

1999

## Report Cover Sheet for Final Report

The following Reporting Requirements **MUST BE MET**

### All Projects

You must submit an **ANNUAL PROGRESS REPORT** by the first Friday in February 1999, detailing the progress of your research. NOTE: IF you are seeking continuation of funding for 2000-2001 for the project, this report will form the basis for CRDC's consideration of ongoing funding. Please complete the budgetary requirements if this is a continuing project.

### Terminating Projects

A **FINAL REPORT** must be submitted within three months of completion of the project. This applies in **ALL** cases including research projects, travel, conference attendances, postgraduate, postdoctoral and funded capital items.

### Tick Report Purpose

**Annual Progress Report** (Due 1<sup>st</sup> Fri Feb. to determine continuation of funding)

**Final Report** (Due 30 September or 3 months after completion of project)

**X**

Actual start date:  
01 July 1996

Anticipated completion date:  
30 June 1999

**OFFICE USE ONLY:**

Date of receipt:

**Project title** (as per original application)

Ecology and management of fusarium wilt in cotton

**CRDC Project Code**  
DAQ 76C

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## Final Report for DAQ76C

### To what extent have the project objectives been achieved?

Each of the six (6) objectives listed as aims in the original project proposal has been largely achieved.

#### 1) Monitor the distribution of *Fusarium* wilt in cotton growing areas.

Cotton growing areas were monitored for *Fusarium* wilt during the life of this project (July 1996-June 1999), in association with Dr S Allen, extension staff and consultants. *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*) was confirmed from several new production areas in Queensland and New South Wales. Analysis of specimens at the Indooroopilly and Toowoomba laboratories has confirmed the presence of *Fov* from a total of 28 farms in Qld and 12 in NSW to date. The disease is likely to be more widespread than this, so it is essential to continue to receive specimens for analysis to monitor the distribution of the disease and to detect any changes in the pathogen or the emergence of new strains.

Production areas in Qld where *Fov* has been confirmed include Cecil Plains, Brookstead, Dalby, Miles, Leyburn and Goondiwindi and one site near Theodore in central Queensland. Both strains of *Fov* were also recorded in the St George area for the first time during the 1998/99 season.

In northern NSW *Fov* has been confirmed at 12 farms in the Mungindi, Boggabilla and Moree areas but not in production areas in the Namoi or Macquarie valleys.

Specimens were also received from Kununurra in Western Australia. *Fusarium* species were isolated from this material but were identified as not being *Fov*.

Other diseases and pathogens such as Black Root Rot, *Rhizoctonia*, *Alternaria* and Bacterial Blight were all observed during the disease surveys. A series of experiments is being conducted in controlled temperature cabinets to investigate the possibility of interactions between *Fov* and the pathogens *Thielaviopsis* (Black Root Rot) and *Rhizoctonia* in cotton seedlings.

#### 2) Characterise and type any *Fusarium oxysporum* isolated from wilted cotton in new areas (including samples from all cotton growing areas in Australia).

##### *Isolation and characterisation*

During the life of this project more than 140 specimens from Qld, NSW and WA have been received at the Indooroopilly and Toowoomba laboratories for analysis. Eighty eight specimens yielded *Fov* which was then analysed for vegetative compatibility group (VCG) and volatile production. Eighty one isolates belonged to VCG 01111 and seven to VCG 01112. No other VCGs were recorded during this study. Many isolates were also prepared for DNA analysis and pathogenicity tests in the glasshouse as appropriate. Other pathogens of cotton such as *Verticillium dahliae*, *Rhizoctonia solani*, *Thielaviopsis basicola* and *Phomopsis* sp. were identified as the cause of disease in many of the specimens which did not yield *Fov*. Results of isolation procedures were communicated directly to the grower or consultant concerned.

##### *Fusarium reference collection and database*

Four hundred and sixty one representative wild type cultures and 27 *nit* mutant cultures of Australian isolates of *Fov* as well as 60 wild type and 28 *nit* mutant cultures of *Fov* isolates from overseas (under AQIS Quarantine permits) have been prepared in pure culture and stored as dried colonised filter paper in the *Fusarium* reference collection held at the Indooroopilly laboratories. Of these, 37 wild types and eight *nit* mutants of Australian isolates and 12 wild type and eight *nit* mutant cultures of overseas isolates have been lyophilised for long term storage. Several other research groups have requested and received characterised isolates of *Fov* from this collection to assist with other research into this disease (eg. Emma Colson, Bruce Lyon, Subbu Putcha).

A database recording every specimen received and every positive isolation of *Fov* made at the Indooroopilly laboratories has been completed. At the time of writing the database contained over 500 entries and is searchable under several fields for example, VCG, cotton variety, state, district or year.

### **DNA fingerprinting and PCR-based detection system**

Dr Suzy Bentley at the CRCTPP has used the DNA Amplification Fingerprinting (DAF) technique to analyse DNA from 270 Australian isolates of *Fov*, 60 overseas isolates, and 30 other *Fusarium* species (for comparison). Dr Bentley has used this information to construct a DNA fingerprint library for the Australian strains of *Fov*. This has enabled the identification of VCG-specific primers which can differentiate between different VCGs of *Fov* and between pathogenic and non-pathogenic strains of *Fusarium oxysporum*. These primers and information on the background genotypes of *Fov* and other *Fusarium* species are currently being used to develop a PCR-based detection system for *Fov* at the CRC for Tropical Plant Protection.

### **3) Investigate the role of weeds and other crops in the development and maintenance of the causal fungus *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*) in the soil.**

Samples of weeds from 14 families and 22 species were examined for the presence of *Fov*. The families were; *Amaranthaceae*, *Asteraceae*, *Azoiaceae*, *Brassicaceae*, *Convolvulaceae*, *Cyperaceae*, *Euphorbiaceae*, *Fabaceae*, *Geraniaceae*, *Malvaceae*, *Mimosaceae*, *Poaceae*, *Portulacaceae* and *Solonaceae*. The species were; *Amaranthus macrocarpus* [dwarf amaranth], *Sonchus oleraceus* [sow thistle], *Trianthema portulacastrum* [black pigweed], *Convolvulus arvensis* [European bindweed], *Convolvulus erubensis* [Australian bindweed], *Cyperus sphacelatus*, *Euphorbia dallachyana*, *Desmodium campylocaulon*, *rhynchosia minima* var *minima* [rhynchosia], *Sesbania cannabini* [sesbania], *Vigna lanceolata* [maloga bean], *Geranium homeanum*, *Anoda cristata* [Anoda weed], *Hibiscus trionum* [bladder ketmia], *Malvastrum spicatum* [wild mulberry], *Neptunia gracilis* [native sensitive plant], *Echinochloa colona* [awnless barnyard grass], *Echinochloa crus-gali* [barnyard grass], *Panicum decompositum*, *Portulaca oleraceae* [pigweed], *Physalis lanceifolia* and *Physalis virginiana* [perennial ground cherry].

There were no external symptoms of wilt observed at the time of collection while vascular and/or root discoloration occurred in only a few specimens. *Fov* was isolated from *Amaranthus macrocarpus* [dwarf amaranth], *Hibiscus trionum* [bladder ketmia] and *Sesbania cannabini* [sesbania]. No other weed species have been found to be hosts of the pathogen to date. However, these three species are important weeds in cotton growing areas and need to be managed to reduce carry-over of the fungus.

### **4) Screen, both in glasshouse and field trials, a range of cotton germplasm for reaction to *Fov*.**

#### **Glasshouse trials**

During the life of this project 30 different breeding lines from the CSIRO and Deltapine breeding programs have been evaluated for disease resistance in glasshouse trials. Pathogenicity test protocols using the root dip method have been optimised and standard benchmark varieties (Siokra I-IV and Sicot 189) are used in each test for comparison. There was good correlation between the data obtained from glasshouse and field screening tests.

Both strains of *Fov* (VCG 01111 and 01112) appear to have equal disease-causing potential on current commercial cotton varieties.

Wild *Gossypium* species have been evaluated for disease resistance in the glasshouse but the results have been inconclusive due to uneven germination rates of seed from the wild species. New protocols are being tested to enable this investigation to be continued in future projects.

#### **Field trials**

Large scale field trials, to evaluate germplasm from both the CSIRO and Deltapine breeding programs for reaction to *Fov* were conducted in each year of the project. Yield data and disease rating data have been analysed and provided to the plant breeders to allow them to make decisions on selection of germplasm for further testing. Thousands of lines have been tested but very few have shown high levels of tolerance to *Fov*. Sicot 189 and DeltaEMERALD are among those varieties which have shown the highest levels of tolerance to *Fov*. However, to date, no lines have shown complete resistance to the pathogen.

The assessment of germplasm also indicated that reselection of disease free plants from within the more tolerant lines could improve tolerance levels. Plots of five lines sown to reselected seed produced between 3 and 10% more yield than plots of the original lines grown in a paired plot trial at the fusarium site on the Downs. There was also evidence that some selections of transgenic cotton varieties may not have the same reaction to fusarium wilt as the untransformed variety.

Field trials with three different stubble treatments were started 1998 and are being continued. In order to be able to monitor the level of *Fov* in the soil under these different treatments a seedling bioassay has been developed for use in the glasshouse. Soil samples have been taken every 3 months for bioassay. This bioassay has been used to evaluate the level of disease in stubble management trials in Qld and NSW (for Steve Allen) and may be used in future trials to evaluate *Fov* population levels in soils from biofumigant crop trials (eg. vetch, Indian mustard). Initial results indicated that the level of *Fov* was lowest where stubble had been left on the surface for about 2 months before incorporation. However, as the trial progresses there seems to be some change in these results and further monitoring needs to continue for several seasons.

#### **5) Assist plant breeders with development of disease tolerant cultivars.**

Thousands of lines have been assessed for reaction to *Fov*. Yield data and disease rating data have been analysed and provided to the plant breeders to allow them to make decisions on selection of germplasm for further testing. In addition, crosses have been made with selected CSIRO material and progeny increased in the glasshouse at Toowoomba over winter. These were grown out in the field trials and further selections and crosses made for increase and evaluation. Data has also been provided to breeders from glasshouse screening trials.

A collection of native *Gossypium* species has been maintained at Indooroopilly and seed is being increased as there are indications that this could be another source of *Fusarium* wilt resistant germplasm.

#### **6) Assist with the development of molecular markers for *Fusarium* wilt tolerance/resistance (Dr Lyon, University of Sydney and Plant Breeders).**

Information on field trials, glasshouse pathogenicity test protocols and pure cultures of characterised strains of *Fov* have been provided to Dr Lyon's program by project staff.

#### **What conclusions (if any) have been reached?**

1. That growers should only plant the most tolerant group of varieties in any area where fusarium wilt has been identified, even if the incidence of the disease is very low.
2. The disease has continued to be recorded in new areas each season, however, several large production areas remain free from the disease.
3. The industry must attempt to retard the spread of the disease to new areas and on affected farms.
4. The disease can be very severe in the seedling phase and more effort is required to manage this phase of the disease.
5. Varieties with greater tolerance to fusarium wilt must be developed.
6. Two distinct strains of the pathogen have been confirmed in Australia. Both appear to have equal virulence on current commercial cotton varieties.

#### **Have you encountered any difficulties with the research project?**

The occurrence of Bonsai Bunchy Top (BBT) throughout the field trials during the 1998/99 season had a significant effect on yield data and the amount of seed obtained from crosses for further selection work. The disease rating itself seemed to be less affected but it is possible that the stress caused by BBT may have exacerbated *Fov* in a number of lines. This disorder is being monitored and some glasshouse work is currently in progress at Toowoomba and Indooroopilly to determine its cause.

#### **What additional Activities have been undertaken during the research project?**

1. Co-supervision of Mr Bo Wang's Ph D studies was completed when Mr Wang submitted his thesis entitled "Studies on the population ecology and infection process of *Fusarium oxysporum* f.sp. *vasinfectum* causing vascular wilt of cotton in Australia" in August 1998. The degree was awarded in March 1999 and Dr Wang has taken up a post-doctoral position in the USA.
2. A presentation on the project was made to the stage 1 review panel during the CRCSCP's 5<sup>th</sup> year review.
3. Project staff have also assisted with planting other CSD trials on the Downs and have been involved with disinfecting of all trial equipment used in fusarium wilt affected areas.
4. Chitinase expressing transgenic cotton, developed by CSIRO staff in Canberra has been screened in field trials.
5. Dr Emma Colson has been assisted with her trials investigating Systemic Induced Resistance to *Fov*.
6. Dr Putcha has been assisted with his field trials investigating biological control of *Fov*.

7. Significant involvement with the Fusarium Working Group, in particular providing information to Mr Greg Salmond and the weeds and diseases group of the CRC National Cotton Extension team, who have been developing information packages on fusarium wilt management. There was also close collaboration the CRDC fusarium wilt awareness project.

8. Efficacy trials were conducted in the glasshouse at Indooroopilly to provide industry with recommendations for disinfecting machinery to reduce the spread of *Fov*. "Farmcleanse" a detergent-based degreasing agent was found to be the most effective disinfecting agent when used in conjunction with physical removal of the soil and plant trash from machinery (eg. by high-pressure hosing). This has been a good outcome for the cotton industry since this agent does not require registration, is biodegradable, has quick break properties and is much safer to use than other fungicides. Other industries in Australia where *Fusarium* wilt is a problem (eg. banana, vegetable) have also adopted this agent for disinfecting machinery and farm implements.

9. The virology group at Indooroopilly has been assisted with collection and maintenance of plants and insects for BBT research.

10. Discussions have been held with Drs Pittaway and Roberts (USQ) about providing support to their proposed project on composting cotton trash. The support would involve identification of *Fov* in trash and compost samples and would involve some Vegetative Compatibility Analyses and DNA finger-printing of isolates.

11. Discussions have been held with Dr David Jones about providing plant pathology support and testing for *Fov* resistance in cotton plants, genetically modified with fusarium wilt resistance genes from tomatoes, if these genes can be isolated and transferred into cotton and if he can obtain funding for this work.

**Are there any other aspects of the project you wish to report on?**

The collaboration and support of Mr Graham Clapham, "Cowan", has been instrumental in most of the objectives dealing with field assessment of germplasm being achieved. His support is gratefully acknowledged.

**What Publications / Published Findings have emanated from the research so far? (Please list)**

DAVIS R D, MOORE N Y and KOCHMAN J K (1996). Characterisation of a population of *Fusarium oxysporum* f.sp. *vasinfectum* causing wilt of cotton in Australia. *Australian Journal of Agricultural Research* 47, 1143-56.

KOCHMAN J K, DAVIS R D, MOORE N Y, BENTLEY S & OBST N R. (1996). Characterisation of the Fusarium wilt pathogen of cotton in Australia. Proceedings of the 8th Australian Cotton Conference, Gold Coast, Queensland, August 1996 pp 651-656.

KOCHMAN J K (1997). Fusarium wilt in cotton. Cotton Insight (official publication of the South Queensland Cotton Growers Association Inc) 14 Feb 1997 p 8.

WANG B, KOCHMAN J K, DALE M & OBST R. (1997). Variation in soil population of *Fov* under different cultivars and crops and the effect of soil type on fusarium wilt of cotton. Abstract in Proceedings of the 11th biennial conference of the Australasian Plant Pathology Society, Perth 1997, p 159 (presented as a poster).

KOCHMAN J K, OBST N R, MOORE N Y, BENTLEY S, DAVIS R D AND O'NEILL W (1997). Fusarium wilt of cotton in Australia. Abstract in Proceedings of the 11th biennial conference of the Australasian Plant Pathology Society Perth 1997, p 295 (presented as a poster).

JOE KOCHMAN, STEVE ALLEN AND GREG SALMOND (1997). Living with fusarium wilt. The Australian Cotton Grower Vol. 18, No. 5, pp 34-36.

KOCHMAN J K (1997). Fusarium wilt of cotton. Slide presentation kit with 21 slides, used by extension staff throughout the Industry (also available on the Internet).

JOE KOCHMAN, STEPHEN ALLEN, NATALIE MOORE AND GREG SALMOND (1998). Fusarium wilt in cotton. 4 page 'wilt alert' pamphlet.

KOCHMAN J K (1998). Living with fusarium wilt. Proceedings of the 9th Australian Cotton Conference, Gold Coast, Queensland, August 1998 pp 549-553.

KOCHMAN J K, OBST N R, MOORE N Y, O'NEILL W T & BENTLEY S (1998). Proceedings of the 9th Australian Cotton Conference, Gold Coast, Queensland, August 1998 pp 571-572.

Moore, N.Y., Kochman, J.K., Obst, N.R., O'Neill, W.T. and Bentley, S. (1998). Fusarium wilt of cotton in Australia. Proceedings of World Cotton Research Conference 2, Athens, Greece, September 1998 p.279.

WANG B, DALE M L, KOCHMAN J K and OBST N R (1999). Effects of plant residue, soil characteristics, cotton cultivar and other crops on fusarium wilt of cotton in Australia. *Australian Journal of Experimental Agriculture*. 39: 203-9.

WANG B, DALE M L, KOCHMAN J K, ALLEN S J and OBST N R (1999). Variations in soil population of *Fusarium oxysporum* f.sp. *vasinfectum* as influenced by fertiliser application and growth of different crops. *Australasian Plant Pathology* 28: 174-181.

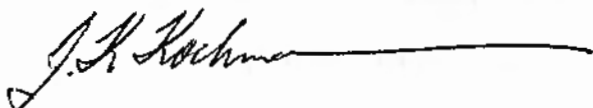
O'NEILL W (1999). New disinfectant gives extra insurance against Fusarium wilt. *The Australian Cottongrower*. 20 (2): 8.

**Have you been involved with any presentations / field days / communication of research to industry? (if so, give brief summaries of these)**

The results of this research have been published in scientific journals, industry journals and the popular press. There has been close association with extension staff and consultants in all sectors of the industry to provide current information on management of fusarium wilt and retarding its spread. The results have also been presented at conferences, field days and grower meetings as listed below.

1. Grower meetings throughout the cotton areas of Queensland and northern New South Wales. These have been organized by CSD, Deltapine and local cotton grower groups.
2. A meeting with Downs consultants at the fusarium trial site to show them results being obtained.
3. The 9th Australian Cotton Conference, Gold Coast, Queensland.
4. The Second World Cotton Research Conference, Athens, Greece. September 1998.
5. The International Fusarium Working Group, CABI Bioscience, Egham, England. September 1998.
6. Experimentalist, Wayne O'Neill, held a workshop for cotton growers and consultants at Dalby to formulate protocols to address movement of vehicles between cotton farms in the Dalby area to minimise the spread of *Fusarium* wilt.
7. Farm walks have been held at trial sites and the Toowoomba and Indooroopilly laboratories have hosted several visits and meetings including; the Fusarium wilt workshop in July 1997, Fusarium working group meetings and a visit by the board of the CRDC in 1999.

If your project is not continuing into the following financial year, you must still submit an annual progress report



J K Kochman  
Supervisors Signature

Date: 27 September 1999