Australian Government
Cotton Research and Development Corporation

Annual, Progress and Final Reports

Part 1 - Summary Details **REPORTS**

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

CRDC Project Number: **DAQ 107C**

Annual Report: ☐ Due 30-September

Progress Report: ☐ Due 31-January

Final Report: ☒ Due 30-September
(or within 3 months of completion of project)

Project Title: **Ecology and development of management strategies for fusarium wilt in cotton**

Project Commencement Date: 01/07/2000 **Project Completion Date:** 30/06/2004

Research Program: Crop Protection

Part 2 – Contact Details

Administrator: Mr Stuart Makings, Acting Senior Project Officer.

Organisation: Queensland Department of Primary Industries and Fisheries, Plant Science, Delivery.

Postal Address: Queensland Department of Primary Industries and Fisheries, Leslie Research Centre,
13 Holberton Street TOOWOOMBA Qld 4350

Ph: 07 4639 8885 **Fax:** 07 4639 8881 **E-mail:** Stuart.Makings@dpi.qld.gov.au

Principal Researcher: Dr J K Kochman, Principal Plant Pathologist

Organisation: Queensland Department of Primary Industries and Fisheries, Plant Science, Delivery.

Postal Address: Queensland Department of Primary Industries and Fisheries, PO Box 102, TOOWOOMBA Qld 4350

Ph: 07 4688 1245 **Fax:** 07 4688 1199 **E-mail:** Joe.Kochman@dpi.qld.gov.au

Supervisor: Dr J K Kochman, Principal Plant Pathologist

Organisation: Queensland Department of Primary Industries and Fisheries, Plant Science, Delivery.

Postal Address: Queensland Department of Primary Industries and Fisheries, PO Box 102, TOOWOOMBA Qld 4350

Ph: 07 4688 1245 **Fax:** 07 4688 1199 **E-mail:** Joe.Kochman@dpi.qld.gov.au

Researcher 2	Dr Suzy Bentley, Senior Research Officer		
Organisation:	CRC for Tropical Plant Protection		
Postal Address:	Molecular Diversity & Diagnostics Research Laboratory, Indooroopilly Research Centre, Plant Pathology Bldg, 80 Meiers Road, INDOOROOPILLY Qld 4068		
Ph: 07 3896 9358	**Fax:** 07 3896 9533	**E-mail:**	S.Bentley@tpp.uq.edu.au

Signature of Research Provider Representative: _____

Part 3.3 – Final Reports (due 3 months after completion of project)

(The points below are to be used as a guideline when completing your final report.

1. Outline the background to the project.

Prior to the 1992-1993 cotton season, Australia was considered to be free from the fungal disease of cotton known as fusarium wilt. The disease is caused by a soil-inhabiting fungus, *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*), and two different strains of the causal pathogen have since been described in Australia (Kochman, 1995; Davis *et al.*, 1996; Kochman, *et al*., 1998). These strains, described as Vegetative Compatibility Groups (VCGs) 01111 and 01112, have caused severe losses to cotton production in Australia, particularly in susceptible varieties.

Fusarium wilt is considered by many growers, ginners, consultants and other industry personnel as the most important constraint to sustainable cotton production to have developed in recent years.

On the Darling Downs in Queensland, Fusarium wilt has spread over a large proportion of the current production area with the worst affected being the Central and Southern Downs. This disease has also been confirmed in several dryland cotton crops. It is estimated to have caused losses of $ 57 million dollars on the Downs in the 1999/2000 season. On some severely affected properties the levels of disease have risen to such an extent that cotton production has not been possible after three seasons, even with the most resistant varieties that are available.

The discovery of *Fov* in many new cotton districts sent shock waves throughout the industry. Districts which have had the disease confirmed from plant samples include: Bourke; the upper Namoi; Warren and Narromine in New South Wales and; St George; Moura and Theodore in Queensland. This project had a number of objectives to address knowledge gaps and obtain data to improve the management of this disease.

2. List the project objectives and the extent to which these have been achieved.

1. Monitor the diversity and distribution of strains of *Fov* in cotton growing areas in Australia. This objective has been achieved.
2. Identify, using glasshouse and field trials, sources of tolerance/resistance to *Fov* and investigate the heritability and genetics of these traits. This objective has been achieved
3. Assist in the development of industry standards to ensure minimum risk of planting seed stock contamination by *Fov*. This objective has been achieved.
4. Assess early stage varietal development germplasm, in collaboration with plant breeders, to ensure that new varieties have the highest tolerance to *Fov* available, prior to their release. This objective has been achieved.
5. Monitor the reaction of varieties to *Fov* and develop a comparative varietal reaction guide for growers. This objective has been achieved.
6. Develop a PCR-based detection system for *Fov* and verify its accuracy. This objective has been achieved.
7. Investigate the role of agricultural practices, such as stubble management, crop rotation, irrigation water treatment and weed management on the ecology of *Fov* and on subsequent disease development. This objective also included some preliminary work on non-pathogenic *Fusarium oxysporum* isolates as possible bio-control agents for *Fov*. This objective has been achieved.
8. Develop and extend new information packages for disease management. This objective has been achieved.

3. Detail the methodology and justify the methodology used.

A number of methodologies have been developed and used for different aspects of this project.

Objective 1

The genetic diversity and geographical distribution of *Fov* in Australia was monitored by direct isolation of the fungus from suspect specimen plants, infected seed and infested soil, followed by Vegetative Compatibility and DNA fingerprinting analyses. The information obtained from the genetic characterisation of the pathogen was then used to develop the DNA diagnostic test for *Fov*.

Fusaric acid production was also used to investigate possible isolate variability. Fusaric acid is produced by strains of *Fusarium oxysporum* that cause vascular wilts in plants including cotton. Pathogenicity of some forma speciales has been positively correlated with the fusaric acid content of diseased plants. The severity of seedling death and symptomatology usually seen in fields infested with *Fov* may be due to the high level of fusaric acid produced by the strains present. Dr Stephen Allen (CSD) and Dr David Nehl (NSW DPI) have observed fields in the Boggabilla/Moree areas in which symptoms of fusarium wilt are less severe than the typical symptoms of disease seen in other fields. Fields in which mild symptoms occur may be due to strains of the pathogen that are low fusaric acid producers. A study, in collaboration with Dr. Barry Blaney (mycotoxicologist DPI&F) was conducted to determine if isolates of *Fov*, from fields where symptoms of fusarium wilt were less severe produce lower levels of fusaric acid than isolates from fields where symptoms are severe.

In preliminary experiments, maize was identified as the substrate to culture isolates of *Fov*, as fusaric acid production was detectible from all but one culture. Fusaric acid was not detectible from three of these cultures grown on millet. Fifty grams of maize were dispensed into 500 mL Erlenmeyer flasks and sterilised. Flasks were inoculated with 2 plugs of fungal culture grown on PDA, one replicate flask per treatment. Flasks were incubated at 27 ºC in the dark and shaken as required to distribute fungal growth. Flasks were incubated for a total of 29 days then samples analysed for fusaric acid production by chemists Barry Blaney and Ian Brock based at ARI in Yeerongpilly, Queensland.

Fusaric acid was extracted from blended samples using a mixture of methanol and phosphate buffer. The extracts were cleaned by liquid/liquid extraction using dichloromethane as solvent and adjusting the pH between alkaline and acid to remove contaminants. The solvent was removed by rotary film evaporation followed by a stream of nitrogen to dryness and the clean extracts dissolved in a small quantity of mobile phase. Fusaric acid was determined by reverse phase HPLC using a C18 column, a mobile phase of methanol and phosphate buffer at pH 7, and UV detection at 271nm. Fusaric acid was quantitated against a solution of the pure standard.

Objectives 2, 4, 5 & 7.

A fusarium field trial site, originally established at Mr Graham Clapham's property 'Cowan' in 1993, was expanded to 24 ha during this project. Small plot trials were established to: identify sources of resistance to *Fov,* assess early stage varietal development germplasm, monitor the reaction of varieties to *Fov* and develop a comparative varietal reaction guide for growers. In addition the site was used to investigate the role of agricultural practices, such as use of particular rotation crops and specific pesticides, on subsequent disease development. Weeds in the trial site were examined for infection by *Fov* and their ability to act as hosts of the pathogen.

Glasshouse pot trials, using soil naturally infested with *Fov*, were used to examine 19 different rotation options over 5 crop cycles with cotton over-sown across all treatments in the final cycle. Glasshouse bioassays were also used to find a relative level of the pathogen in soil. Fifty seeds of a susceptible variety were planted into soil in plastic flats for each assay. The proportion of plants surviving after six weeks was used as an indicator of pathogen population within soil. Pot trials were also used to compare reactions of varieties to infection by *Fov*.

Non-pathogenic strains of *Fusarium oxysporum* have been reported to successfully reduce the incidence of fusarium wilt in numerous crops in glasshouse and field trials, following application of the fungus to soil at planting. Twenty non-pathogenic strains of *Fo*, recovered from wild cotton (*Gossypium sturtianum*, *G. australe*, *G. nelsonii* and *G. bickii*) growing in Mt Isa, Qld, Alice Springs, NT and Flinders Ranges, SA, were obtained from Dr. Bo Wang (CSIRO, Canberra) for assessment.

In addition to these more than 100 non-pathogenic *Fo* isolates were recovered from field soil collected from the Darling Downs of Queensland. Experiments commenced in this project to develop a suitable bioassay to examine their potential to reduce the incidence of fusarium wilt (VCG 01111) of cotton in glasshouse trials.

A bioassay was developed where seedling trays were half filled with soil and 20g of millet grain, colonised by a non-pathogenic strain of *Fo*, were spread evenly over the soil. The inoculum was then covered with a thin layer of soil and 20 cotton seeds of each variety (Siokra 1-4 and Delta Emerald) were placed on top. Seeds were then covered with another layer of soil, watered in and then covered with vermiculite. Prior to challenging seedlings with the pathogen surface sterilised root samples were plated onto media to determine if seedlings were colonised with strains of non-pathogenic *Fo*. Four-week-old seedlings were transplanted into pots containing pasteurised potting mix inoculated with *Fov* colonised millet that had been mixed throughout the soil. External symptoms of infection such as wilting were observed 4-6 weeks after planting. Plants were harvested 6-8 weeks after transplanting and rated internally for *Fov* infection. A second bioassay, using soil naturally infested with *Fov*, has been developed and experiments using it are currently in progress as part of Project DAQ130C.

Objective 3.

The probability of seed transmission of *Fov* was investigated in the laboratory. Seed cotton was hand picked from *Fov* infected and non-infected plants from cotton growing areas in southern Queensland in 2000, 2001 and in 2002. Fuzzy seed was also obtained in 2001and 2002 from a cotton gin that had processed cotton picked from fields where the disease had caused significant losses. The cotton was picked from the more susceptible varieties. Hand picked cotton was ginned with a laboratory gin and a proportion was acid-delinted. Both acid-delinted and fuzzy seed were then used in a number of experiments to determine whether *Fov* could be detected in the sample and what effect the treatments had on survival of the pathogen in the seed.

Several methods were employed to detect and identify the pathogen in seed. Samples of either 100 or 200 seeds were placed onto 1.5% water agar or onto Nash Snyder selective medium agar for *Fusarium* (Nash and Snyder, 1962) in Petri plates. There were five seeds per plate and 5000 seeds were plated. Agar plates were incubated at room temperature (22-25˚C) for at least seven days on water agar and 10-12 days on Nash Snyder medium. Fungi that grew from the seed were identified by microscopic examination, using taxonomic characters such as spore types and spore formation. *Fusarium* species were identified using international standard identification manuals (Burgess *et al* 1994; Nelson *et al.*1983).

Fungi identified as *Fusarium oxysporum (Fo)* in the cotton gin samples were subjected to further testing. Pathogenic isolates of *Fo* are indistinguishable from non-pathogenic isolates under microscopic examination. However, pathogenic strains of *Fov* can be distinguished from from non-pathogenic *Fo* using DNA Amplification Fingerprinting (DAF) (Bentley *et al*, 2000) and this technique was used to identify any *Fov* cultures within the *Fo* cultures isolated.

During 2001 ginned, as well as acid delinted seed samples from infected plants were plated onto water agar at regular intervals after hand picking. They were examined microscopically 7-10 days after plating for the presence of *Fo* cultures. Plating ceased two months after no *Fo* was detected. Seed samples were stored in the laboratory in paper bags at room temperature (19-25 ºC).

Allen & Kochman (2001) reported the complete suppression of the pathogen when seed was treated with metalaxyl. This was surprising as metalaxyl has no reported activity against *Fusarium* species and did not stop growth of the pathogen when incorporated into agar media. Further studies were conducted with two formulations of metalaxyl (Apron XL and Mantle).

Fuzzy seed that had been professionally fumigated with methyl bromide as a requirement for export, was also tested for *Fo* and *Fov* and compared with samples of non-fumigated seed from four samples in 2001 and 12 samples in 2002.

In 1983 Hillocks reported that infection levels in seed ranged from 2% in a resistant variety to 21% in a susceptible variety. Seed cotton was hand picked from diseased plants of 12 Deltapine varieties,

varying in susceptibility to *Fov*, from a trial during April 2001 to determine if similar results would be obtained with Australian isolates of the fungus. Seed was ginned, acid delinted, plated and then inspected for *Fo*.

Objective 6.

A collection of Australian and overseas isolates of *Fov*, that represents all the different races and vegetative compatibility groups (VCGs) that occur, and isolates originating from different geographical regions, was established at DPI&F laboratories in Indooroopilly. This collection of isolates of *Fov* was extensively characterised using a number of different molecular techniques, including DNA amplification fingerprinting (DAF) analysis, restriction enzyme (RE) haplotyping analysis of the intergenic spacer (IGS) region of the ribosomal DNA (rDNA), DNA sequencing of the IGS region of the rDNA, and sequencing of other genes such as the mitochondrial small sub-unit (mtSSU) and α-elongation factor (α-EF) genes. The Australian isolates of *Fov* were compared to overseas isolates of *Fov*, other formae speciales of *F. oxysporum*, non-pathogenic isolates of *F. oxysporum*, and other species of *Fusarium*, to determine the genetic relatedness amongst these groups. The information generated from the genetic characterisation of Australian and overseas isolates of *Fov* was used to develop a PCR-based DNA diagnostic test for each of the Australian strains of *Fov*. The intergenic spacer (IGS) region of the ribosomal DNA (rDNA) was targeted as the basis for a diagnostic test because (i) it is a stable marker, (ii) it is present in multiple copies (allowing more sensitive detection), and (iii) the presence of both conserved and variable regions allows discrimination at different (taxonomic) levels of specificity. Both conventional gel-based and real-time fluorescent detection assays have been developed for the Australian strains of *Fov*. The gel-based assay enables direct detection of *Fov* from mycelial, plant and seed specimens whereas the real-time assay enables direct detection from these samples and also soil samples. The new real-time tests offers much improved specificity compared to the conventional PCR assay, greater sensitivity and much higher sample throughput. The TaqMan® assay format offers the following advantages over the gel-based diagnostic test for *Fov*: (i) increased specificity, (ii) increased sensitivity, (iii) faster sample processing (2-3 hours with no post PCR processing such as running gels), (iv) reduced risk of contamination, (v) quantitation, and (vi) it is amenable to high sample throughput.

Objective 8.

New information on fusarium wilt management, obtained during this project, has been extended to the Cotton Industry in a number of formats and forums. These include presentations by project staff at: field days, grower meetings, cotton consultant meetings, Industry Development Officer meetings, seed company meetings, national and international conference. In addition, staff have been interviewed by the rural press in a variety of formats. Papers and brochures have been provided to growers via the Technical Resource Centre of the Australian Cotton CRC at ACRI. Project staff are also members of the Fusarium Management Committee (FUSCOM) and assisted with the development of the Integrated Disease Management Guidelines.

References cited in section 3.

Allen, S.J. and J.K. Kochman. 2001. Eliminating seed-borne inoculum of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton. Proceedings of the Beltwide Cotton Conference, Volume 1: 139-140 (2001). National Cotton Council, Memphis, TN.

Bentley, S., J.K. Kochman, N.Y. Moore, J.A. Pattermore, L. Gulino and W.T. O'Neill. 2000. DNA diagnostics for fusarium wilt of cotton. In: Proceedings of the 10th Australian Cotton Conference, Brisbane, Queensland. Australian Cotton Growers Association, Wee Waa, Australia. 455-461.

Burgess, L.W., B.A. Summerell, S. Bullock, K.P. Gott and D. Backhouse. 1994. Laboratory Manual for *Fusarium* Research 3rd Edition. University of Sydney, NSW, Australia.

Hillocks, R.J. 1983. Infection of cotton seed by *Fusarium oxysporum* f.sp. *vasinfectum* in cotton varieties resistant or susceptible to fusarium wilt. Tropical Agriculture 60: 141-143.

Nash, S.M. and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of bean root rot *Fusarium* in field soils. Phytopathology 52: 567-572.

Nelson, P.E., T.A. Tousson and W.F.O. Marasas. 1983. Fusarium species: An illustrated manual for identification. Pennsylvania University Press, University Park. USA. 193 pp.

4. Detail and discuss the results including the statistical analysis of results.

Objective1

In excess of 660 specimens, suspected of being fusarium wilt, have been examined during the course of the project. These specimens were received from all cotton growing areas from growers, consultants and researchers. Approximately 50% of these tested positive for *Fov* with most being strain VCG 01111 which was the original strain identified from the Darling Downs. No additional pathogenic strains have been identified to date. A reference collection of preserved strains of *Fov* is being maintained at the DPI&F laboratories at Indooroopilly. A database recording every specimen received and every positive isolation of *Fov* made at the Indooroopilly laboratories, has been completed. The database is searchable under several fields for example, VCG, cotton variety, state, district or year.

As was expected, fusaric acid production varied with isolates from approximately 2 mg/kg up to more than 300 mg/kg. These levels are similar to those recorded by other workers. Data presented in Table1 indicate the levels of Fusaric acid production do not appear to correlate with the virulence of *Fov* isolates in any way and acid production is not likely to be a useful tool to identify pathogen diversity.

Table 1: Comparison of fusaric acid production and pathogenicity of isolates when inoculated into pasteurised potting mix and planted with Siokra 1-4.

VCG	Isolate	Disease severity in field	Fusaric acid mg/kg wet material[A] (low-moderate-high)	Vascular browning In pot tests[AB]
01112	Standard #24597	Severe	256 (moderate) f	1.3 a
	#24599	Severe	313 (moderate) f	2.8 bc
	NPFo #24646	None	12 (low) cd	-
01111	'Korolea'Boggabilla, NSW	Mild	5 (low) b	3.0 c
	'Midkin' Moree, NSW	Mild	16 (low) d	-
	'Cockatoo' Moree, NSW	Mild	27 (low)d	2.3 ab
	Standard #24596	Severe	2 (low)a	3.8 c
	#24516	Severe	6 (low) b	3.3 bc
	#24650	Severe	7 (low) bc	2.4 ab
	Bourke, NSW	Severe	16 (low) ab	3.3 bc
	Carroll, NSW	Severe	72 (low) e	-
	Brewarrina, NSW	Severe	382 (mod) f	2.1 ab

[A] = Numbers followed by the same letter are not significantly different from one another.
[B] = Where 1 = base of stem infected below soil level to 5 = dead plant

Objectives 2, 4 & 5.

The identification of sources of tolerance/resistance to *Fov*, the assessment of early stage varietal development germplasm and the development of a varietal reaction guide for growers have been mainly achieved by using field trial data obtained at Mr Graham Clapham's property 'Cowan', situated near Cecil Plains. Initial glasshouse tests on germplasm reaction to *Fov* produced similar results to those obtained from field trials and, because field trials provided data on many aspects of fusarium wilt management, much of the research effort was concentrated in this area.

Some 24-25 ha of trials have been planted at 'Cowan' during each year of the project. Usually, these were planted in October and harvested in May. There were more than 7700 plots in these trials during the 2003-4 season. There were some promising advances in the levels of resistance to *Fov* in germplasm and lines and single plant selections and crosses were made between more resistant germplasm. The site was also used to improve the resistance of some varieties by means of reselection of more resistant material from the trial plots.

A resistant line, identified in one of the first trials, has been extensively used by the plant breeders to develop varieties with improved resistance to Fusarium wilt. The new variety Sicot F1 is the result, after extensive breeding by CSIRO and testing at 'Cowan'. This variety produced 9.5 bales with a 43% gin turnout (about 25% more yield than the standard) in the variety comparison trials at

'Cowan'. More germplasm, with improved resistance, is still in the developmental stage and not ready for commercial release.

It has proved difficult to gain an understanding of the genetics and heritability of resistance. When the progeny of crosses between susceptible and 'resistant' lines have been challenged with *Fov* the segregation ratios suggest a continuum of reaction from susceptible to 'resistant'. This would preclude any resistance identified to date being imparted by single dominant genes. Crosses between 'resistant' varieties can produce progeny with: increased resistance, similar levels of resistance or full susceptibility. There may be problems with homogeneity for *Fov* resistance in the lines used in crosses. However, the data and observations to date indicate that resistance is quantitative and polygenic.

Data from this project have been instrumental in advising the breeders and industry on the reaction of early stage varieties to *Fov*. Much of the information on the reaction of the first transgenic varieties to fusarium wilt was obtained from this project and the data continue to be used by both CSIRO and Deltapine breeding programs to enhance the Fusarium wilt resistance of their varieties.

FUSCOM has utilised the data from this project to develop the 'F rank' system so that growers can compare the reaction of varieties to *Fov*. There is no direct correlation between high F rank and yield and poor seed quality (Seedling Vigour Index) can reduce the 'F rank' of a variety markedly. The 'Frank' data from the 2003/04 season are presented in Table 2. the data indicate that there may have been some problems with seed quality of some of the varieties as some of the seed appeared to be carryover stock. The proportion of plants of the standard variety, with a zero and 1 rating, will have a marked affect on the 'F rank' rating of other varieties in a comparison trial. If the standard has a low proportion of zeros and 1s, then the 'F rank of better varieties will be skewed upwards. Figure 1 shows that the proportion of plants, rating zero and 1s, in the standard Sicot 189 has been falling significantly, from around 40% to less than 5%, over the last 4 years. This may be due to a number of factors, including disease pressure in the trial site or quality of the seed. The quality of the standard seed will need to be monitored each season to ensure reliable 'F rank' data is produced from trials.

Figure 1. The percentage of plants rating zero and 1 in the standard Sicot 189 at 'Cowan' during four seasons from 2001 to 2004.

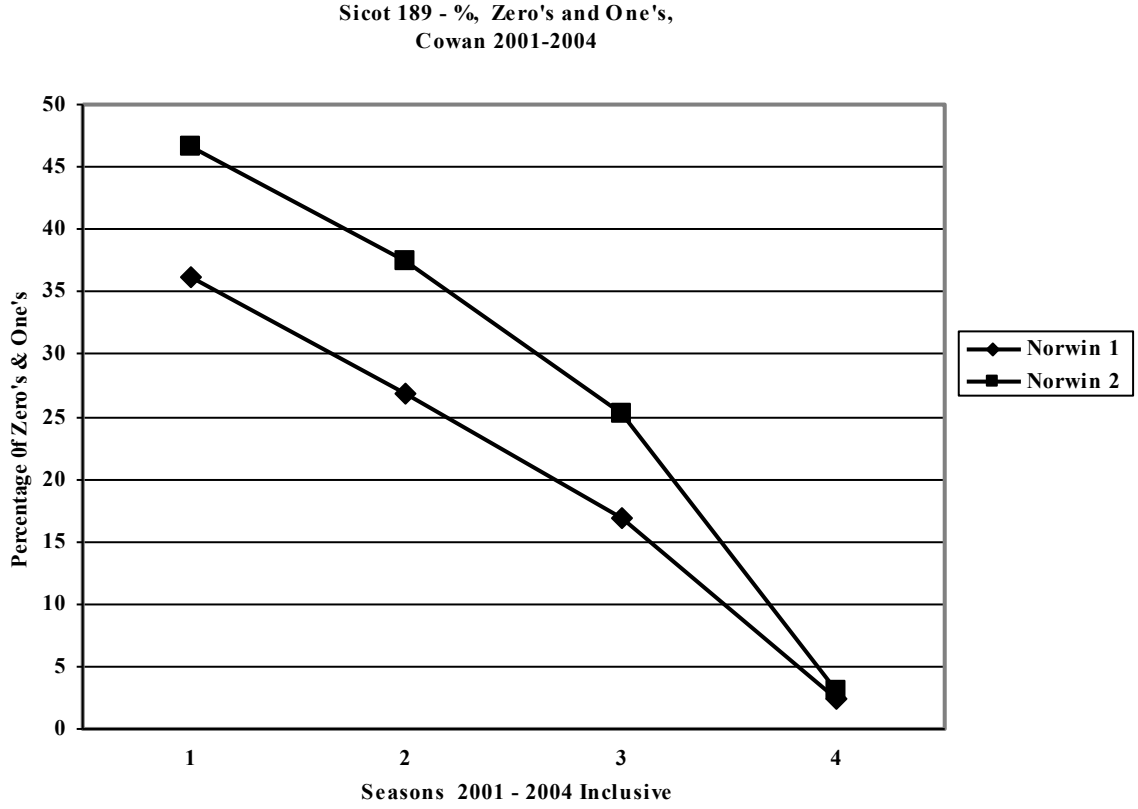

Table 2: F rank, yield and gin turnout of cotton varieties in the Fusarium Assessment Trial, conducted at "COWAN", Norwin during season 2003/04. Data was analysed using spatial analysis.

Cotton Variety	% of plants rated 0 & 1	" F " RANK	KGS / HA Seed cotton	Mean Gin Turnout %
CSX 69	15.55	362	4890 a*	43.1
Sicot 14B	11.83	275	4349 a	40.5
CSX 420	10.64	247	4378 a	44.8
Sicala 45	10.46	243	3727	42.8
NuEMERALD RR	9.14	213	4617 a	43.0
CSX 842	9.07	211	3932	44.9
CSX 416	8.82	205	4254 a	41.9
CSX 405	8.50	198	4345 a	42.9
CSX 102	7.83	182	4010	45.1
DPX 03Q301DR	7.68	179	3982	44.6
CSX 409	7.15	166	3567	42.9
DP 510 RR	7.09	165	4040	43.0
DeltaEMERALD	6.89	160	3815	43.5
DP 579 BGII	6.83	159	3857	39.0
DP 570 BGII	6.81	158	3886	40.3
Siokra V-18	6.70	156	2921	45.7
Sicot 71	6.42	149	3573	46.3
NuEMERALD	6.06	141	4109 a	42.8
DPX 03Q085	6.06	141	3672	42.8
Sicot 80	4.98	116	3487	44.2
CSX 414	4.61	107	2808	43.7
DPX 03Q208D	4.56	106	3444	41.5
CSX 47 RR	4.35	101	3021	45.0
(Standard) Sicot 189	**4.31**	**100**	**3609**	**43.5**
DP 502 RR	3.64	85	2922	46.4
CSX 407	3.29	77	2654	39.7
DeltaDIAMOND	3.12	73	2199	47.2
Siokra V-17	2.98	69	2071	43.9
Sicala V-2 RR	2.93	68	2985	43.1
DPX 03Q307DR	2.91	68	3223	40.1
CSX 415	2.77	64	3405	41.0
DPX 00Q01 R	2.40	56	2515	45.6
DPX 00S01	2.16	50	2843	43.2
DP 556 BGII/RR	2.15	50	2750	42.9
DP 546 BGII/RR	1.87	44	2191	41.9
DPX 03Q027	1.81	42	1852	43.4
DPX 03Q075	1.81	42	2593	45.3
DPX 03Q043	1.64	38	2107	44.8
Sicot 289i (Bulk Plots)	1.52	35	3605	43.4
DP 560 BGII	1.49	35	1949	42.9
DPX 03Q066	1.48	34	2471	43.4
DeltaOPAL RR	1.37	32	2011	40.7
DP 576 BGII	0.98	23	2669	40.6
DPX 03Q109	0.36	8	2194	44.1
Siokra V-16	0.00	0	786	42.4

***Values followed by the same letter are not significantly different from one another**

**There is some question on the quality of the seed of some varieties which may have affected their F rank.

Objective 3.

Data from seed studies showed that *Fo* was not detected in any of the seed, hand picked from healthy plants, even those growing in known *Fov* infested fields, in any season. Furthermore, in 2002 and again in 2004, *Fo* was not detected in seed hand picked from plants showing wilt symptoms in several localities in Queensland and northern New South Wales.

The level of *Fusarium oxysporum* was generally low (8-11%) in seed from infected plants and from ginned cotton, harvested from known infested fields, even though some seed samples yielded high levels of *Fusarium* species.

Results from studies on persistence of *Fov* in seed showed that the fungus did not persist for more than six months when infected seed was stored in paper bags in the laboratory. Ginned fuzzy cotton, hand picked in May 2000, had *Fusarium* species in 56% of the fuzzy seed and 11% in the acid delinted seed. This seed was used in a number of tests and there was a continuous decline in the level of *Fusarium* in the seed. In October 2000 no *Fusarium* was isolated from the acid delinted seed while 26% of the fuzzy seed yielded mainly *Fusarium equiseti* (Corda) Sacc. There was no *Fo* recorded in the fuzzy seed either.

Forty seven percent of acid delinted and 93% of fuzzy seed, hand picked in March 2001, yielded *Fusarium* species (Table 3). Eleven percent of these isolates were identified as *Fo*. By September *Fo* could not be detected in either delinted or fuzzy seed. Other species of *Fusarium* persisted but at lower levels than originally detected.

In addition to the seed treatments already reported as being effective in suppressing *Fo* in seed (Allen and Kochman 2001), *Fo* was not detected in any acid delinted seed treated with either formulation of metalaxyl, at the standard rate. Untreated seed yielded 2% *Fo*.

A number of specific aspects of the methyl bromide fumigation studies are commercial-in-confidence so only general results can be provided. Non-fumigated fuzzy seed yielded an average of 10% and 6% *Fo* colonies in 2001and 2002 respectively. Fumigation with methyl bromide was found to eliminate all *Fo* when used at the appropriate concentration for the appropriate length of time. Other species of *Fusarium* did survive fumigation but their number was much reduced in the fumigated seed.

The variety was a significant factor in the level of *Fo* recovered in plating tests. The proportion of seed that yielded *Fo* varied from 0 in the most resistant varieties to 10% in the most susceptible variety.

Many of the results obtained with the Australian isolates of *Fov* in seed cotton are very similar to those already reported overseas. Dr David Nehl (NSW DPI) and Dr Joe Kochman (QDPI&F) have used these results in a proposal to revise the cotton seed production protocols, should *Fov* be identified in seed production areas. This proposal has been assessed by FUSCOM and has been circulated to Industry bodies.

Table 3. The percentage of seed, yielding *Fusarium* species, when plated at various intervals after harvest in 2000 and 2001.

2000					2001				
Month	Acid delinted		Fuzzy		Month	Acid delinted		Fuzzy	
	Fusarium species	**Fo**	*Fusarium* species	**Fo**		*Fusarium* species	**Fo**	*Fusarium* species	**Fo**
May	11	9	56	11	March	47	11	93	13
October	1	0	28	0	May	25	5	82	7
Jan 2001	0	0	25	0	August	26	2	76	1
					September	26	0	80	0
					November	24	0	80	0

Objective 6.

A PCR based diagnostic assay has been developed to detect the two Australian strains of *Fov*. Initially, a conventional PCR (polymerase chain reaction) test, which relied on gel-based detection of unique fragments of the intergenic spacer (IGS) region of the ribosomal (r) DNA was developed. This diagnostic test proved accurate for identification of 1000's of isolates of *Fov* from pure mycelial DNA extracts, plant material, seed and preliminary testing of soil samples. However, more intensive soil testing during the test validation phase, revealed a small number of false positive reactions due to cross-specificity with unknown strains of *F. oxysporum* occurring in the soil microflora. The PCR reaction parameters of the conventional gel-based PCR test were fully evaluated and exploited to enhance the reaction specificity in soil testing, to no avail.

Consequently, the test format was converted to a real-time fluorescent-based TaqMan® MGB probe assay so that the limited DNA sequence variability amongst closely related strains of *F. oxysporum* could be better exploited. The specificity of the real-time test has been examined on our collection of Australian isolates of *Fov*, overseas isolates of *Fov*, both pathogenic and non-pathogenic strains of *F. oxysporum* (including more than 200 isolates from cotton growing soils) and other species of *Fusarium*. Amongst the hundreds of isolates tested, 5 cross-specificities have been identified. These are with three overseas strains of *Fov*, which are race A and have the same DNA sequence in the region of the PCR probe and if they were ever to occur in Australia, it is presumed that they would also be pathogenic to cotton (and would need to be to detected with the test anyway), one with an uncharacterized isolate of *F. oxysporum* from cotton-growing soils and one with an isolate of *F. anthophilum,* obtained from the *Fusarium* collection held at the University of Sydney. This isolate of *F. anthophilum* has a different sequence in the region of the probe, and this species has not been shown to be pathogenic to plants and has rarely been isolated in Australia (Brett Summerell, personal communication).

To date, 1706 samples have been tested with the real-time PCR test, of which 455 were positive for *Fov*, 1246 were negative and 5 were cross-specificities (0.29%). Most importantly, there were no false negative results recorded – in all cases where fusarium wilt was known to occur the disease was correctly detected and identified. All samples have been correctly diagnosed and results correlated with those from traditional identification based on morphology, vegetative compatibility and pathogenicity tests.

It is also important to note that no problems have been encountered with direct detection of *Fov* from mycelia, plants or seed samples. The false positive reactions occur only when testing soil samples. Direct DNA-based detection of plant pathogens from soil is notoriously difficult, due to problems with PCR inhibition from co-extracted soil compounds, the implications of uneven pathogen distribution on sampling strategies, and variation in disease threshold levels. In this project, each of the objectives has been addressed as far as possible, given the budget and staff available. All aspects relating to the development of the *Fov* diagnostic test have been optimised and standardized so that the test can now be used for routine testing and further research.

Because the real-time *Fov* diagnostic test allows sensitive detection of *Fov*, and is amenable to quantitation and high sample throughput, it will be a valuable research tool for further studies relating to the ecology and epidemiology of fusarium wilt. It will enable quantification of the amount of *Fov* in different soils in relation to disease incidence which will improve our understanding of disease threshold levels under different growing conditions and also pathogen distribution, reservoirs of infection and the role of alternative hosts in the disease cycle.

Objective 7.

Observations in the summer field trials indicated that later planting (late October, early November) on the Darling Downs, reduces incidence of *Fov* in the seedling phase of crop development and there is lower disease incidence in the mature cotton. Other researchers have made similar observations and some growers are adopting this as a routine practice where fusarium wilt is a problem. It is likely that reduction of seedling stress, by avoiding cold shock conditions, could be a major factor in reducing disease incidence.

Results from a summer field crop rotation experiment at Cecil Plains on the Darling Downs has shown that significantly more cotton plants survived until maturity and there were significantly more plants rating 0 and 1 (i.e. 5 % or less vascular discolouration) following the bare fallow treatment (Table 4). Greater cotton seedling death and more severe disease occurred in the treatments where soybean and mungbean had previously been sown.

Table 4: Assessment of plant survival, disease incidence and yield of cotton after various rotations.

Rotation	Emergence count	Final plant count	% 0s & 1s	Yield (bales/ha)
Fallow	127 a	67 a	33 a	6.5 a
Maize	119 a	46 b	16 b	5.7 ab
Sorghum	102 b	30 c	7 c	4.7 abc
Cotton	81 c	28 c	5 c	3.8 bc
Soybean	73 c	13 d	6 c	3.1 c
Mungbean (replanted)*	22 (76)	0 (23)	0 (19)	0 (2.9)

* mungbean – replanted 6 weeks later due to 95 % initial seedling losses; the number in brackets shows counts of the replanted variety - data was not included in the statistical analysis.

Small glasshouse pot trials, using soil naturally infested with *Fov*, were used to examine 19 different rotation options over 5 crop cycles with cotton oversown across all treatments in the final cycle. Rotation cycles that included a fallow treatment either one or two crops before growing cotton generally resulted in less severe fusarium wilt (lower MDI, mean disease index) compared to cycles where a fallow treatment was not included or occurred early in the cycle (Table 5).

Table 5: **Mean Disease Index (MDI) of cotton following different rotation sequences.**

Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	MDI
Soybean	Wheat	Fallow	Fallow	Cotton	0.8 ab
Fallow	Fallow	Fallow	Fallow	Cotton	1.2 abc
Cotton	Wheat	Mungbean	Fallow	Cotton	1.3 abc
Sorghum	Chickpea	Fallow	Wheat	Cotton	1.6 bc
Cotton	Fallow	Cotton	Fallow	Cotton	2.6 def
Maize	Oats	Cotton	Fallow	Cotton	2.9 def
Cotton	Cotton	Cotton	Cotton	Cotton	3.1 ef
Mungbean	Fallow	Cotton	Wheat	Cotton	3.3 fg
Sunflower	Barley	Mungbean	Oats	Cotton	4.5 hi

Root material was left in pots between cycles but stubble was removed. Results showed that crops with larger root systems (sunflower, broccoli, lucerne, maize, sorghum) had more disease (higher MDI) in the following cotton compared to crops with smaller root systems (fallow, chickpea, fieldpea, millet, pigeonpea). This reflects the role of residue and organic matter in pathogen survival and disease incidence. Isolations of *Fov* have been made from mature plants growing in these pot trials including; sunflower, maize, sorghum (roots only), mungbean, fieldpea, pigeonpea, vetch, chickpea, lucerne (stems and roots). *Fov* has not however been isolated from any seed from rotation crops of mungbean, sorghum, chickpea, soybean and millet that have been produced on the Darling Downs.

Preliminary data from these rotation experiments have highlighted the importance of a fallow, prior to sowing cotton, for the management of this disease.

A small number of trial plots with and without Temik were included because growers had reported increase of some seedling diseases where they had used this product. There was no indication that applying Temik at various rates had any effect on the incidence of fusarium wilt.

There were some interesting results from preliminary experiments with non-pathogenic isolates of *Fo* as possible bio-control agents for *Fov*. Seedlings were successfully colonised by non-pathogenic *Fo*. Two isolates appeared to promote plant growth, however in a repeat experiment there was no effect.

Problems were encountered with this initial bioassay. These were that *Fov* infection was low and inconsistent meaning that it was very difficult to assess whether isolates had an effect or not. In addition to this pasteurised potting mix does not contain mycorrhizal fungi, which are necessary for adequate nutrition and growth of cotton. Therefore this bioassay was reviewed and a second bioassay was developed. The investigation of non-pathogenic *Fo* strains for the control of *Fov* is continuing in project DAQ 130C.

Objective 8.
Information generated from this project has been disseminated throughout the industry via the Australian Cotton CRC's National Cotton Extension Team network. Distribution has been through grower meetings and field-days, regional "Cotton Tales" newsletters and through local Cotton Consultants Association branch meetings. In addition, staff have been interviewed by the rural press in a variety of formats. Papers and brochures have been provided to growers via the Technical Resource Centre of the Australian Cotton CRC at ACRI. Project staff are members of the Fusarium Management Committee (FUSCOM) and assisted with the development of the Integrated Disease Management Guidelines.

Project staff have presented papers at national and international conferences and at CRC reviews. Staff have also presented seminars for agricultural students at Universities. Much of the information is published in conference proceedings and is freely available to the Cotton Industry.

5. Provide a conclusion as to research outcomes compared with objectives. What are the "take home messages"?

The research objectives have been largely achieved and have provided the cotton industry with some significant outcomes for the management of fusarium wilt. Many of the current strategies to manage the disease have been developed, in collaboration with other researchers, as a result of this research. Specific highlights include:

- The development of the 'F rank' system to allow growers to compare *Fov* resistance of varieties.
- The identification of germplasm with resistance to the pathogen which has lead to breeding and release by CSIRO of Sicot F1, a variety with significantly improved resistance to *Fov* and acceptable yield and quality characteristics.
- The identification of some agricultural practices that may reduce the incidence of the disease.
- The development of a PCR based diagnostic test for identification of Australian strains of *Fov*.
- The management strategies have not increased pesticide usage.

A number of growers have commented that the outcomes of this research have made them much more confident that they will be able to continue growing cotton profitably.

6. Detail how your research has addressed the Corporation's three Outputs - Economic, Environmental and Social?

The research has addressed all three of the Corporation's outputs. The development of sustainable and cost effective management strategies for *Fov* will provide confidence to growers to continue producing the crop. This will maintain and provide jobs for rural communities and in turn help to maintain the social fabric of these communities. The research has concentrated on identification of resistance to the disease combined with improved agricultural practices. Pesticide use has not increased as a result of the strategies developed thus far, which is an excellent result for the environment.

7. Provide a summary of the project ensuring the following areas are addressed:

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.)

The identification of germplasm with resistance to the pathogen has lead to breeding and release by CSIRO of Sicot F1, a variety with significantly improved resistance to *Fov* and acceptable yield and quality characteristics. Other sources of resistance identified in the project continue to be used in breeding programs to develop varieties wit even better resistance to *Fov*.

There are no patents or licences applied for.

b) other information developed from research (eg discoveries in methodology, equipment design, etc.)

The 'F rank' system has been developed to allow growers to compare *Fov* resistance of varieties.

Agricultural practices that may reduce the incidence of the disease have been identified.

The development of a PCR based diagnostic test for identification of Australian strains of *Fov*. There have been discussions about the delivery of the test with CRCTPP, CRDC and other interested parties. The test is currently available for use by Australian researchers and negotiations are underway for delivery of the test for end-users.

c) are changes to the Intellectual Property register required?

No

8. Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

(b) for the future presentation and dissemination of the project outcomes.

(c) for future research.

A new project DAQ 130C will exploit and extend the technologies developed in this project. This project will continue on from projects DAQ107C, 3.1.8 AC and 2.2.12AC (Australian Cotton CRC). Its aims are to: monitor cotton diseases in Queensland as well as monitoring the diversity and distribution of strains of *Fov* in cotton-growing areas in Australia; identify, sources of resistance to *Fov* and investigate the heritability and genetics of these traits; assess varieties in early stage development, in collaboration with plant breeders, to ensure that new varieties have the highest resistance to *Fov* available, prior to their release; investigate the role of crop rotation on the ecology of *Fov* and on subsequent disease development; investigate non-pathogenic *Fusarium oxysporum* populations in soil to determine if they can modify the ecology of the pathogenic populations and subsequent disease development in cotton and; develop and extend new information packages for disease management.

9. List the publications arising from the research project and/or a publication plan. (NB: Where possible, please provide a copy of any publication/s)

Kochman, J.K., Moore, N.Y., Obst, N.R., O'Neill, W.T., Salmond, G. and Bentley, S. (2000). "Management strategies for Fusarium wilt of cotton". Proceedings of the 10th Australian Cotton Conference, Brisbane, 16-18 August 2000, pp 443-453.

Bentley, S., Kochman, J.K., Moore, N.Y., Pattemore, J.A., Gulino, L.M. and O'Neill, W.T. "DNA Diagnostics for fusarium wilt of cotton". Paper in proceedings of the 10th Australian Cotton Conference, Brisbane, 16-18 August 2000, pp 455-461.

Allen, S.J. and J.K. Kochman. 2001. Eliminating seed-borne inoculum of *Fusarium oxysporum* f. sp. *vasinfectum* in cotton. Proceedings of the Beltwide Cotton Conference, Volume 1: 139-140 (2001). National Cotton Council, Memphis, TN.

Kochman J, Swan L, Moore N, Bentley S, O'Neill W, Mitchell A, Obst N, Lehane J, Gulino L L and Salmond G (2002). The Fusarium threat – are we making the progress? Proceedings of the 11th Australian Cotton Conference, Brisbane, 13-15 August 2002, pp 643-652.

Kochman, J.K., L.J. Swan, W.T. O'Neill, and S. Bentley (2003). Detection, persistence and control of *Fusarium oxysporum* f.sp. *vasinfectum* in cotton seed in Australia. Proceedings of the Beltwide Cotton Conference, Nashville, Tennessee, USA, 6-10 January, 2003, pp185-190. National Cotton Council, Memphis, TN.

Salmond G.R. and Kochman J.K. (2003). Disease management information within the Australian cotton industry - an on-going research, extension and industry collaboration. World Cotton Research Conference - 3, 10-13 April 2003. Capetown, South Africa.

J.K. Kochman, L.J. Swan, S. Bentley, N. Moore, L.J. Smith, W. O'Neill, J. Lehane, L. Gulino and G. Salmond. (2004). A decade of living with fusarium wilt of cotton. Proceedings of the 3rd Australian Soilborne Disease Symposium, 8-11 February 2004, Rowland Flat, South Australia, pp. 79-80.

J Kochman, L J Swan, S Bentley, L J Smith, W O'Neill, J Lehane, G Salmond and J Hare. (2004). Fusarium wilt and other cotton diseases in Queensland during the 2003-2004 season. Proceedings of the 2004 Cotton Consultants Australia Annual General Meeting, 18-19 May 2004, Narrabri NSW, section 5, 4 pages.

Peter Reid, Stephen Allen, Joe Kochman, Warwick Stiller, Greg McNamara, John Lehane and Greg Constable (2004). Sicot F-1: a variety with increased resistance to Fusarium wilt. Proceedings (CD Version) of the 12th Australian Conference, Gold Coast, 10-12 August 2004, Breeding and technology section.

10. Have you developed any online resources and what is the website address?

No. All information is assessable via the Technical Resource Centre of the Australian Cotton CRC at ACRI.

11. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. Where possible include a statement of the costs and potential benefits to the Australian cotton industry or the Australian community.

The results from this project have had an impact already, on growers' returns and value of properties, rural businesses and communities that service the cotton industry as well as processors and exporters. These results have been vital to plant breeders to assist them in producing less susceptible varieties. The research results obtained during this project have been widely disseminated throughout the industry as soon as they were collected.

Recently, there have been independent economic evaluations or impact assessments commissioned by the both the Australian Cotton CRC and the CRC for Tropical Plant Protection (CRCTPP). The report to the Australian cotton CRC, by the BDA group, indicates the benefits of Fusarium wilt research to the Australian Cotton Industry at $184 million in present value terms. The report to the CRCTPP, by Clements and Eyles, estimated the present value of benefits of the fusarium wilt diagnostic at $17.8 million, because of its importance in allowing the fuzzy-seed export trade to the USA to continue. Based on these figures, this project, in collaboration with other projects on fusarium wilt, has made a very significant contribution to the triple bottom line of the Cotton Industry in Australia.

Part 4 – Final Report Executive Summary

Fusarium wilt is considered by many growers, ginners, consultants and other industry personnel as the most important constraint to sustainable cotton production to have developed in recent years. The discovery of *Fov* in many new cotton districts sent shock waves throughout the industry. Districts which have had the disease confirmed from plant samples include: Bourke; the upper Namoi; Warren and Narromine in New South Wales and; St George; Moura and Theodore in Queensland. This project had a number of objectives to address knowledge gaps and obtain data to improve the management of this disease.

In excess of 660 specimens, suspected of being fusarium wilt, have been examined during the course of the project. Approximately 50% of these tested positive for *Fov* with most being strain VCG 01111 which was the original strain identified from the Darling Downs. No additional pathogenic strains have been identified to date. A reference collection of preserved strains of *Fov* is being maintained at the DPI&F laboratories at Indooroopilly. A database, which includes all records isolations of *Fov* made at the Indooroopilly laboratories, has been completed and is searchable under several fields such as, VCG, cotton variety, state, district or year. Fusaric acid production by various isolates of *Fov* does not appear to correlate with the virulence of *Fov* isolates in any way and acid production is not likely to be a useful tool to identify pathogen diversity.

Seed studies showed that *Fo* was not detected in any of the seed, hand picked from healthy plants in any season. Furthermore, in 2002 and again in 2004, *Fo* was not detected in seed hand picked from plants showing wilt symptoms in several localities in Queensland and northern New South Wales. The level of *Fusarium oxysporum* was generally low (8-11%) in seed from infected plants and from ginned cotton, harvested from known infested fields. Results from studies on persistence of *Fov* in seed showed that the fungus did not persist for more than six months when infected seed was stored in paper bags in the laboratory. Hence, it is unlikely that acid delinted, fungicide treated seed will transmit *Fov* and Dr David Nehl (NSW DPI) and Dr Joe Kochman (QDPI&F) have used these results to revise the cotton seed production protocols, should *Fov* be identified in seed production areas. This draft protocol has been circulated to Industry bodies for comment.

Many of the current strategies to manage the disease have been developed as a result of the project work carried out at Mr Graham Clapham's property 'Cowan'. The "Cowan" trial site is recognised by the cotton industry as a high disease incidence site, providing unbiased information on disease management practices. Some specific outcomes include: (i) the development of the 'F rank' system to allow growers to compare resistance of varieties to *Fov*, (ii) the identification of germplasm with resistance to the pathogen which has lead to breeding and release by CSIRO of Sicot F1, a variety with significantly improved resistance to *Fov* and with acceptable yield and quality characteristics and, (iii) the identification of some agricultural practices, such as planting date and crop rotations, that may reduce the incidence of the disease. The management strategies that have been developed have not increased pesticide usage.

A PCR based diagnostic assay has been developed to detect the two Australian strains of *Fov*. The TaqMan® assay format offers the following advantages over the gel-based diagnostic test for *Fov*: (i) increased specificity, (ii) increased sensitivity, (iii) faster sample processing (2-3 hours with no post PCR processing such as running gels), (iv) reduced risk of contamination, (v) quantitation, and (vi) it is amenable to high sample throughput. To date, 1706 samples have been tested, of which 455 were positive for *Fov*, 1246 were negative and 5 were cross-specificities (0.29%). Most importantly, there were no false negative results recorded – in all cases where fusarium wilt was known to occur, *Fov* was correctly detected and identified. All samples have been correctly diagnosed and results correlated with those from traditional identification based on morphology, vegetative compatibility and pathogenicity tests.

The results from this project have had an impact on growers' returns, value of properties, rural businesses and communities that service the cotton industry as well as processors and exporters. These results have been vital to plant breeders to assist them in producing less susceptible varieties. The research results obtained during this project have been widely disseminated throughout the industry.