4.2 Experiments

4.2.1 *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 superimposed 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

4.2.2 *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.

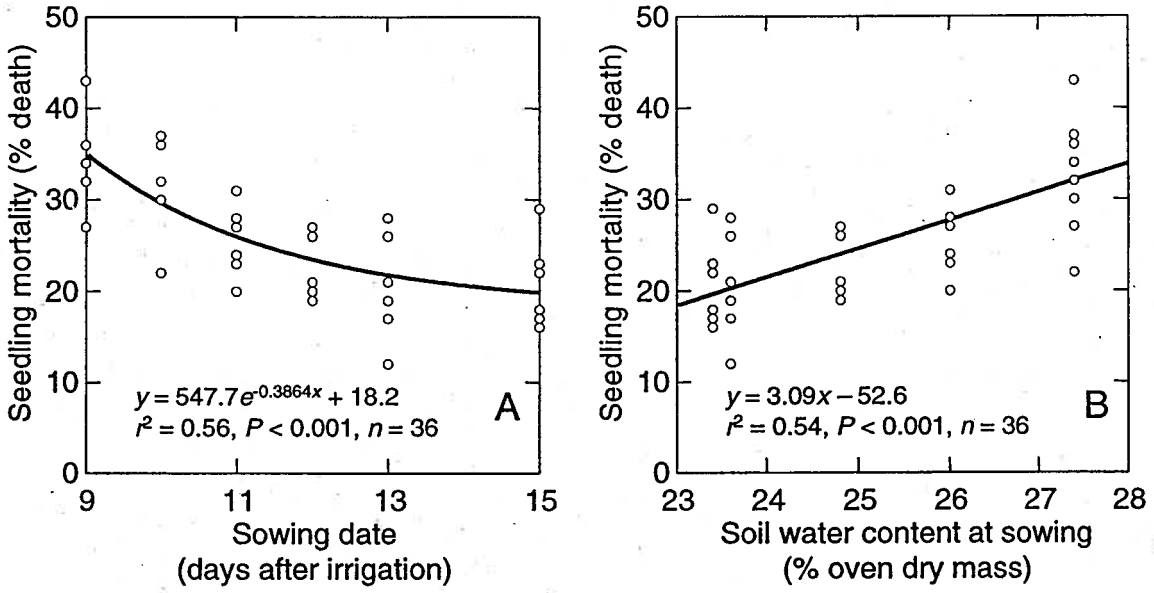

Figure 17. Seedling mortality of cotton (var. 289RRi), sown at various dates after irrigation in a field at the Australian Cotton Research Institute in 2003, decreased as sowing was delayed after irrigation (A) and increased with increasing soil moisture content (B)

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.

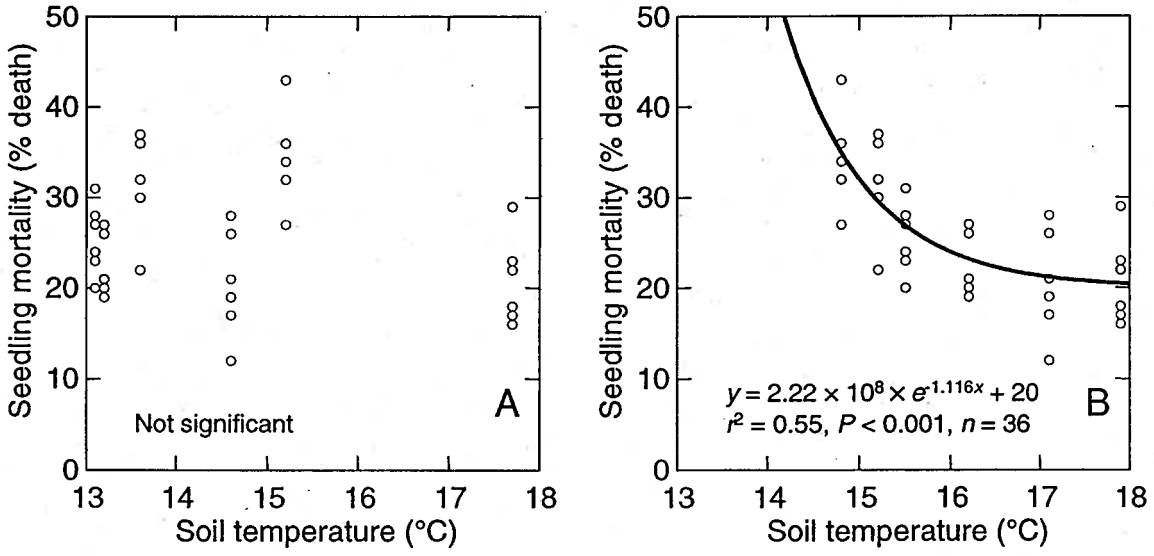

Figure 18. Seedling mortality of cotton (var. 289RRi), sown at various dates after irrigation in a field at the Australian Cotton Research Institute in 2003, was not related to soil temperature at 9:00 am on the day of sowing (A) but decreased as the mean of soil temperatures at 9:00 am on the day of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

4.2.3 *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 Dynasty™ 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 Peridiam™. The untreated control received Peridiam™ 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).

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

Table 9. 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).

Table 8. 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.

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 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

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.

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 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™	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™	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™	51 ef
Dynasty™	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 Dynasty™ 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 Dynasty™

Seed treatment fungicide	Seedling mortality (% death)
PCNB + metalaxyl-M	31
Dynasty™	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 Dynasty™ 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

4.2.4 *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 °C and the minima by an average of 0.3 °C (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 1.2°C and the minima by 0.8°C 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 3.7°C and 3.2°C 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).

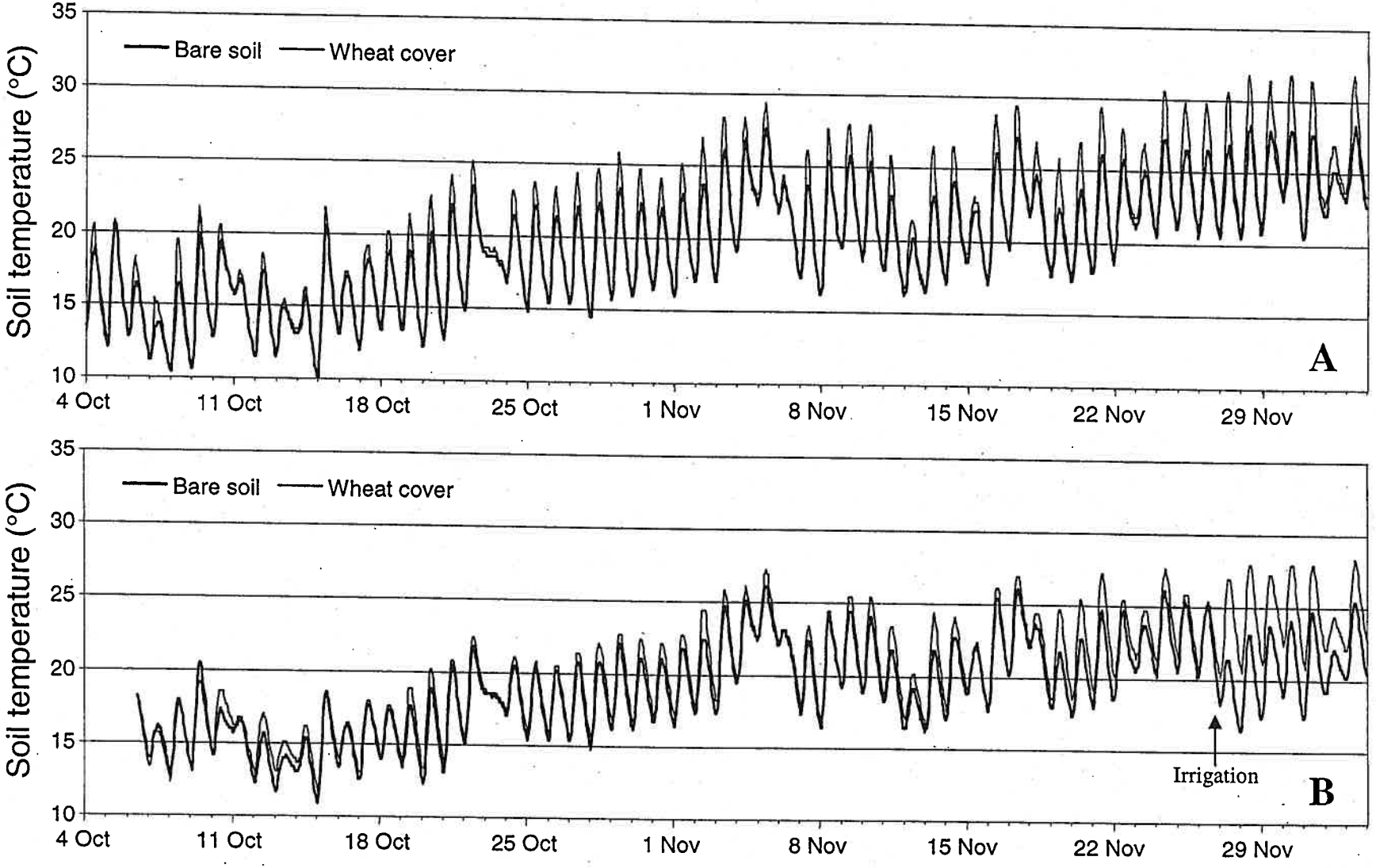

Figure 19. Effect of cover crops, of wheat on the shoulders of the bed, on soil temperature in two cotton crops near Hillston in the 2001-02 season, with (A) replicated small plots of shoulder wheat and (B) a single large plot of shoulder wheat

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).

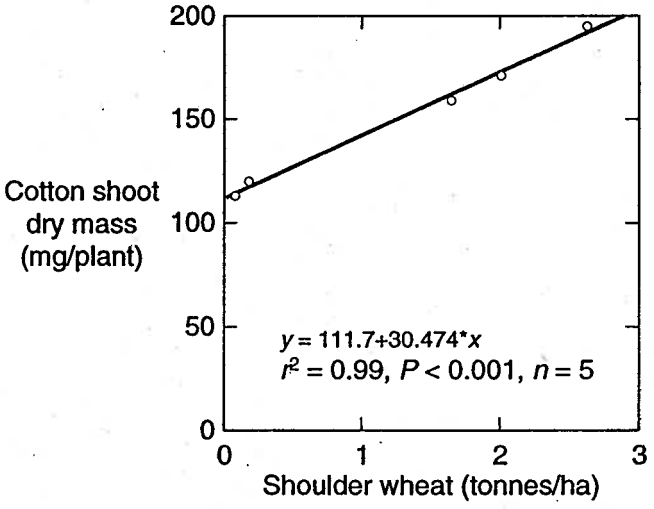

Figure 20. Cotton growth increased according to the dry mass of a cover crop of wheat on the shoulders of the bed, in a field at the Australian Cotton Research Institute in 2002-03.

4.2.5 *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 Brotomax™ 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-S-methyl 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).

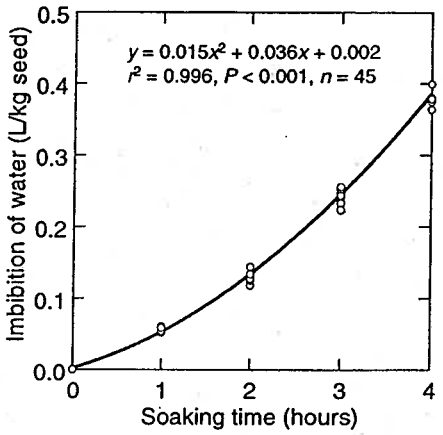

Figure 21. Imbibition of water by cotton seed (cv. Sicala V2) over time (data estimated gravimetrically and pooled for two studies)

In previous experiments, soaking cotton seed for three hours in a solution of acibenzolar-S-methyl (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).

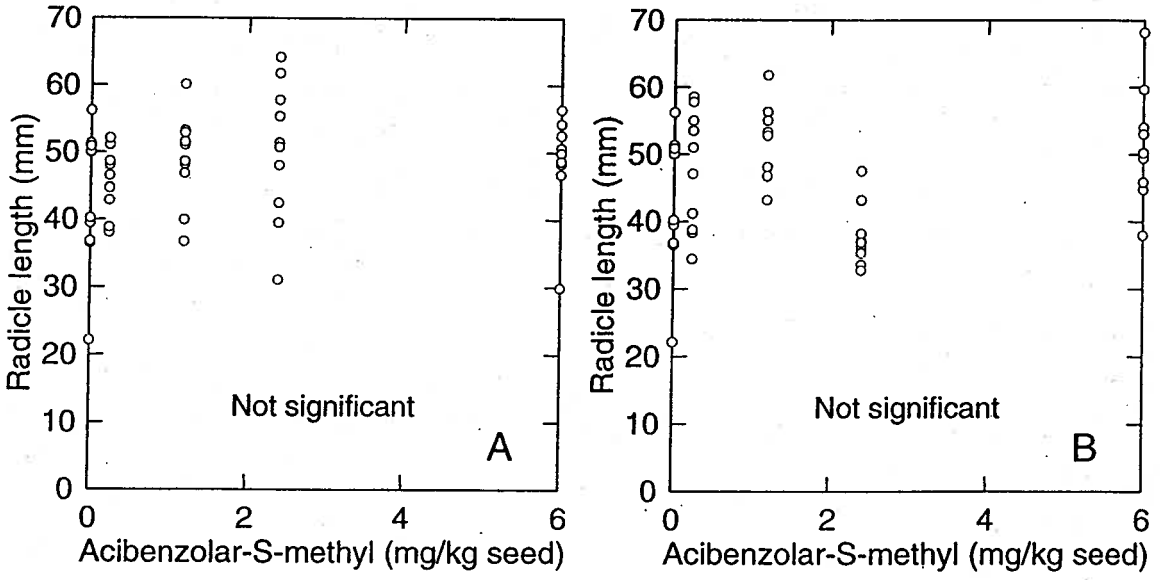

Figure 22. Application of acibenzolar-S-methyl, formulated as Bion® (A) or Boost® (B), to cotton seed (cv. Sicot 189) with the standard seed treatment fungicides (PCNB and metalaxyl-M) had no effect on germination and radicle growth under sterile conditions.

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-S-methyl (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-S-methyl (Bion™) 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 (Bion™) seed treatment on Fusarium wilt in cotton in a field near Boggabilla in the 2001-02 season

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-S-methyl (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. Benlate™ at 600 g/ha); triadimenol (Baytan™ C FS at 330 mL ha); toclofos methyl (Rizolex™ at 120 g/ha). The fungicides and acibenzolar-S-methyl 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)	[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.

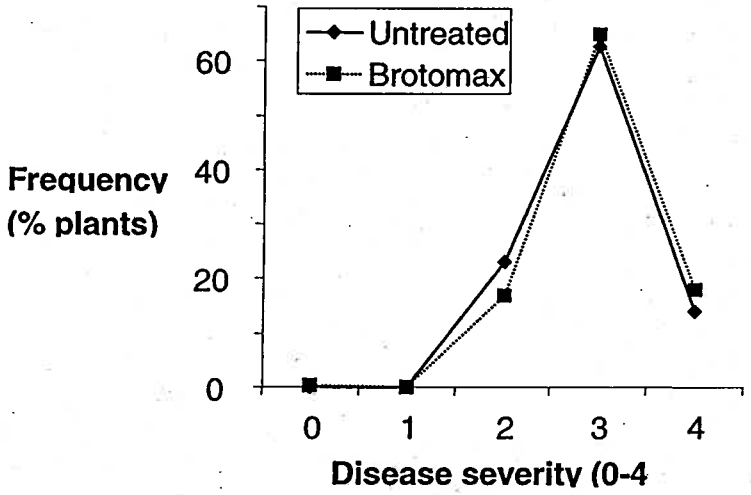

Figure 23. Severity of Verticillium wilt in cotton in a field on the Breeza plain, 2002

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

Plant hormones 2003-04

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-S-methyl, 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, Peridiam™ blue) and sown in single-row, 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-S-methyl, 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.

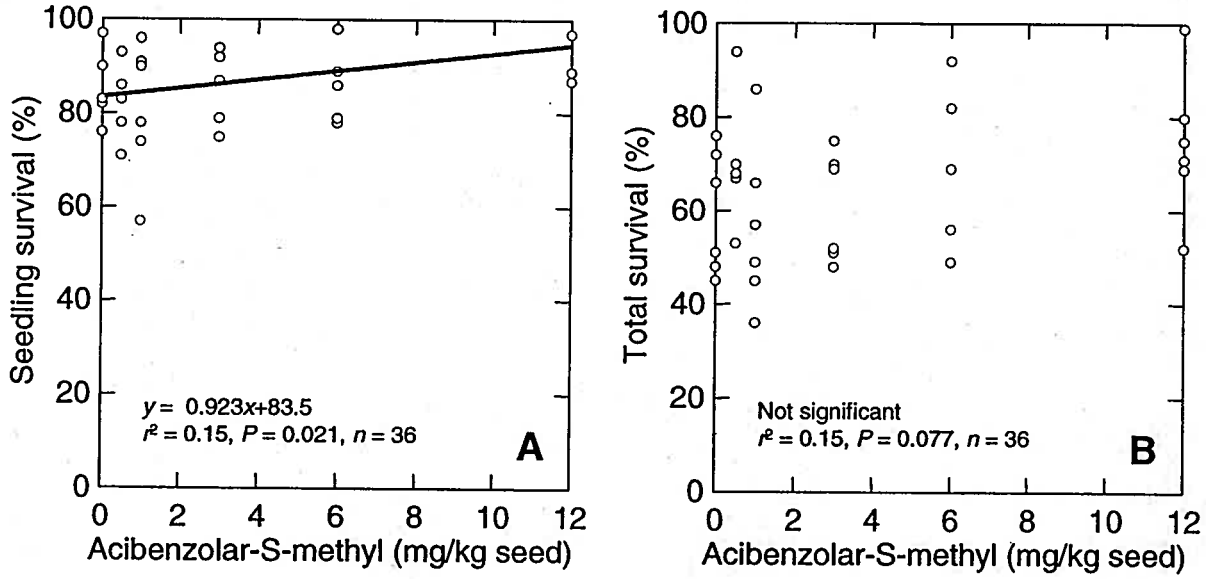

Figure 24. Application of acibenzolar-S-methyl to cotton seed at sowing increased survival of cotton seedlings in the original plants stand, through to the end of the season (seedling survival, A), but had no effect on the proportion of the original plant stand that had survived all season with little 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, Peridiam™ 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 Brotomax™ 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 Brotomax™ 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™ (20 mL/kg seed)	11.6
	Not significant ($P = 0.927$)

4.2.6 *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).

4.2.7 *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).

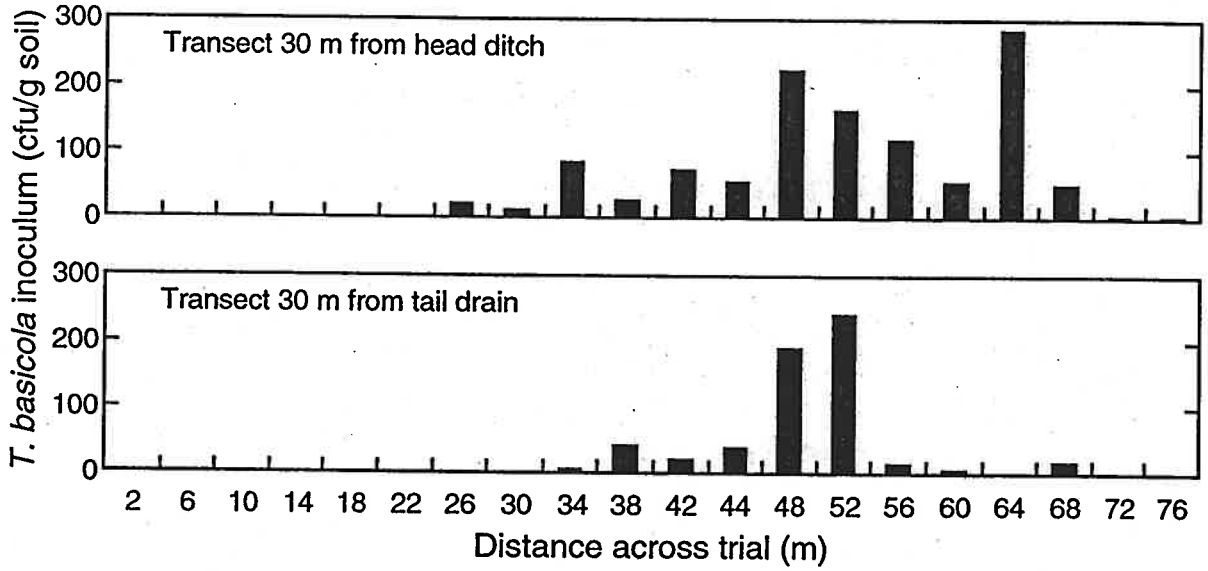

Figure 25. Initial distribution of inoculum of *T. basicola* across the trial site used in a long-term biofumigation experiment at ACRI (soil sampled 20 June 2002).

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)
Bare	Wheat	Bare
Wheat	Canola	Vetch
Bare	Bare	Wheat
Vetch	Vetch	Canola
Canola	Vetch	Canola
Bare	Wheat	Bare
Wheat	Canola	Vetch
Canola	Bare	Wheat
Vetch	Vetch	Canola
Wheat	Canola	Vetch
Canola	Bare	Wheat
Bare	Vetch	Canola
Vetch	Canola	Vetch
Vetch	Wheat	Wheat
Wheat	Bare	Bare
Bare		
Canola		
Canola		
Bare		
Vetch		
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)
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.

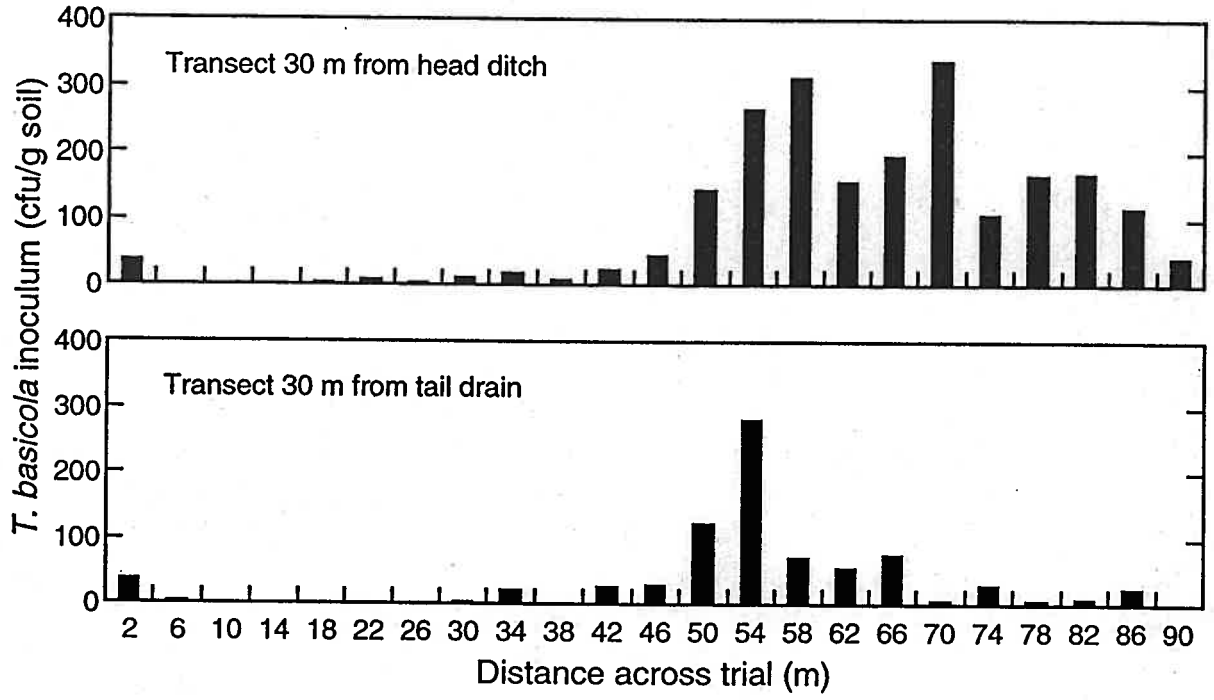

Figure 26. Distribution of inoculum of *Thielaviopsis basicola* across the trial site used in the long-term biofumigation experiment at ACRI (soil sampled 8 July 2003)

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	[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	[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)

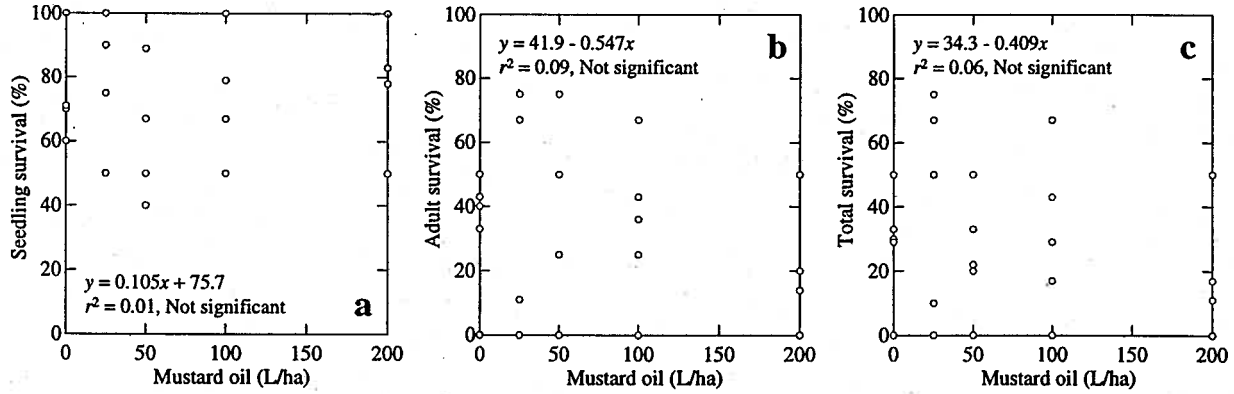

Figure 27. Lack of effect of increasing rates of application of mustard oil to the soil on the severity of Fusarium wilt of cotton in an infested field near Boggabilla in 2002-03. Seedling survival is the percentage of the original plant stand, in October, that was still alive at the end of the season. 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.

4.2.8 *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).

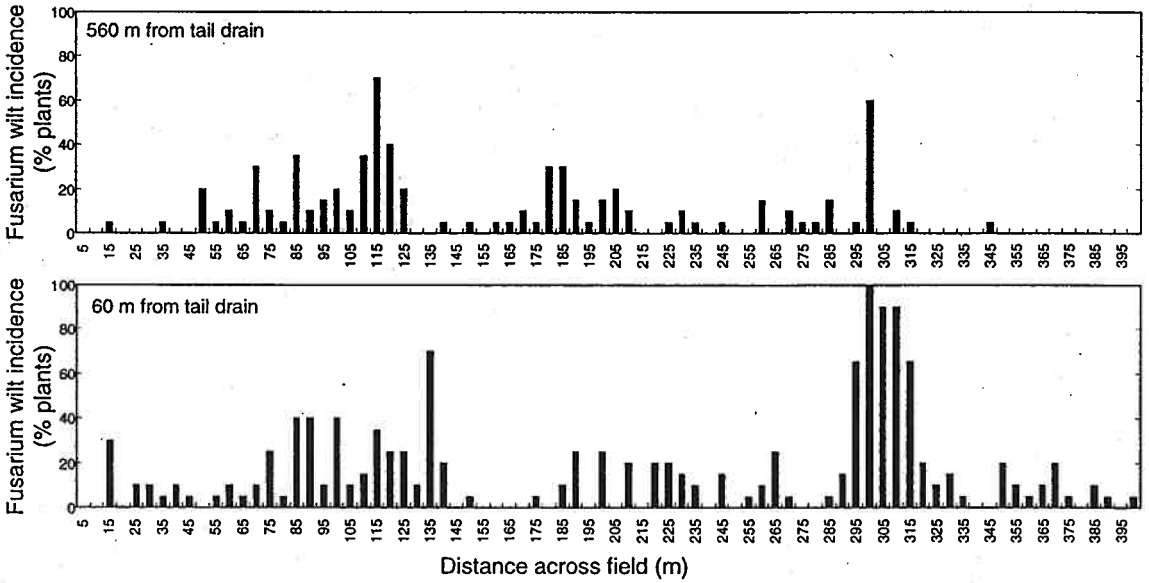

Figure 28. Incidence of Fusarium wilt of cotton in two transects of a field near Boggabilla in March 2003

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.

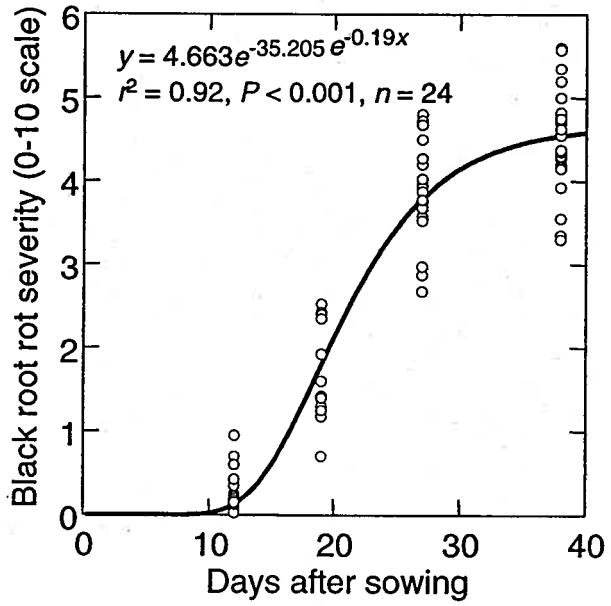

Figure 29. Progress of black root rot of cotton in a commercial cotton field near Wee Waa in the 2003-04 season.

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).

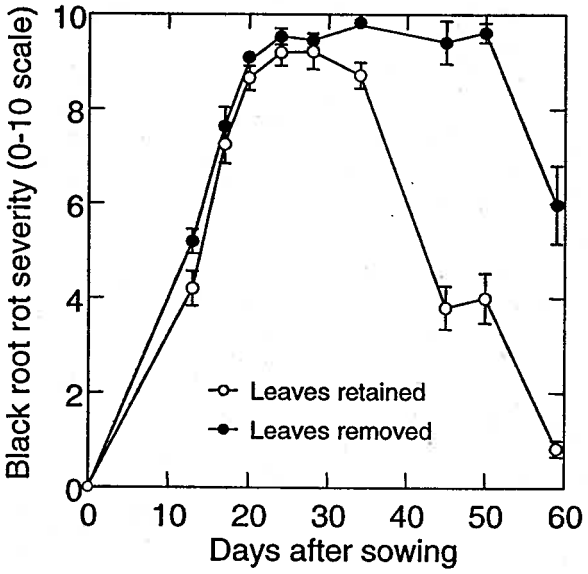

Figure 30. Progress of black root rot of cotton in a field at the Australian Cotton Research Institute in the 2003-04 season, with and without trimming of the leaves at each assessment.

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.

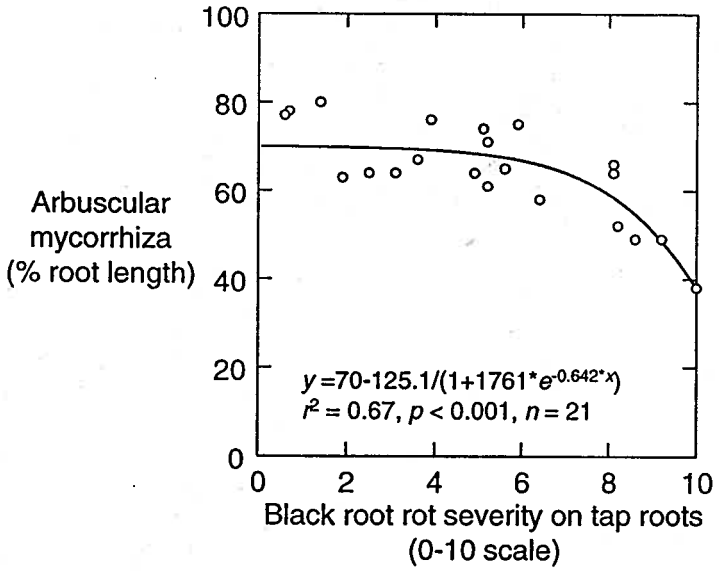

Figure 31. Colonisation of cotton roots by arbuscular mycorrhizal fungi declined as the severity of black root rot on the tap roots increased, especially when more than half the tap root was affected (>5 on 0-10 scale), in the Cotton CRC Farming Systems Trial at Warren on 21 November 1998.

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.

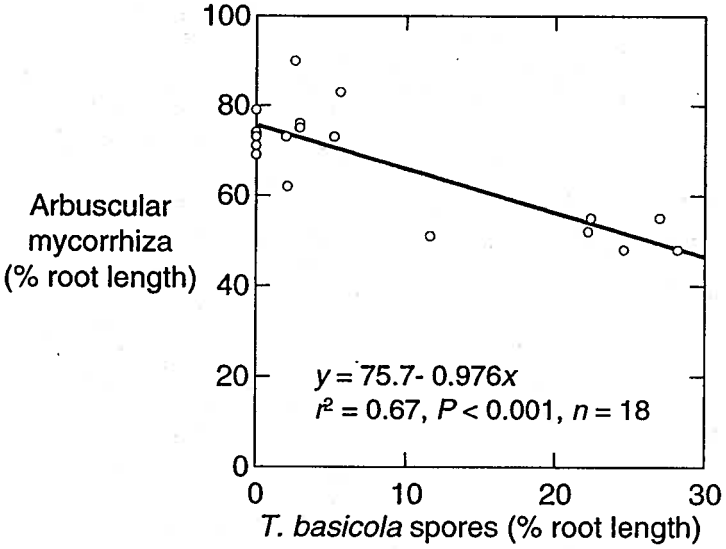

Figure 32. Colonisation of cotton roots by arbuscular mycorrhizal fungi declined as the severity of black root rot on the tap roots increased, especially when more than half the tap root was affected (>5 on 0-10 scale), in the Cotton CRC Farming Systems Trial at Warren on 21 November 1998.

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.

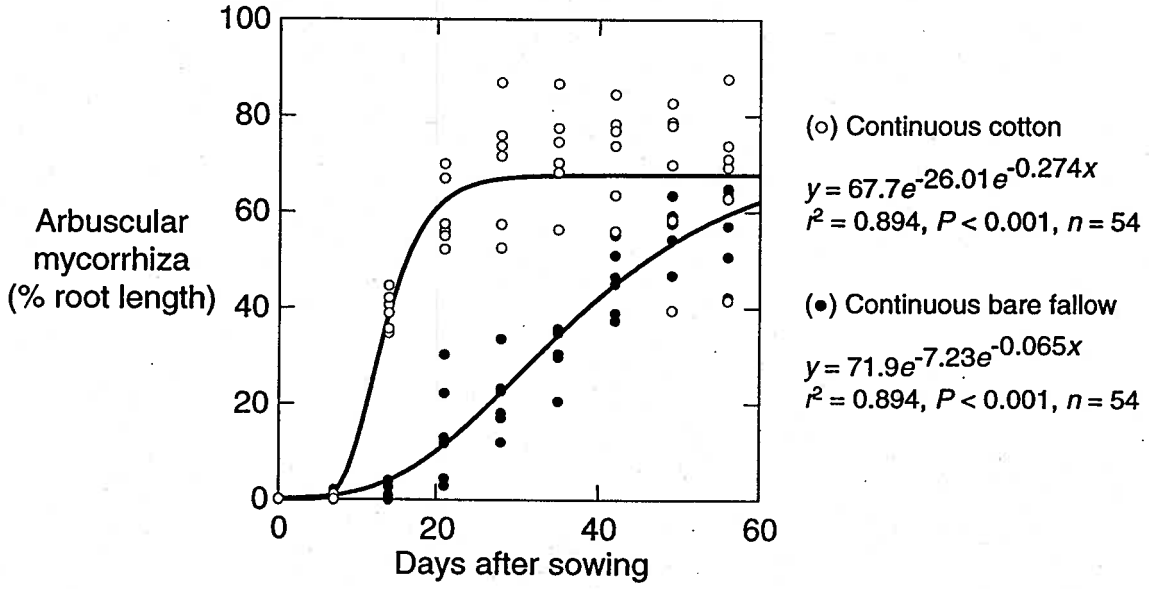

Figure 33. Delayed colonisation of cotton in bioassays of the infectivity of soil collected April 2004 from plots with either continuous cotton or four years of weed-free fallow, in a field at the Australian Cotton Research Institute

4.2.9 *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.

This section presents the results of a report prepared for Dr Robyn Heath, University of Melbourne. This report is presented as an Appendix in the CRDC project MU1C *Transgenic cotton for the control of Fusarium wilt.*

Assessment of the impact of defensin genes in transgenic cotton on black root rot and mycorrhizas

A study conducted in collaboration with the University of Melbourne as part of the CRDC project MU1C "Transgenic cotton for the control of Fusarium Wilt"

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Introduction
Black root rot of cotton, caused by *Thielaviopsis basicola*, occurs in all major cotton growing regions in Australia (Nehl et al. 2004). The fungus produces thick-walled chlamydospores that are very persistent in the soil. The severity of the disease increases with each crop of cotton, irrespective of fallows and rotation with non-host crops. The disease is pandemic in Australia (Nehl et al. 2004) and there are no effective controls. The objective of the research presented here was to evaluate the potential for control of black root rot by transformation of cotton with defensin genes from tobacco. Given that cotton is highly dependent on symbiosis with arbuscular mycorrhizal fungi, the potential for the defensin genes to alter mycorrhizal development was also evaluated.

Materials and methods

General
Seeds of three lines of transgenic cotton (cv. Coker) and one of non-transgenic cotton (cv. Coker) were supplied by Dr Robyn Heath, University of Melbourne. The four lines of cotton were labelled A, B, C and D before dispatch from Melbourne so that their identity remained anonymous for the duration of the experiments. Line A was the untransformed control and the other three lines were transformed with a defensin gene (Hexima Ltd, Melbourne). In the PC2 laboratory at the Australian Cotton Research Institute (ACRI), the seeds were sorted manually to remove visibly damaged seed (which was then destroyed by autoclaving) and to remove exceptionally small or large seeds. The seeds were sealed in zip-lock plastic bags before transferring to the PC2 glasshouse.

Soil that was naturally infested with *Thielaviopsis basicola* was collected from the centre of the bed (0-15 cm in depth) in field C4 at the Australian Cotton Research Institute (ACRI) at a position approximately 50 m from the tail drain on 9 April 2003. The soil was passed through a sieve (aperture 12mm), transferred to a concrete mixer and homogenized, and stored in open plastic bags in the shade until use. On 10 April half of that soil was steamed for two hours (70-80°C). Pots (polystyrene foam cups, 750 mL volume, 95 mm inside-diameter at the top, 175 mm high, with three drain holes punched in the base) were filled to within approximately

2 cm of the top with unsteamed soil on 10 April, and with steamed soil on 14 April. Half of these pots were transferred to a PC2 glasshouse at ACRI for the first experiment and half were stored for a week before transfer to the PC2 glasshouse for the second experiment. The temperature control of the glasshouse was set to run at 18 to 23°C on a 12 hour day/night cycle.

Experiment 1

The experiment was a 2 ×4 × 2 factorial design with five replicates, as follows: steamed or unsteamed soil × four cotton lines × two harvest dates, at 21 and 35 days after sowing (DAS). On 15 April 2003, five seeds were sown in each pot using a seed-dibber to obtain a uniform depth of 40 mm from the top of the pot (dibber described by Nehl, 1996, PhD thesis). The pots were placed in plastic trays (150 mm deep) and watered by filling the tray with water (filtered bore water from ACRI) to a depth of 5-6 cm and left to soak overnight to ensure that all the soil was wet. The pots were subsequently watered at 3, 5, 7, 9, 16, 19, 22, 25, 28 and 31 days after sowing (DAS), by filling the trays with water to 2 cm deep and allowing them to soak for approximately 10 minutes. After each watering, the pots were transferred back to the glasshouse bench, which allowed free drainage. Water was withheld between 9 and 16 DAS due to cloudy weather that slowed the rate of drying of the soil. To prevent cross-contamination separate trays were used for watering the steamed and unsteamed soil. At 7 DAS the seedlings were thinned by pulling them from the soil, leaving two plants in each pot. Any seedlings that subsequently emerged were also removed and the date of emergence was recorded.

In the harvest at 21 DAS, most of the soil mass was removed from the pots and carefully teased away from the roots, with the shoots still attached. The roots and adhering rhizosphere soil were inserted into a sterile flat bottle, the shoots were cut from the roots at a point equivalent to soil level (the end point of chlorophyll in the stem) and the bottle (previously tared) and roots were weighed to determine the mass of roots and soil. The bottles were capped and transferred to the PC2 laboratory and 95 mL of sterile water agar (1 g/L) was added. The bottles were re-capped, shaken vigorously (15 min) and a 1 mL aliquot of this suspension .was dispensed into sterile 9 cm Petri dishes (8 replicates). The concentration of inoculum of *T. basicola* in the aliquot was determined by adding 25 mL of a selective medium, TBCEN agar (Nehl et al. 2004). The roots in the bottle were then washed carefully and, for each plant, the percentage length of the tap root with characteristic blackening was estimated on a scale of 0 to 10, where 0 = no blackening, 1 was >0 and <=10 %, 2 was >10 and <=20%, and so on. The roots were then bunched longitudinally, cut at intervals of approximately 1 cm, and a sub-sample of root pieces (0.3 to 0.5 g fresh weight) was transferred to a vial and sufficient ethanol (70 %) was added to completely submerge the root sample. The root were later cleared and stained (Koske and Gemma, 1989) and the percentage of the roots, by length, with arbuscules and/or chlamydospores was estimated by the gridline intersect method of Giovannetti and Mosse (1980). The shoots were weighed and dried in an oven (70°C, 48 h) before determining their dry mass.

In the harvest at 42 DAS, the plants were removed from the soil by submerging the pots horizontally in a bucket of clean water, such that air could just enter the top of the pot, and gently agitating until the soil such that it washed into the bucket with the majority of the root system kept intact by supporting it on one hand under the water. The severity of black root rot, percentage of the roots with arbuscules and/or chlamydospores, and shoot mass were assessed in the same manner as in the harvest at 21 DAS.

Experiment 2

This was a repeat of Experiment 1. The cotton was sown on 22 April 2003, and watered at 3, 9, 12, 15, 21, 24, 27, 30 and 33 days after sowing (DAS). The methods were otherwise the same as in Experiment 1. Mycorrhizal colonisation of cotton in line D and line C at 42 days in Experiment 2 could not be assessed due to a problem with the staining procedure, which resulted in plasmolysis of cortical cells and uptake of the stain by these cells. This problem did not affect assessment for the presence of chlamydospores of *T. basicola.*

Statistical analysis

Two way analysis of variance (AOV) was conducted using the program Systat (Version 10, Hearne Scientific) with cotton line and soil treatment as factors. For those parameters that were reduced to zero (i.e. no variance) in steamed soil, a one way AOV was conducted on the data from unsteamed soil only.

Results and discussion

Cotton seedlings emerged faster in the first experiment than in the second experiment (Figure 34), probably because cool overcast weather was experienced during the first week after sowing in Experiment 2 but not in Experiment 1 (Figure 35). For each cotton line, emergence at 11 days after sowing in steamed soil was not significantly different to that in unsteamed soil, except for Line C, which established poorly in Experiment 1 (Tables 40 and 41). In steamed soil in Experiment 1, emergence was greatest in Line D and the least in the control, Line A (Table 40). The same pattern of emergence in steamed soil was also observed in Experiment 2 (Table 41). These differences probably reflect variation in the vigour of the different cotton lines, as pathogens were largely absent in the steamed soil. However, line D emerged at a faster rate than the control, especially in Experiment 2 (Figure 34).

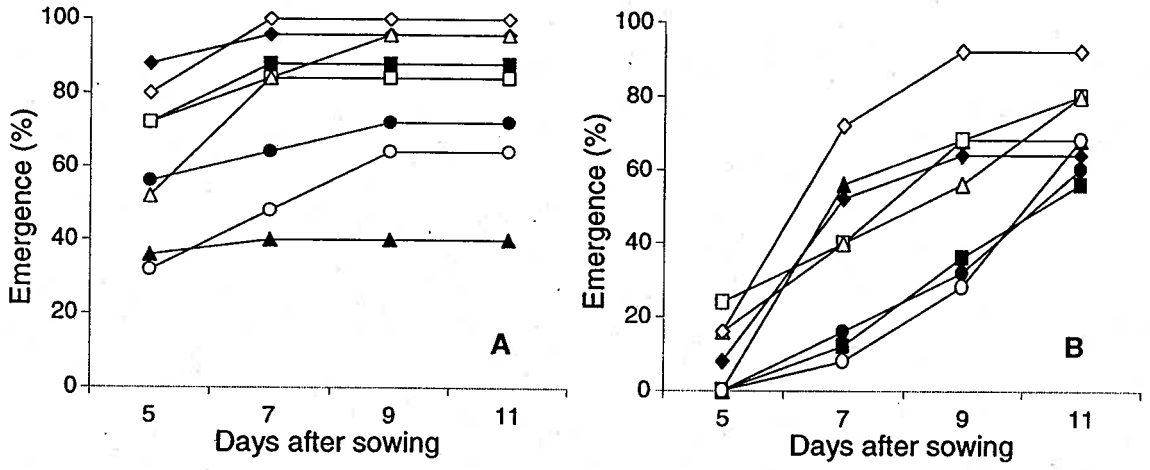

Figure 34. Emergence of experimental lines (● A, ■ B, ▲ C, ◆ D) of cotton (cv. Coker) transformed with defensin genes, and sown in steamed (open symbols) or unsteamed (closed symbols) potted soil, in Experiment 1 (A) and Experiment 2 (B)

In both Experiments, cotton growth was increased dramatically by steaming the soil (Tables 40 and 41; Figure 36). The steaming process completely eliminated *T. basicola* from the soil, as no disease was observed in steamed soil in Experiment 1. In Experiment 2, a small amount of unsteamed soil was accidentally used to 'top-up' the height of soil in pots prior to sowing and this contamination resulted in a small amount of disease in some plants in the steamed soil (Table 41). This contamination was not substantial enough reduce the growth of cotton in the steamed soil treatment (Table 41, Figure 2).

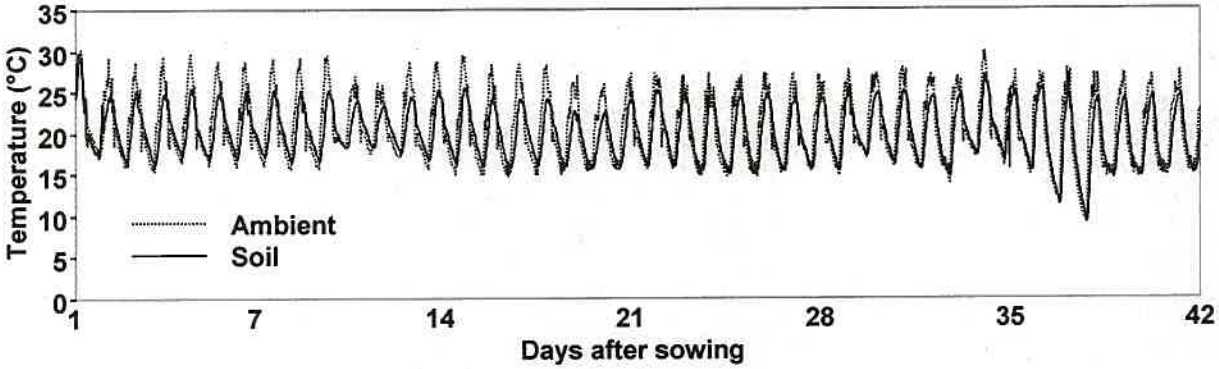

Figure 35. Temperatures in the glasshouse during Experiment 1

Some differences in susceptibility to black root rot were observed among the four lines but these differences were not consistent, especially in lines B and C. In the unsteamed soil in Experiment 1, the disease index was not significantly different among the four cotton lines at both 21 and 42 days after sowing (Table 40). At 21 days in Experiment 2, the severity of black root rot was lower in line B than in the control (line A) but by 42 days it was higher (Table 41).

Figure 36. Cotton in steamed (LHS) and unsteamed (RHS) soil at 35 days after sowing in Experiment 2

Line D gave the most consistent indication of enhanced resistance due to transformation. In both experiments, the presence of chlamydospores of *T. basicola* on the roots at 42 DAS was substantially lower in line D than in the control (Tables 40 and 41). This difference wasn't reflected by a significantly lower density of inoculum in the selective-medium assay. However, chlamydospores are important for survival of the pathogen in the field and the selective medium may have detected less-persistent propagules, such as endospores. In Experiment 2 at 42 DAS, line D had greater root and shoot growth and a reduced severity of symptoms on tap roots, than in the control. It could be argued that Line D happens to have greater vigour than the control because in the steamed soil line D's dry mass at 21 DAS was greater than that of the control in both experiments. However, this difference in dry mass was not apparent at 42 DAS (Tables 40 and 41), suggesting that the lower level of disease in line D, at 42 DAS in Experiment 2, resulted in improved seedling growth.

Arbuscular mycorrhizal colonisation was not significantly different among the four cotton lines, except at 42 days in Experiment 2 (Tables 40 and 41). Overall, the degree of colonisation by arbuscular fungi was relatively low in comparison to that which occurs in the field (Nehl et al. 1996). However, the temperatures in the glasshouse were set to favour black root rot. The mean soil temperature was 20°C, which was likely to have been too cool for rapid mycorrhizal colonisation of cotton (Smith and Roncadori, 1986).

In summary, there is evidence that one of the lines transformed with defensin genes exhibited enhanced resistance against the black root rot pathogen, *T. basicola*, and reduced its reproductive potential. Furthermore, transformation with the defensin genes appears to have no adverse effects on mycorrhiza development in the transformed lines. Further assessment of the potential for these genes to control black root rot is warranted, particularly in Line D.

Acknowledgements
Sincere thanks are extended to Mr David Shann and the Cotton Production Unit of CSIRO Plant Industries for assistance with access to the PC2 glasshouse and to Tracey Mor and Peter Lonergan for providing technical assistance. Financial assistance from the Cotton Research and Development Corporation and NSW Department of Primary Industries is gratefully acknowledged.

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Table 40. Growth, mycorrhizal colonisation and development of black root rot (caused by *Thielaviopsis basicola*) in experimental lines of cotton (cv. Coker) transformed with defensin genes, in steamed and unsteamed potted soil (Experiment 1)

	Steamed soil				Unsteamed soil				
	Line A	Line B	Line C	Line D	Line A	Line B	Line C	Line D	Probability
11 DAS									
Emergence (%)	48c	84ab	84ab	100a	64bc	88ab	40c	96ab	$P \leq 0.026$
21 DAS									
Shoot fresh mass (g/plant)	1.4ab	1.5ab	1.2bc	1.6a	0.83de	0.69e	0.74e	1.1cd	$P \leq 0.018$
Shoot dry mass (g/plant)	0.23bc	0.24ab	0.20cd	0.28a	0.17de	0.14e	0.14e	0.21bc	$P \leq 0.034$
Root fresh mass (g/plant)	2.5ab	2.1b	2.3ab	2.8a	1.2cd	0.8d	0.9cd	1.4c	$P \leq 0.032$
Disease severity (0-10 scale)	0	0	0	0	7.8	7.1	5.2	8.1	NS
T. basicola inoculum (cfu/g rhizosphere soil)	0	0	0	0	155b	361a	201ab	109b	$P \leq 0.009$
T. basicola inoculum (cfu/g root)	0	0	0	0	709b	1337a	738b	504b	$P \leq 0.010$
T. basicola chlamydospores (% root length)	0	0	0	0	4	4	1	3	NS
Arbuscular root (% root length)	0	0	0	0	11	8	10	8	NS
42 DAS									
Shoot fresh mass (g/plant)	2.46b	2.75a	2.47ab	2.74a	0.73d	0.82cd	0.71d	1.06c	$P \leq 0.046$
Shoot dry mass (g/plant)	0.67a	0.66a	0.55b	0.64a	0.18c	0.21c	0.09d	0.24c	$P \leq 0.037$
Root fresh mass (g/plant)	2.69ab	2.85ab	2.56b	3.12a	0.65c	0.85c	0.71c	1.04c	$P \leq 0.009$
Disease severity (0-10 scale)	0	0	0	0	10	10	10	9	NS
T. basicola chlamydospores (% root length)	0	0	0	0	22a	20a	11b	12b	$P \leq 0.043$
Arbuscular root (% root length)	0	0	0	0	21	28	22	28	NS

Values in rows followed by the same letter are not significantly different by pairwise comparison of means with Fisher's LSD test at the state probability level. NS = not significant, DAS = days after sowing

Table 41. Growth, mycorrhizal colonisation and development of black root rot (caused by *Thielaviopsis basicola*) in experimental lines of cotton (cv. Coker) transformed with defensin genes, in steamed and unsteamed potted soil (Experiment 2)

	Steamed soil					Unsteamed soil				
	Line A	Line B	Line C	Line D		Line A	Line B	Line C	Line D	Probability
11 DAS										
Emergence (%)	68ab	80ab	80ab	92a		60b	56b	68ab	64ab	$P \leq 0.029$
21 DAS										
Shoot fresh mass (g/plant)	1.45b	1.44b	1.31b	1.82a		0.81cd	0.72d	0.77cd	0.92c	$P \leq 0.027$
Shoot dry mass (g/plant)	0.20b	0.22b	0.18bc	0.29a		0.14de	0.11e	0.14de	0.16cd	$P \leq 0.030$
Root fresh mass (g/plant)	1.91b	1.69bc	1.46c	2.60a		0.82d	0.69d	0.62d	0.86d	$P \leq 0.044$
Disease severity (0-10 scale)	1.0d	0.7d	0.5d	1.9cd		7.0a	2.9bcd	4.2abc	5.4ab	$P \leq 0.039$
T. basicola inoculum (cfu/g rhizosphere soil)	5c	35bc	49bc	47bc		135a	88ab	69b	72ab	$P \leq 0.043$
T. basicola inoculum (cfu/g root)	16b	109ab	133ab	119ab		370ab	348ab	511a	406ab	$P = 0.022$
T. basicola chlamydospores (% root length)	0	0	0	0		11	6	11	13	NS
Arbuscular root (% root length)	0	0	0	0		9	9	12	16	NS
42 DAS										
Shoot fresh mass (g/plant)	3.1a	3.2a	3.3a	3.2a		1.9b	2.0b	1.3c	2.1b	$P \leq 0.019$
Shoot dry mass (g/plant)	0.48a	0.52a	0.52a	0.51a		0.20cd	0.22bc	0.11d	0.29b	$P \leq 0.049$
Root fresh mass (g/plant)	1.9ab	2.0a	1.8ab	2.2a		1.0c	1.0c	0.7c	1.5b	$P \leq 0.038$
Disease severity (0-10 scale)	0.5d	0.8d	0.1d	0.8d		6.8b	6.1b	8.5a	4.5c	$P \leq 0.024$
T. basicola chlamydospores (% root length)	2c	4abc	3bc	2bc		7.8a	6.1ab	5.0abc	2.2bc	$P \leq 0.027$
Arbuscular root (% root length)	3c	2c	1c	3c		15b	20a	ND	ND	$P \leq 0.034$

Values in rows followed by the same letter are not significantly different by pairwise comparison of means with Fisher's LSD test at the state probability level. NS = not significant. DAS = days after sowing, ND = not determined

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5. CONCLUSIONS (RESEARCH OUTCOMES VERSUS 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.**

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.

6. COTTON R&D CORPORATION OUTPUTS

The research conducted in this project has contributed to the Corporation's economic, environmental and social outputs by modifying the integrated disease management (IDM) strategy for 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 in-furrow 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 Dynasty™ 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-S-methyl 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

7. PROJECT 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 Dynasty™ 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 Brotomax™ 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.

7.1 Technical advances

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.

7.2 Other developments

7.3 Changes to intellectual property register

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.

8. FURTHER DEVELOPMENT

8.1 Technological

The design concept for a trash retaining drop box (commercial in confidence) will require engineering development and testing in the field.

Acibenzolar-S-methyl requires further evaluation to determine the rates and application methods that will optimise its potential for activation of resistance in cotton against black root rot.

8.2 Outcome deployment

It is anticipated that revised disease management guidelines, incorporating results from CRDC projects DAN153C, DAN154C, DAN176C and DAN177C, will be prepared and endorsed for release at the Australian Cotton Conference in August 2006.

Revisions to the existing management strategy for seedling disease, black root rot and Fusarium wilt, previously published in the CRDC guidelines, *Integrated Disease Management* in 2002, have been formulated to include recommendations (highlighted in bold font) arising from the research conducted in this project and CRDC projects DAN122C and DAN153C, as follows.

Control strategy for seedling disease

PLANNING

- Use a variety with good seedling vigour
- Use effective seed treatment fungicides

GROUND PREPARATION

- Plant into well prepared, high, firm beds
- Carefully position fertiliser in the bed – not under the plant line!

PRE-PLANTING

- Plant into moisture rather than planting dry and watering-up

AT PLANTING

- **Plant as late as possible, within the planting window, to minimise the chances of exposure to cool wet conditions that favour disease**
- **After pre-irrigation, delay planting until soil water content is at the lower end of the range needed for stand establishment**
- **Time** planting **to coincide with soil temperatures >15C in the first week**
- Be careful with the use of herbicides at planting

ROTATIONS

- Incorporate rotation crop residues as soon as possible after harvest (especially legume crop residues)
- **Incorporate green-manure and biofumigation crops at least four weeks before sowing cotton**

Control strategy for black root rot

PLANNING

- Choose varieties that have the capacity to 'catch up' later in the season, **including genetically modified varieties that have high fruit retention**
- **Pending it's registration for use on cotton against black root rot, treat all seed sown with Boost® (a.i. acibenzolar-S-methyl)**
- **Design or modify irrigation reticulation systems to return water from infested fields directly to the storage used for their supply or install settling ponds before on-flow to other fields.**

GROUND PREPARATION

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

EARLY SEASON

- **Plant as late as possible, within the planting window, to minimise the period of peak symptoms**
- If choosing to sow early, sow when temperatures are warm and rising (a soil temperature of 16°C is OK, 20°C is better), temperature measurements should be taken in the fields where black root rot occurs.
- Replanting decisions should be made on the basis of stand losses, not the size of the seedlings.
- Watch for early onset of water stress (i.e. because the root system is weak) and irrigate accordingly, but avoid waterlogging.

LATE SEASON

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

AFTER HARVEST AND AT ALL TIMES

- Practice good farm hygiene. Farmcleanse (used at 10%) is effective against *T. basicola* and is a useful aid to decontaminate vehicles after mud is removed adhere to the principal of: COME CLEAN, GO CLEAN
- **Minimise the exit of floating residues, of all crops, in tail water in affected fields eg. trash-retaining drop boxes (refer to intellectual property register)**

ROTATION

- Rotate with non-host crops (eg. cereals, canola) for **up to three seasons,** if possible **(for fields that were on a 1:1 cotton:wheat rotation, consider 3:3 cotton:wheat)**
- **If drought is forcing fallows of two seasons or more, allocate the longest periods of fallow to heavily infested fields**
- Biofumigation with woolly pod vetch or mustard between consecutive cotton crops or after a wheat fallow. The success of biofumigation depends upon the growth of the biofumigation crop and good incorporation (at least four weeks before cotton).
- Avoid rotation with legumes, **including pigeon pea,** excepting woolly pod vetch, and control alternative weed hosts, **especially bell vine, wild gooseberry, *Datura* spp. native rosella, velvet leaf and phasey bean.**
- Flooding of fields for 30 days during summer reduces the population of *T. basicola* dramatically. This option will be limited by the topography of fields and the availability of water.

Control strategy for Fusarium wilt

PLANNING

- If your farm is free from this disease, try to keep it that way! – See 'Farm Hygiene'; 'Come clean-Go clean'
- Use the most resistant cotton varieties available, especially if Fov occurs in your district
- **Pending it's registration for use on cotton against black root rot, treat all seed sown with Boost® (a.i. acibenzolar-S-methyl)**
- Ensure that seed is treated (eg. **Dynasty™ or** Quintozene® and Apron®)

PLANTING

- Plant to avoid unnecessary stress to germination and early growth eg. not in cold conditions.

IN CROP

- Control weeds during and between crops
- Avoid mechanical inter-row cultivations if possible during the crop (eg. use shielded sprayer to control weeds)
- Manage the crop to avoid stresses such as waterlogging, over-fertilisation, root damage
- Maintain farm hygiene and awareness of incoming traffic through the season
- Conduct regular inspections to allow early detection of any suspicious looking plants. If any are found, send immediately to QDPI for analysis. Educate farm workers what to look for and encourage reporting
- If *Fov* is confirmed, rogue and burn for small patches

- Solarisation may also be an appropriate treatment for small affected patches detected early in the season.
- Isolate affected areas from irrigation flows and traffic to avoid spreading the fungus. Minimise tail-water from affected fields.

LATE SEASON

- Ensure that harvesting machinery is clean
- If Fov has been confirmed on your farm notify all relevant parties so that measures can be taken to avoid spreading the fungus to other fields on your property and to other regions

AFTER HARVEST

- After harvest, retain crop residues on the surface for as long as possible before incorporation

ROTATIONS

- Selection and management of rotation crops is important as the pathogen is able to survive in association with the residues of non host crops.
- **Do not use green manure crops or biofumigation (e.g. vetch, mustard, canola) in areas where Fusarium wilt occurs**
- Summer flooding, where possible, has been shown to be effective but does not eradicate the pathogen.

8.3 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.

9. COMMUNICATION OF RESULTS

9.1 Publications

Refereed scientific papers

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

Mondal AH, Nehl DB, Allen SJ (2004) First report of *Thielaviopsis basicola* on soybean in Australia. *Australasian Plant Pathology* **33**, 451-452.

Nehl DB, Allen SJ, Kochman JK (2003) Fusarium wilt of cotton in Australia: a fatal fungal affliction? *Microbiology Australia* **24**, 8-11.

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

Book chapters and books

Allen SJ, Nehl DB, Moore N (2002) '*Integrated Disease Management.*' (Cotton Research and Development Corporation: Narrabri).

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

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Nehl DB, Brown JF, Allen SJ (2001) Bacterial stunt. In *Compendium of Cotton Diseases, 2nd Edition* (Eds Kirkpatrick T, Rothrock C) pp. 56-57. (APS Press: St Paul, Minnesota)

Refereed conference papers

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

Baker JA, Al-Jaaidi S, Nehl DB, Backhouse D, Katz A, Pereg-Gerk L (2004) Transformation of *Thielaviopsis basicola*: a tool to understand the host-pathogen interactions at a molecular level. In '3rd Australian Soilborne Disease Symposium'. pp. 133-134. (South Australian Research and Development Institute: Rowland Flat)

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Australian Cottongrower articles

Johnson A, Nehl DB (2004) Verticillium wilt: not dead and not forgotten. *The Australian Cottongrower* **25**, 8-10.

Nehl DB (2003) *Pythium* comes in from the cold. *The Australian Cottongrower* **24**, 12-15.

Nehl DB, Allen SJ, Mondal AH, Lonergan PA (2001) Managing the black root rot menace. *Australian Cottongrower* **22**, 52-55.

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

Conference proceedings

Harvey JA, Aitken EAB, Nehl DB (2002) Genetic diversity of *Thielaviopsis basicola*. In 'Proceedings of the 11th Australian Cotton Conference'. pp. 685-687. (Australian Cotton Growers Research Association: Brisbane, Australia)

Harvey JA, Nehl DB, Aitken EA (2003) Geographical distribution of *Thielaviopsis basicola* in Australia. In '8th International Congress of Plant Pathology'. pp. 258. (International Society for Plant Pathology: Christchurch)

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

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Nehl DB, Mondal AH (2001) Biofumigation to control black root rot in cotton. In 'Conference Handbook, 13th Biennial Conference, Australasian Plant Pathology Society'. pp. 156. (Australasian Plant Pathology Society: Cairns, Australia)

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Cotton CRC Information Sheets

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Rourke K, Nehl DB (2001) 'Black root rot update, March 2001.' Information sheet p. 2. (Australian Cotton Cooperative Research Centre: Narrabri, NSW)

Other non-refereed publications

Allen SJ, Nehl DB (2002) Diseases. In *Australian Dryland Cotton Production Guide, Third Edition* (pp. 69-70. (Cotton Research and Development Corporation: Narrabri, Australia)

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

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

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Nehl DB (2001) Manipulation of the soil biota in cotton farming systems. In *'Proceedings of the Cropping Systems Forum, 2001'*. (Cotton Research and Development Corporation: Narrabri, NSW, Australia)

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Media releases

Cross A, Nehl DB, "Black Root Rot concern for cotton," *Media Release*, 2001,

Cross A, Nehl DB, "Warning on Fusarium Wilt," *Media Release*, 2001,

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Johnston T, Nehl DB, "Cotton disease survey yields mixed results," *Media Release*, 2002,
Johnston T, Nehl DB, "Cold start prompts early disease spread," *Media Release*, 2003,
Johnston T, Nehl DB, "Drought threatens fungi in fallowed fields," *Media Release*, 2004,
Nehl DB, Cross A, "Cotton and vetch," *Media Release*, 2001,

9.2 On-line resources

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

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

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

Allen SJ, Nehl DB, Moore N (2002) *'Integrated Disease Management.'* Cotton Catchment Communities CRC: Narrabri, http://cotton.crc.org.au/Publicat/Path_cot.htm

Nehl DB, Allen SJ (2002) *'Symptoms of diseases and disorders of cotton in Australia.'* Cotton Catchment Communities CRC: Narrabri, http://cotton.crc.org.au/publicat/Path1cot.htm

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Nehl DB, Lonergan PA (2003) *'Lachlan and Murrumbidgee Disease Update, 2003.'* Cotton Catchment Communities CRC: Narrabri, http://cotton.crc.org.au/Assets/PDFFiles/Disease/cg1410b4.pdf

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10. LIKELY IMPACT OF RESEARCH OUTCOMES (COST-BENEFIT)

Seedling disease presents a threat to cotton production in the cool production areas of NSW, including the Lachlan and Murrumbidgee Valleys and, to a lesser extent, the Macquarie and upper Namoi Valleys. Inadequate control of seedling disease may be viewed as an impediment to expansion of cotton production in the Murrumbidgee Valley, where existing irrigators are considering cotton as an alternative crop to rice. The adoption of measures to decrease the impact of seedling disease on cotton production in these areas will enable continued productivity, profitability and sustainability of the industry.

Black root rot continues to threaten cotton production across NSW. Its ongoing dispersal into the Lachlan and Murrumbidgee Valleys is of particular concern, as the cool conditions experienced in these areas are likely to favour rapid development of disease. The implementation of on-farm containment procedures, such as trash retention on fields, will help prevent further spread of this pathogen within and between farms.

Fusarium wilt is a major threat to cotton production in Australia. The adoption of measures to control and constrain Fusarium wilt will be invaluable to both prevent disease progress and enable breeders and researchers to build upon the existing control strategy for Fusarium wilt. Pending its registration, use of acibenzolar-S-methyl (Bion®) as a seed treatment should assist in slowing disease progress and subsequent dispersal of the pathogen. Heightened awareness of the potential for further spread of Fusarium wilt has contributed to the successful deployment of the "come clean, go clean" strategy across NSW.

The disease surveys have highlighted the changing status of cotton diseases over time and provide valuable insights into the factors affecting disease incidence and severity. This information enables i) research initiatives to be prioritised and ii) the deployment of research outcomes from this project, and other projects, to be deployed as integrated disease management.

11. BUDGET

Total funds contributed to DAN154C by the CRDC.

Year	DAN154C
2001-02	147,943
2002-03	140,053
2003-04	130,000
Total	$417,996

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