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## Part 1 - Summary Project Details

# Final Report

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| **Report Due Date:** | **29-Sept-00** | **CRDC Project Number** | **DAQ 99C** |

**Project Title:** (< 15 words)

Ecology and development of management strategies for fusarium wilt in cotton.

## Part 2 - Project Contact Details


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## Part 3 - Final Report Format

**1. Outline the background to the project.**

Wilt caused by the fungus *Fusarium oxysporum* f.sp. *vasinfectum (Fov)* was first identified in Australia in 1993. Since then, this destructive disease of cotton has continued to spread and is now (1998/99 season) found throughout the Darling Downs, in the Goondiwindi/Boggabilla area, Mungindi area, north of Moree and at Theodore. The disease has caused very serious losses in some areas with fields being ploughed out in some instances. It is becoming a very serious constraint to cotton production in many areas.

Results obtained during the project DAQ76C indicate that all varieties tested to date are susceptible to infection by the fungus once there is a sufficient level of the fungus in the soil. There is a marked range of susceptibility to the disease and varieties have been ranked in three susceptibility groups. Varieties in the most susceptible group require less fungal spores in the soil to become infected than the other two groups and these varieties are usually totally killed out in infected parts of fields.

The disease can attack plants at any time of the growing season. It has been shown to be very significant in seedling death at the start of the season, particularly in adverse conditions, often killing the majority of seedlings of very susceptible varieties. It is also found to cause significant plant deaths during the boll-filling phase of crop production.

Although much data has been collected on the ecology and management of the disease, many aspects require further and ongoing investigation. Management of the disease will be largely dependent on the availability of more tolerant varieties, whether they are developed by traditional selection and breeding methods, transgenic means or systemic resistance inducing agents. More information is required on agricultural practices such as stubble management and rotations in relation to decreasing the pathogen population in the soil. Biological control agents appear to show some promise and evaluation in larger field trials is needed to determine their efficacy in conjunction with more resistant varieties.

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

*1. Complete planting of field trials by December and complete assessment by July 2000.*
Field trials were replanted at "Cowan" at the end of November, following the destruction of the trial originally planted in October, by hail. The lateness of the replant precluded the collection of meaningful yield data, otherwise the objective was achieved.

*2. Complete disease surveys to determine the extent of the fusarium wilt affected area by the end of May 2000.* Objective was achieved.

*3. Complete the monitoring of pathogen diversity by July 2000.* Objective was achieved.

*4. Assess and select germplasm with disease tolerance to allow plant breeders to progress the development of cultivars with higher levels of fusarium wilt tolerance, by July 2000.*
Objective was achieved

*5. Identify unique DNA sequences for the two Australian pathotypes of Fov by July 2000.*
Objective was achieved.

*6. Develop information packages and extend to the industry as results become available.*
Objective was achieved.

**3. Detail the methodology and a justification for the methodology used.**

This research project integrated a range of methodologies which are as follows.

***Glasshouse and field trials were used to rate germplasm for reaction to Fov.*** Standardised techniques for germplasm screening, developed during project 76C, were used in glasshouse trials and a fusarium nursery site, established at 'Cowan' on the Darling Downs, was used for the field trials. Data on germplasm reaction was forwarded to plant breeders in both of the Australian breeding programs.

***Disease surveys***. These were conducted throughout the cotton growing areas of Queensland, by surveying crops twice (early and late) during the season.

**10. List the publications arising from the research project.**

Bentley S, Kochman J K, Moore N Y, Pattemore J A, Gulino L and O'Neill W T. (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. pp. 455-461.

Kochman J, Moore N, Obst N, O'Neill W, Salmond G and Bentley S. (2000). Management strategies for Fusarium wilt of cotton. In: Proceedings of the 10th Australian Cotton Conference, Brisbane, Queensland. Australian Cotton Growers Association Wee Waa, Australia. pp. 439-453.

Kochman J. (2000). Fusarium wilt in cotton-the 1999/2000 season. Cotton Consultants Australia Inc. Annual General Meeting Report, 17 May 2000, Narrabri. Paper 3, 4p.

Moore N Y and O'Neill W T. (2000). Detergent based degreaser for disinfecting machinery to reduce the spread of Fusarium wilt in cotton. Australian Cotton CRC Information Sheet. March 2000. Available through the Australian Cotton CRC webpage on the internet at: http://www.cotton.pi.csiro.au

Salmond G. (2000). Fusarium wilt update. Australian Cotton CRC Information Sheet. March 2000. Available through the Australian Cotton CRC webpage on the internet at: http://www.cotton.pi.csiro.au

J K Kochman
Principal Plant Pathologist
28 September 2000

## Part 5 - Plain English Summary

**You must submit a Plain English Summary of yourcompleted research project that is not commercial in confidence, and that can be published by the Cotton Research & Development Corporation in print or on the world wide web. An electronic copy of the plain English summary must also be forwarded by E-mail (angela@crdc.org.au).**

The discovery this season of *Fov* in many cotton districts previously thought to be free from the disease has sent shock waves throughout the industry. Districts where this disease has been confirmed now include: The Darling Downs, Goondiwindi, Talwood, Theodore, Baralaba, St George and Dirrinbandi in Queensland, and Boggabilla, Mungindi, Moree, Bourke, Boggarbri, Carroll (upper Namoi), Warren and Narromine in New South Wales. The disease has not been found in the production areas of Emerald in Queensland, Tandou and Hillston in New South Wales, Western Australia or the Northern Territory.

The monitoring of disease outbreaks and pathogen diversity indicate that, to date, only two strains of the pathogen have been identified in Australia. As a result of the work on characterisation, unique DNA sequences have been identified for both races and this work is being used as the basis of developing a diagnostic kit for the fungus in soil. This is an outcome that the industry has been requesting for some time.

As with Fusarium wilts of other crops, host plant resistance is the primary strategy for long-term management of this disease. The data from this project show that there is germplasm with significantly more resistance than the current best commercial varieties. However, indications are that a rating on plant survival alone will not suffice in selecting resistant material in this germplasm. It appears that a combination, of high plant survival and a high proportion of the surviving plants showing no or little vascular discolouration, is required for resistant germplasm selection.

The unexpected problems with resistance to *Fov* in some INGARD® varieties are partly explained by the data obtained in this project. The fact that the only varieties which have been transformed to be the carrier varieties for *Bt* and Roundup-Ready genes are very susceptible to *Fov*, indicates added problems for plant breeding programs that need to be overcome.

The issue of integrity of disease resistance in all new varieties, including transgenic breeding lines, is of major concern to the industry. Varieties such as those transformed with the *Bt* gene are understandably desirable in requiring less pesticide sprays for control of *Helicoverpa* species but the impact of the introduction of this gene on other qualities in the plant, such as resistance to diseases, is a complex issue that requires more understanding. The mechanisms and heritability of resistance to *Fov* in cotton plants are not well understood. Research projects that have just commenced, aim to better understand this issue by studying the segregation for resistance and susceptibility in transformed and untransformed breeding lines through various generations.

Information packages have been delivered, via Australian Cotton CRC information sheets, at conferences, workshops, ACGRA meetings, media interviews and the popular press. Presentations were made at 13 Grower meetings addressing Fusarium wilt problems. Locations started from Warren in the south to Theodore and Emerald in the north and Dirrinbandi and St George in the west. More than 900 growers and consultants have attended, some of them several times.

- ***Monitoring of pathogen diversity and identification of unique DNA sequences for the two Australian pathotypes***. Traditional mycological techniques, Vegetative Compatibility Group (VCG) analyses, DNA fingerprinting, RFLP and DNA sequence analysis of the ribosomal (r)DNA were all techniques used to characterise the pathogen and monitor its diversity. Polymerase Chain Reaction (PCR) was used to specifically detect the unique DNA sequences for the two Australian pathotypes.

***Development of information packages and extension to the industry.*** Information packages have been delivered, via Australian Cotton CRC information sheets, at conferences, workshops, ACGRA meetings, media interviews and the popular press. Presentations were made at 13 Grower meetings addressing Fusarium wilt problems. Locations started from Warren in the south to Theodore in the north and Dirrinbandi and St George in the west. More than 900 growers and consultants have attended, some of them several times.

## 4. Detail results including the statistical analysis of results.

Disease surveys were conducted as planned during the reporting period. Fusarium wilt, verticillium wilt, black root rot, *Rhizoctonia*, alternaria leaf blight and bacterial blight were all observed during the disease surveys. The incidence of fusarium wilt and verticillium wilt has been particularly high on the Downs this season, largely as a result of the cold conditions in November, December and early January.

Some 180 cotton specimens from Qld, NSW and WA were received. Of these, 57.3% were positive for *Fov*. Of the positive specimens, 97 belonged to VCG 01111 and 5 belonged to VCG 01112. New recordings were made in the districts of Baralaba and Dirranbandi in Qld and in Bourke, Boggabri, Carroll (Upper Namoi) and Warren in NSW. No positive recordings of *Fov* were made from Emerald in Qld, Walgett, Wee Waa, Narrabri, Tandou or Hillston in NSW or from WA. *Verticillium* was recovered from 25 specimens. Other fungal pathogens identified included *Phomopsis*, *Rhizoctonia*, *Alternaria*, *Lasiodiplodia theobromae* and *Colletotrichum gossypii*. Various other *Fusarium* species and *Tricothecium roseum* were also recovered on more than one occasion. Several cultures were lodged with the Plant Pathogen Herbarium at Indooroopilly for future reference. Details of all diagnostic specimens received have been entered into a searchable database. This database presently contains more than 670 entries in total.

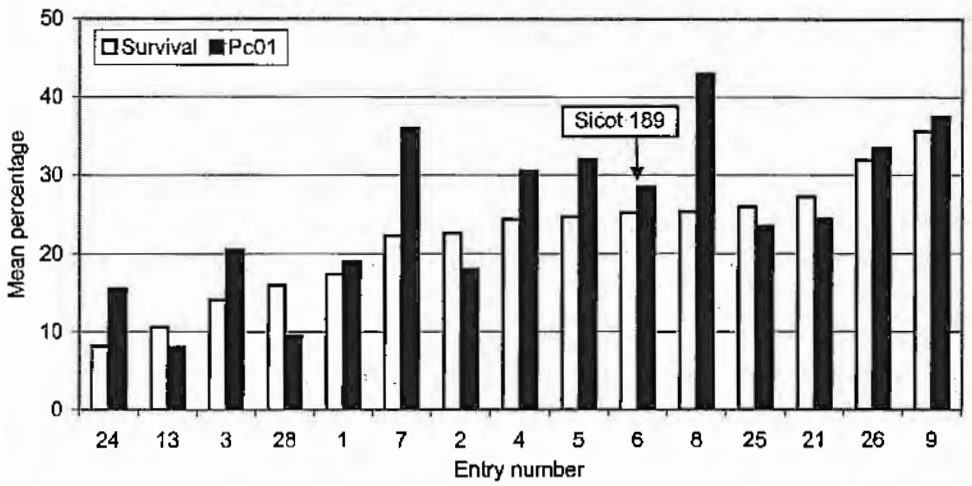
One hundred and twenty three varieties and breeding lines from both CSIRO and DeltaPine breeding programs were assessed in the glasshouse for reaction to *Fov*. In addition, over 800 lines were assessed for reaction to *Fov* in field trials.

There was a range of reactions in the germplasm to *Fov*, both in the glasshouse and field trials. Despite the some of the worst seasonal conditions for cotton growing there were still some very interesting and useful results from the field trials. All of these data have been forwarded to the plant breeders. A selection of data has been used to illustrate some of the findings.

In field trials data were collected on the proportion of plants producing cotton in each line (as a percentage of initial emergence) and the stems of 50 surviving plants in each line were cut determine the extent of vascular discolouration, (0 = no discolouration, 1 = upto 25% of stem discoloured, 2 = upto 50% of stem discoloured, 3 = upto 75% of stem discoloured and 4 = upto 100% of stem discoloured), caused by the disease.

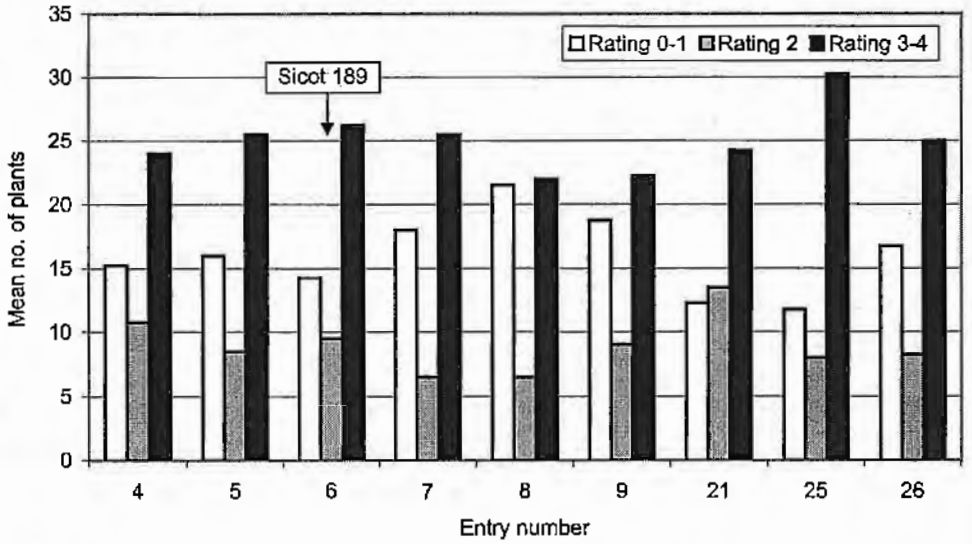
In Trial A, assessing one group of germplasm in a replicated multi-row trial, there were significant differences in the percentage of plants surviving and in the percentage of plants rating 0,1 in stem cuts (Figure 1). Entry numbers 9 and 26 have a significantly higher number of plant surviving ($P = 0.05$) than the other entries. However, entries 8 and 7 have the highest proportion of their surviving plants with 0,1 stem-cut ratings. When the total stem cut ratings were analysed, it was found that the numbers of surviving plants with a rating of 3, 4 were higher than the 0, 1 rating (Figure 2), for all entries. This indicates that a rating on plant survival alone would **not** suffice in selecting resistant material in this germplasm.

**Fig 1. Percentage of plants producing cotton (Survival) and plants of rating 0 and 1 (Pc01) in a *Fusarium* trial at Cowan (Trial A)**




Commercial varieties: 1- Delta Pearl; 2- Delta Opal; 3- Siokra V-17; 4- Sicala V-2; 5- Sicala 40; 6- Sicot 189

**Fig 2. Average number of plants in grouped stem cut ratings in a *Fusarium* trial at Cowan (Trial A)**




Commercial varieties: 4- Sicala V-2; 5- Sicala 40; 6- Sicot 189

Other germplasm was assessed for reaction to *Fov* in a single row strain trial (Trial B). The data indicated that survival of some of the lines exceeded 80%, approximately twice that of the best commercial check varieties. Stem cut ratings showed that some entries contained a significantly higher (P = 0.05) percentage of plants rating 0,1 than Delta EMERALD (Figure 3). When the total stem cut ratings were analysed, it was found that the numbers of surviving plants with a rating of 3, 4 were lower than the number of plants with 0, 1 rating (Figure 4), for all entries except entry 1.

Fig 3. Percentage of plants rating 0 and 1 (Pc0,1) in a *Fusarium* trial at Cowan (Trial B)

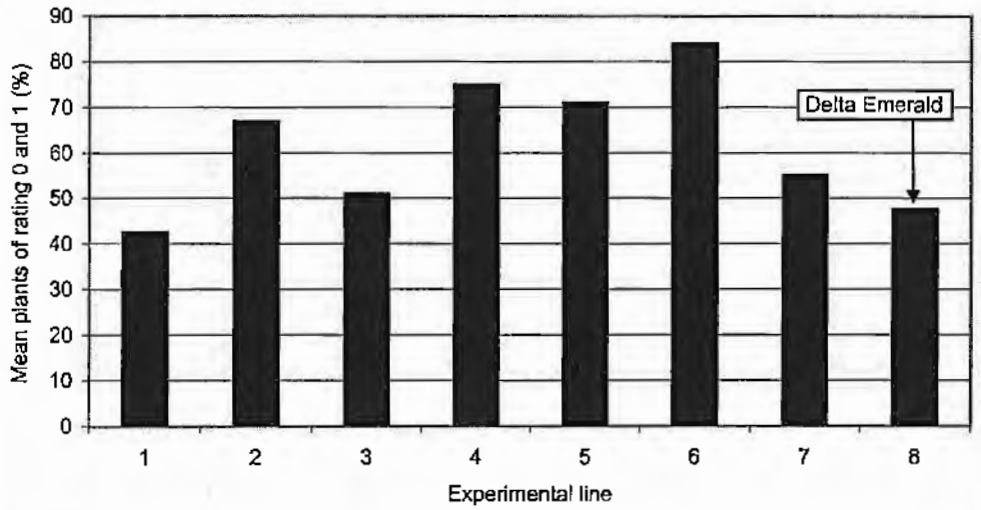
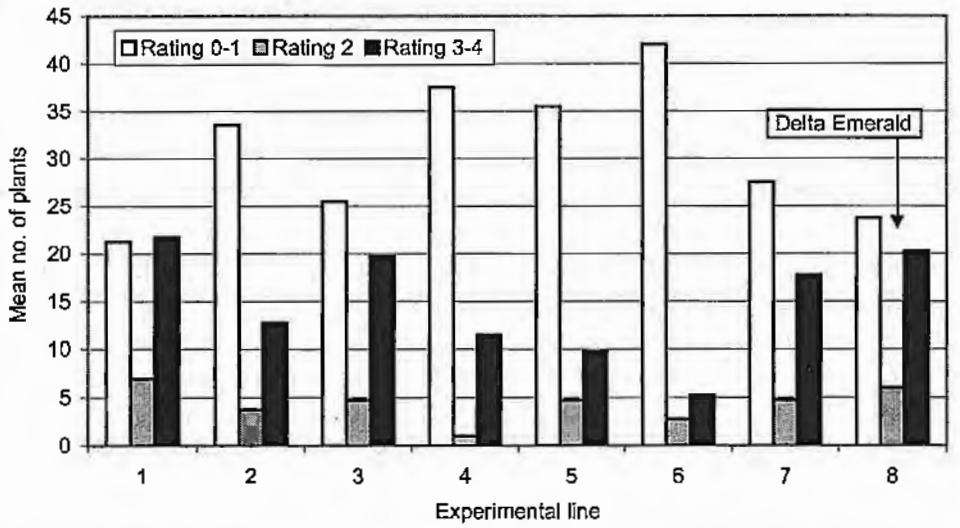
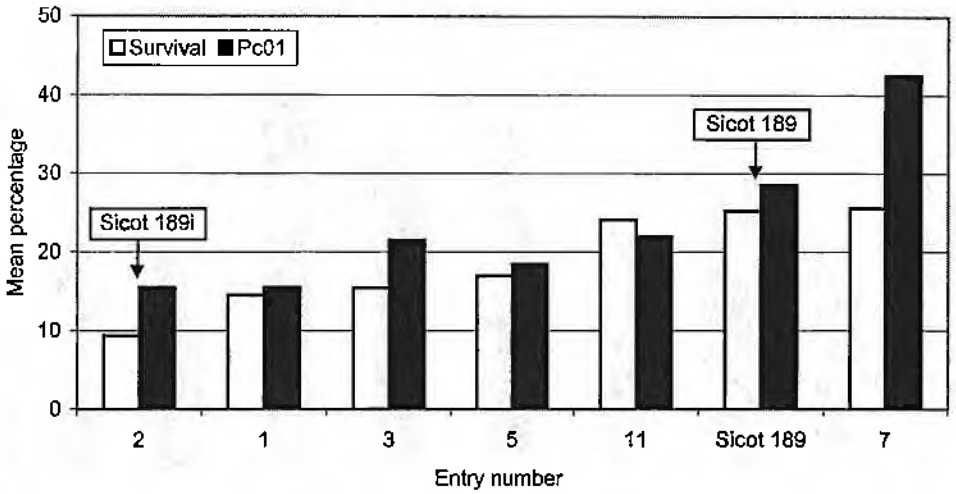



Fig 4. Average number of plants in grouped stem cut ratings in a *Fusarium* trial at Cowan (Trial B)




Investigations of the reaction of a range of *Bt* transformed germplasm (Trial C) confirmed the preliminary observations reported in DAQ 76C. A number of the transformed lines and varieties had significantly fewer plants surviving than their conventional counterparts as well as having fewer plants with 0,1 stem cut ratings (Figure 5). There were also significant differences between selections of the same line, confirming the need for careful selection and testing of lines before the release of a variety. Although a different rating system is used in glasshouse trials similar data was obtained for variation within transgenic lines.

Fig 5. Percentage of plants producing cotton (Survival) and plants of rating 0 and 1 (Pc01) in a *Fusarium* trial at Cowan (Trial C)




Commercial varieties: 1- Sicala V-2i; 2- Sicot 189i; 3- Nucotn 37; 5, 7, 11- Selections of the same Ingard line

The trials also included: preliminary investigations with chemical treatments (Tachigaren) on seed and some over row spray treatments ; BTH, a systemic resistance inducing agent, other biological agents and waste products, bio-fumigation with Hairy Vetch and stubble management practices were also assessed for their effects on *Fov*. There were no significant differences observed in *Fov* control between treated and untreated plots in these trials. Although there were no significant differences observed between the stubble management practices investigated during this project this season, there have been differences observed in previous seasons. These stubble management trials should continue for several more seasons.

Differences were observed in the biological control agent trials, managed for Dr Putcha.

## 5. Discuss the results, and include an analysis of research outcomes compared with objectives.

The discovery this season of *Fov* in many cotton districts previously thought to be free from the disease has sent shock waves throughout the industry. Districts where this disease has been confirmed now include: The Darling Downs, Goondiwindi, Talwood, Theodore, Baralaba, St George and Dirrinbandi in Queensland, and Boggabilla, Mungindi, Moree, Bourke, Boggarbri, Carroll (upper Namoi), Warren and Narromine in New South Wales. The disease has not been found in the production areas of Emerald in Queensland, Tandou and Hillston in New South Wales, Western Australia or the Northern Territory.

New occurrences of the disease during 1999/2000 are unlikely to be the result of spread this year. There is evidence that low levels (fungal spore numbers) of *Fov* in the soil will not cause wilt symptoms to appear in even the most susceptible varieties but high levels of *Fov* in the soil will kill even the most resistant varieties that are currently available.

The monitoring of disease outbreaks and pathogen diversity indicate that to date only two strains of the pathogen have been identified in Australia. As a result of the work on characterisation, unique DNA sequences have been identified for both races and this work is being used as the basis of developing a diagnostic kit for the fungus in soil. This is an outcome that the industry has been requesting for some time.

As with Fusarium wilts of other crops, host plant resistance is the primary strategy for long-term management of this disease. Whilst both cotton breeding programs in Australia (CSIRO and Deltapine Australia) are continuing major efforts to breed for resistance to the Australian strains of *Fov*, the best of the current commercial varieties (Sicot 189 and DeltaEMERALD) have only partial, and not complete, resistance. This means that some losses can be expected when growing these varieties on severely affected farms, but not as much as if susceptible varieties are grown. If highly susceptible varieties (eg. Siokra 1-4, DeltaJEWEL) are grown in affected areas, disease levels in the crop and in the soil will rapidly increase.

The data show that there is germplasm with significantly more resistance than the current best commercial varieties. However, indications are that a rating on plant survival alone will **not** suffice in selecting resistant material in this germplasm. It appears that a combination, of high plant survival and a high proportion of the surviving plants showing no or little vascular discolouration, is required for resistant germplasm selection.

The unexpected problems with resistance to *Fov* in some INGARD® varieties are partly explained by the data obtained in this project. The fact that the only varieties which have been transformed to be the carrier varieties for *Bt* and Roundup-Ready genes are very susceptible to *Fov*, indicates added problems for plant breeding programs that need to be overcome.

The issue of integrity of disease resistance in all new varieties, including transgenic breeding lines, is of major concern to the industry. Varieties such as those transformed with the *Bt* gene are understandably desirable in requiring less pesticide sprays for control of *Helicoverpa* species but the impact of the introduction of this gene on other qualities in the plant, such as resistance to diseases, is a complex issue that requires more understanding. The mechanisms and heritability of resistance to *Fov* in cotton plants are not well understood. Research projects that have just commenced, aim to better understand this issue by studying the segregation for resistance and susceptibility in transformed and untransformed breeding lines through various generations.

**6. 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 and future research needs.**

*Fov* is now considered by many growers, ginners, consultants and other industry personnel as the most important constraint to sustainable cotton production in Australia 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 areas being the Central and Southern Downs. The first records of this disease in dryland cotton crops were confirmed this season. It is estimated to have caused losses of AUS$57 million on the Darling Downs alone, this season. It is becoming more severe and causing increasing losses in other cotton growing areas. On some severely affected properties the levels of disease have risen such that cotton production has not been possible after three seasons, even with the most resistant varieties that are available. If successful management strategies are not developed, cotton growing will not be viable in affected areas.

The identification of germplasm, with improved levels of resistance to *Fov*, is already being used in breeding programs to provide new varieties with better resistance to fusarium wilt. Data on ecology and distribution of the fungus has also been used to develop information packages that detail methods, which can be employed by the industry, for retarding the spread of the disease.

There is a particular problem with notification of new disease outbreak notification, which has been referred to the Fusarium working group and the ACGRA. Unless growers, with confirmed disease outbreaks on their properties, agree to having this information made known to the industry, DPI officers are obliged to keep this information confidential under their code of conduct. This could reduce the effectiveness of management strategies for this disease.

**7. Describe the project technology (eg. commercially significant developments, patents applied for or granted, licenses, etc).**

Data on germplasm identified as more resistant to *Fov* has been available to plant breeders. Where proprietry germplasm is involved, the information on its reaction to *Fov* is provided confidentially to the owners.

**8. Provide a technical summary of any other information developed as a part of the research project. Include discoveries in methodology, equipment design, etc.**

A PCR based soil diagnostic test is being developed using data obtained, by collaborative partners in the CRCTPP, from this project. This diagnostic tool will require significant testing and ground truthing before a useful diagnostic kit can be developed.

**9. State the recommendations on the activities or other steps that may be taken to further develop, disseminate, or to exploit the project technology.**

A new project, which will continue and exploit the findings of this work, has been submitted to CRDC and approved. The project's objectives are:

**1. Monitor the diversity and distribution of strains of *Fov* in cotton growing areas in Australia.** This is required to ensure that any evaluation of lines or varieties for reaction to *Fov* is conducted with the whole spectrum of diversity within the pathogen's population.

**2. Identify, using both glasshouse and field trials, sources of tolerance/resistance to *Fov* and investigate the heritability and genetics of these traits.** Tolerant varieties will be the cornerstone of any management strategy. More and different sources of tolerance to *Fov* need to be identified and their mode of action understood, to determine if they are useful for plant breeders.

**3. Assist in the development of industry standards to ensure minimum risk of planting seed stock contamination by *Fov*.** The need for this is self evident.

**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.** The varieties which had been identified as the most tolerant have been severely affected in some areas. Many of the samples received have been transgenic varieties and there is substancial evidence that some transformed varieties are more susceptible to the disease than their conventional counterparts. If all varieties can be assessed during their developmental stages, the risk of releasing highly susceptible new varieties should be greatly reduced.

**5. Monitor the reaction of varieties to *Fov* and develop a comparative varietal reaction guide for growers.** An independent disease assessment guide has been requested by many growers and consultants.

**6. Develop a PCR-based detection system for *Fov* and verify its accuracy.** Currently a soil bio-assay is used to detect *Fov* in soil. This takes 6-8 weeks and a molecular based system should greatly reduce this time frame. The work on pathogen detection systems at CRC for Tropical Plant Protection is being used for this part of the project.

**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.** Better agricultural practices, together with more tolerant varieties, will provide the best strategy to manage this disease.

**8. Develop and extend new information packages for disease management.** A multi-media campaign to inform of the procedures to limit the spread of this disease will be maintained. The need for continued best management practice on farm will be highlighted.