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: **CSP155C**

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: AFLP diversity of Fov in cultivated cotton fields and genotyping of G. hirsutum x G. sturtianum backcross lines

Project Commencement Date: 1 Oct 2002 **Project Completion Date:** 30 Sept 2004

Research Program: - Please Select One -

Part 2 – Contact Details

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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. Postgraduates please note the instructions outlined at the end of this Section.)

1. Outline the background to the project.

Fusarium wilt was first recorded in Australia in 1993 and subsequently has spread through eastern cotton growing districts. The speed with which the disease spread has engendered urgency in developing better disease management strategies and improved commercial cultivars. CRDC projects CSP113C (principal researcher Bo Wang) and CSP120C (principal researcher Augusto Becerra) are exploring two critical aspects of Fusarium wilt in Australia. CSP113C is surveying and genotyping the *Fusarium oxysporum* (*Fo*) strains associated with native *Gossypium* species and screening indigenous native cottons for novel sources of disease resistance. CSP120C is developing new breeding materials that incorporate native germplasm with promising levels of resistance and evaluating the feasibility of transferring these genes to cultivated cottons. In both cases, preliminary results have produced some key results that we believe merit immediate attention. However, the extra work envisioned cannot be undertaken with the resources at hand. Therefore, this proposal requests funds for a full time technical officer for 12 months to support both projects. The "additional" objectives that could be achieved are listed below.

CSP113C produced two important results: (1) the identification of a wild *Fo* lineage that is more similar genetically to the two cotton field pathogens (VCGs 11 and 12) than are any of the overseas isolates tested to date and (2) the identification of a possible third *Fov* strain from several cotton fields. If both results are substantiated, and they are still tentative pending rigorous confirmation, they have serious implications for Fusarium wilt management.

The genetic similarity between VCGs 11 and12 and specific lineage of wild *Fo*, some of which are mildly pathogenic on Siokra1-4, supports the hypothesis that VCGs 11 and 12 arose in Australia. If this is substantiated, it suggests that continuous monoculture of cotton may have selected for pathogenic strains among a pool of benign or mildly pathogenic native *Fo* strains, raising the possibility that it could occur again (see below). We would like to substantiate this result by comparative gene sequencing of the native lineages, VCGs 11 and 12, and overseas *Fov* strains. An important sub-component of this process will be to identify the mating type of all the strains, and VCGs 11 & 12. If both mating types are present, it may be possible that rare sexual reproduction could generate new pathotypes. Simultaneously we would like to genotype (using AFLPs) a wide range VCG 11 & 12 isolates collected from across the eastern cotton growing districts to ascertain whether new genotypes are being generated.

If a third *Fov* strain exists, it should be incorporated into breeding programs as early as possible and its prevalence ascertained and monitored. However, before this happens, we need to fully characterize this new genotype to (1) confirm that it is *Fusarium oxysporum*, (2) test whether it is pathogenic on cultivated cotton, and (3) establish how closely related this third strain is to VCGs 11 & 12.

The objectives of CSP120C have been significantly altered to accelerate work with *G. hirsutum* X *G. sturtianum* hybrids—the original grant objectives involved the *G. hirsutum* X K-genome hybrids. This displacement of objectives reflected the early promise of *G. sturtianum* as a possible source of Fusarium wilt resistance. As evident in the CSP113C August 2002 report, *G. sturtianum* accessions are more Fusarium wilt resistant than the other widespread native *Gossypium* species, *G. australe*. More importantly, replicated glasshouse disease assays have confirmed that *G. hirsutum* X *G. sturtianum* hybrids are significantly more resistant than their *G. hirsutum* parent (see CSP120C August 2002 report). On this basis, we believe it is important to continue this work. Twenty-three backcross families have been tested in the glasshouse and 88 families have been evaluated at the Norwin disease nursery. Selecting lines for future work requires genotyping a substantially larger number of individuals than proposed originally. The genotyping is required to identify the lines worthy of continued development. At the same time, we believe it is important to continue to develop the K-genome hybrids, to the extent possible, as they may also prove of value in the future, and thus more technical assistance is necessary.

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

The purpose of this project was to expand the scope of CSP113C and CSP120C (see additional objectives below). The additional technical assistance would allow . . .

- Bo Wang (CSP113C) to examine the level of genetic diversity among *Fov* isolates from cotton fields and to establish the evolutionary relationships between the *Fov* isolates of cotton fields, wild pathogenic *Fo* isolates, and overseas *Fov* isolates; and
- Augusto Becerra (CSP120C) to meet the original objectives of CSP120C, while incorporating the extensive work necessary to assay 50 *G. hirsutum* x *G. sturtianum* aneuploids families for disease resistance and to genotype the individuals.

Additional objectives—CSP113C

- **Extract *Fov* isolates from diseased cotton plants collected from 9 cotton fields from each of 3 cotton growing districts.**
  - *Completed: A total of 1189 isolates were recovered from diseased cotton plants collected from 21 fields across QLD and NSW.*

- **Assay genetic diversity among cotton-field-*Fov* isolates by genotyping (with AFLPs) 20 representative isolates from each farm sampled.**
  - *Completed: A total of 152 cotton* Fov *isolates were genotyped with four* Taq*I X* Mse*I AFLP primer combinations*

- **Sequence 2 genes from 2 representatives of each of 10 "wild" *Fov* lineages, 4 cotton-field-*Fov* isolates, and representatives of each of the 6 overseas races to determine the evolutionary relationships among the "wild" Australian *Fov*, the overseas, and the cotton-field-*Fov* isolates.**
  - *Completed: Four genes have been sequenced for 28 "wild"* Fov*, 4 VCG-11, 4 VCG-12, and all 8 overseas* Fov *races.*

- **Genotyping and pathogenicity testing of "third *Fov*" variant**
  - *Completed: Eight "unusual* Fov *variants have been genotyped (using gene sequences) and tested for pathogenicity.*

Additional objectives—CSP120C

- **Field evaluation of 30 *G. hirsutum* x *G. sturtianum* aneuploid BC_2F_2 families for *Fov* resistance.**
  - *Completed: 364 lines have been evaluated over two seasons in field nurseries.*

- **Glasshouse evaluation of an additional 23 *G. hirsutum* x *G. sturtianum* aneuploid BC families for *Fov* resistance.**
  - *Completed: A total of 47 lines have been tested for* Fov *resistance in glasshouse trials.*

- **AFLP genotyping of the 46 best *G. hirsutum* x *G. sturtianum* aneuploid BC families tested in the glasshouse**
  - *Completed. 47 lines have been genotyped using AFLPs*

3. Detail the methodology and justify the methodology used.

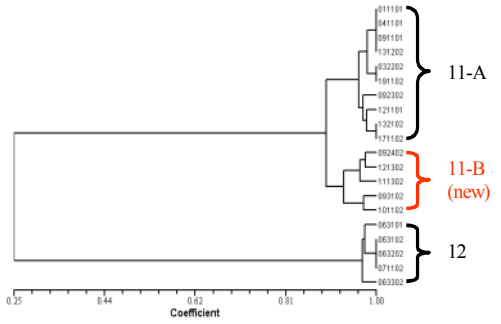
The relevant methodologies and their justification are fully delineated in the final reports for CSP113C and CSP120C

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

The *Fov* strains in cotton fields are more diverse than originally thought.

A total of 152 cotton field *Fov* isolates were genotyped with four *Taq*I X *Mse*I AFLP primer combinations. This preliminary study shows that VCG 11 is more diverse genetically than previously thought (Fig. 1). In addition, some isolates from St George are distinct from the VCG 11 & 12 isolates. Given the strange disease symptoms noticed by extension staff in this area, it appears that additional novel genotypes of *Fov* may be present. The long-term management consequences of this are not yet clear, but monitoring of the genetic structure of *Fov* populations in cotton fields is continuing under CSP 156C.

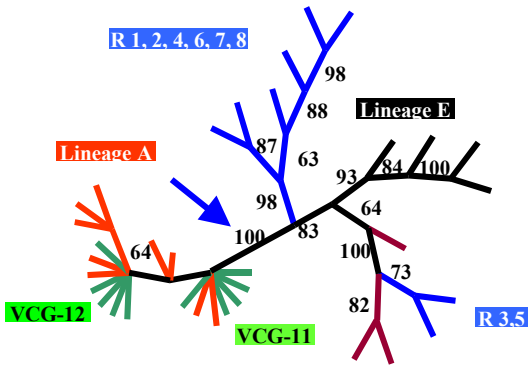
Fig. 1 Variation of cotton field *Fov* populations revealed by four AFLP primer combinations.



The *Fov* strains have not been introduced from overseas; they are closely related to indigenous non-pathogenic *Fo* strains found in non-agricultural Australian soils.

Four genes have been sequenced from 28 *Fusarium oxysporum* isolates representing the range of *Fusarium oxysporum* diversity (identified with AFLPs) present in Australia and from representatives of the 8 overseas *Fov* races. The results confirm that VCGs 11 & 12 have not been introduced from overseas, but are local isolates closely related to lineage A (Fig. 2). These results confirm earlier data based on AFLPS.

Fig. 2. Parsimony optimized topology of DNA sequence from 4 genes (mtRSS, PP, NR, EF1α). A heuristic search returned a five equally likely trees with consistency indices of 0.92 and a retention indices of 0.98.



Field evaluation of *G. hirsutum* X *G. sturtianum* aneuploid families BC2F2 families have not identified any superior introgressant lines.

In 2000/2001, 2 lines were evaluated. In 2001/2002, 191 lines were evaluated in *Fusarium* field nurseries. This year (2002/2003), 173 single plant selections have been planted for field evaluation. Based on the glasshouse results, ~ 50 families from single plant selections were planted in the Queensland *Fusarium* field nurseries in the 2002/2004 season. Another set of single plant selections have been planted in the field nurseries for the 2004/2005 season. To date no promising lines have been identified.

Five *G. sturtianum* chromosomes are significantly associated with Fusarium wilt resistance.

Having confirmed earlier reports of Fusarium wilt resistance in *G. sturtianum*, 47 *G. sturtianum* x *G. hirsutum* (Gos-5271) BC_2F_3 aneuploid families carrying a subset of the *G. sturtianum* chromosomes were tested for *Fov* resistance. Twelve of these lines were particularly promising, exhibiting disease levels significantly better than their *G. hirsutum* parent (Figure 3). Based these results we focused on identifying the genomic location of the resistance observed in *G. sturtianum* and identifying hybrids in which this resistance has been transferred to *G. hirsutum*. A suite of 181 *G. sturtianum* chromosome-specific molecular markers were selected to assess the relationship between *G. sturtianum* chromosome transmission. Logistic regression identified five putative *G. sturtianum* chromosomes (8 *G. sturtianum* LGs) that improve resistance to Fusarium wilt This result constitutes direct evidence that the incorporation of several genomic regions of the C genome into the cultivated cotton could significantly improve the levels of *Fov* disease-resistance response observed in the recipient cultivated cotton genotype.

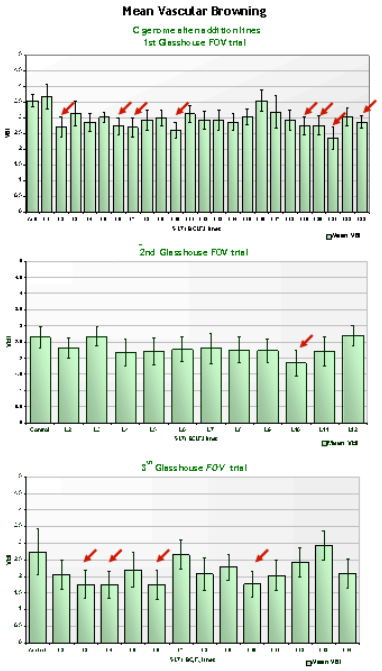


Figure 3: Mean vascular browning (VB) for 47 BC_3 C genome chromosome addition lines. The vertical bars indicated the confidence interval. The arrows show significant differences between the control (CPI 138969) and the addition lines to Fov resistance at $_{90\%}$CI

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

- The *Fov* isolates in cotton fields are more diverse genetically than originally thought. This suggests that continuing monitoring (ongoing in CSP156C) is appropriate. Early results from CSP156C have documented that some new genotypes are appearing in cotton fields. Whether this poses a problem for disease management or is part of the ecology of crop pathogens is currently under investigation.
- The observation that the Australian *Fov* strains are indigenous to Australia reinforces the efficacy of our current quarantine protocols, but the presence of closely related non-pathogenic strains of Fusarium in cotton fields could complicate the development of diagnostic tools. This issue has been discussed in the Fuscom meetings, and relevant isolates have been sent to Suzy Bentley to incorporate into validation trials.
- All analyses indicate the *G. sturtianum* has useful levels of Fusarium wilt resistance, but field trials and genetic analyses indicate the transferring these genes to cultivated cottons will be very difficult. Under CSP159C, the research effort has been redirected to other equally promising sources of Fusarium wilt resistance that have recently come to light and that are more amenable to genetic manipulation.

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

This project, and the projects it supported (CSP113C and CSP120C) are designed to improve the profitability and competitiveness of cotton production in Australia by providing a better understanding of the origin and natural biology of *Fusarium* that, in turn, will contribute to more effective disease management strategies. If novel resistance genes are discovered and can be transferred to elite cotton cultivars, the profitability of cotton production in *Fusarium* infected fields will be improved.

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.)

not applicable

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

The genotyping systems developed in this project provide a means of rapidly and reliably discriminating among various *Fov* genotypes. We continue to develop better glasshouse assays that allow preliminary assessments for Fusarium wilt resistance without tying up precious quarantine glasshouse space. Although this does not eliminate the need for field nursery confirmation for promising accessions, highly susceptible lines can be identified and eliminated earlier in the process.

Screening the wild Australian *Gossypium* accessions has identified resistant and susceptible accessions. The resistant accessions are important sources of novel resistance genes, while the susceptible accessions are critical for developing the experimental populations needed for gene discovery. The genetic linkage map of the *G. sturtianum* genome will be a critical resource in identifying location of the resistance genes and then identifying and isolating them

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.

Much of the data gathered under this grant has already by been presented in Australian and International venues. As the final data sets are analysed, the project outcomes will be presented at appropriate venues, most notably the Fuscom research symposia. In addition to the presentations and publications listed below, several other manuscripts are being prepared for peer-reviewed journals

Brubaker, C.L., and others. Cotton Genomics at CSIRO Plant Industry. Proceedings of the Beltwide Cotton Conference, San Antonio, Texas, USA. [5-9 Jan 2004]

Wang, B., A. Becerra, D. Beasley, H. McFadden, A. Davidson, C. L. Brubaker, and J.J. Burdon. 2003. CRC Annual Review Booklet.

Brubaker, C. L. and A. Becerra. 2002. Genetic linkage mapping of the diploid *Gossypium* species. Cotton Science 14 Supplement: Proceedings of the 3rd International Cotton Genome Initiative Workshop, Nanjing, China. [3-6 June 2002]

Becerra Lopez-Lavalle, L.A., H. G. McFadden, and C.L. Brubaker. 2002. Genetic characterization of chromosome inheritance in *G. hirsutum* X C genome alien chromosome addition lines: Fusarium wilt resistance in wild Australian *Gossypium*. Proceedings of the 11th Australian Cotton Conference, Brisbane, Queensland, Australia. [13-15 Aug 2002]

Wang, B., C.L. Brubaker, and J.J. Burdon. 2002. Potential *Fusarium* pathogens of cotton associated with native *Gossypium* species. Proceedings of the 11th Australian Cotton Conference, Brisbane, Queensland, Australia. [13-15 Aug 2002]

Brubaker, C.L. "Fusarium wilt of cotton in Australia." 15 Oct 2003, National Key Lab of Crop Genetics and Germplasm Enhancement Cotton Research Institute, Nanjing Agricultural University, Nanjing, China

(c) for future research.

The information, molecular markers, and experimental populations will be used to more fully understand the genetics of *Fov* resistance in cotton and the evolution of virulence in Fusarium wilt pathogens, with the ultimate hope of identifying the specific genes controlling resistance. This work is continuing under CSP159C and CSP155C.

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)

- Journal papers:

McFadden, H., D. Beasley, AND C. L. BRUBAKER. 2004. Assessment of *Gossypium sturtianum* and *G. australe* as Potential Sources of Fusarium Wilt Resistance to Cotton. Euphytica 138: 61-72.

Wang, B., Brubaker, C. L., and Burdon, J. J. 2004. *Fusarium* species and Fusarium wilt pathogens associated with native *Gossypium* populations in Australia. Mycological Research 108: 35-44.

Wang, B., Brubaker, C. L., Woods, M. J., Matheson, B. A., and Burdon, J. J. Wild *Fusarium oxysporum* f. sp. *vasinfectum* associated with native *Gossypium* populations in Australia. Phytopathology. [in review]

Wang, B., Priest, M. J., Davidson, A., Brubaker, C. L., Woods, M. J., and Burdon, J. J. Endophytic fungi of native *Gossypium* species in Australia. Australian Journal of Botany. [in preparation]

- Conference papers

Wang, B., Brubaker, C. L., and Burdon, J. J. 2003. Incidence of Fusarium wilt pathogens in the rhizosphere of Australian native cottons. Handbook of the 8th International Congress of Plant Pathology, 363. (Christchurch, New Zealand).

Wang, B., Brubaker, C. L., and Burdon, J. J. 2002. Potential Fusarium pathogens of cotton associated with native Gos*sypium* species. Proceedings of the 11th Australian Cotton Conference, (CD version) (Brisbane, Queensland).

Wang, B., Brubaker, C. L., and Burdon, J. J. 2001. Incidence of *Fusarium* spp. in rhizosphere soil of wild cottons in the Mt Isa area of Queensland. Handbook of Australasian Plant Pathology Society 13th Biennial Conference, 386. (Cairns, Queensland).

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

NO

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.

Results of this project are of great importance to the cotton industry. Firstly, identification of resistance genes in native *Gossypium* germplasm provides an alternative resistance source for breeding programs. Secondly, knowledge about wild *Fov* associated with native *Gossypium* species highlights the need to screen new cotton areas for wild *Fov* before extensive stands of cotton are grown there. Thirdly, clarification of the local origin of *Fov* in Australian cotton fields suggests that careful monitoring to insure that other latent *Fusarium oxysporum* pathotypes in Australia do not increase in virulence. Collectively these results will assist the development improved cotton cultivars and provide information that can be used to improve farm management practices so that the effects of Fusarium wilt can be mitigated. This will contribute to the long-term sustainability of cotton production in Australia.

Part 4 – Final Report Executive Summary

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), is a destructive disease of cotton (*Gossypium hirsutum* L) in almost all cotton producing countries of the world. First reported in 1993, this disease is now widespread in Australia and is causing substantial losses. There are also 17 native *Gossypium* species or wild cottons in Australia, some of which have ranges that overlap cotton-growing regions. Wild crop relatives are a traditional source of novel resistance genes for many plant diseases, and preliminary studies of Australian *Gossypium* species suggested they may contain some useful levels of Fusarium wilt resistance. At the same time, however, it was possible that the native species could be harbouring potential cotton pathogens. The main objectives of this project was to explore the risk and the potential of the Australian *Gossypium* species.

Screening the Australian *Gossypium* species identified a range of accessions that will be useful in the continuing efforts to develop new cotton cultivars with improved levels of Fusarium wilt resistance. Although there was considerable variation in Fusarium wilt resistance among the Australian *Gossypium* species, *G. sturtianum* emerged as a possible source of novel resistance genes. Subsequent analyses confirmed that *G. sturtianum* was resistant to Fusarium wilt, but genetic analyses have established that transferring the *G. sturtianum* genes to cultivated cotton will be extremely difficult.

Simultaneously, it has become clear that while the native *Gossypium* species are not harbouring cotton field pathogens. However, surveys of the pathogen in cotton fields suggest that pathogen is continuing to evolve and continuing vigilance would be appropriate.