Macquarie Valley may favour a much more rapid increase in the incidence of Fusarium wilt, and hence a more rapid increase in dispersal, than in warmer production areas. While the transects have provided good information on the progress of Fusarium wilt in the border rivers region, there is not enough data from transects in the Macquarie Valley to be certain of climatic influences. Monitoring of the seven transects established in the Macquarie Valley will continue when cropping resumes after the drought.

In a field near Dalby that had been out of cotton for eight seasons 37% of plants had symptoms of Fusarium wilt (S.J. Allen, personal communication). This observation confirms expectations, based on the experience with Fusarium wilt in California, that this pathogen is able to survive in the soil for very long periods of time in the absence of host plants. Hence, it seems unlikely that very long fallows, forced by the drought, will eliminate or reduce the incidence of Fusarium wilt.

Many farms do not have the pathogen and efforts to minimise the spread of Fusarium wilt should not be relaxed. It is important that growers and consultants confirm and declare if the disease is present in an area. The Fusarium wilt diagnostic service provided by the QDPI is funded by the cotton industry and is free to growers. The majority of samples submitted return a negative result and some growers who are withholding samples could be worried unnecessarily. Early detection of the disease is essential for the establishment of an effective control strategy, especially including the use of less-susceptible cotton varieties.

Verticillium 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 increased recently in the Namoi Valley, probably due to declining use of resistant varieties
- Monitoring of a severe case of Verticillium wilt established that it was not caused by a new aggressive strain of *V. Dahlia*
- Extension needs to emphasise the value of resistant varieties on an ongoing basis to prevent resurgence of this disease
- Verticillium wilt has not been reported in some areas and occurs at very low levels in others
- Farm hygiene should be practiced to minimise further spread of Verticillium wilt

The 2001-02 season was very favourable to the Verticillium wilt pathogen and the mean incidence of the disease in commercial crops in NSW was 6.7% of plants, compared to an average of 3.7% during the previous five seasons (Figure 14). The warm conditions experienced through much of the 2002-03 season were less favourable to the Verticillium wilt pathogen than in 2001-02 and the mean incidence of the disease in commercial crops in NSW fell slightly to 5.6 % of plants (Figure 14). In the 2001-02 and 2002-03, the incidence of Verticillium wilt in most regions was below 4% (Table 4) but the average for NSW (Figure 14) was heightened by the Namoi valley, where over 11% of plants were infected in both those seasons (Table 4). In the 2003-04 season, the mean incidence of the Verticillium wilt was very low in all regions of NSW and Queensland, except the Namoi valley (Table 4).

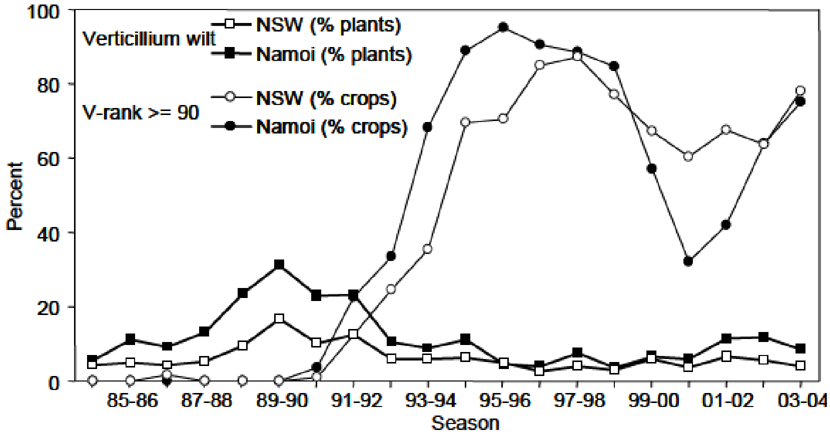
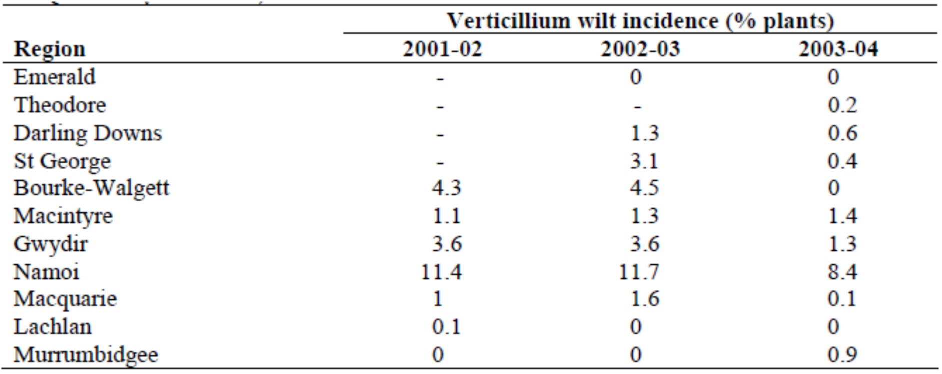


Figure 14. Relationship between the incidence of Verticillium wilt in farms surveyed annually by NSW Department of Primary Industries and the deployment of resistant cotton varieties (V-rank of 90 or more) in the Namoi Valley and NSW.

Verticillium wilt has not been reported in the Emerald region or at Menindee. The disease as observed for the first time in cotton in the Lachlan Valley in 2001-02 and in the Murrumbidgee Valley in 2003-04 in the annual disease surveys conducted as part of the this project. However, these were isolated instances and the disease is not yet widespread in those areas. Even in the older production areas there are still farms where the disease has not been observed. The importance of farm hygiene measures in preventing the spread of Verticillium wilt should be emphasised.

Table 4. Incidence of Verticillium wilt of cotton in farms surveyed annually in NSW and Queensland (data for Qld courtesy of S.J Allen)



	Verticillium wilt incidence (% plants)		
Region	**2001-02**	**2002-03**	**2003-04**
Emerald	-	0	0
Theodore	-	-	0.2
Darling Downs	-	1.3	0.6
St George	-	3.1	0.4
Bourke-Walgett	4.3	4.5	0
Macintyre	1.1	1.3	1.4
Gwydir	3.6	3.6	1.3
Namoi	11.4	11.7	8.4
Macquarie	1	1.6	0.1
Lachlan	0.1	0	0
Murrumbidgee	0	0	0.9

There are two factors that determine the severity of Verticillium wilt within fields: climatic conditions and cropping history. In the 2002-03 seasons, severe Verticillium wilt in some fields resulted in premature defoliation of plants late in the season (symptoms that could be confused with Fusarium wilt). These fields generally had a history of high levels of Verticillium wilt. In some fields, susceptible varieties had very little Verticillium wilt while in other fields the disease was rampant in the same variety, reflecting the presence of the pathogen due to prior cropping history. Previous research has shown that the repeated use of resistant varieties can lower the level of inoculum of *V. dahliae* in the soil (see Final Report for DAN121C, *Diseases of Cotton VI*).

The recent increase in the incidence of Verticillium wilt in the Namoi valley (Figure 14) probably reflects a dramatic decline in the use of resistant varieties in that region. Between 1994-05 and 1998-99, approximately 90% of the Namoi valley was sown to resistant varieties. However, by the 2000-01 season, the use of resistant varieties in the Namoi Valley had fallen to 32% of crops sown (Figure 14). Resistant varieties have subsequently been deployed in greater numbers which appears to have led to the lower incidence of Verticillium wilt in the 2003-04 season.

A severe patch of Verticillium wilt was observed in a susceptible variety of cotton (Siokra V16i) in a field in the upper Namoi valley in the 2001-02 season. Plants were defoliating and dying and initially the symptoms suggested the presence of Fusarium wilt. *Fov* was not present in the stems of affected plants. To determine whether or not the severe patch represented the presence of a new aggressive strain of *V. dahliae*, soil was collected from beneath dead and dying plants within the patch, from beneath healthy plants 50 m away from the patch, and from the Verticillium wilt nursery at ACRI. The soil was potted and separately sown with either a susceptible variety (Siokra 1-4) and a resistant variety (Sicala V2). These plants were grown under cool glasshouse conditions for eight weeks and then the incidence and severity of Verticillium wilt was assessed. Plants in the soil from the severe patch did not develop symptoms any more severe than those in the other two soils, nor did any of them die. Verticillium wilt was present in plants in the original patch but became sequentially less severe in the following two crops of cotton. It was concluded that this case did not represent an outbreak of a new aggressive strain of *V. dahliae*. However, examination of any future, similar cases should account for the possibility that new strains may appear.

Boll rots

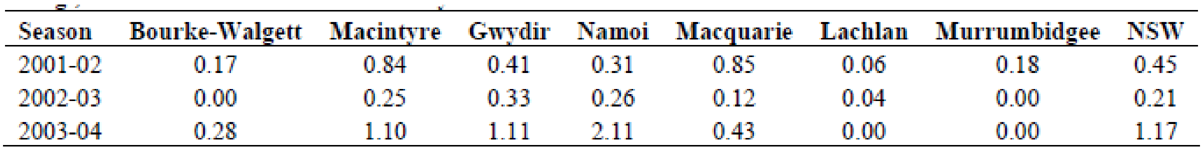
- Cotton boll rots were observed at very low levels across NSW in the 2001-02, 2002-03 and 2003-04 seasons
- Cotton boll rots can cause high losses in association with seasonal conditions, especially heavy rainfall events but such losses are localised and non-repeating

In the 2001-02 season, the incidence of boll rots was generally low, averaging 0.45% of bolls for NSW production areas (Table 5), compared with 2.8 % in the previous season. The generally dry conditions during the second half of the season resulted in a low incidence of the disease. However, heavy local rainfall events in February resulted in severe losses in a few crops, particularly in some Pima crops in the Macquarie valley. Phytophthora boll rot was predominant, with the incidence of other boll rots being less than 0.1% across NSW. A few bolls infected with *Sclerotinia sclerotiorum* were observed in one field in the Lachlan valley.

The dry conditions during the summer of 2002-03 were unfavourable for Phytophthora boll rot, and its incidence was one of the lowest on record; averaging 0.13% of bolls for NSW production areas. The incidence of other boll rots was less than 0.07% across NSW, bringing the total for both types of boll rot to 0.21% (Table 5).

Phytophthora boll rot was the predominant type in the 2003-04 season. In NSW the average incidence of all boll rots was 1.17%, which was higher than in the previous season, but still relatively low. Early maturing crops at Emerald were exposed to extended periods of wet weather in late January and early February and the incidence of boll rots was 6.9% (S.J. Allen, personal communication).

Table 5. The incidence of cotton boll rots, caused by *Phytophthora nicotianae* var. *parasitica* and other fungi, observed in annual disease surveys of NSW



Season	Bourke-Walgett	Macintyre	Gwydir	Namoi	Macquarie	Lachlan	Murrumbidgee	NSW
2001-02	0.17	0.84	0.41	0.31	0.85	0.06	0.18	0.45
2002-03	0.00	0.25	0.33	0.26	0.12	0.04	0.00	0.21
2003-04	0.28	1.10	1.11	2.11	0.43	0.00	0.00	1.17

In most seasons, boll rots were observed in more than 60% of fields inspected (Figure 15). Rainfall is the primary determinant of boll rots and the lowest levels were observed during the droughts in 1995 and 2003. The incidence of boll rots is consistently low in the Lachlan and Murrumbidgee Valleys, where summers rainfall is generally lower than in other areas.

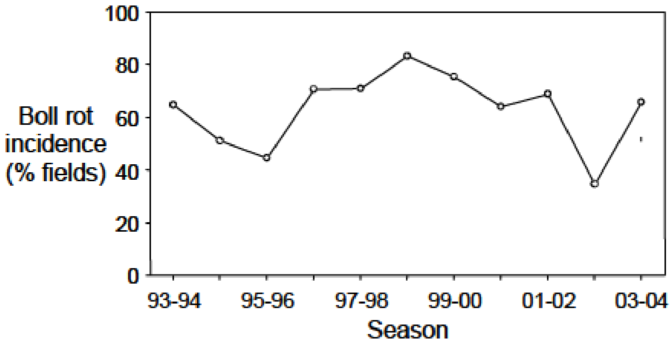


Figure 15. The incidence of cotton boll rots, caused by *Phytophthora nicotianae* var. *parasitica* and other fungi, among fields surveyed annually in NSW

Alternaria leaf spot

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

There were no substantial symptoms of Alternaria leaf spot in cotton seedlings in the November surveys in all three seasons of this project. Symptoms of Alternaria leaf spot were observed in 98% of fields surveyed in March 2002 but the severity was generally very low; 0.16% of leaf area affected, which was similar to the previous season. Alternaria leaf spot was observed in most field fields surveyed in March 2003 but the severity was generally very low (0.14% leaf area affected). Alternaria leaf spot was observed in most field fields surveyed throughout NSW in March 2003 but the severity was again very low. In some crops the disease was relatively severe, causing defoliation, in association with premature senescence (Figure 16).

Figure 16. Alternaria leaf spot of cotton (right) was associated with crop maturity in the half of a field at Croppa Creek (left) that was sown early but not in the other half of the field, which was replanted and therefore, less mature at the time of inspection in Feb 2003.

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
- There were no significant interactions between carryover of cotton trash and any of the diseases monitored in the disease surveys

Cotton bunchy top was not observed in any of the cotton crops inspected during in the annual disease surveys conducted in the 2001-02 and 2002-03 seasons. A single ratoon-plant with the leaf mottle symptoms was observed in a non-survey field. In 2003-04 season, the disease was observed in 1.3 and 0.1% of plants in two fields respectively in Theodore (S.J. Allen, personal communication) and a single plant with symptoms of cotton bunchy top was observed in NSW. While ever the current low levels of infestation persist, cotton bunchy top poses little threat to cotton production.

Upland varieties of cotton in Australia are resistant to bacterial blight, caused by *Xanthomonas axonopodis* pv. *Malvacearum*. Pima crops are susceptible. The disease was not observed in any crops inspected in the annual disease surveys in NSW as part of this project, which included some crops of Pima in the Lachlan and Murrumbidgee Valleys. Sudden wilt is caused by ‘ordinary’ species of Fusarium that are usually non-pathogenic and it is often associated with water logging or root-wounding from cultivation. Affected plants wilt, defoliate and die. Plants may produce regrowth in some situations. Sudden wilt does not re-occur in the same places in the following crop. Sudden wilt was observed in isolated plants in several crops that were inspected in March in all three seasons of this project.

Some pathogens may survive on crop residues and cotton residues on the soil surface have been quantified each year since the inception of the disease surveys in 1983. There were no significant correlations between trash carryover and the incidence of Verticillium wilt, boll rots, late-season Alternaria leaf spot, black root rot or seedling mortality.

Seedling disease nurseries

- Late incorporation of woolly pod vetch has been used successfully to increase the severity of seedling disease in cotton for experimental purposes
- The technique’s effectiveness may depend on having adequate soil moisture to enable colonisation of the vetch residues by seedling pathogens prior to sowing cotton

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 but is reported to have little impact on cotton seedling mortality if it is incorporated at least four weeks before sowing cotton (Rothrock et al. 1995). Since the 1995-96 season, woolly pod vetch 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).

To test the extent the effectiveness of this practice in creating a seedling disease ‘nursery’, replicated four-row plots of vetch, sown along the top of the beds, were incorporated on either 21 August or 23 September 2002. The field was irrigated on 25 September. Cotton seed with the standard fungicides (Apron® + PCNB) was sown on 3 October and the field was irrigated again on 4 October. These treatments were repeated in another section of the

same field in the following year, except that the field was not irrigated after in corporation of the vetch in September.

In the 2002 season, cotton seedling mortality was increased substantially by late incorporation of the vetch (Table 6). At the time cotton was sown, there was little visible residue of the early-incorporated vetch, even though its dry mass was substantial, whereas the residue of the late-incorporated vetch was plentiful in the beds. In the second year, the late incorporation of vetch did not have a significant impact on cotton seedling mortality (Table 6). Since the field was not irrigated after the incorporation of vetch in September and the soil was very dry, conditions in the soil were probably not favourable for colonisation of the vetch residues by *Rhizoctonia* and *Pythium*. This observation may explain the lack of effect by vetch on mortality of cotton (Table 6). However, cotton seedling mortality at six weeks after sowing in that field (32 to 34%) was substantially higher than the average for the Namoi Valley (25%) in that season (Table 1), indicating that the site was suitable of for evaluation of seedling disease in other experiments. Furthermore, in a seed treatment experiment that was super-imposed over these vetch plots, the mortality of cotton seed that lacked fungicide was increased significantly by the late incorporation of vetch (see Table 7.3.4seedtreatment).

Table 6. Effect of early and late incorporation of vetch on seedling mortality of cotton (cv. Sicot 289RRi) sown at the Australian Cotton Research Institute on 3 October 2002 and 29 September 2003 (DAS = days after sowing)

		Cotton seedling mortality (% death)	
Vetch incorporated	**Vetch dry matter (t/ha)**	**21 DAS**	**42 DAS**
2002			
Early (21 Aug 2002)	5.0	20	28
Late (23 Sep 2002)	5.9	34	41
Probability ($n = 6$)	$P = 0.016$	$P = 0.001$	$P = 0.002$
2003			
Early (20 Aug 2003)	1.7	26	34
Late (22 Sep 2003)	5.7	25	32
Probability ($n = 6$)	$P < 0.001$	Not significant	Not significant

Timing of sowing

- Delaying the date of sowing as late as possible within the planting window can avoid conditions that favour seedling disease
- Sowing should be timed to coincide with the onset of periods of weather that will result in a mean soil temperature of 16°C during the first week from sowing
- Sowing should be delayed after pre-irrigation until soil water content is at the lower end of the range that is adequate for seedling establishment in any particular soil

Cool conditions early in the season favour seedling disease, black root rot and Fusarium wilt. Furthermore, cotton seedlings are most susceptible to seedling disease in their first two to three weeks of life and become resistant thereafter. If sowing is delayed then the length of time seedlings are exposed to conditions that favour soilborne pathogens may be sufficiently reduced to impact upon the severity and/or incidence of disease.

Experiments on the effects of fungicide seed treatment and delayed sowing were conducted in 2002-03 and 2003-04 in the seedling disease nursery (late incorporation of vetch) at the Australian Cotton Research Institute. In both seasons, seed the standard fungicide seed treatment (PCNB and metalaxyl-M) decreased seedling mortality substantially (Table 7).

Delayed sowing did not affect seedling mortality in 2002-03 in either treatment. In 2003-04, disease pressure was greater, with 84 % death of plants in the untreated seed sown early. In that year, delaying sowing until the end of October decreased seedling mortality substantially in the untreated seed and resulted in a 33% increase in yield over the untreated seed that was sown early (Table 7). Yield in the early-sown plots was increased by 42% by treatment of seed with fungicides. While delaying sowing, by itself, did not provide greater protection than the fungicides, the principle demonstrated here should be applicable to cooler cotton-growing regions, such as the Lachlan and Murrumbidgee Valleys, where seed treatment fungicides alone are not providing adequate control of seedling disease in some years (Table 1, Figure 2).

Table 7. Decreased seedling mortality and increased yield of cotton with seed treatment and delayed sowing at the Australian Cotton Research Institute

			Seedling mortality(% death)		**Yield**
Season	**Sowing date**	**Seed treatment***	**21 DAS**	**42 DAS**	**(ba/ha)**
2002-03	Early (3 Oct)	Untreated	58a	60a	-
		PCNB + metalaxyl-M	36b	40b	-
	Late (31 Oct)	Untreated	50a	52a	-
		PCNB + metalaxyl-M	36b	38b	-
			$P < 0.001$	$P < 0.001$	
2003-04	Early (29 Sep)	Untreated	84a	85a	6.0c
		PCNB + metalaxyl-M	44c	49c	8.5a
	Late (27 Oct)	Untreated	59b	61b	8.0b
		PCNB + metalaxyl-M	48c	47c	8.9a
			$P \leq 0.032$	$P \leq 0.032$	$P \leq 0.031$

Values in columns with the same letter are not significantly different by pairwise comparison of means using Fisher' LSD at the stated probability level.

Delaying the sowing date will increase the probability that the period when cotton plants are most susceptible to seedling pathogens will coincide with climatic conditions that do not favour seedling disease. A further option available to growers is to time the date of sowing following pre-irrigation to avoid cool wet conditions in the soil. In a field experiment at the Australian Cotton Research Institute in 2003-04, cotton was sown at successive dates following pre-irrigation. Seedling mortality declined as sowing was successively delayed after the pre-irrigation (Figure 17a). This decline coincided with decreasing soil water content on the day of sowing (Figure 17b). In that soil, sowing was able to be delayed until soil water content fell below 24% with no loss in stand establishment. Limits for adequate soil water content at other sites would have to be determined on a soil by soil basis.

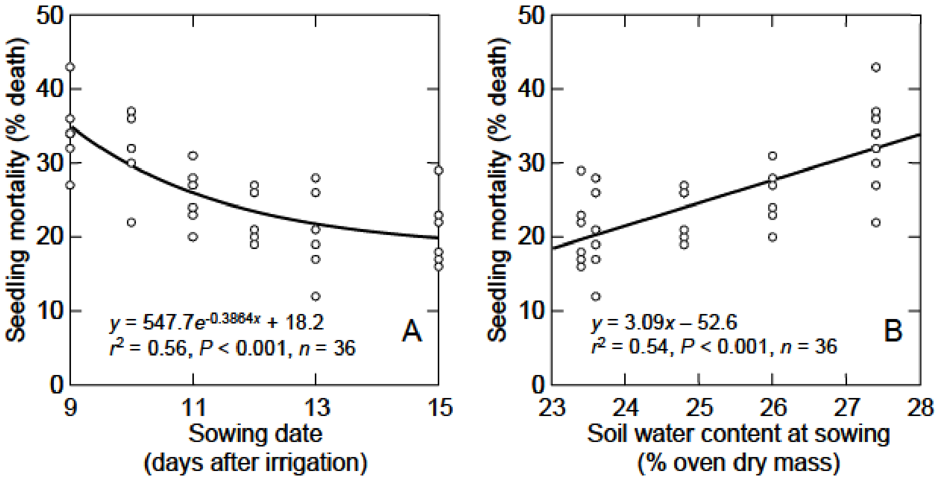


Figure 17. Seedling mortality of cotton (var. 289RRi), sown at various dates after irrigation in a field at the Australian Cotton Research Institute in 2003, decreased as sowing was delayed after irrigation (A) and increased with increasing soil moisture content (B)

Soil water content and soil temperature are not independent parameters. However, seedling mortality was not correlated with soil temperature on the day of sowing (Figure 18a). Soil temperature during the days following sowing was related to subsequent temperatures, with 55% of the variation in seedling mortality being explained by the mean soil temperature over the first week from sowing (Figure 18b). Seedling mortality increased exponentially as the mean soil temperature in the week from sowing fell below 16°C. This experiment illustrates the need to time sowing to coincide with the onset of periods of warm weather.

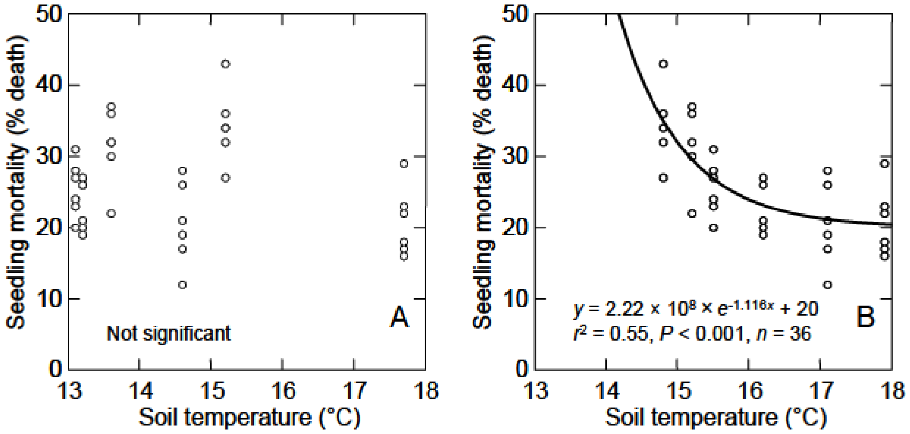


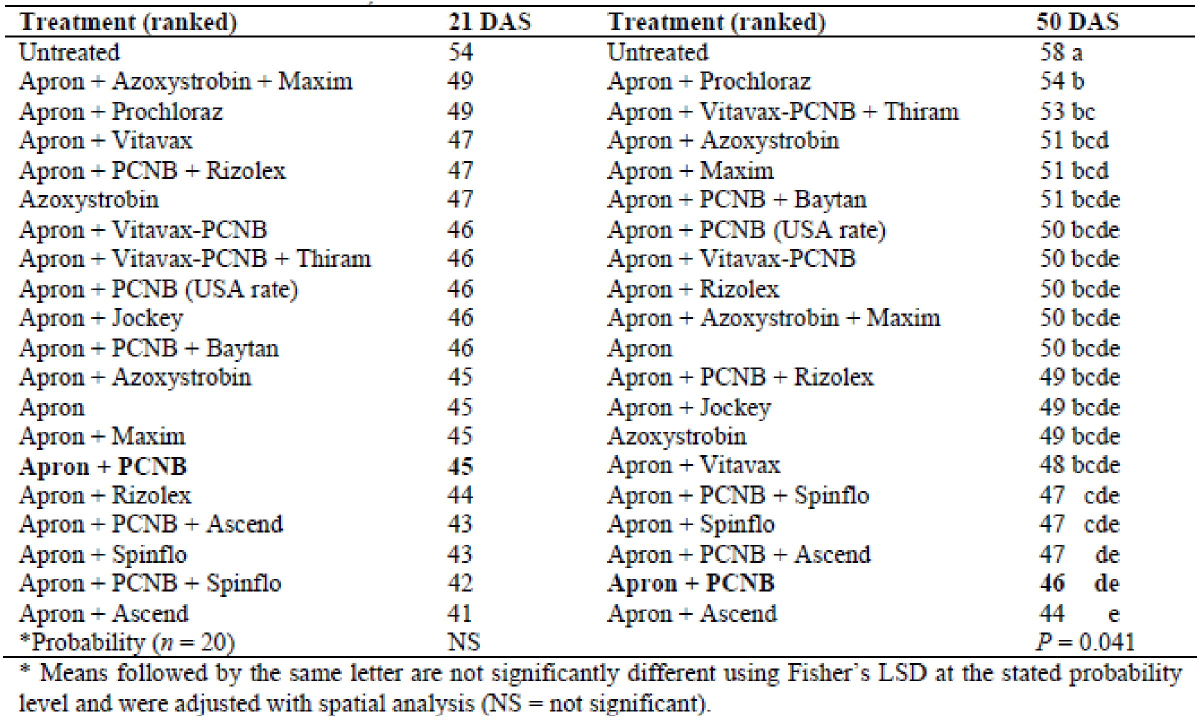
Figure 18. Seedling mortality of cotton (var. 289RRi), sown at various dates after irrigation in a field at the Australian Cotton Research Institute in 2003, was not related to soil temperature at 9:00 am on the day of sowing (A) but decreased as the mean of soil temperatures at 9:00 am on the day of sowing and the following six days increased (B)

Fungicides and other products for control of seedling disease

- Seed treatment experiments showed that seedling pathogens, such as *Rhizoctonia* and *Pythium*, vary in dominance from field to field and year to year.
- A few fungicide combinations gave slightly greater protection than the standard fungicides in some years but not others.
- The fungicide DynastyTM consistently performed as well as the standard fungicides
- The non-fungicidal products, including acibenzolar-S-methyl, were not effective

Seed treatment experiments were conducted in cotton fields in the 2001-02, 2002-03 and 2003-04 seasons. These experiments were made possible by the collaboration of Cotton Seed Distributors, who provided, treated and packaged the seed, Deltapine Australia who planted trials at Dalby, Goondiwindi and the CSIRO Cotton Production Unit who planted trials at Hillston and Warren, and cooperating growers. In each year, fungicides and other treatments (Table 8) were applied to cotton seed in twenty different combinations, with the carrier PeridiamTM. The untreated control received PeridiamTM only. Seeds were counted into lots of 100 or 150 seeds and respectively sown into single-row plots 10 or 15 m long using a cone seeder. There were 10 replicate plots for each seed treatment, in a completely randomised block design. Seedling establishment was assessed at approximately three and six weeks after sowing, and the means for each treatment were compared by analysis of variance with spatial correction (statistical program ASREML).

Table 8. Seedling mortality (% death) of cotton (var. Sicot 53) sown on 25 Sep 2001 at the Australian Cotton Research Institute with various fungicides applied as seed treatments (DAS = days after sowing; standard seed treatment in bold)



Treatment (ranked)	21 DAS	Treatment (ranked)	50 DAS
Untreated	54	Untreated	58 a
Apron + Azoxystrobin + Maxim	49	Apron + Prochloraz	54 b
Apron + Prochloraz	49	Apron + Vitavax-PCNB + Thiram	53 bc
Apron + Vitavax	47	Apron + Azoxystrobin	51 bcd
Apron + PCNB + Rizolex	47	Apron + Maxim	51 bcd
Azoxystrobin	47	Apron + PCNB + Baytan	51 bcde
Apron + Vitavax-PCNB	46	Apron + PCNB (USA rate)	50 bcde
Apron + Vitavax-PCNB + Thiram	46	Apron + Vitavax-PCNB	50 bcde
Apron + PCNB (USA rate)	46	Apron + Rizolex	50 bcde
Apron + Jockey	46	Apron + Azoxystrobin + Maxim	50 bcde
Apron + PCNB + Baytan	46	Apron	50 bcde
Apron + Azoxystrobin	45	Apron + PCNB + Rizolex	49 bcde
Apron	45	Apron + Jockey	49 bcde
Apron + Maxim	45	Azoxystrobin	49 bcde
Apron + PCNB	**45**	Apron + Vitavax	48 bcde
Apron + Rizolex	44	Apron + PCNB + Spinflo	47 cde
Apron + PCNB + Ascend	43	Apron + Spinflo	47 cde
Apron + Spinflo	43	Apron + PCNB + Ascend	47 de
Apron + PCNB + Spinflo	42	**Apron + PCNB**	**46 de**
Apron + Ascend	41	Apron + Ascend	44 e
*Probability (*n* = 20)	NS		*P* = 0.041

* Means followed by the same letter are not significantly different using Fisher's LSD at the stated probability level and were adjusted with spatial analysis (NS = not significant).

In the 2001-02 season, seedlings sown at ACRI with the standard fungicide treatment (Apron® + PCNB) had a much higher seedling mortality (48%, Table 9) than the average for the Namoi valley (27%, Table 1). In contrast, in the field at Hillston, seedling mortality with the standard fungicide treatment (33 to 38%, Table 10) was similar to the mean for the Lachlan Valley in that season (34%, Table 1). This enhanced seedling mortality at ACRI indicated that the incorporation of woolly pod vetch as a green manure crop shortly before sowing cotton, was successful in creating a seedling disease 'nursery' at that site. At both Hillston and ACRI, all fungicide treatments reduced seedling mortality significantly and most combinations gave a level of control equal to that of the standard treatment (Table 9, Table 10). Treatment with Apron® by itself controlled disease as well as the standard treatment, suggesting that *Pythium* may have been the dominant pathogen in both experiments.

Table 9. Seed treatments evaluated for their effects on cotton seedling mortality during the course of this research project

Source	Product	Active ingredient		Product applied	Target organisms
Syngenta	Apron® XL350ES	Metalaxyl-M	350g/L	0.43 mL/kg seed 0.214 mL/kg seed (½ rate)	*Pythium*
Syngenta	Maxim® 100 FS	Fludioxonil	100 g/L	0.25 mL/kg seed	*Rhizoctonia*, *Fusarium* spp.
Syngenta	Azoxystrobin 100 FS	Azoxystrobin	100 g/L	1.5 mL/kg seed	*Rhizoctonia*
Syngenta	Boost®	Acibenzolar-S-methyl	500 g/L	24 µL /kg seed (12 mg a.i.) 12 µL /kg seed (6 mg a.i.) 6 µL /kg seed (3 mg a.i.) 2 µL /kg seed (1 mg a.i.)	Non-fungicidal
Syngenta	*Dynasty™	Metalaxyl-M + Fludioxonil + Azoxystrobin	37.5g/L 12.5 g/L 75 g/L	2.0 mL/kg seed	*Rhizoctonia*, *Fusarium* spp., *Pythium*
Bayer	Quintozene®	PCNB	500 g/L	2.15 mL/kg seed (Australia) 3.74 mL/kg seed (USA)	*Rhizoctonia*
Bayer	Spinflo™	Carbendazim	500 g/L	1.5 mL/kg seed	*Rhizoctonia*
Bayer	Exp. formulation	Prochloraz	199 g/L	1.0 mL/kg seed	*Fusarium* spp.
Bayer	Jockey®	Fluquinconazole	167 g/L	4.5 mL/kg seed	Various cereal pathogens, not *Rhizoctonia* or *Fusarium* spp.
Bayer	Baytan® C 154 FS	Triadimenol + Cypermethrin	150 g/L 4 g/L	1.5 mL/kg seed	*Rhizoctonia*, *Thielaviopsis*
Bayer	BAY001	a.i. not advised		1.0 mL/kg seed	-
Hannafords	Thiram® 42S	Thiram	420 g/L	2.0 mL/kg seed	*Rhizoctonia*, *Fusarium* spp., *Pythium*
Hannafords	Vitavax® 200 FF	Carboxin Thiram	200 g/L 200 g/L	5.0 mL/kg seed	*Rhizoctonia*, *Fusarium* spp., *Pythium*
Hannafords	Vitavax®-PCNB FF	Carboxin + PCNB	200 g/L 200 g/L	5.0 ml/kg seed	*Rhizoctonia*
Sumitomo	Rizolex® 50 WP	Toclofos-methyl	500g/kg	4.0 g/kg seed	*Rhizoctonia*
Uniroyal	Ascend® 30	TCMTB	300 g/L	2.9 mL/kg seed	*Rhizoctonia*, *Fusarium* spp., *Pythium*
Agrometados	Brotomax®	Brotomax	1 L/L	9 mL/kg seed	Non-fungicidal

* The application rate for Dynasty™ gave rates for its individual components that were equal to those for Apron®, Maxim® and azoxystrobin applied separately.

Table 10. Seedling mortality (% death) of cotton (var. Sicot 53) sown on 2 Oct 2001 at Hillston with various fungicides applied as seed treatments (DAS = days after sowing; standard seed treatment in bold)

Treatment (ranked)	30 DAS	Treatment (ranked)	50 DAS
Untreated	41 a	Untreated	45 a
Azoxystrobin	35 ab	Azoxystrobin	38 b
Apron + Maxim	34 b	**Apron + PCNB**	**38 bc**
Apron + Azoxystrobin + Maxim	34 bc	Apron + Maxim	38 bc
Apron	34 bc	Apron	38 bc
Apron + Vitavax 200 FF	34 bc	Apron + Vitavax	36 bcd
Apron + PCNB	**33 bcd**	Apron + Prochloraz	36 bcd
Apron + Prochloraz	33 bcd	Apron + Jockey	36 bcd
Apron + PCNB (USA rate)	33 bcd	Apron + Ascend	35 bcd
Apron + Jockey	32 bcd	Apron + Vitavax-PCNB	35 bcd
Apron + Vitavax-PCNB	32 bcd	Apron + Rizolex	35 bcd
Apron + Ascend	31 bcd	Apron + PCNB + Rizolex	34 bcd
Apron + PCNB + Spinflo	31 bcd	Apron + Azoxystrobin + Maxim	34 bcd
Apron + Rizolex	30 bcd	Apron + PCNB (USA rate)	33 bcd
Apron + PCNB + Baytan	30 bcd	Apron + Spinflo	33 bcd
Apron + PCNB + Rizolex	30 bcd	Apron + PCNB + Spinflo	33 bcd
Apron + Spinflo	29 bcd	Apron + PCNB + Baytan	32 cd
Apron + Vitavax-PCNB + Thiram	28 cd	Apron + PCNB + Ascend	31 d
Apron+ PCNB + Ascend	28 cd	Apron + Vitavax-PCNB + Thiram	31 d
Apron + Azoxystrobin	27 d	Apron + Azoxystrobin	31 d
*Probability ($n = 20$)	$P = 0.014$		$P = 0.001$

* Means followed by the same letter are not significantly different using Fisher's LSD with spatial analysis at the stated probability level and were adjusted with spatial analysis.

In the experiment at Dalby in the 2001-02 season, the mean seedling mortality assessed on 27 Dec 2001 was 48% but there were no significant differences among the treatments (data not presented), suggesting that factors other than seedling disease affected mortality. In the 2002-03 season, even though the average seedling mortality for the Namoi valley (35%) was higher than usual (Table 1), seedling mortality at ACRI with the standard fungicide treatment was substantially higher (49% for Apron® + PCNB, Table 11) indicating again that woolly pod vetch was able to successfully create a seedling disease nursery.

Apron® and Quintozene® did not control seedling disease when used individually (Table 11), suggesting that *Pythium* and *Rhizoctonia* were both responsible for seedling death. Many of the other combinations of fungicides reduced seedling mortality but none were significantly better than the standard treatment.

In the same experiment, sown at Hillston on 26 Sep 2002, mean seedling mortality assessed on 14 Nov 2002 was 16% which was much lower than the average for the Lachlan Valley in that season (52%, Table 1) and there were no significant differences among the treatments (data not presented). The very low seedling mortality at this site may have been due to the relatively short history of cotton cropping in that field. In the experiment at Dalby, sown with the same treatments on 10 Oct, mean seedling mortality assessed on 26 Nov 2002 was 27 % and there were no significant differences between treatments. The lack of differences may have reflected the relatively late planting date, thus avoiding cool conditions, and the potential for treatment differences may have been confounded further by the presence of self-sown seedlings from the previous year's crop.

In the 2003-04 season, the seedling disease trial at ACRI seedling mortality ranged from 39 to 48% in the standard treatment (Table 12), which was again well above the Valley average (25%, Table 1) due to late incorporation of woolly pod vetch. Mortality of the untreated seeds was exceptionally high (Table 12), reflecting the cool wet conditions that occurred during October 2003.

The only treatments that controlled seedling disease effectively in that experiment were those that contained Apron®. PCNB did not reduce mortality by itself, nor did it increase the level of control achieved by Apron®. These results clearly indicate that *Pythium*, was the dominant fungal pathogen of cotton in that field, in that season. While seed treatment with acibenzolar-S-methyl (Boost®) can induce a degree of resistance in cotton against black root rot and Fusarium wilt (refer to section on systemic acquired resistance below), it did not reduce seedling mortality (Table 12). This suggests that the resistance mechanisms activated by acibenzolar-S-methyl were either (i) of insufficient quality to be effective against *Pythium* or (ii) activated too late to affect pre- and post-emergent damping off. Similarly, Brotomax, a putative agent for activation of systemic acquired resistance, did not reduce seedling mortality (Table 12). In contrast to the experiment at ACRI, Apron® had no effect on seedling mortality at the Warren site, whereas PCNB gave a level of control equal to that of the industry standard (Table 13). Hence, *Rhizoctonia* was the dominant pathogen causing damping off in the experiment at Warren. Seedling mortality with the standard treatment at the Warren site (40 to 49 %, Table 13) was similar to that of the Lachlan Valley in that season (48%, Table 1).

Table 11. Seedling mortality (% death) of cotton (var. Sicot 289RRi) sown on 3 Oct 2002 at the Australian Cotton Research Institute season with various fungicides applied as seed treatments (DAS = days after sowing; standard seed treatment in bold)

Treatment (ranked)	**(%death) 21 DAS**	**Treatment (ranked)**	**(%death) 42 DAS**
Untreated	57 a	Untreated	59 a
Apron	57 ab	PCNB	59 a
Apron + Prochloraz	55 abc	Apron	58 a
PCNB	55 abc	Apron + Prochloraz	58 ab
Apron + Ascend	53 abcd	Apron + Vitavax	53 abc
Apron + BAY001	52 abcd	Apron + BAY001	53 abc
Apron + Jockey	49 abcd	Apron + Jockey	53 abcd
Apron + Vitavax	49 abcde	Apron + Ascend	53 abcd
Apron + PCNB + BAY001	47 abcdef	**Apron + PCNB**	**49 bcde**
Apron (½ rate) + Azoxystrobin + Maxim	48 bcdef	Apron + Maxim	49 cde
Apron + PCNB + Ascend	46 cdef	Apron (½ rate) + Azoxystrobin + Maxim	48 cde
Apron + PCNB	**45 def**	Apron + Azoxystrobin	48 cde
Apron + Maxim	44 def	Apron + PCNB + Ascend	47 cde
Apron + Azoxystrobin	44 def	Apron + PCNB + BAY001	46 cde
Apron + Vitavax-PCNB	41 ef	Apron + PCNB + Rizolex	46 cde
Apron + Vitavax-PCNB + Thiram	41 ef	Apron + Vitavax-PCNB + Thiram	45 cde
Azoxystrobin	41 ef	Apron + Vitavax-PCNB	44 de
Apron + PCNB + Rizolex	40 ef	Apron + PCNB (USA rate)	44 de
Apron + Rizolex	38 f	Azoxystrobin	44 de
Apron + PCNB (USA rate)	38 f	Apron + Rizolex	43 e
*Probability (*n* = 20)	*P* < 0.01		*P* < 0.01

* Means followed by the same letter are not significantly different using Fisher's LSD with spatial analysis at the stated probability level and were adjusted with spatial analysis.

Table 12. Seedling mortality (% death) of cotton (var. Sicot 289RRi) sown on 29 Sep 2003 at the Australian Cotton Research Institute with various fungicides applied as seed treatments (DAS = days after sowing; standard seed treatment in bold)

Treatment (ranked)	**(%death) 21 DAS**	**Treatment (ranked)**	**(%death) 42 DAS**
Brotomax	92 a	Brotomax	93 a
Boost (6 mg)	88 ab	Boost (6 mg)	90 ab
Boost (12 mg)	86 ab	Boost (12 mg)	89 ab
Untreated	83 b	Untreated	88 ab
Azoxystrobin	82 b	PCNB	84 b
Boost (1 mg)	81 b	Azoxystrobin	84 b
Boost (3 mg)	81 b	Boost (1 mg)	84 b
PCNB	81 b	Boost (3 mg)	83 b
Apron	46 c	Apron	54 c
Apron + PCNB + Boost (1 mg)	43 cd	Apron + PCNB + Boost (12 mg)	51 cd
Apron + PCNB + Boost (12 mg)	40 cde	Apron + PCNB + Boost (1 mg)	50 cd
Apron + PCNB	39 cde	Apron + PCNB + Boost (3 mg)	48 cde
Apron + PCNB + Brotomax	38 de	**Apron + PCNB**	**48 cde**
Apron (½ rate) + Maxim	37 de	Apron (½ rate) + Maxim	47 cde
Apron + Ascend	37 de	Apron + PCNB + Boost (6 mg)	45 de
Apron + PCNB + Boost (6 mg)	36 de	Dynasty^{TM}	45 de
Apron + PCNB + Boost (3 mg)	34 e	Apron + PCNB + Brotomax (100%)	45 de
Apron + PCNB (USA rate)	33 e	Apron + Ascend	44 de
Apron (½ rate) + Azoxystrobin	33 e	Apron + PCNB (USA rate)	44 de
Dynasty^{TM}	33 e	Apron (½ Rate) + Azoxystrobin	40 e
*Probability (*n* = 20)	*P* < 0.01		*P* < 0.01

* Means followed by the same letter are not significantly different using Fisher's LSD with spatial analysis at the stated probability level and were adjusted with spatial analysis.

Table 13. Seedling mortality (% death) of cotton (var. Sicot 289RRi) sown on 30 Sep 2003 at Warren with various fungicides applied as seed treatments (DAS = days after sowing; standard seed treatment in bold)

Treatment (ranked)	(%death) 28 DAS	Treatment (ranked)	(%death) 58 DAS
Brotomax	67 a	Brotomax	74 a
Boost (12 mg)	66 a	Boost (12 mg)	74 ab
Boost (1 mg)	65 a	Boost (1 mg)	73 ab
Apron + Ascend	63 ab	Boost (3 mg)	71 ab
Boost (3 mg)	62 ab	Apron + Ascend	71 ab
Apron	61 abc	Apron	70 abc
Untreated	61 abc	Boost (6 mg)	69 abc
Boost (6 mg)	60 abc	Untreated	68 abcd
Apron(½ rate) + Maxim	51 bcd	Apron(½ rate) + Maxim	61 bcde
Apron + PCNB + Boost (12 mg)	48 cde	Apron + PCNB + Boost (12 mg)	61 bcde
Apron + PCNB + Brotomax	47 de	Apron + PCNB + Brotomax	58 cdef
Apron + PCNB + Boost (3 mg)	47 de	Apron + PCNB + Boost (1 mg)	58 cdef
Apron + PCNB + Boost (6 mg)	45 de	Apron + PCNB + Boost (3 mg)	56 def
Apron + PCNB + Boost (1 mg)	44 de	Apron + PCNB + Boost (6 mg)	56 ef
Azoxystrobin	41 de	Azoxystrobin	53 ef
Apron + PCNB	**40 de**	Dynasty^{TM}	51 ef
Dynasty^{TM}	38 e	**Apron + PCNB**	**49 ef**
Apron(½ rate) + Azoxystrobin	37 e	Apron + PCNB (USA rate)	48 f
PCNB	37 e	Apron (½ rate) + Azoxystrobin	47 f
Apron + PCNB (USA rate)	37 e	PCNB	45 f
*Probability ($n = 20$)	$P < 0.01$		$P < 0.01$

* Means followed by the same letter are not significantly different using Fisher's LSD with spatial analysis at the stated probability level and were adjusted with spatial analysis.

The same seed treatments were used in experiments sown at Goondiwindi and Dalby in late October 2003. In these experiments there were no significant differences among any of the treatments, again suggesting that late sowing avoids seedling disease pressure.

In a field near Warren, a grower sowed replicated plots, eight rows wide, in 2003 with either the standard fungicide treatment (Apron® + PCNB) or Dynasty® (Table 14). There were no significant differences between these two treatments. However, in an adjacent experiment using Rizolex® as an in-furrow spray there were not significant differences either (Table 28). This suggests that there was little disease pressure from *Rhizoctonia* in that field and does not necessarily indicate the effectiveness of the fungicides against *Rhizoctonia*. Since DynastyTM contains metalaxyl-M the two fungicide treatments were probably equally effective against *Pythium* and there may have been other factors affecting stand establishment in that field.

Table 14. Seedling mortality of cotton (cv. Sicot 71) at 20 days after sowing at Warren with either the standard fungicide treatment or DynastyTM

Seed treatment fungicide	Seedling mortality (% death)
PCNB + metalaxyl-M	31
Dynasty^{TM}	28
	Not significant, $P = 0.178$

In the 2003-04 season, a seed treatment experiment was superimposed over the vetch plots described above (Table 6). This experiment was located at the tail drain end of the plots. The mortality of cotton seed that lacked fungicide was increased substantially by the late incorporation of vetch (Table 15) confirming the use of late incorporation of vetch to create a disease nursery. Disease severity in untreated seed close to the tail drain (Table 15) was substantially greater than that further from the tail drain (2003, Table 6) Addition of acibenzolar-S-methyl to the standard seed treatment fungicides (PCNB + metalaxyl-M) did not affect seedling mortality in comparison to the standard treatment. Seed treatment with DynastyTM was equal to that of the standard fungicides.

Table 15. Effect of early and late incorporation of vetch and seed treatment fungicides on seedling mortality of cotton (cv. Sicot 289RRi) at 21 days after sowing at the Australian Cotton Research Institute in 2003

Vetch incorporated	Seed treatment	Cotton seedling mortality (% death
Early (20 Aug 2003)	Untreated	68b
	PCNB + metalaxyl-M	30c
	PCNB + metalaxyl-M + acibenzolar-S-methyl	28c
	Dynasty™	26c
Late (22 Sep 2003)	Untreated	85a
	PCNB + metalaxyl-M	32c
	PCNB + metalaxyl-M + acibenzolar-S-methyl	30c
	Dynasty™	36c
	[A]Probability ($n = 6$)	$P < 0.004$

[A]Values followed by the same letter are not significantly different by pairwise comparison of means using Fishers LSD at the stated probability level

Cereal cover crops

- Experiments with cereal cover crops in this project confirmed previous observations of their potential to increase early-season growth of cotton
- Cotton growth was correlated positively with dry matter production in the cereal cover crop
- To avoid problems with establishment of cotton, cover crops need careful placement on the shoulders of the bed, in well prepared beds, with the cotton planting line remaining clear
- The potential for cover crops to reduce the severity of black root rot will require trials to be conducted in locations with sufficiently even distribution of the pathogen

Research in CRDC project DAN100C, *Detection, distribution and control of early season growth disorder of cotton*, indicated that mulches of hay had potential to increase early season growth and fruit maturity of cotton, particularly in fields affected by bacterial stunt. Further research in CRDC project DAN122C, *Black root rot and slow early season growth of cotton*, confirmed the potential for cover crops to increase growth and yield of cotton in the absence of disease, and to potentially reduce either the severity of black root rot or its impact. The research in the two projects indicated that the benefits of wheat cover crops to early season growth of cotton were due to a combination of factors, including:

- improved soil structure
- moderation of soil temperature extremes but higher soil temperature during irrigation
- retention of soil moisture near the soil surface due to reduced evaporation
- reduced wind chill

Research on the potential for cover crops to reduce the severity of black root rot and decrease seedling mortality, particularly in the cooler cotton production areas was continued in this project.

In an experiment at Hillston, replicated plots of wheat were sown along a single 12-row run in a field known to be infested with *T. basicola*. Wheat (cv. Janse) was sown on 20 June 2001 as a single row on the shoulders of the bed (henceforth *shoulder wheat*) either side of the cotton planting line. Shortly after emergence of the wheat, herbicide was applied to half the plots to create the 'bare' treatment. The rest of the wheat continued to grow (albeit poorly) and was killed with herbicide just prior to sowing of cotton was sown on 3 October 2001. Temperature data-loggers were installed, soil samples were collected to assay the population density of *T. basicola*, and the field was irrigated on 4 October. No differences in plant stand or in the severity of black root rot were observed (Table 16). However, the population of *T. basicola* in that part of the field was variable (half the plots had no spores of *T. basicola* at all) and below 50 cfu/ g soil, which is an approximate minimum level required to produce substantial symptoms on cotton roots. The shoulder wheat in this field did not grow as well as it did elsewhere on that farm. Nevertheless a 10% increase in early shoot growth was observed (Table 16). The cover crop increased the daily soil temperature maxima by an average of 2.1 ºC and the minima by an average of 0.3 ºC (Figure 19a). Since the disease pressure was low, the increase in growth with the cover crop was probably due to the warmer soil temperatures and/or reduced wind chill on the seedling plants, as cold winds were experienced during October.

Table 16. Effect of shoulder wheat, in replicated small plots, on black root rot and growth of cotton in a field at Hillston in the 2001-02 season

Treatment	Spore population 3.10.01 (cfu/g soil)	Plant stand 31.10.01 (plants/m)	Black root rot severity 31.10.01 (0-10 scale)	Shoot dry mass 31.10.01 (mg/plant)	Shoot dry mass 21.11.01 (mg/plant)
Bare	15	10.1	2.0	78	820
Wheat	33	9.8	2.0	86	750
	NS	NS	NS	$P = 0.014$	NS

NS = not significant

In a second field on the same farm at Hillston in the 2001-02 season, shoulder wheat was grown under similar conditions but was only sown in one large block across half the field. Cotton was sown at approximately the same time as in the first field. On 6 October, single temperature probes with data loggers were installed each side of the junction between the wheat block and the bare soil, at a distance of 50 m from the tail drain. The field was irrigated on 10 October. Since there were no replicate plots, the effect of the shoulder wheat on cotton growth and establishment was assessed along a transect 40 m from, and parallel to the trail drain, with five replicate sampling positions either side of the junction between the shoulder wheat and the rest of the field. In this field, the cover crop of shoulder wheat increased the early season growth of cotton by 25% at the end of October (Table 17). Three weeks later the plants in the wheat cover area were 150% bigger than those in the bare area of the field. Although the plots were not replicated, the difference between the two areas was very distinct well beyond the small area that was sampled. There was no *T. basicola* detected in samples taken previously elsewhere in this field, and symptoms of black root rot were not observed at the sampling site where the soil temperature and cotton growth was measured.

Table 17. Effect of shoulder wheat, in a single large plot, on growth of cotton in a field at Hillston in the 2001-02 season

	Shoot dry mass 31.10.01 (mg/plant)	Shoot dry mass 21.11.01 (g/plant)
Bare	66	0.30
Cover	82	0.75
	$p = 0.009$	$p = 0.001$

The shoulder wheat increased the daily soil temperature maxima by 1.2ºC and the minima by 0.8ºC on average (Figure 19b). This additional heat in the soil was probably not sufficient to account for the growth increase with shoulder wheat in this field. In comparison to the first field, the wheat in this second field was taller and had a thicker stand, which probably reduced the wind-chill factor to a greater extent than in the first field. The relative increase in soil temperature due to the cover crop was greatest after an irrigation, at the end of November; being an average of 3.7ºC and 3.2ºC higher for the daily maxima and minima for seven days (Figure 19b).

A third experiment using wheat as a cover crop for cotton was conducted on a farm near Narromine in the Macquarie Valley in the 2001-02 season. In this experiment wheat was grown in eight-row plots for the length of the field, with five replicates, and then sprayed with herbicide prior to sowing cotton (Sicala V2RR) on 4 Oct. In this experiment the cotton did not establish well, probably due to difficulties in planting through the dead wheat plants, which were not well aligned with the shoulders of the beds. The soil in the bare plots was cultivated to a good tilth for sowing, whereas the soil in the wheat-cover plots was cloddy with cotton stalks from a previous crop lying on the surface. In this experiment plant stand was reduced by 27.5% by the wheat cover (Table 18). The distribution of black root rot among the plots was very variable and there was no difference in the severity of black root rot between the treatments. Cotton shoot growth was reduced by 24% in the wheat-cover plots, although this difference was not reflected by plant height (Table 18).

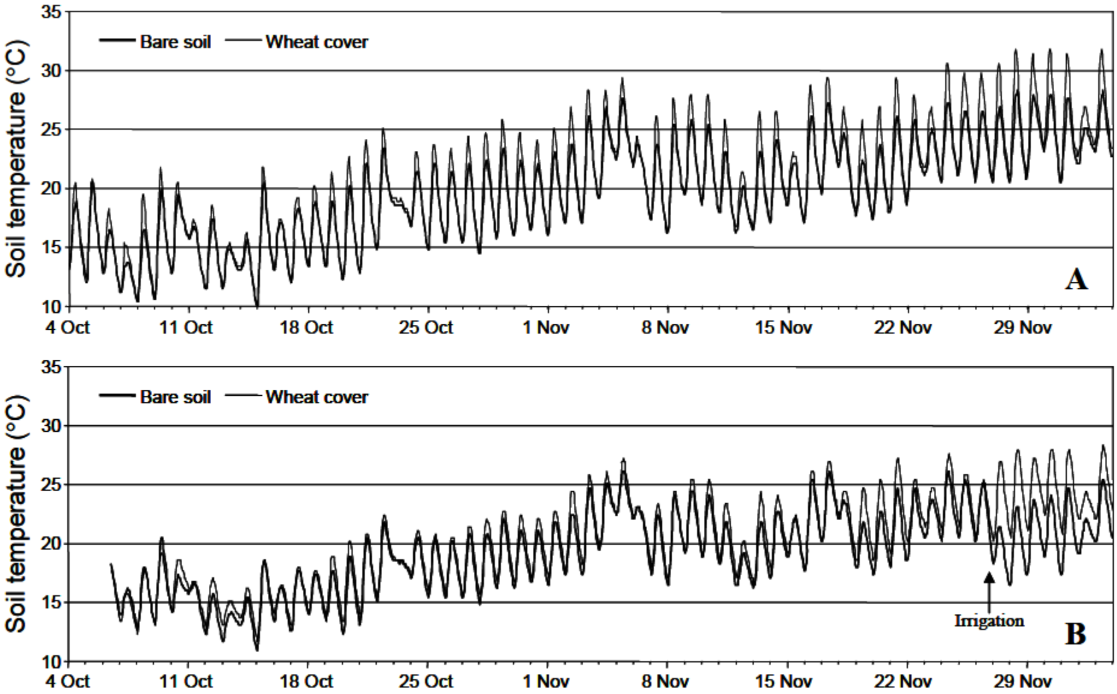


Figure 19. Effect of cover crops, of wheat on the shoulders of the bed, on soil temperature in two cotton crops near Hillston in the 2001-02 season, with (A) replicated small plots of shoulder wheat and (B) a single large plot of shoulder wheat

Table 18. Effect of shoulder wheat, in replicated eight-row plots, on plant establishment, black root rot and growth of cotton at 29 days after sowing in a field near Narromine in the 2001-02 season,

Treatment	Plant stand (plants/m)	Black root rot severity (0-10 scale)	Relatively healthy lateral roots (no./plant)	Shoot dry mass (g/plant)	Shoot height (mm/plant)
Bare soil	12.0	5.0	11.0	155	53
Wheat cover	8.7	6.8	7.3	117	59
Probability* (*n* = 5)	*P* = 0.004	NS	NS	*P* = 0.004	NS

* NS = not significantly different

A cover crop of shoulder wheat was included as a treatment in the long-term biofumigation experiment commenced at ACRI in the 2002-03 season. The growth of the wheat was variable (ranging from 0.08 to 2.63 tonnes/ha), probably because no nitrogen fertiliser was applied to the wheat and some of these plots had little available nitrogen where they overlapped those of experiments in previous seasons. In the analysis of variance of all treatments in the long-term biofumigation experiment, the shoulder wheat did not have any significant effects on establishment, growth and disease of cotton (Table 33). However, within that treatment, at 27 days after sowing, there was a very strong, positive correlation between cotton growth and the dry matter of the shoulder wheat (Figure 20), confirming previous observations of growth increases in cotton sown into cereal cover crops (see final reports for DAN100C and DAN122C).

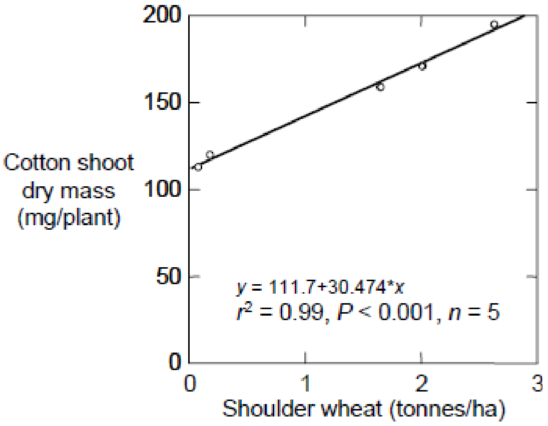


Figure 20. Cotton growth increased according to the dry mass of a cover crop of wheat on the shoulders of the bed, in a field at the Australian Cotton Research Institute in 2002-03.

Systemic acquired resistance

- A practical method for application of acibenzolar-S-methyl to cotton seed was developed, using 6 mg/kg seed, which was equivalent to the rate in previous, successful experiments using seed soaking
- Application of acibenzolar-S-methyl to cotton seed in combination with standard seed treatment fungicides was shown to have no phytotoxic effects on germination of cotton seed and subsequent seedling growth
- Seed treatment with acibenzolar-S-methyl consistently activated resistance against Fusarium wilt of cotton, although the effects were not major when disease severity was moderately low
- Acibenzolar-S-methyl increased seedling establishment in one experiment in a field infested with the Fusarium wilt pathogen but not in other experiments.
- Acibenzolar-S-methyl did not activate resistance against Verticillium wilt of cotton
- The potential for an extended, active 'shelf life' of acibenzolar-S-methyl, when applied to seed in combination with the standard fungicides, was demonstrated
- Foliar application of BrotomaxTM and salicylic acid was ineffective against Fusarium wilt and Verticillium wilt of cotton
- Seed treatment with plant hormones was ineffective against Fusarium wilt of cotton

Development of seed treatment rates for acibenzolar-S-methyl

Previous research (Final Report DAN122C) had indicated that the non-fungicidal chemical acibenzolar-S-methyl has the capacity to activate the resistance of cotton against black root rot when applied as a seed treatment by soaking the seeds in solutions of acibenzolar-Smethyl immediately prior to sowing. While this method proved to be effective, it would be more practical if acibenzolar-S-methyl could be mixed with the standard seed treatments applied to commercial seed, thus eliminating the task of soaking the seed and associated problems with delivery of seed through the planter; imbibition alone was shown to reduce the delivery of seed by certain cotton planters (see Final Report DAN153C).

To determine appropriate rates for application of acibenzolar-S-methyl with the standard seed coatings, the equivalent amount of acibenzolar-S-methyl absorbed during the seed soaking process had to be calculated. Imbibition of water by cotton seed (cv. Sicala V2) was determined gravimetrically in two experiments and the data pooled to fit a polynomial model for the rate of absorption of water (Figure 21).

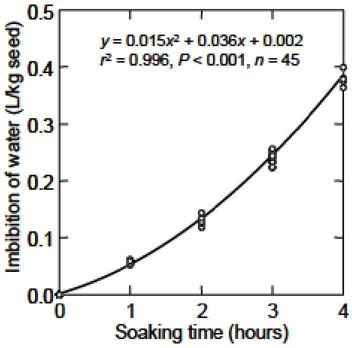


Figure 21. Imbibition of water by cotton seed (cv. Sicala V2) over time (data estimated gravimetrically and pooled for two studies)

In previous experiments, soaking cotton seed for three hours in a solution of acibenzolar-Smethyl (25 mg/L) was sufficient to activate heightened resistance against *T. basicola* (see DAN122C Final Report). Since 1 kg of cotton seed imbibes 0.24 L of water in three hours (Figure 21), uptake of acibenzolar-S-methyl in those experiments was equivalent to 6 mg/kg seed.

To test whether or not adding acibenzolar-S-methyl to the seed coating might be phytotoxic, a range of rates equivalent to soaking with the 25 mg solution of acibenzolar-S-methyl and lower rates were evaluated in combination with the standard seed treatment fungicides (Table 19). Bion® was a granular formulation and was ground finely in a mortar and pestle before applying to 200 g black seed (cv. Sicot 189) in a plastic bag, agitating the bag until the powder was sufficiently dispersed on the seed and then adding 1.7 mL of liquid QAP (PCNB, metalaxyl-M, Peridiam™ blue), with agitation until seeds were evenly coated. Boost® was applied in a similar manner, except the Boost® was allowed to dry on the seeds before addition of the QAP. The seeds were germinated on moist paper towelling in sterile germination chambers that allowed the radicle to grow downwards such that its length could be measured easily. The germination chambers each contained 10 seeds and there were five replicate chambers for each rate. Chambers were incubated at 23°C and root length was assess after 137 hours.

Table 19. Rates of acibenzolar-S-methyl and equivalent rates for formulations applied, with the fungicides PCNB and metalaxyl-M, as a seed coating to cotton seed to evaluate the potential for phytotoxicity in germinating seedlings

Seed-soaking equivalent (Acibenzolar-S-methyl ppm in solution)	Acibenzolar-S-methyl (mg/kg seed)	Formulation equivalent	
		Bion (mg/kg seed)	Boost (µL/kg seed)
0	0	0	0
1	0.24	0.48	0.48
5	1.2	2.4	2.4
10	2.4	4.8	4.8
25	6.0	12	12

Application of both formulations of acibenzolar-S-methyl in combination with PCNB and metalaxyl-M had no detrimental effect on germination and growth of cotton seedlings under sterile conditions (Figure 22).

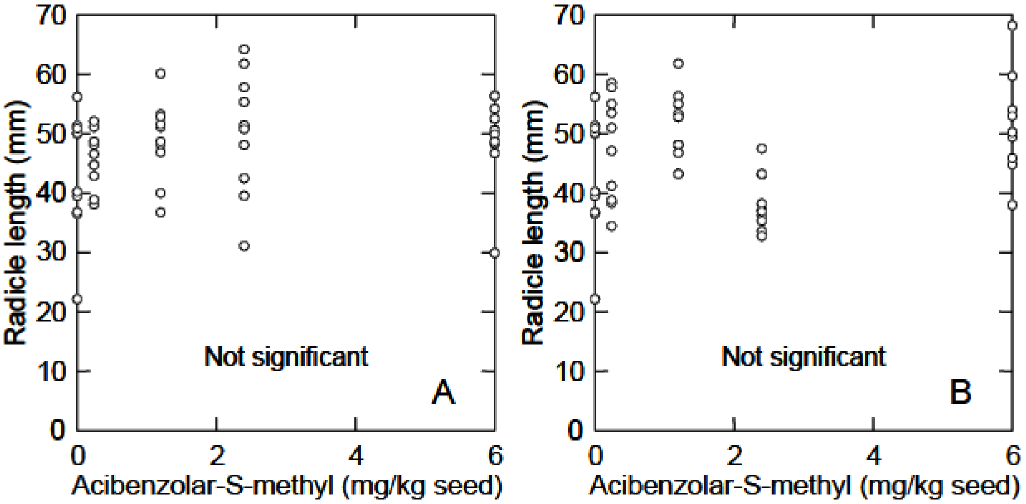


Figure 22. Application of acibenzolar-S-methyl, formulated as Bion® (A) or Boost® (B), to cotton seed (cv. Sicot 189) with the standard seed treatment fungicides (PCNB and metalaxyl-M) had no effect on germination and radicle growth under sterile conditions.

To test whether or not seed treatment with these formulations might activate some resistance against black root rot of cotton, seed was treated with either Bion® or Boost® at rates equivalent to 0.24 and 1.2 mg acibenzolar-S-methyl/kg seed. For each treatment five seeds were sown in each of five replicate pots filled with soil collected from a cotton field at the Australian Cotton Research Institute. The pots were grown in the glasshouse and black root rot was assessed at 28 days after sowing. Unfortunately, the soil used contained little inoculum of *T. basicola* and disease symptoms were observed on only a few plants (data not presented). However, progressive assessment of plant establishment indicated that none of the formulations affected seedling emergence (Table 20).

Table 20. Application of acibenzolar-S-methyl, formulated as Bion® or Boost®, to cotton seed (cv. Sicot 189) with the standard seed treatment fungicides (PCNB and metalaxyl-M) had no effect on germination and radicle growth under sterile conditions (DAS = days after sowing)

	Seedling emergence (%)		
Seed treatment	**8 DAS**	**11 DAS**	**13 DAS**
Control	36	60	64
Bion (2.4 mg/kg seed)	28	68	96
Bion (12 mg/kg seed)	40	64	84
Boost (2.4 µL/kg seed)	72	88	88
Boost (2.4 µL/kg seed)	44	80	88
	Not significant ($P = 0.234$)	Not significant ($P = 0.448$)	Not significant ($P = 0.113$)

The above investigation demonstrated that application of acibenzolar-S-methyl to cotton seed at the rate of 6 mg/kg is equivalent to the rate used successfully against black root rot by soaking seed. Furthermore, application of acibenzolar-S-methyl in combination with standard seed treatment fungicides had no phytotoxic effects on germination of seed and subsequent growth. Applying acibenzolar-S-methyl as a seed coating would be a much more practical method than seed soaking.

Acibenzolar-S-methyl 2001-02

To assess the potential for soaking seeds in acibenzolar-S-methyl to control Verticillium wilt, seeds of cotton (cv. NuPearl RR) were soaked for three hours in a solution of acibenzolar-Smethyl (25 mg L-1) and sown at 15 seeds/m in four-row plots in a completely randomised block design in the Verticillium wilt nursery at ACRI on 9 October 2001. Disease incidence was assessed after harvest by cutting all the stems, at approximately cotyledon level, in two 10 m lengths of cotton row in each plot and assessing disease severity on a scale from 0-4 (described above for the Brotomax experiments). Acibenzolar-S-methyl had no effect on the total incidence of Verticillium wilt (Table 21). Acibenzolar-S-methyl did decrease the severity of black root rot of cotton in this experiment and these results were reported separately in the Final Report for CRDC funded project DAN153C, *Managing black root rot of cotton*, including a reduction in stand establishment at the beginning of the season.

Table 21. Severity of Verticillium wilt of cotton, with and without seed treatment by soaking in a solution of acibenzolar-S-methyl at sowing, at the Australian Cotton Research Institute in 2001-02

Acibenzolar-S-methyl (µg/mL)	Verticillium wilt incidence (% plants)
0	92
25	97
	Not significant

To evaluate the potential for acibenzolar-S-methyl to control Fusarium wilt, experiments were conducted in severely-infested fields near Boggabilla. A liquid formulation of acibenzolar-Smethyl (BionTM) was mixed with the QAP (PCNB, metalaxyl and Peridium Blue) and applied to ‘black’ seed on the day of sowing or, to test its shelf-life, ten days before sowing. QAP alone was used as the control. In both seed treatments, acibenzolar–S-methyl induced systemic resistance against Fusarium wilt substantially (Table 22). The potential for application of acibenzolar–S-methyl within standard seed coatings has now been demonstrated. Acibenzolar-S-methyl also increased stand significantly in that experiment (Table 22). Such effects on seedling establishment are generally not observed with acibenzolar-S-methyl (see final report DAN122C).

Table 22. Effect of acibenzolar-S-methyl (BionTM) seed treatment on Fusarium wilt in cotton in a field near Boggabilla in the 2001-02 season Values in columns with the same letter are not significantly different by pairwise comparison of means at the stated probability level. Parameters measured are the same as in Table 3.

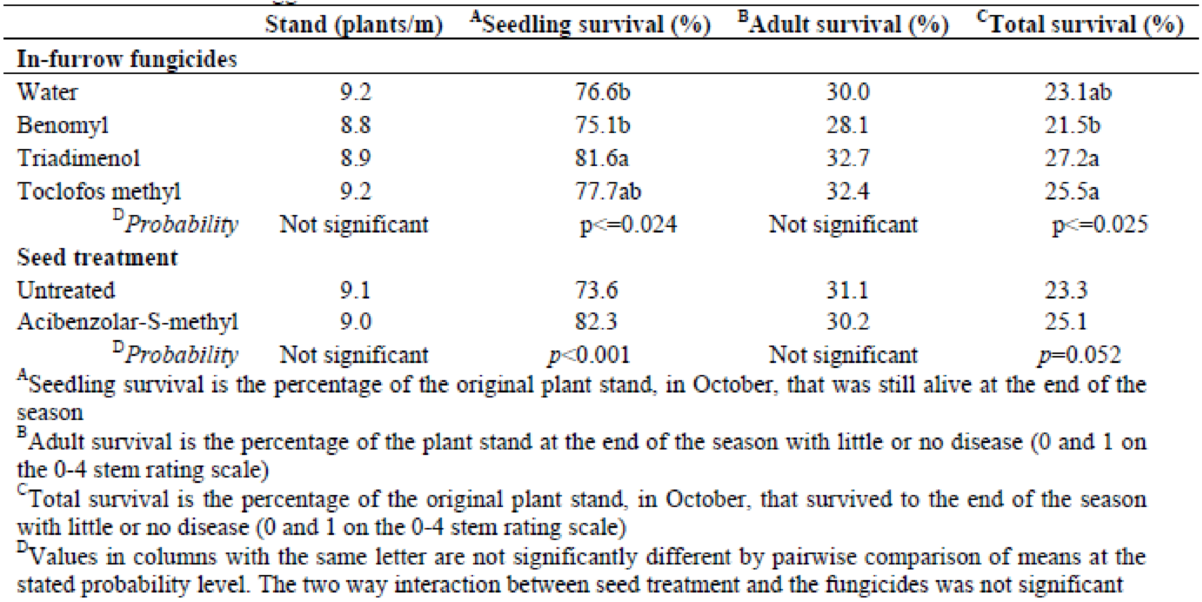
Treatment	Seedling stand (plants/m)	Seedling survival (%)	Healthy adult plants (%)	Plants healthy all season (%)
Control (QAP only)	10.0b	78b	33b	26b
Bion™ at sowing	10.7a	87a	37a	33a
Bion™ 10 days before sowing	11.5a	84ab	39a	33a
	$p \leq 0.031$	$p = 0.018$	$p < 0.05$	$p < 0.05$

Values in columns with the same letter are not significantly different by pairwise comparison of means at the stated probability level. Parameters measured are the same as in Table 3.

In a second experiment at Boggabilla, cotton seed was soaked in a solution of acibenzolar-Smethyl (25 µg/L) for three hours prior to sowing, with untreated seed as the control and sown with an air seeder. These two seed treatments were split into a further four in-furrow fungicide treatments (sprayed in solution at the rate of 87L/ha) in single-row plots 15 m long as follows: water as control; benomyl (a.i. BenlateTM at 600 g/ha); triadimenol (BaytanTM C FS at 330 mL ha); toclofos methyl (RizolexTM at 120 g/ha). The fungicides and acibenzolar-Smethyl had no effect on seedling establishment (Table 3). Acibenzolar-S-methyl and triadimenol increased the survival of seedlings (i.e. beyond December) by 7% and 12%

respectively, but had on effect on the number of healthy adult plants. Triadimenol increased the number of plants that were healthy all season in comparison to benomyl (total survival (Table 23) but not in comparison to the control. Acibenzolar-S-methyl almost increased the number of plants that were healthy all season (Table 23). There were significant differences among rows in the experiment and the data require spatial analysis.

Table 23. Effect of acibenzolar-S-methyl seed treatment and in-furrow fungicides on Fusarium wilt in cotton in a field near Boggabilla in the 2001-02 season Stand (plants/m) ASeedling survival (%) BAdult survival (%) Total survival (%) In-furrow fungicides



	Stand (plants/m)	[A]Seedling survival (%)	[B]Adult survival (%)	[C]Total survival (%)
In-furrow fungicides				
Water	9.2	76.6b	30.0	23.1ab
Benomyl	8.8	75.1b	28.1	21.5b
Triadimenol	8.9	81.6a	32.7	27.2a
Toclofos methyl	9.2	77.7ab	32.4	25.5a
[D]*Probability*	Not significant	p<=0.024	Not significant	p<=0.025
Seed treatment				
Untreated	9.1	73.6	31.1	23.3
Acibenzolar-S-methyl	9.0	82.3	30.2	25.1
[D]*Probability*	Not significant	*p*<0.001	Not significant	*p*=0.052

[A] Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season

[B] Adult survival is the percentage of the plant stand at the end of the season with little or no disease (0 and 1 on the 0-4 stem rating scale)

[C] Total survival is 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)

[D] Values in columns with the same letter are not significantly different by pairwise comparison of means at the stated probability level. The two way interaction between seed treatment and the fungicides was not significant

Brotomax™ 2001-02

A liquid product known as Brotomax™ is reported to induce systemic acquired resistance (SAR) against plant pathogens in a number of crops. The potential for Brotomax™ to induce SAR against Verticillium wilt was examined in a field trial on the Breeza plain in the 2001-02 cotton growing season. Cotton (variety V17) was sown in the second week of October. The experiment used a completely randomised block design in part of a field where severe Verticillium wilt had been observed in the previous year. There were two treatments: Brotomax™ and the untreated control. Brotomax™ was applied as a 1% solution sprayed over whole plants until run-off, on 6 November 2001. Brotomax™ was applied again as a 1% solution sprayed all over the plants until run-off, on 18 January 2001. The severity of Verticillium wilt was assessed by cutting stems and assessing vascular discolouration on the 0-4 scale (as described in Methodology) Climatic conditions during the first half of the 2001-02 season were very favourable for development of Verticillium wilt. The disease was more severe at the Breeza plain site, than in the Verticillium wilt nursery at the Australian Cotton Research Institute. There was little difference in the incidence and severity of Verticillium wilt between treatments (Figure 23). Although Brotomax™ was not effective in this experiment, it may be worth investigating the use of different rates and/or timing of application.

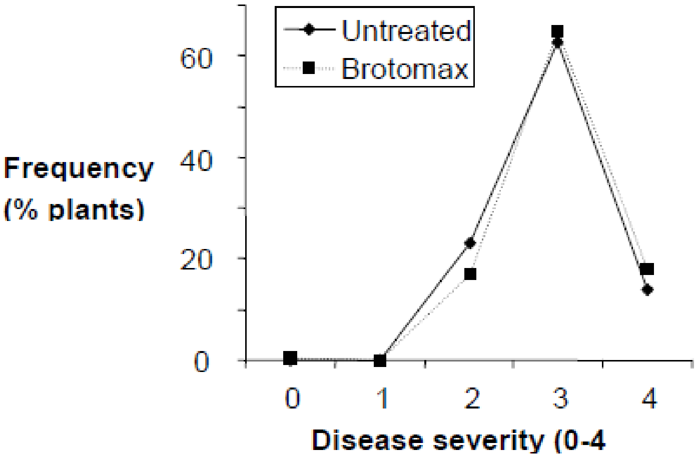


Figure 23. Severity of Verticillium wilt in cotton in a field on the Breeza plain, 2002

Brotomax™ and salicylic acid 2002-03

In further evaluation of Brotomax™, either Brotomax™ (1% solution), salicylic acid (10 mMol solution) or water were sprayed on to the foliage of cotton plants at the seedling stage and at flowering in the 2002-03 season in a field at Boggabilla, in one-row plots that were 20 m long in a completely randomised block design. The field was severely infested with the Fusarium wilt pathogen. Plant stand was recorded on 29 October 2002 and the incidence of Fusarium wilt was recorded by cutting stems and assessing vascular discolouration on the 0-4 scale (as described in Methodology) on 7 April 2003. This experiment was repeated in the Verticillium wilt nursery at ACRI in the same season. At these sites, neither product influenced the development of Fusarium wilt (Table 24) and Verticillium wilt (Table 25) significantly in comparison to the control.

Table 24. Lack of effect of foliar application of putative activators of systemic acquired resistance on the incidence of Fusarium wilt of cotton at Boggabilla in the 2002-03 season

	[A]Seedling survival (%)	[B]Total survival (%)
Control	68	13
Brotomax	65	13
Salicylic Acid	69	16
Probability:	Not significant	Not significant

[A] Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season

[B] Total survival is 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)

Table 25. Lack of effect of foliar application of putative activators of systemic acquired resistance on the incidence of Verticillium wilt and yield of cotton at the Australian Cotton Research Institute in the 2002-03 season

	Verticillium wilt incidence (% plants)	Lint yield (ba/ha)
Control	30	4.2
Brotomax	32	4.1
Salicylic Acid	29	4.0
Probability:	Not significant	Not significant

To test reports by growers and consultants that application of gibberellic acid to cotton seed may improved seedling vigour in the presence of Fusarium wilt, cotton seed (cv. Sicala 289RR) was treated with the product Early Harvest® (3.9 mL/ kg seed; a.i. kinetin 0.09%, gibberellic acid 0.03%, indole butyric acid 0.045%) and sown in single-row replicated plots in an infested field near Moree on 10 October 2003. Plant stand was assessed at 17 d after sowing and disease severity was assessed at the end of the season using the 0-4 scale for vascular discolouration of stems. The plant hormones in Early Harvest® PGR had no significant effects on seedling establishment, or the incidence and severity of Fusarium wilt at the end of the season (Table 26).

Table 26. Lack of effect of seed treatment with gibberellic acid on stand establishment and the incidence and severity of Fusarium wilt of cotton in a field near Moree in the 2003-04 season

	Stand establishment (plants/m)	**[A]Seedling survival (%)**	**[B]Total survival (%)**
Untreated	7.7	82	63
Early Harvest® PGR	7.5	89	72
Probability:	Not significant	Not significant	Not significant

[A] Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season

[B] Total survival is 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)

Acibenzolar-S-methyl and Brotomax 2003-04

To evaluate the most effective rate for application of acibenzolar-S-methyl to cotton seed, for control of Fusarium wilt, cotton seed (cv. Sicala 289RR) was treated with acibenzolar-Smethyl, applied to black seed at six rates (0, 0.5 1, 3, 6 and 12 mg/kg) in combination with the standard fungicide seed treatment (PCNB, metalaxyl-M, PeridiamTM blue) and sown in singlerow, replicated plots in a completely randomised block design, in a field near Moree on 10 October 2003. Plant stand was assessed at 17 days after sowing and disease severity was assessed at the end of the season using the 0-4 scale for vascular discolouration of stems. Acibenzolar-S-methyl had no effect on stand establishment at 17 days after sowing (data not presented) and most of the seedlings survived until the end of the season (seedling survival, Fig. 24a). Seedling survival increased significantly with increasing rates of acibenzolar-Smethyl, although the correlation was relatively weak; only 15% of variation in seedling survival could be explained by variation in the rate of acibenzolar-S-methyl. However, mean seedling survival at 12 mg acibenzolar-S-methyl per kg of seed was significantly ($P = 0.46$) higher than in the control (85%). The severity of Fusarium wilt at this trial site was moderate, with an average of 65% of plants surviving with little or no disease all season (Fig. 24b). The lack of significant major effects by acibenzolar-S-methyl is consistent with the relatively low severity of disease in this experiment. Data from sites where the average total survival is greater than 70% are not used in evaluation of the Fusarium wilt resistance of commercial cultivars due to the potentially high variance of the data. Nevertheless, the results of this experiment do demonstrate the capacity for acibenzolar-S-methyl to activate heightened levels of resistance in cotton against Fusarium wilt.

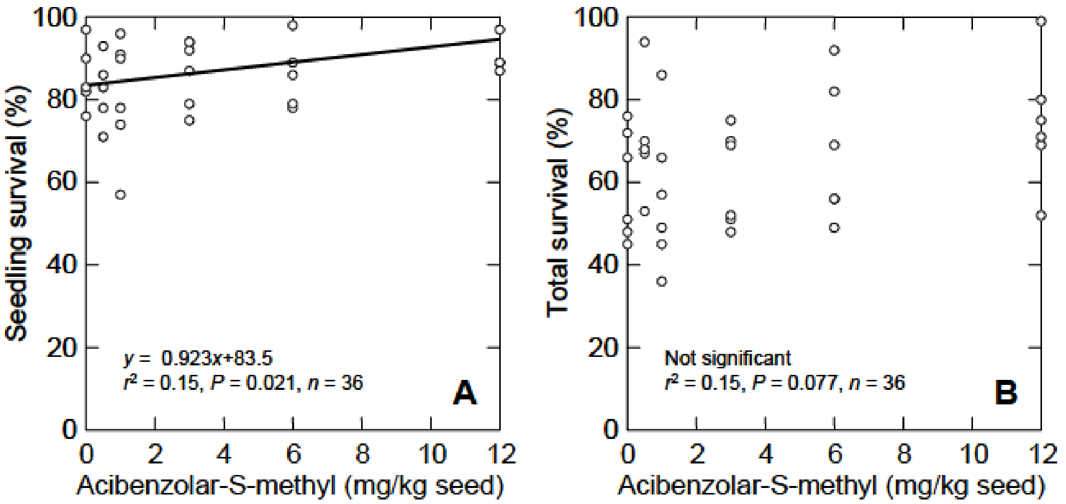


Figure 24. Application of acibenzolar-S-methyl to cotton seed at sowing increased survival of cotton seedlings in the original plants stand, through to the end of the season (seedling survival, A), but had no effect on the proportion of the original plant stand that had survived all season with little or no symptoms of Fusarium wilt (B), in a field near Moree NSW in the 2003-04 season.

In the 2003-04 season, cotton (cv. Siokra 1-4) was sown in the Verticillium wilt nursery at the Australian Cotton Research Institute on 29 September (15 seeds/m). 'Black' seed was treated immediately before sowing with either i) the standard fungicide treatment (PCNB, metalaxyl- M, PeridiamTM blue) applied as 20 mL/kg seed, ii) the standard fungicide treatment plus acibenzolar-S-methyl (6 mg/kg seed) or iii) Brotomax alone at the rate of 20 mL/kg seed.

Plant stand was assessed at 22 days after sowing and there were no significant differences (Table 27). For acibenzolar-S-methyl, this result was consistent with most other trials to date, in which there has been no enhancement of seedling establishment. Importantly, these experiments demonstrate that acibenzolar-S-methyl does not decrease stand establishment when applied as part of the seed dressing. The apparent decreases in seedling establishment when acibenzolar-S-methyl is applied by soaking seeds just before sowing were shown to be an artefact of the swelling of seeds with imbibition (DAN153C) and was not apparent when an air seeder was used (Table 23). The data from these experiments confirm that acibenzolar-S-methyl does not have a phytotoxic effect on seedlings that affects establishment. The effect of acibenzolar-S-methyl and BrotomaxTM on Verticillium wilt was not assessed due to the confounding effect of establishment of large numbers of self-sown cotton plants during the course of the experiment.

Table 27. Lack of effect of seed treatment with acibenzolar-S-methyl and BrotomaxTM on stand establishment of cotton in a field at the Australian Cotton Research Institute in the 2003-04 season

	Stand establishment (plants/m)
Untreated	11.2
Acibenzolar-S-methyl (6 mg/kg seed)	11.4
Brotomax^{TM} (20 mL/kg seed)	11.6
	Not significant ($P = 0.927$)

In-furrow fungicides

- In-furrow application of several fungicides did not control seedling disease
- The benefit to growers from using in-furrow fungicides that are registered for control of seedling disease will depend upon the relative disease pressure exerted by their target pathogens in any given year or field
- In-furrow application of the fungicides benomyl, triadimenol and toclofos methyl was ineffective in controlling Fusarium wilt

Seedling disease

In two field trials in the Murrumbidgee valley, Rizolex® had no impact on seedling mortality (Table 28). This *does not* mean that this fungicide is ineffective against *Rhizoctonia*. Conditions were cold at the start of the trial in the 2001/02 season and some seedlings were still emerging at 27 days after sowing. Most of the seedlings (with or without Rizolex®) had very few symptoms of post-emergent infection by *Rhizoctonia* (i.e. on the hypocotyls). The strains of *Rhizoctonia* that infect cereals are different to those that infect cotton. Since the fields in these trials had only recently changed from cereal cropping to cotton, the levels of the strains of that are specific to cotton (*Rhizoctonia* AG4) may have been low. All the seed in these trials was treated with Quintozene®, which is active against *Rhizoctonia*. Since the addition of Rizolex® did not reduce seedling mortality any more than the standard seed treatment, it appears that *Rhizoctonia* was not a dominant cause of seedling mortality in these trials. In some years, at some sites, *Pythium* can be the dominant pathogen causing seedling disease and seed treatment fungicides are recommended for cotton in all regions. The value of using in-furrow fungicides, such as Rizolex®, will vary according to pressure from *Rhizoctonia* in a given year, in a given field.

Table 28. Effect of in-furrow application of toclofos-methyl (Rizolex®, at recommended rate) on seedling mortality of cotton in the Murrumbidgee valley.

	Seedling mortality (%)	
Treatment	27 DAS	48 DAS
2001/02 season		
Untreated	61	63
Rizolex®	60	61
2003/04 season		
Untreated	44	ND
Rizolex®	45	ND

DAS = days after sowing, ND = not determined

In the 2003-04 season, in a field trial at a farm north of Warren, toclofos-methyl (Rizolex®, Sumitomo) was applied as an in-furrow spray in plots that ran the full length of the field. The field was sown on 1 October at 14 seeds/m. The field had heavier soil near the tail drain and lighter soil at the head ditch end of the plots. Seedling mortality at 20 days after sowing averaged 32 and 37% in the lighter and heavier soils respectively. At 20 days after sowing, Rizolex® had no effect on seedling establishment at either end of the field (Table 29).

Table 29. Effect of in-furrow application of toclofos-methyl (Rizolex®, at recommended rate) on seedling mortality of cotton in the Macquarie valley in 2003

	Stand establishment (plants/m)	
Treatment	21 Oct	19 Dec
Heavier soil		
Untreated	8.6	7.3 (0.37)
Rizolex®	9.0	8.3 (0.35)
Probability	NS	$P = 0.049$
Lighter soil		
Untreated	9.9	ND
Rizolex®	9.2	ND
Probability	NS	

Data in brackets were transformed ($\sqrt{1/x}$) for normality before analysis of variance, NS = not significant, ND = not determined

By 80 days after sowing, seedling mortality had increased to 44% in the heavier soil, with the Rizolex® treatment increasing plant stand by one plant/m, or 12% (Table 29). While the increased stand with Rizolex® was not large, it should be borne in mind that all the seed in these trials was treated with PCNB (Quintozene®, Bayer Crop Science) which is active against *Rhizoctonia*. The results *do not* show that Rizolex® is ineffective against *Rhizoctonia* but add further evidence indicating that the predominance of seedling pathogens varies from site to site. It seems that *Pythium* may have been the more dominant pathogen in the field used in this experiment, although other factors are involved in stand establishment. The benefit to growers from using in-furrow fungicides, such as Rizolex®, will vary according to pressure from *Rhizoctonia* in a given year, in a given field.

Fusarium wilt

In a field experiment at Boggabilla in the 2001-02 season, in-furrow application of the fungicide triadimenol at planting gave a significant increase in seedling survival in a crop heavily affected by Fusarium wilt but this effect did not translate to an increase in total survival in comparison to the control (Table 23). The other fungicides in that experiment did not have significant effects on Fusarium wilt and none of them increased seedling establishment at the start of the season (Table 23).

Biofumigation

- A long-term trial of biofumigation with vetch and canola at a site with a low level of *T. basicola* in the soil was commenced at the Australian Cotton Research Institute
- In trial at Hillston, common vetch (Vicia sativa) appears not to have biofumigation potential for black root rot although cold winter conditions may have prevented sufficient growth
- Vetch, mustard and canola were increased the severity of Fusarium wilt in trials at Boggabilla and should not be used as biofumigation crops on farms with Fusarium wilt
- Mustard meal and mustard oil were not effective as biofumigation agents against Verticillium wilt or Fusarium wilt

Biofumigation involves planting a ‘green manure’ crop that releases compounds that are toxic to pests or pathogens in the soil. Conventional soil fumigation is not a practical option for cotton diseases in Australia because of the scale of production and because fumigants do not penetrate heavy soils very well. Biofumigation offers a safe, self-generating method of distributing a natural fumigant throughout the soil profile. There are several types of plants that can be used, including canola, mustards and certain species of vetch. Vetch has been used successfully as a biofumigant for black root rot in cotton in the USA and has the added benefit of providing nitrogen to the following cotton crop. The use of woolly pod vetch as a biofumigation crop for black root rot is becoming popular due to the potential nitrogen return. However, the impact of biofumigation crops on Fusarium wilt had not been evaluated prior to this project.

Biofumigation crops

Black root rot. In a field infested with the black root rot fungus (*T. basicola*) at Hillston, common vetch (*Vicia sativa*, cv. Blanche Fleur) was sown on 19 June 2001. The population of *T. basicola* in the soils was assessed in cores collected from gaps in the stand on 14 July 2001 and, when cotton was sown, on 3 October 2001. The vetch treatment had no effect on the level of spores or the severity of disease in the subsequent cotton crop (Table 30). Cold conditions were experienced after the vetch was sown and it is estimated that the biomass of the vetch crop was approximately 2 tonne/ha, which may have been too little to give a substantial biofumigation effect.

Table 30. Lack of effect of a green manure crop of common vetch (*Vicia sativa*, cv. Blanche Fleur) on the population of *Thielaviopsis basicola* and the severity of black root rot of cotton in a field at Hillston in the 2001-02 season.

	Spore population before vetch (cfu/g soil)	Spore population after vetch (cfu/g soil)	Plant stand 31.10.01 (plants/m)	Black root rot severity 31.10.01 (0-10 scale)
Bare	719	362	9.1	8.7
Vetch	671	278	9.7	8.4
	NS	NS	NS	NS

NS = not significant, cfu = colony forming units

Long-term biofumigation for black root rot

Control of black root rot of cotton has two objectives: reversal of a severe infestation and prevention of the build-up of the pathogen in the soil in the first place. Previous work on biofumigation crops for control of black root rot of cotton has included trials in heavily infested soils. In 2002 a long-term experiment was commenced at the Australian Cotton Research Institute to determine whether or not the use of biofumigation crops could prevent the increase of black root rot from an initially low level of the pathogen in the soil. Before commencing the experiment, soil was assayed for inoculum of *T. basicola* along two transects of the trial site, which had rows 120 m long; one transect at 30 m from the head ditch and one at 30 m from the tail drain. The assays indicated that an area of infested soil existed at one end of the trial site, with more inoculum in the head-ditch transect than in the tail-drain transect (Figure 25).

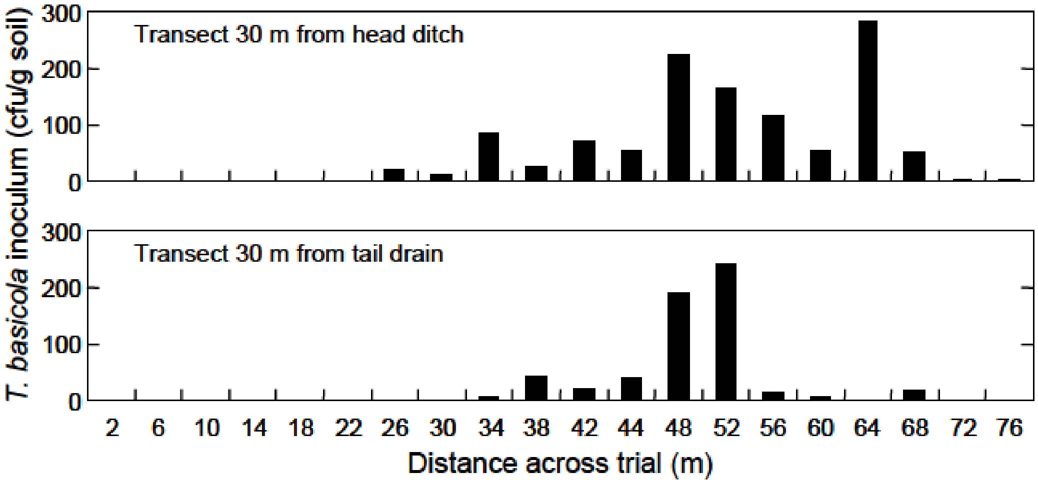


Figure 25. Initial distribution of inoculum of *T. basicola* across the trial site used in a long-term biofumigation experiment at ACRI (soil sampled 20 June 2002).

The experiment was commenced with four-row plots running the length of a field with beds 1 m wide, in 2002-03 (Table 31). The treatments were woolly pod vetch (cv. Namoi) and Canola (cv. BQ mulch) as green manure crops, and wheat (cv. Yallaroi). The wheat was sown in two rows, one on each shoulder of the bed and sprayed with herbicide prior to sowing cotton between the rows in the centre of the bed.

In the 2003-04 season, the trial design was altered because cultivation of bare plots was causing too much interference with adjacent wheat plots. Pairs of four-row plots were merged into eight-row plots and the trial was split into two tiers of plots, Tier 1 being the half closest to the head ditch and Tier 2 being the half closest to the tail drain. (Table 31). In both 2002 and 2003, delays in harvesting the previous cotton crops prevented timely sowing of the biofumigation crops; 14 June in 2002 and 23 May 2003. Consequently, dry matter production of the vetch and canola was relatively low. Dry matter of the vetch and canola, at the time of incorporation (21 August 2002, 19 August 2003), was not measured in 2002 and was less than 1 t/ha in 2003 (Table 32).

Table 31. Plot design for green manure crops (vetch, canola) and a wheat cover-crop sown on the shoulders of the beds in a cotton field at the Australian Cotton Research Institute 2002-03 2003-04 Plots (120 m long) Tier 2 (plots 60 m long)) Tier 1 (plots 60 m long)

2002-03 Plots (120 m long)
Bare
Wheat
Bare
Vetch
Canola
Bare
Wheat
Canola
Vetch
Wheat
Canola
Bare
Vetch
Vetch
Wheat
Bare
Canola
Canola
Bare
Vetch
Wheat
Bare
Bare

2003-04 Tier 2 (plots 60 m long))	 Tier 1 (plots 60 m long)
Wheat	Bare
Canola	Vetch
Bare	Wheat
Vetch	Canola
Vetch	Canola
Wheat	Bare
Canola	Vetch
Bare	Wheat
Vetch	Canola
Canola	Vetch
Wheat	Wheat
Bare	Bare

Table 32. Dry matter production of green manure crops (vetch, canola) and a wheat cover-crop sown on the shoulders of the beds in a cotton field at the Australian Cotton Research Institute

	Above-ground dry mass (t/ha)	
	2002-03	2003-04
Vetch	-	0.93
Canola	-	0.66
Wheat	1.31	1.63

In the 2002-03 season there were no differences in the severity of black root rot of cotton or cotton growth (Table 33). The mean level of symptoms observed of tap roots of cotton was moderately high in the 2002-03 season but variable, reflecting the variable distribution of the pathogen in the soil (Figure 25). The growth of biofumigation crops and the shoulder wheat was also variable due to a nitrogen deficit in some plots that was carried over from the previous cropping history of the field. In the shoulder wheat plots, cotton dry mass and wheat dry mass were positively correlated and these observations are described in the section on cover cropping (Figure 20).

Table 33. Lack of effect of biofumigation crops on the severity of black root rot and growth of cotton, on 30 October 2002, in a field at the Australian Cotton Research Institute Black root rot severity on tap roots (0-10 scale) Shoot dry matter (g/plant)

	Black root rot severity on tap roots (0-10 scale)	Shoot dry matter (g/plant)
Bare	6.7	0.13
Wheat	5.9	0.15
Mustard	5.7	0.12
Vetch	6.0	0.15
	Not significant	Not significant

In 2003-04, problems with the cotton planter resulted in seed being sown excessively deep and the trial had to be replanted with cotton. Consequently disease severity was not assessed in that year. By Feb 2004, the population of *T. basicola* in the soil had increased substantially across the trial site (Figure 26) particularly in the area where inoculum was high at the initiation of the trial (Figure 25). This trial is being continued in order to address the initial question of the capacity for biofumigation crops to slow the increase of *T. basicola* in soil.

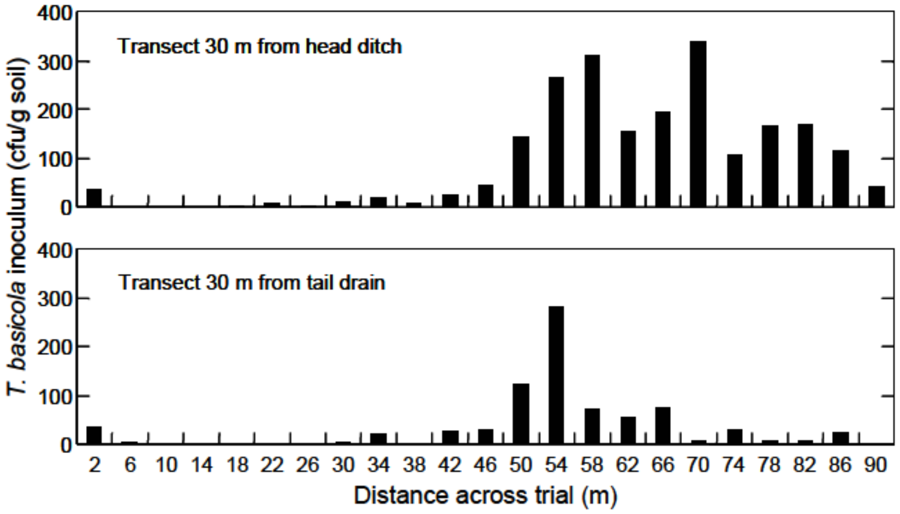


Figure 26. Distribution of inoculum of *Thielaviopsis basicola* across the trial site used in the long-term biofumigation experiment at ACRI (soil sampled 8 July 2003)

Fusarium wilt.

Two experiments to assess the potential for control of Fusarium wilt using biofumigation crops were conducted in a severely infested field near Boggabilla (Figure 10). Indian mustard (cv. 651) and vetch (cv. Capello) were sown by hand on 11 May 2001, slashed on 27 August and incorporated two days later. Cotton (cv. Sicot 70) was sown on 8 October 2001 at 10.5 seeds/m in two-row plots, 20 m long, in a completely randomised block design, using unsown plots as the control and six replicates. Stand establishment was assessed on 29 October in the middle 16 m of each plot and stems were cut for assessment of vascular discolouration on 30 April 2002. A duplicate experiment was conducted in the same manner using canola and chickpea, in adjacent plots. The chickpea failed to germinate and the cotton in these plots was not assessed.

Following the mustard and vetch green manure crops, the severity of Fusarium wilt was increased substantially in comparison to the control (Table 34). A similar increase in the severity of Fusarium wilt was observed when canola was grown as a green manure crop prior to cotton (Table 35). Biofumigation crops clearly have the potential to increase the severity of Fusarium wilt and should not be used until proven to be effective.

Table 34. Increased Fusarium wilt in cotton sown six weeks after incorporation of woolly pod vetch and Indian mustard in a field near Boggabilla in the 2001-02 season Bare Vetch Mustard

	Bare	Vetch	Mustard	[A]Probability
Initial plant stand (plants/m)	7.9a	7.5a	5.8b	$P \leq 0.001$
[B]Seedling survival (%)	60a	36b	46b	$P \leq 0.014$
[C]Adult survival (%)	22.1a	4.4b	3.9b	$P \leq 0.001$
[D]Total survival (%)	13.5a	1.7b	1.8b	$P \leq 0.001$

[A] Values in rows with the same letter are not significantly different by pairwise comparison of means using the Scheffé test at the stated probability level. (DAS = days after sowing).

[B] Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season

[C] Adult survival is the percentage of the plant stand at the end of the season with little or no disease (0 and 1 on the 0-4 stem rating scale)

[D] Total survival is 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)

Table 35. Increased Fusarium wilt in cotton sown six weeks after incorporation of canola in a field near Boggabilla in the 2001-02 season Bare Canola

	Bare	Canola	[A]Probability
Initial plant stand (plants/m)	8.1a	5.3b	$P = 0.003$
[B]Seedling survival (%)	52	46	$P = 0.044$
[C]Adult survival (%)	15	6.5	Not significant
[D]Total survival (%)	8.0	3.0	$P = 0.020$

[A] Values in rows with the same letter are not significantly different by pairwise comparison of means using the Scheffé test at the stated probability level. (DAS = days after sowing).

[B] Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season

[C] Adult survival is the percentage of the plant stand at the end of the season with little or no disease (0 and 1 on the 0-4 stem rating scale)

[D] Total survival is 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)

Soil amendment with mustard products

When the tissues of brassicas, such as canola and mustard, are physically disrupted, glucosinolates in these tissues are rapidly converted to isothiocyanates (ITC) that are released into the atmosphere. The ITC released after brassica crops are incorporated are the most active compounds in the biofumigation effect using brassicas. Mustard oil and mustard meal made from cultivars with a high glucosinolate content may release substantial amounts of ITC. The potential to control Verticillium wilt and Fusarium wilt using mustard products was evaluated in the 2002-03 season.

Verticillium wilt.

Experiments using mustard meal and mustard oil were conducted in the Verticillium wilt nursery at the Australian Cotton Research Institute, Narrabri Mustard meal (product name 'Naturafume') and mustard oil (product name 'Voom') were kindly provided by Mr Prem Akhil. In the first experiment, mustard meal was applied to the soil in a randomised block design with two treatments and four replicates. Plots were two rows wide × 15 m long, with a row of buffer on either side and at least 10 m buffer at either end. Mustard meal (1000 kg/ha) was spread by hand on the soil surface in a band approximately 15 cm wide along the planting line on each row on 24 September 2002 and immediately incorporated into the soil using rotary harrows. Cotton (cv. Sicala V3RRi) was sown (15 seeds/m) in all plots on 3 October 2002. The severity of Verticillium wilt was assessed on 15 May 2003 by cutting the stems of every cotton plant in each plot and scoring for the presence or absence of symptoms of Verticillium wilt. In the second experiment in the Verticillium wilt nursery, a 5% emulsion of mustard oil in filtered bore-water was injected into the soil at 10 cm in depth at the rate of 400L/ha, which was equivalent to 20 L mustard oil/ha. The experimental design and procedures were otherwise the same as for the mustard meal experiment.

Application of the mustard oil and mustard meal prior to sowing cotton had no effect on the incidence of Verticillium wilt (Tables 36 and 37).

Table 36. Plant establishment and incidence of Verticillium wilt of cotton after pre-planting amendment of soil with mustard meal (1 tonne/ha) in the Verticillium wilt nursery at the Australian Cotton Research Institute in 2002-03

	Treatment		
	Untreated	**Mustard meal**	*Probability*
Stand (plants/m, 15 May 2002)	6.6	5.1	Not significant ($p = 0.166$)
Verticillium wilt (% plants)	40	30	Not significant ($p = 0.146$)

Table 37. Plant establishment and incidence of Verticillium wilt of cotton after pre-planting injection of mustard oil (20 L/ha) into soil in the Verticillium wilt nursery at the Australian Cotton Research Institute in 2002-03

	Treatment		
	Untreated	**Mustard oil**	*Probability*
Stand (plants/m, 15 May 2002)	5.2	5.7	Not significant ($p = 0.324$)
Verticillium wilt (% plants)	29	21	Not significant ($p = 0.448$)

Fusarium wilt.
Two experiments using mustard oil were conducted in a commercial field near Boggabilla that was severely infested with the Fusarium wilt pathogen. In the first experiment, mustard oil was applied in a randomised block design with 2 treatments × 8 replicates in single row plots 10 m long, on 27 September 2002. In each treated plot, a furrow (10 cm wide × 15 cm deep) was dug by hand along the planting line, 400 mL of a 5% emulsion of mustard oil (50 mL oil/L distilled water) was sprayed into the base of the furrow using a garden sprayer, the excavated soil was immediately replaced in the furrow and the beds were raked-over to provide a level surface suitable for planting. This rate was equivalent to 20 L of mustard oil per hectare. In the control plots 400 mL of distilled water was applied in the same manner as for the treated plots. Cotton (cv. Sicot 70) was sown (13 seeds/m) across the plots by the grower on 8 October 2002. The plots were arranged in two tiers of eight, with no buffers, such that cotton was sown across the whole experiment in single pass of the eight-row planter. Plant stand was assessed on 29 October, 27 November and 7 April by counting all plants within each 10 m plot. Disease severity was assessed on 7 April 2003 by cutting the stems of every cotton plant in each plot and counting the number of plants with little (less than 5% of the stem in cross-section) or no symptoms of Fusarium wilt.

In the second experiment, mustard oil was applied in a completely randomised block design with 5 rates × 5 replicates. Plots were 2 m long and arranged in 5 tiers in 5 adjacent rows, with 1 m of buffer between each tier. Mustard oil was applied in the same manner as in the first experiment except that 500 mL of emulsion, containing either 0, 5, 10, 20 or 40 mL of mustard oil in distilled water, was poured into furrows in each 2 m plot. These rates were equivalent to 0, 25, 50, 100 and 200 L of mustard oil per hectare. Cotton (cv. Sicot 70) was sown (13 seeds/m) across the whole experiment by the grower on 19 November 2002. Plant stand was assessed on 189 December and 7 April by counting all plants within each 10 m plot. The severity of Fusarium wilt was assessed in the same manner as in the first experiment.

In both experiments the application of mustard oil had no significant effects on the stand establishment or the incidence and severity of Fusarium wilt (Table 38, Figure 27). The severity of Fusarium wilt was substantially less in the second experiment (Figure 27), even though it was located immediately adjacent to the first experiment, suggesting that the delayed planting date for this experiment avoided cool early season conditions that may favour infection of cotton.

Table 38. Plant establishment and severity of Fusarium wilt of cotton after pre-planting amendment of soil with mustard oil (20 L/ha) into soil in a field severely infested with the Fusarium wilt pathogen at Boggabilla in 2002-03

	Treatment		
	Untreated	Mustard oil	Probability
Stand 29 October (plants/m)	6.4	7.8	Not significant ($p = 0.714$)
Stand 27 November (plants/m)	4.8	5.6	Not significant ($p = 0.428$)
Stand 7 April (plants/m)	4.3	5.2	Not significant ($p = 0.441$)
[A]Seedling survival (%)	66.5	66.4	Not significant ($p = 0.769$)
[B]Adult plant survival (%)	22.9	22.2	Not significant ($p = 0.841$)
[C]Total survival (%)	15.6	15.0	Not significant ($p = 1.000$)

[A] Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season

[B] Adult survival is the percentage of the plant stand at the end of the season with little or no disease (0 and 1 on the 0-4 stem rating scale)

[C] Total survival is 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)

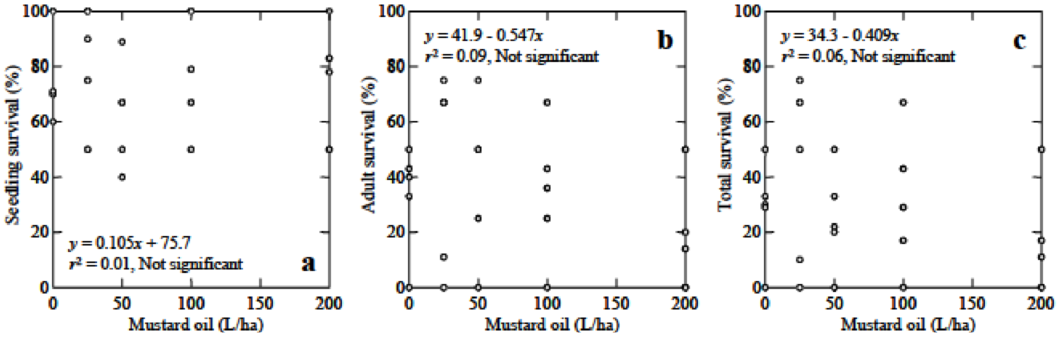


Figure 27. Lack of effect of increasing rates of application of mustard oil to the soil on the severity of Fusarium wilt of cotton in an infested field near Boggabilla in 2002-03. Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season. Adult survival is the percentage of the plant stand at the end of the season with little or no disease (0 and 1 on the 0-4 stem rating scale). Total survival is 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).

The failure of the mustard products to produce a measurable biofumigation effect against Verticillium wilt and Fusarium wilt of cotton may be due to inadequate penetration of the soil, rather than a lack of activity by the ITC released from the products. In this regard, such products, although of a non-synthetic derivation, face the same hurdle to deployment as conventional fumigants.

Plant-pathogen-soil interactions

- Transects of a field with varying soil type suggest that some soils may be less conducive to development of Fusarium wilt of cotton
- Symptoms of black root rot on tap roots develop slowly during the first two weeks after sowing, reach a plateau level of infection from three to five weeks and then decline as the tap root expands with warmer conditions
- Any factor that slows cotton growth may give the impression that black root rot is more severe if it delays the sloughing of blackened cells in the outer layers of cotton tap roots
- The peak activity of the black root rot pathogen, *T. basicola*, and seedling pathogens are mutually exclusive, providing further evidence that *T. basicola* does not kill cotton seedlings
- Mycorrhizal colonisation was correlated negatively with both symptoms of black root rot on tap roots and production of spores by *T. basicola* on lateral roots
- Mycorrhizal fungi survived long bare fallows of 28 and 35 months in substantial numbers in fields at Bourke
- Mycorrhizal fungi survived a bare fallow of four years in substantial numbers in a field experiment at the Australian Cotton Research Institute

Fusarium wilt and soil type

In order to quantify the potential for soil type to influence the severity of Fusarium wilt of cotton, the incidence of vascular discolouration was assessed in two transects across part of a cotton crop on 31 March 2003. This field was selected because i) that part of the field was known to vary in soil texture and colour and ii) Fusarium wilt had been observed in the field in the 1996-97 cotton season, therefore, having had sufficient time to spread through the field.

Fusarium wilt was relatively widespread across the two transects (Figure 28). The patch of dying plants observed 60 m from the tail drain in 1996-97 coincided with an area of high incidence in 2003 at approximately 300 m along the transect at 60 m from the tail drain (Figure 28).

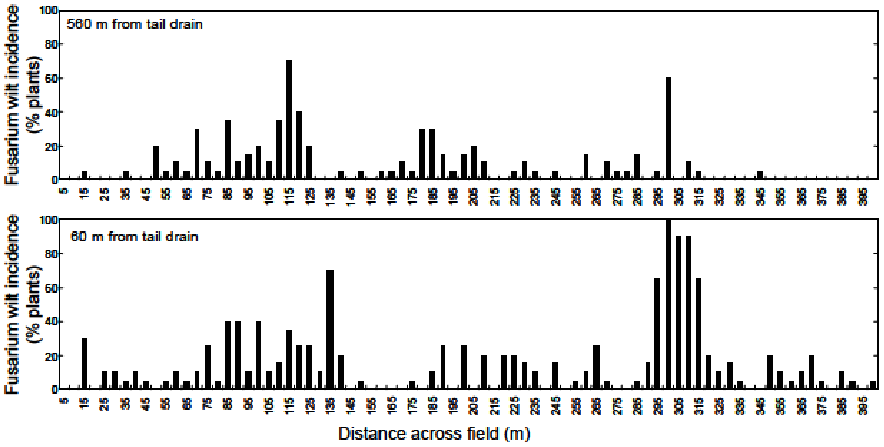


Figure 28. Incidence of Fusarium wilt of cotton in two transects of a field near Boggabilla in March 2003

The Fusarium wilt fungus is known to move easily down furrows with the flow of irrigation and the incidence of Fusarium wilt at 60 m from the tail drain tended was correlated significantly with the incidence at 500 m further up the same furrows ($r_2 = 0.107$, $P = 0.003$). However, this relationship was weak, with only 11% of the variation at 60 m from tail drain being explained by variation at 560 m from the tail drain. Incidence at 60 m from tail drain is likely to have been influenced by lateral flow of inoculum along the tail drain but the variable soil type in this field may also have been a factor. The low incidence of Fusarium wilt in the interval between 130 and 175 m in the560-m transect (Figure 28) coincided with a visibly obvious area of red soil, whereas the other parts of the transect passed over brown to grey heavy clay soil. Anecdotal observations indicate that black root rot is less severe in areas of red sandy soil in infested fields and the same may hold true for Fusarium wilt. Soil cores were taken for future analysis.

Black root rot and seedling disease

The belief that black root rot kills cotton seedlings is widely held amongst growers and consultants, despite repeated evidence from the cotton disease surveys that there is no relationship between stand establishment and the incidence of black root rot (Figure 4). The reason for this lack of relationship becomes clear when observations of the timing of symptoms are compared. In the experiments evaluating the effects of fungicides on cotton seedling mortality, plant death generally increases by no more than a few percentage points after the first assessment at 21 days after sowing (Tables 9 to 13). The pathogens causing seedling death, namely *Pythium* and *Rhizoctonia*, are active at both the pre-emergent and post-emergent stages. In contrast, the development of symptoms of black root rot in cotton is relatively slow. When the progress of black root rot was assessed in replicated plots in a commercial cotton field near Wee Waa in the 2003-04 there was little sign of infection at 12 days after sowing (Figure 29). Disease increased exponentially thereafter, reaching a peak at approximately 38 days.

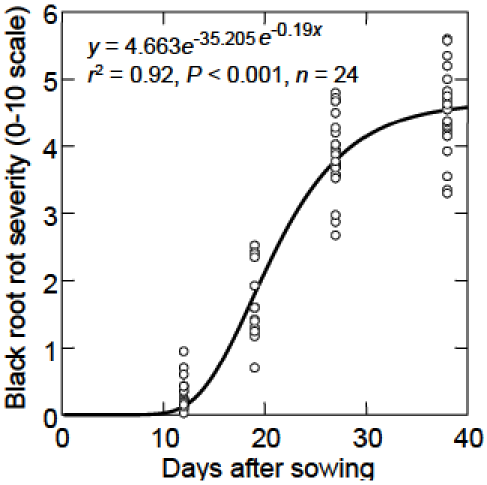


Figure 29. Progress of black root rot of cotton in a commercial cotton field near Wee Waa in the 2003-04 season.

In a similar study at the Australian Cotton Research Institute in the 2003-04 season, the progress of black root rot was monitored in replicated plots by Dr Anowar Mondal. In half the plots, the leaves were trimmed with scissors at each assessment to deliberately prevent plant growth and the expansion of cotton roots. In both treatments the progress of disease was initially exponential, with little disease occurring in the first two weeks after sowing (Figure

30). The severity of black root rot reached a peak at about three weeks after sowing. With the onset of warmer seasonal conditions, the tap roots of plants that were not trimmed expanded and sloughed off the infected layers of the root cortical tissue. This process was delayed in the plants that had their leaves trimmed, as their growth was inhibited (Figure 30). This experiment clearly demonstrated that black root rot causes a temporary infection of the root cortex and that external influences on plant growth can determine whether or not the seedlings grow out of the plateau stage of symptoms.

Since the symptoms of black root rot do not peak until around three weeks after sowing, the assumption that that black root rot is responsible for seedling death, most of which occurs prior to that point in time, is illogical. The observation that the plateau stage of severe infection by *T. basicola* occurs in the interval from 21 to 35 days after sowing (Figure 30) provides theoretical evidence in support of the observations that delayed sowing can avoid the environmental conditions that favour black root rot and reduce its severity (see Final Report, DAN153C), as well as seedling disease (Table 7, Figure 18).

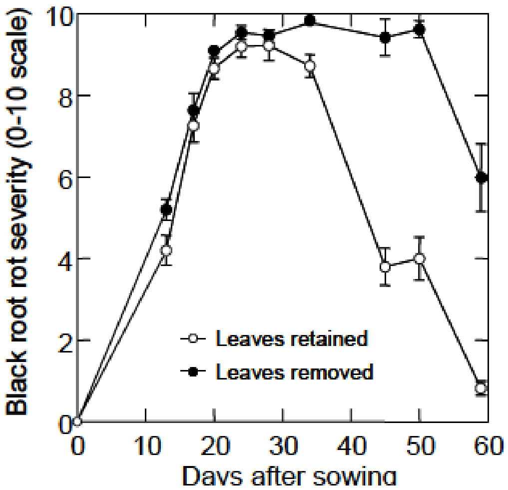


Figure 30. Progress of black root rot of cotton in a field at the Australian Cotton Research Institute in the 2003-04 season, with and without trimming of the leaves at each assessment**.**

Black root rot and mycorrhizas

The increase in the distribution of black root rot in the cotton CRC farming systems experiment at Warren was previously described in the Final Report for DAN122C. In the Final Report for the Cotton CRC project NSWA2.5.2 *Maximising mycorrhizal infection in cotton*, data on mycorrhizal colonisation of cotton in the rotation treatments was presented. However, neither of these reports presented an analysis of the relationship between mycorrhizal colonisation and the severity of black root rot. In 1996 in the farming systems trial at Warren, cotton roots were well colonised by mycorrhizal fungi but colonisation decreased when more than half the tap root was affected by black root rot (Figure 31). This non-linear relationship probably reflects the fact that two different infection courts are being compared, namely mycorrhizal colonisation in lateral roots and black root rot symptoms on tap roots.

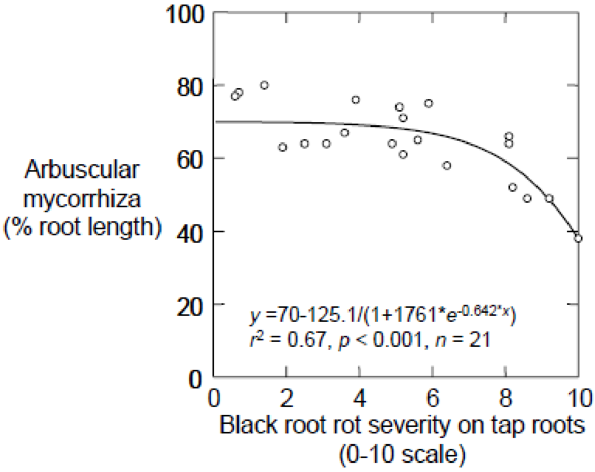


Figure 31. Colonisation of cotton roots by arbuscular mycorrhizal fungi declined as the severity of black root rot on the tap roots increased, especially when more than half the tap root was affected (>5 on 0-10 scale), in the Cotton CRC Farming Systems Trial at Warren on 21 November 1998.

A negative relationship ($r_2 = 0.47$) between the severity of black root rot on tap roots and mycorrhizal colonisation of the lateral roots of cotton in a field at Goondiwindi in 1998 was also reported previously in the Final Report for DAN122C. That report did not include a comparison of mycorrhizal colonisation with the presence of chlamydospores of *T. Basicola* on the lateral roots, presented herewith (Figure 32). In this instance, the comparison is between two different organisms in the same infection court (lateral roots) and the relationship is linear. The implication is that colonisation by *T. basicola* directly impedes colonisation by mycorrhizal fungi. However, the indirect effect of black root rot on cotton growth may also limit the capacity of the plant to provide carbohydrate to support mycorrhizal colonisation. In either case, the lack of mycorrhizal development in cotton plants with severe symptoms of black root rot is likely to contribute to the delay in early season growth caused by this disease.

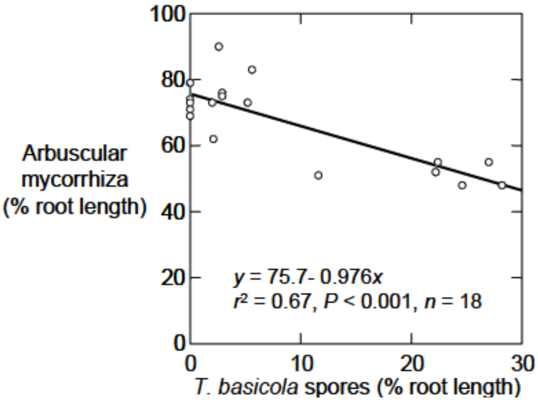


Figure 32. Colonisation of cotton roots by arbuscular mycorrhizal fungi declined as the severity of black root rot on the tap roots increased, especially when more than half the tap root was affected (>5 on 0-10 scale), in the Cotton CRC Farming Systems Trial at Warren on 21 November 1998.

Mycorrhizas and very long fallows

Mycorrhizal symbiosis (also known as VAM) is ubiquitous in cotton. Cotton is highly dependent on the mycorrhizal fungi for the supply of phosphorus and zinc from the soil. The fungi cannot grow in the soil without a living host. Repeated field experiments have previously shown that sufficient numbers of mycorrhizal fungi survive in the soil during bare fallows of 18 months. With the drought experienced during this project, some cotton fields have experienced bare fallows of two years or more and there has been concern that the mycorrhizal fungi will not survive until the next crop of cotton.

To assess the status of mycorrhizal fungi in soils that had experienced very long bare fallows during the drought, test strips of linseed and cotton (2 m long) were sown in March 2004 in 12 eight-row plots in each of two fields at Bourke (Table 39). Mycorrhizal colonisation of cotton roots usually peaks at around six weeks after sowing in commercial crops, at levels around 50% or higher. Given that both fields experienced very long bare fallows, the cotton and linseed were well colonised by mycorrhizal fungi at the time of sampling (Table 39). Cool temperatures were experienced from the end of April onwards, which may have slowed the rate of spread of colonisation within the root systems. The barley in field 75 only emerged 4-5 days before sampling and was not yet colonised.

Table 39. Mycorrhizal (VAM) colonisation of roots of cotton and linseed after very long fallows at Bourke

	Field 45/46	Field 75
Fallow length	28 months	35 months
Effective sowing date*	24 March	30 March
Sampling time	42 DAS	36 DAS
Linseed VAM (%)	29 ± 3.1	37 ± 2.5
Cotton VAM (%)	28 ± 1.6	35 ± 1.2

* Seed was sown in both fields on 19 March into dry soil and then irrigated at the dates indicated.

It seems likely that the very dry conditions experienced at Bourke contributed to the preservation of mycorrhizal fungi in these soils. Given the length of time that the mycorrhizal fungi survived in these fields without any host plants, a substantial amount of viable fungus should be available to colonise the following cotton crop. A study to test the adequacy of these surviving populations of mycorrhizal fungi was initiated. Replicated eight-row plots of barley were sown during winter 2004, to act as ‘nurse’ crops for the mycorrhizal fungi and the effect on mycorrhizal development in the subsequent cotton crops is to be monitored during the 2004-05 season.

An experiment to investigate the effect of prolonged bare fallows on survival of mycorrhizal fungi in soil was initiated at the Australian Cotton Research Institute in the 1997-98 season. The experiment consisted of plots with cotton every summer (continuous cotton) or continuous bare fallow. In the first few years the fallow plots had a substantial seed bank of weeds but by 2000 the plots were virtually weed free. These plots have been utilised in collaborative research at the University of Sydney, including a BSc Honours student, two PhD students and a post doctoral fellow. To measure the effects of the continuous bare fallow on the survival of mycorrhizal fungi a bioassay was conducted in potted soil collected on 20 April 2004, at which point the plots had experienced four years of weed-free bare fallow and eight years without a crop. Soil was potted, sown with cotton (cv. Sicala V2), maintained in the glasshouse, with watering from the base, and mycorrhizal colonisation was assessed every seven days for eight weeks.

Colonisation of cotton roots by arbuscular mycorrhizal fungi progressed rapidly in the soil from continuous cotton plots, reaching a plateau level of around 65%, at about 28 days after sowing (Figure 33). Colonisation was delayed in the soil from the bare fallow plots but approached the plateau level of the continuous cotton plots by eight weeks after sowing. A delay in colonisation suggests that the inoculum potential of the mycorrhizal fungi was depleted. Since substantial colonisation did eventually develop in all pots with soil from the bare fallow plots (Figure 33). Given the capacity for cotton to compensate for early-season delays in growth, it is difficult to predict whether or not the level of inoculum surviving in the bare fallow plots would have resulted in a yield loss. Evaluation of the survival of mycorrhizal fungi in this experiment will be continued in subsequent seasons.

The popular belief that bare fallows of 17 to 18 months reduce mycorrhizal development in cotton was shown previously to be misconceived (CRC project NSWA2.5.2 *Maximising mycorrhizal infection in cotton*). Observations in this project indicate that mycorrhizal fungi can survive in substantial numbers for periods of up to four years bare fallow.

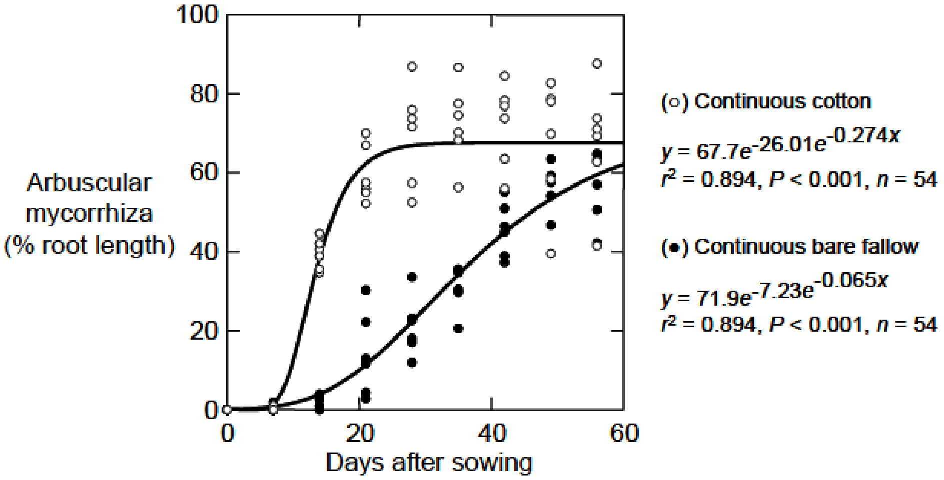


Figure 33. Delayed colonisation of cotton in bioassays of the infectivity of soil collected April 2004 from plots with either continuous cotton or four years of weed-free fallow, in a field at the Australian Cotton Research Institute

Outcomes

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 seedling disease, black root rot and Fusarium wilt to provide more effective options for control of these diseases. 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:

- Ongoing, 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 soilborne pathogens, such infurrow fungicides for Fusarium wilt
- Increased awareness of the potential for biofumigation crops to worsen Fusarium wilt.
- Increased awareness by growers and consultants of the need to avoid environmental conditions that favour seedling disease and black root rot by delaying sowing and pre-empting temperatures on a rising trend
- Independent confirmation of the performance of DynastyTM seed treatment, which has a safer user profile and less environmental impact than formulations containing PCNB

- Development of method for application of acibenzolar-S-methyl with standard seed coatings
- Further confirmation of the potential for seed treatment with acibenzolar-S-methyl to induce resistance against Fusarium wilt
- Decision by Syngenta Crop Protection to proceed with registration of acibenzolar-Smethyl for seed treatment of cotton in Australia
- Continued use of resistant varieties to maintain low incidence of Verticillium wilt.
- Confirmation that mycorrhizal fungi at Bourke had survived extended bare fallows, leading to savings in the application of phosphorus fertilisers, as proposed by commercial suppliers

Conclusion

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. Growers generally responded to changes in the level of seedling mortality by altering sowing rates in the following season. The disease surveys confirmed that seedling mortality increases with increasing latitude, with the southern regions of NSW being particularly at risk. Seedling mortality was not correlated with the incidence of black root rot.

Black root rot. The Australian cotton industry is currently experiencing a widespread, chronic epidemic of black root rot. Black root rot has been observed on all of the farms surveyed regularly in the Macintyre, Gwydir, Namoi and Macquarie Valleys, and in 78% of fields and 39% of plants within those farms. If the pathogen continues to increase its distribution within these four valleys at its current rate, then approximately 95% of fields, and 90% of plants within those fields, will be infested by 2011. 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 Valley. Black root rot was detected in the Murrumbidgee Valley for the first time in November 2003, as part of the surveys in this project. *T. basicola* is not distributed evenly within fields and assays of soil do not necessarily detect the pathogen, even when it is located nearby in the field. Many farms do not have black root rot and farm hygiene should be practiced to minimise further spread.

Fusarium wilt. The Australian cotton industry is currently at the onset of a widespread, epidemic of Fusarium wilt. Fusarium wilt has been reported on a total of 75 farms in NSW and has been observed on 30% of the farms surveyed regularly across NSW. If the pathogen continues to disperse at its current rate, then approximately 90% of farms in NSW will be affected by 2012. The Macintyre Valley has been the affected the most 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. 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. Transects of an infested field in the Macquarie Valley suggest that Fusarium wilt may progress much more quickly in cooler cotton growing regions but the absence of crops, due to the drought, has prevented further confirmation of this hypothesis. 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 increased recently in the Namoi Valley, probably due to declining use of resistant varieties. Monitoring of a severe case of Verticillium wilt established that it was not caused by a new aggressive strain of *V. dahliae*. Extension should emphasise the value of resistant varieties on an ongoing basis to prevent resurgence of this disease. Verticillium wilt has not been reported in some areas and occurs at very low levels in others and, therefore, farm hygiene should be practiced to minimise further spread.

Boll rots. Cotton boll rots were observed at very low levels across NSW in the 2001-02, 2002-03 and 2003-04 seasons. Cotton boll rots can cause high losses in association with seasonal conditions, especially heavy rainfall events from January to early March. The threat from boll rots to the cotton industry is low due to the localised and seasonally specific nature of their occurrence.

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. There were no significant interactions between carryover of cotton trash and any of the diseases monitored in the disease surveys.

Objective 2. To continue to develop and/or evaluate control strategies for Verticillium wilt, Fusarium wilt, Alternaria leaf spot and seedling diseases of cotton.

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

			Implications	
Strategy	**Focus**	**Result**	**Science**	**Industry**
Crop residue management	Evaluation of vetch residue impact on seedling mortality	Late incorporation of vetch increases activity of *Rhizoctonia* and *Pythium* causing greater seedling death	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	Sowing late within the planting window can avoid conditions favouring seedling disease	Increased understanding of factors contributing to severity of seedling disease	New management tool for seedling disease
Timing of sowing	Timing of sowing after pre-irrigation	Seedling mortality decreased with increasing delay after sowing	Increased understanding of effect of soil temperature and water content on seedling disease	Better decision-making for timing of sowing after pre-irrigation to avoid conditions favourable to seedling disease
Fungicides	Seed treatment prior to sowing	Seedling pathogens shown to 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

Objective 2 continued

Strategy	Focus	Result	Implications	
			Science	Industry
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 with Dynasty™	Dynasty™ performed as well as the standard fungicides	Confirmation of effectiveness of components of Dynasty™ in seedling disease control	Independent evaluation of effectiveness of Dynasty™
Fungicides	Seed treatment with non-fungicidal products	Non-fungicidal products did not control seedling disease	Confirmation that acibenzolar-S-methyl does not activate resistance against seedling pathogens	Better decision-making regarding trial use of non-fungicidal products
Fungicides	In-furrow application for seedling disease	No effect on seedling disease	Confirmation that pressure from different species of seedling pathogens is variable	Benefit of in-furrow fungicides depends activity of target pathogens
Fungicides	In-furrow application for Fusarium wilt	No effect on Fusarium wilt	Fungicides benomyl, triadimenol and toclofos methyl not effective against Fusarium wilt	Confirmation of existing assertion that furrow fungicides do not control Fusarium wilt
Cover crops	Shoulder wheat	Warmer soil temperatures and increased growth of cotton seedlings	Indication that cover crops have potential to modify environmental conditions that favour soilborne pathogens	Potential tool for coping with cold early season conditions in cool production areas
Systemic acquired resistance (SAR)	Development of seed treatment methods	Appropriate rate for acibenzolar-S-methyl (Bion®) developed	No phytotoxic effects by acibenzolar-S-methyl incorporated in seed coating	New tool for application of acibenzolar-S-methyl to cotton seed
SAR	ASM for Fusarium wilt	Severity of Fusarium wilt consistently decreased. No effect on Verticillium wilt. Occasional decrease in seedling mortality.	Pathogen-specific SAR response that persists with dilution through time and in growing tissues	New tool for control of Fusarium wilt of cotton
SAR	Foliar application of salicylic acid and Brotomax™	Salicylic acid and Brotomax™ ineffective against Fusarium wilt and Verticillium wilt	Confirmation that SAR not activated	Commercial claims questionable
SAR	Seed treatment with plant hormones	No effect on Fusarium wilt	Confirmation that SAR not activated	Commercial claims questionable
Biofumigation	Vetch, mustard and canola for Fusarium wilt	Severity of Fusarium wilt increased substantially	Fusarium wilt pathogen multiplies rapidly on high-N crop residues	Avoid using green-manure crops in areas affected by Fusarium wilt
Biofumigation	Mustard meal and mustard oil	No effect on Fusarium wilt or Verticillium wilt	Active compounds not released in sufficient quantity or not able to penetrate soil	Commercial claims questionable

Objective 3. To initiate investigations of host-pathogen interactions involved in soilborne diseases 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:

			Implications	
Strategy	**Focus**	**Result**	**Science**	**Industry**
Identify suppressive soil types	Transects of a field infested with the Fusarium wilt pathogen	Some areas free of the disease despite long presence of the pathogen in the field	Understanding of factors affecting disease severity	Identification of soil types with greatest risk, leading to targeted control measures
Identify timing of conditions favouring disease	Monitor disease progress in black root rot in the field	Peak periods of activity of black root rot and seedling pathogens are mutually exclusive.	Importance of timing in assessing disease emphasised.	Delayed sowing can minimise period of exposure to peak in black root rot. Seedling disease should not be confused with black root rot
Mycorrhizas and disease	Comparison of black root rot and mycorrhizal colonisation	Mycorrhizal colonisation decreased in association with black root rot	Linear negative relationship between colonisation of fine roots by *T. basicola* and mycorrhizal fungi identified	Decreased mycorrhizal colonisation is a symptom of the disease
Survival of mycorrhizal fungi	Extended bare fallows	Substantial numbers of mycorrhizal fungi surviving up to 35 months bare, dry fallow	Mycorrhizal fungi have evolved to cope with climatic vagaries of the Australian landscape, including drought-induced fallows	Mycorrhizal fungi generally survive in adequate numbers. Commercial claims for replacement with fertiliser questionable
Defensin genes for disease resistance	Evaluation of transgenic lines from University of Melbourne	Enhanced resistance against *T. basicola* and reduced reproductive potential	Defensin genes have potential to control *T. basicola* with no adverse effect on mycorrhizal colonisation of cotton	Potential tool for breeding resistant cotton varieties

Objective 4. Facilitate delivery and deployment of cotton disease management strategies.
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 (8); presentations at conferences (9), industry and research meetings (31), and grower meetings and field days (15); media releases (7) and media interviews (6); lectures to the cotton production course (18 hours). Modifications to the existing disease management strategy for seeding disease, black root rot and Fusarium wilt have been devised and are presented in Section 8, below.

Extension Opportunities

Technical Development

A practical method for application of acibenzolar-S-methyl to cotton seed was developed, using 6 mg/kg seed, which was equivalent to the rate in previous, successful experiments using seed soaking. for future research.

Intellectual Property

A design concept for a trash-retaining drop box (commercial in confidence) box has been submitted separately to the CRDC for commercial evaluation.

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 Fusarium wilt 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.

Future Research

The annual disease surveys and observational studies of the incidence of Fusarium wilt should be continued. The impact of and seedling disease and black root rot in the new cotton areas in southern NSW should be monitored. Disease surveys should identify environmental and cultural factors influencing the emergence or re-emergence of disease threats.

Research should focus on continued development and evaluation of control strategies for seedling disease, black root rot and Fusarium wilt, including seed treatment fungicides, systemic acquired resistance, rotation crops, biofumigation crops, cover crops and delayed sowing. Host-pathogen-soil interactions (including herbicides) contributing to the severity of black root rot of cotton should be investigated, with the aim of identifying features that might be exploited for disease control. The use of varieties with disease resistance, where available, and the minimisation of the spread of soilborne pathogens has been beneficial, and should remain a high priority for the industry. Long-term field experiments on the role of black root rot, mycorrhizal fungi (VAM) and other soil organisms in the soil ecosystem (soil 'health') should be continued.

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Part 4 – Final Report Executive Summary

The disease surveys have indicated that seedling disease, black root rot and Fusarium wilt continue to threaten the productivity of cotton production in NSW and Queensland. No single control measure gives adequate protection against either black root rot or Fusarium wilt and seedling disease is severe in the cool production areas of NSW. An integrated disease management approach is required and, therefore, a range of potential tools for control of cotton diseases were evaluated in glasshouse and field experiments. Key findings include:

Disease surveys

Seedling mortality

- Seedling mortality was greatest with cool wet conditions early in the season, reflecting the major impact of seedling disease (caused by *Rhizoctonia* and *Pythium*)
- In NSW, seedling mortality was relatively low during most of the 1990's but increased dramatically in 2000, 2001 and 2002
- The risk of seedling disease increases with increasing latitude, with the southern regions of NSW being particularly prone
- Growers tend to respond to changes in the level of seedling mortality by altering sowing rates in the following season
- Seedling mortality is not increased by black root rot

Black root rot

- The Australian cotton production industry is currently experiencing a widespread, chronic epidemic of black root rot
- Black root rot has been observed on all of the farms surveyed regularly in the Macintyre, Gwydir, Namoi and Macquarie Valleys, and in 78% of fields and 39% of plants within those farms
- If the pathogen continues to increase its distribution within these four valleys at its current rate, then approximately 95% of fields, and 90% of plants within those fields, will be infested by 2011
- 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 Valley
- Black root rot was detected in the Murrumbidgee Valley for the first time in November 2003, as part of the surveys in this project *T. basicola* is not distributed evenly within fields and assays of soil do not necessarily detect the pathogen, even when it is located nearby in the field
- Many farms do not have black root rot and farm hygiene should be practiced to minimise further spread

Fusarium wilt

- The Australian cotton industry is currently experiencing a widespread, chronic epidemic of Fusarium wilt
- Fusarium wilt has been reported on a total of 75 farms in NSW
- Fusarium wilt has been observed on 30% of the farms surveyed regularly across NSW
- If the pathogen continues to disperse at its current rate, then approximately 90% of farms in NSW will be affected by 2012
- The annual disease surveys need to assess congruent transects within each season to enable comparisons between the incidence of early-season and late-season diseases and/or symptoms

- The Macintyre Valley has been the affected the most 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 closely followed the incidence of the disease among farms
- 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 an immediate reduction in the incidence of Fusarium wilt in the following crop
- Transects of an infested field in the Macquarie Valley suggest that Fusarium wilt may progress much more quickly in cooler cotton growing regions but the absence of crops, due to the drought, has prevented further confirmation of this hypothesis
- Fusarium wilt has not been reported on the majority of farms in NSW and farm hygiene should be practiced diligently to minimise further spread

Verticillium 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 increased recently in the Namoi Valley, probably due to declining use of resistant varieties
- Monitoring of a severe case of Verticillium wilt established that it was not caused by a new aggressive strain of *V. dahliae*
- Extension needs to emphasise the value of resistant varieties on an ongoing basis to prevent resurgence of this disease
- Verticillium wilt has not been reported in some areas and occurs at very low levels in others
- 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 2001-02, 2002- 03 and 2003-04 seasons
- Cotton boll rots can cause high losses in association with seasonal conditions, especially heavy rainfall events but such losses are localised and non-repeating

Alternaria leaf spot

- Alternaria leaf spot was consistently observed at low levels in virtually all 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
- There were no significant interactions between carryover of cotton trash and any of the diseases monitored in the disease surveys

Control measures

Seedling disease nurseries

- Late incorporation of woolly pod vetch has been used successfully to increase the severity of seedling disease in cotton for experimental purposes
- The technique's effectiveness may depend on having adequate soil moisture to enable colonisation of the vetch residues by seedling pathogens prior to sowing cotton

Timing of sowing

- Delaying the date of sowing as late as possible within the planting window can avoid conditions that favour seedling disease
- Sowing should be timed to coincide with the onset of periods of weather that will result in a mean soil temperature of 16°C during the first week from sowing
- Sowing should be delayed after pre-irrigation until soil water content is at the lower end of the range that is adequate for seedling establishment in any particular soil

Fungicides and other products for control of seedling disease

- Seed treatment experiments showed that seedling pathogens, such as *Rhizoctonia* and *Pythium*, vary in dominance from field to field and year to year.
- A few fungicide combinations gave slightly greater protection than the standard fungicides in some years but not others.
- The fungicide DynastyTM consistently performed as well as the standard fungicides
- The non-fungicidal products, including acibenzolar-S-methyl, were not effective

Cereal cover crops

- Experiments with cereal cover crops in this project confirmed previous observations of their potential to increase early-season growth of cotton
- Cotton growth was correlated positively with dry matter production in the cereal cover crop
- To avoid problems with establishment of cotton, cover crops need careful placement on the shoulders of the bed, in well prepared beds, with the cotton planting line remaining clear
- The potential for cover crops to reduce the severity of black root rot will require trials to be conducted in locations with sufficiently even distribution of the pathogen

Systemic acquired resistance

- A practical method for application of acibenzolar-S-methyl to cotton seed was developed, using 6 mg/kg seed, which was equivalent to the rate in previous, successful experiments using seed soaking
- Application of acibenzolar-S-methyl to cotton seed in combination with standard seed treatment fungicides was shown to have no phytotoxic effects on germination of cotton seed and subsequent seedling growth
- Seed treatment with acibenzolar-S-methyl consistently activated resistance against
- Fusarium wilt of cotton, although the effects were not major when disease severity was moderately low
- Acibenzolar-S-methyl increased seedling establishment in one experiment in a field infested with the Fusarium wilt pathogen but not in other experiments.
- Acibenzolar-S-methyl did not activate resistance against Verticillium wilt of cotton
- The potential for an extended, active 'shelf life' of acibenzolar-S-methyl, when applied to seed in combination with the standard fungicides, was demonstrated

- Foliar application of BrotomaxTM and salicylic acid was ineffective against Fusarium wilt and Verticillium wilt of cotton
- Seed treatment with plant hormones was ineffective against Fusarium wilt of cotton

In-furrow fungicides

- In-furrow application of several fungicides did not control seedling disease
- The benefit to growers from using in-furrow fungicides that are registered for control of seedling disease will depend upon the relative disease pressure exerted by their target pathogens in any given year or field
- In-furrow application of the fungicides benomyl, triadimenol and toclofos methyl was ineffective in controlling Fusarium wilt

Biofumigation

- A long-term trial of biofumigation with vetch and canola at a site with a low level of *T. basicola* in the soil was commenced at the Australian Cotton Research Institute
- In trial at Hillston, common vetch (Vicia sativa) appears not to have biofumigation potential for black root rot although cold winter conditions may have prevented sufficient growth
- Vetch, mustard and canola were increased the severity of Fusarium wilt in trials at Boggabilla and should not be used as biofumigation crops on farms with Fusarium
- wilt
- Mustard meal and mustard oil were not effective as biofumigation agents against Verticillium wilt or Fusarium wilt

Interactions

Plant-pathogen-soil interactions

- Transects of a field with varying soil type suggest that some soils may be less conducive to development of Fusarium wilt of cotton
- Symptoms of black root rot on tap roots develop slowly during the first two weeks after sowing, reach a plateau level of infection from three to five weeks and then decline as the tap root expands with warmer conditions
- Any factor that slows cotton growth may give the impression that black root rot is more severe if it delays the sloughing of blackened cells in the outer layers of cotton tap roots
- The peak activity of the black root rot pathogen, *T. basicola*, and seedling pathogens are mutually exclusive, providing further evidence that *T. basicola* does not kill cotton seedlings
- Mycorrhizal colonisation was correlated negatively with both symptoms of black root rot on tap roots and production of spores by *T. basicola* on lateral roots
- Mycorrhizal fungi survived long bare fallows of 28 and 35 months in substantial numbers in fields at Bourke
- Mycorrhizal fungi survived a bare fallow of four years in substantial numbers in a field experiment at the Australian Cotton Research Institute

Transgenic disease resistance

- One of the cotton lines transformed with defensin genes exhibited enhanced resistance against the black root rot pathogen, *T. basicola*, and reduced its reproductive potential.
- Transformation with the defensin genes appears to have had no adverse effects on mycorrhiza development in the transformed lines.