Cotton RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

Part 1 - Summary Details **REPORTS**

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CRDC Project Number: **CSP 120C**

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: Genetic characterization of homoeologous recombination and chromosome inheritance in G. hirsutum x K genome alien chromosome addition lines

Project Commencement Date: 4 Jan 2001 **Project Completion Date:** 31 Dec 2003

Research Program: 5 Breeding and Biotechnology

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1. Outline the background to the project.

Native to Australia are 17 species of wild *Gossypium* species, commonly called native cottons, which have been the focus of increased attention in recent years. The commercial release of genetically modified cotton cultivars prompted calls for assessments of the probability that transgenes could escape from cultivated cottons into a wild species and transform the wild species into an aggressive weed. The native Australian cottons also carry genes of potential agronomic utility, e.g., gossypol free seeds and *Fusarium* wilt resistance that have not been incorporated into cotton breeding programs.[1,2]; Kochman, personal communication). Under grant CSP47C, it was demonstrated that natural barriers between cultivated cottons and the native cottons growing near the Queensland and New South Wales cotton growing areas reduce the risk of transgene escape to zero. At the same time, however, it was shown that under glasshouse conditions, these natural barriers could be overcome. As a result, fertile hybrids carrying chromosomes of the native Australian have been developed and the relative feasibility of euploid and polyploid (or hexaploid bridging) breeding strategies were evaluated.[3] Fertile hybrids, however, are the only the first step. Successful transfer of genes between species, whether desirable (*e.g.,* agronomic traits) or undesirable (*e.g.*, escaped of transgenes to wild species) only occurs when the donor and recipient chromosomes interact and recombine.

While euploid interspecific hybrids (or trispecies bridging crosses) maximize the interactions between the chromosomes of the donor species and those of the recipient cultivated species, progeny are only rarely recovered and often cannot be backcrossed further without time consuming embryo rescue and in vitro culture of hybrid embryos.[4] In contrast, hexaploid bridging gives rise to reasonable numbers of backcross progeny without extensive intervention. The fertility of the backcross progeny increases with each successive generation, but the donor chromosomes are sequentially shed during recurrent backcrossing and the frequency of donor and recipient chromosome interactions is lower. Theoretically, however, this allows more recombinants to be recovered because the much larger number of progeny more than compensates for the lower frequency of recombination.

The validity of this approach was tested using 129 backcross progeny of a cross between a synthetic hexaploid [2x (*G. hirsutum* x *G. australe*)] and cultivated cotton (*G. hirsutum*) (CSP85C). The initial cross produced an F_1 pentaploid hybrid that contained the four sets of *G. hirsutum* chromosomes and one set of the *G. australe* chromosomes. This was backcrossed to *G. hirsutum* producing 18 BC1 progeny that contained all the *G. hirsutum* chromosomes but only some of the 13 *G. australe* chromosomes. The BC_1 progeny were backcrossed again to *G. hirsutum* producing 111 BC2 progeny. Each of these BC_2 progeny contains a subset of the *G. australe* chromosomes in their respective BC1 parent. All 18 BC1 progeny and their parents were screened using 698 *G. australe*-specific molecular markers (AFLPs); the 111 BC_2 progeny are currently being screened. A subset of these markers is simultaneously being mapped in a *G. nelsonii* x *G. australe* F_2 population to provide an independent assessment of marker linkages.[5] The comparison of the BC_2 and F_2 data will reveal the extent of interspecific gene exchanges between the *G. hirsutum* and the *G. australe* chromosomes.

Although final assessment of *G. hirsutum—G. australe* chromosomal recombination must wait for the complete BC_2 data set analysis, The analysis of the BC_1 progeny and comparison of these results

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[1]Brubaker, C. L., C. G. Benson, C. Miller, and D. N. Leach. 1996. Occurrence of terpenoid aldehydes and lysigenous cavities in the 'glandless' seeds of Australian *Gossypium* species. *Australian Journal of Botany* 44: 601-612.

[2] J. Kochman, personal communication.

[3] Brubaker, C. L., A. H. D. Brown, J. McD. Stewart, M. J. Kilby, and J. P. Grace. 1999. Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. *Euphytica* 108: 199-213.

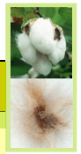
[4] Vroh Bi, I., J. P. Baudoin, B. Hau, and G. Mergeai. 1999. Development of high-gossypol cotton plants with low-gossypol seeds using trispecies bridge crosses and in vitro culture of seed embryos. Euphytica 106: 243-251.

[5] Brubaker, C. L. and A. H. D. Brown. 2003. The use of multiple alien chromosome addition aneuploids facilitates genetic linkage mapping of the *Gossypium* G genome. *Genome* 46**:** 774-791.

with the *G. nelsonii* x *G. australe* F_2 segregation data have already produced useful results.[6] Genetic analysis of 668 *G. australe* alleles among the 18 BC_1 individuals 634 loci resolved 13 blocks of non-recombinant loci that correspond to the 13 *G. australe* chromosomes. Thus the number and the identity of the chromosomes in each BC_1 individual was determined. By gathering these data for successive backcross generations, the frequency and the fidelity of transmission for each *G. australe* chromosome can be more accurately assessed. Knowing the *G. australe* chromosome complement of each BC_1 individual also makes it possible to identify chromosomes carrying genes for traits of interest. For example, among the 18 BC_1 individuals, all plants carrying **chromosome 5** (Table 1) have **brown lint**, and the two plants that retain the **pink flower** colour of *G. australe* are the only plants that have retained **chromosome 8**.

Table 1: Distribution and number of G. australe chromosomes among 18 BC1 aneuploid progeny from 5n [*G. australe* x *G. hirsutum*] x *G. hirsutum*. Associations with brown lint and pink flower traits are indicated in boxes

Linkage Group	668	689	677	701	681	682	689	700	701	702	703	704	705	706	707	708	709	754	No. of BC1s
5					X		X	X			X	X	X				X		7
6					X	X		X			X		X			X	X	X	8
12				X		X	X	X	X					X				X	7
4			X			X					X	X							4
2			X	X		X	X		X	X	X			X	X				9
8		X										X							2
9		X		X					X							X	X		5
7		X	X	X	X	X	X	X	X			X	X	X	X	X			13
11	X		X				X	X	X	X			X					X	8
1	X	X		X	X		X					X	X		X		X		9
10	X	X	X				X	X			X	X		X		X	X		10
3	X	X	X			X			X		X		X					X	8
13	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	18
No. of Linkage Groups	5	7	7	6	5	7	8	7	7	3	7	7	7	5	5	5	5	5	



The juxtaposition of the rate of chromosome loss versus the frequency of *G. hirsutum—G. australe* interspecific chromosomal recombination is the critical parameter governing the success of hexaploid bridging programs as well as estimating the risk of transgene escape, should these intergenomic hybrids arise in nature. It is now well documented that the probability that hybrids involving the wild cotton species in Queensland and New South Wales is functionally zero, and thus these considerations are moot, but the situation in the north-western cotton growing regions of Australia is less certain.[7]

The northwest corner of Australia contains 12 species of unique wild cotton species known as the Kimberley, or K genome, cottons. In an earlier analysis, the risk potential of the K genome species was also estimated to be low, but unlike the wild cotton species in eastern cotton districts, hybrids between the K genome cottons and *G. hirsutum* are sometimes partially fertile, raising the possibility of gene exchange.[7] To date this only occurs when *G. hirsutum* is the female, and since this hybrid seed would be harvested with the crop, the risk of escape is very low. All attempts to obtain hybrids between *G. hirsutum* and the K genome species with the K genome species as the female have failed, but only 88 pollinations have been attempted.[8] The possibility that commercial cotton production involving transgenic cotton cultivars will expand in the northwest cotton growing regions in the next five years suggests that it would be prudent to examine the barriers between *G. hirsutum* and relevant K genome species more rigorously. Because fertile hybrids are a remote possibility, using molecular markers, as is being done under CSP85C for *G australe*, to ascertain the rate of chromosome loss versus the frequency of *G. hirsutum*—K genome interspecific chromosomal recombination will be also contribute to accurate estimates of the risk of transgene escape.

Concurrently, the development of K genome chromosome-specific molecular markers may be useful to cotton breeders. *F. oxysporum* Schlecht. ex Fr. f. sp. *vasinfectum* (Atk.) Snyd. & Hans. (*Fov*) is the causal organism of Fusarium wilt of cotton and has been reported to be present in most of the major cotton-growing areas of the world (Fig. 1). Until recently, it had not been reported in Australia, however, during maturation of the 1993 cotton crop, wilted plants were observed in several fields on the Darling Downs.[9] The increase in *Fusarium* wilt in certain cotton districts has heightened

[6] Brubaker, C. L. and A. H. D. Brown. 2003. The use of multiple alien chromosome addition aneuploids facilitates genetic linkage mapping of the *Gossypium* G genome. *Genome* 46**:** 774-791.
[7] Brown, A. H. D., C. L. Brubaker, and M. J. Kilby. 1997. Assessing the risk of cotton transgene escape into wild Australian *Gossypium* species. In G. D. McLean, P. M. Waterhouse, G. Evans, and M. J. Gibbs [eds] The Commercialisation of Transgenic Crops: Risk, Benefit and Trade Considerations [Proceedings of a workshop held in Canberra Australia 11-13 March 1997] pp. 83-94. Cooperative Research Centre for Plant Science and Bureau of Resource Sciences, Canberra, Australia.
[8] Brubaker, C. L., A. H. D. Brown, J. McD. Stewart, M. J. Kilby, and J. P. Grace. 1999. Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. *Euphytica* 108: 199-213.
[9] Kochman, J. K., 1995 Fusarium wilt in cotton - a new record in Australia. *Australasian Plant Pathology* 24: 74.

interest in the genetics of *Fusarium* resistance. In a preliminary survey of the wild Australian cotton species, Joe Kochman has shown that some but not all the K genome and C genome species are resistant to the two major *Fusarium* pathotypes. Under CSP47C, thirty interspecific hybrids among the K genome were synthesized. Some crosses are between susceptible and resistance species and could provide a means of elucidating the genetic control of *Fusarium* resistance in the wild Australian cotton species, an aspect of the project that would be done in collaboration with Joe Kochman of the Queensland DPI and Jeremy Burdon of the CSIRO Division of Plant Industry (new CRDC grant proposal under review).

Figure 1: World distribution of *Fov*

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

<u>Objective 1</u>: Reciprocal crosses between *G. hirsutum* and selected K genome species.

We experienced difficulty maintaining the K-genome species under glasshouse conditions in Canberra, despite spending considerable time resowing lines. At this point our seed stocks were nearly depleted. If the K-genome seed stocks were lost, they would have been to be recollected from the wild.

This objective was originally proposed as means of addressing lingering concerns (mostly from Environment Australia) regarding the possibility that GMO cottons could transfer transgenes to the native K genome cottons native to the Kimberley plateau. Subsequent to the submission of this grant, C. L. Brubaker prepared a report on behalf of the OGTR reviewing the data bearing on this issue: C. L. Brubaker, *An evaluation of the scientific data bearing on the potential for gene flow between GM cottons and Gossypium K-genome species indigenous to Western Australia and the Northern Territory*. This report concluded that " . . . the available data adequately establish that the risk of transgene transfer from a GM cotton to *Gossypium rotundifolium* [the only species deemed to be at risk] is acceptably low." This report appears to have put the issue to rest. At the most recent OGTR-sponsored meeting of the Cotton Review Panel (5,6 December 2002; Canberra, ACT), none of the represented stakeholders requested any further scientific research into this issue.

Given the experimental difficulties, the lack of interest in the data and the need for cost savings this objective was set aside and replaced (see CSP120C Jan 2003 report).

<u>Objective 2</u>: Develop synthetic polyploid germplasm from *G. hirsutum* X K genome hybrids when the K genome species is resistant to *Fusarium* wilt, and <u>Objective 3</u>: Develop segregating F2 or BC1 progeny of crosses between K genome species susceptible and those resistant to *Fusarium* wilt.

Given the difficulties with culturing these species, insufficient accessions have been screened for Fusarium wilt resistance to allow us to identify suitable parents for crosses. Fortunately, the screening of the *G. sturtianum* accessions has been highly successful, and suitable lines have been identified in this species. The initial hybrids have already been synthesized, and in fact the first (F1) generation is already growing, and a suitable F2 family for genetic analyses has been generated. Our experience, suggests that the K genome species are not likely to be useful in developing Fusarium wilt resistant cultivars, unlike *G. sturtianum* which has shown robust resistance which is expressed in hybrids with *G. hirsutum*.

Given the experimental difficulties and the need for cost savings this objective was set aside and replaced (see CSP120C Jan 2003 report).

New objective 1: Assessing the *Fov* resistance of Gos-5271, a *G. hirsutum* x *G. sturtianum* synthetic hybrid.

This was done in a series of replicated trials in two locations in collaboration with Dr Helen McFadden and Dean Beasley (work completed). A manuscript describing the results is currently in press in the journal *Euphytica*: McFadden, H., D. Beasley & C. L. Brubaker. Assessment of *Gossypium sturtianum* and *G. australe* as potential sources of Fusarium wilt resistance to cotton.

New objective 2: Develop chromosome-specific markers for *G. sturtianum*.

We developed a suite of *G. sturtianum* chromosome-specific molecular markers to track the fidelity and frequency of *G. sturtianum* chromosome transmission aneuploid backcross lines. Using these markers, we evaluated aneuploid backcross lines, in the hope of identifying specific chromosomes carrying genes the improve Fusarium wilt resistance [refer to new objective 8]. (work completed)

New objective 3: Genetic analysis of *G. sturtianum* parental stocks for F_2 families and development of F2 segregating families for genetic linkage mapping.

Thirty-six *G. sturtianum* accessions were evaluated using 24 *Eco*RI/*Mse*I and 26 *Pst*I/*Mse*I primer combinations to identify suitable C-genome accessions for locus ordering. Based on these results two intraspecific *G. sturtianum* F2 families have been developed. The first has been genotyped using 343 AFLP loci. This population is being used to develop a reference genetic linkage map. A second F2 family, segregating for Fusarium wilt resistance, has been generated as well. This second population will be planted soon and used for genetic analyses of Fusarium wilt resistance in cotton. (work completed)

New objective 4: Glasshouse and evaluation of 46 *G. hirsutum* x *G. sturtianum* aneuploid BC families for *Fov* resistance.

We evaluated Fusarium wilt resistance in advanced backcross hybrids between *G. hirsutum* and *G. sturtianum* [refer to new objective 1]. (work completed)

New objective 5: Field evaluation of 30 *G. hirsutum* x *G. sturtianum* aneuploid BC_2F_2 families for *Fov* resistance.

Based on the results of new objective 4, we undertook field evaluations of the *G. hirsutum* x *G. sturtianum* advanced backcross aneuploid stocks. In 2000/2001, 2 lines were evaluated. In 2001/2002, 191 lines were evaluated in *Fusarium* field nurseries. In 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 (2003/2004) in the Queensland *Fusarium* field nurseries and single plant selections were undertaken in April 2004 for the following season.

New objective 6: Genetic analysis of *G. hirsutum* x K genome BC_1 individuals.

To assess the frequency and fidelity of the K-genome chromosome transfer to *G. hirsutum*, DNA material has been obtained from 35 BC_1 to develop a K genome BC_1 aneuploid map using 24 *Pst*I-*Mse*I primer combinations. (all data collected; analysis underway).

New objective 7: Generate a K genome map.

We generated 86 F_2 progeny from a cross between *G. populifolium* x *G. enthyle* (Hyb-638) for analysis of chromosome recombination in *G. hirsutum* x K genome aneuploid hybrids. These progeny have been genotype using 24 *Pst*I/*Mse*I primer combinations. The analysis is currently in progress.

<u>New objective 8</u>: AFLP genotyping of the 46 *G. hirsutum* x *G. sturtianum* aneuploid BC families.

Based the results abstracted in Objective 5, 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*. (work completed).

3. Detail the methodology and justify the methodology used.

Frequency and fidelity of alien chromosome transmission

Direct crosses between wild Australian diploid species and cultivated tetraploids can be realized for some combinations. The success in transferring potential useful traits depends on generating large numbers of fertile progeny and optimising recombination between the tertiary pool chromosomes (C, G, and K), and the A and D chromosomes of the cultivated tetraploid cottons. Two methods ('tetraploid' and 'hexaploid' bridging) have been proposed to generate self-fertile backcross progeny between the tertiary pool and the cultivated tetraploids.[10,11] Tetraploid bridging (or tri-species bridging crosses) maximizes the interactions between the chromosomes of the donor species and those of the recipient cultivated species, but progeny are only rarely recovered and often cannot be backcrossed further without time consuming embryo rescue and *in vitro* culture of hybrid embryos.[12] In contrast, hexaploid bridging gives rise to reasonable numbers of backcross progeny without extensive intervention (Fig. 1).[10] The fertility of the backcross progeny increases with each successive generation, but the donor chromosomes are sequentially shed during recurrent backcrossing and the frequency of donor and recipient chromosome interactions is lower due to autosyndesis. Theoretically, however, this allows more recombinants to be recovered because the much larger number of progeny, which compensates for the lower frequency of recombination.[10] Attempts to introgress genes from the Australian C genome (*G. sturtianum*) has been made using a tetraploid bridging strategy[12,13], in this study we have adopted a hexaploid bridging approach for the C and K genome as a mean of transferring them into the cultivated cottons. The plasticity of the hexaploid bridging approach has made possible the construction of a number of alien chromosome additions lines dissecting the C and G genomes, which offers a potential powerful tool for genetic analysis and evolutionary studies.

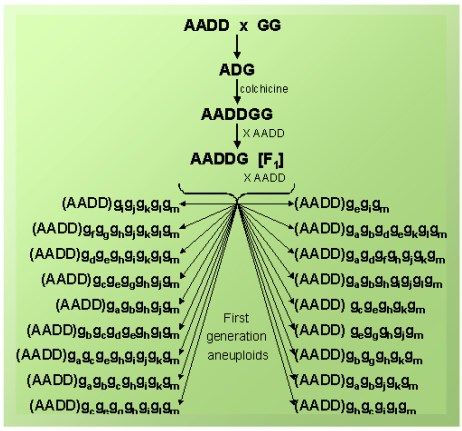


Figure 2: Hexaploid bridging approach

To track the frequency and fidelity at which each C and K genome chromosome is transmitted into the AD genome species (*G. hirsutum*) chromosome-specific markers can be used alternatively to a cytogenetic approach. DNA fingerprinting with wide genome coverage such as AFLP can be used to develop chromosome-specific markers to track the inheritance of these alien chromosomes in the alien chromosome addition lines. A suite of *G. sturtianum* and *G. anapoides* AFLP chromosome-specific markers to track the frequency and fidelity of the C and K genome transmission

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[10] Brubaker, C. L., A. H. D. Brown, J. McD. Stewart, M. J. Kilby, and J. P. Grace. 1999. Production of fertile hybrid germplasm with diploid Australian *Gossypium* species for cotton improvement. *Euphytica* 108: 199-213.

[11] Stewart, J. M. 1995. Potential for crop improvement with exotic germplasm and genetic engineering. In G. A. Constable and N. W. Forrester [eds.], Challenging the future: Proceedings of the World Cotton Conference-1 (Brisbane Australia; February 14-17 1994) 313-37. CSIRO, Melbourne.

[12] Vroh Bi, I., J. P. Baudoin, B. Hau, and G. Mergeai. 1999. Development of high-gossypol cotton plants with low-gossypol seeds using trispecies bridge crosses and in vitro culture of seed embryos. Euphytica 106: 243-251.

[13] Vroh Bi I., Baudoin J.P., Mergeai G. 1998. Cytogenetics of cotton plants involved in the introgression of « glandless-seed and glanded-plant » trait from *Gossypium sturtianum* Willis into upland cotton (*Gossypium hirsutum* L.). *Plant Breeding* 117, 235-241.

in a *G. hirsutum* x *G. sturtianum* and *G. hirsutum* x *G. anapoides* hexaploid bridging families needed to be developed to screen first (BC_1) and second (BC_2) generation aneuploids. The AFLP molecular marker system was selected because of the large number of loci that can be screened in a single assay allowing the examination of more of the genome per unit of effort, which is useful for any study on genotype relationships. Brubaker and Brown also demonstrated that dominant maker system such as AFLP is equally efficient as co-dominant maker system to track alien chromosomes in hexaploid bridging aneuploid series.[14]

Alien chromosome associations

The distribution of *G. sturtianum* and *G. anapoides* markers among the aneuploids allowed us to determine the number and the identity of the chromosomes in each generation. By gathering these data for two successive backcross generations, the frequency of transmission for each *G. sturtianum* and *G. anapoides* chromosome could be determined. Also, knowing the distribution of the C and K genome chromosome-specific markers among aneuploid lines have the potential to: (i) resolve chromosomal location of those markers that do not segregate in a core F_2 mapping C and K genome mapping populations or that could not be mapped due to ambiguity in determining cis/trans phase or distortions in markers segregation, and (ii) resolve the association between agronomically important traits and alien chromosomes additions.

The *Fov* disease test was conducted 8 weeks after inoculation. Diseased plants were identified by the dark brown discolouration of the vascular tissues. Disease severity was assessed by rating the plants on a scale of 0-5 according to their vascular wilt symptoms, in which 0, healthy and 5, dead. Mean disease incidence and its standard error was calculated for VB, and used to determine a confidence interval (CI) with a P= 0.1. This disease assessment scale correlates with the one developed by the Fuscom committee for field trials.

The data analysis of association between *Fov* disease resistance response and the presence of C genome chromosomes in the *G. hirsutum* background was done using logistic regression using the relationship between *Fov* response variable [*e.g.*, VB; 0&1(resistant) and 4&5(susceptible)] and a set of explanatory variables (*e.g.*, C-genome chromosomes present in the *G. hirsutum* milieu) because the dependent (response) variable is categorical (VB scores ranging from 0-5). Logistic regression is most often used when the dependent variable is dichotomous, that is, takes only two values (0-1, C-genome chr. – non C-genome chr.), but can also be applied when the dependent variable is ordinal or nominal. SAS has procedures for modelling all three cases, dichotomous, ordinal and nominal. In our study we used logistic regression to model the relationship between *Fov* disease response and C genome chromosome inheritance to identify chromosomes of *G. sturtianum* significantly associated with *Fov* disease resistance.

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

<u>New objective 1</u>: Assessing the *Fov* resistance of Gos-5271, a *G. hirsutum* x *G. sturtianum* synthetic hybrid.

We confirmed earlier reports of Fusarium wilt resistance in *G. sturtianum*, and established that hybrids between *G. hirsutum* and *G. sturtianum* are more resistant that their *G. hirsutum* parent (in conjunction with Helen McFadden, Queensland DPI, and CSP113C). Gos-5271, and its *G. hirsutum* and *G. sturtianum* parents, were assayed in four trials (the first three trials are summarized in Table 2). Using standard control cotton cultivars [Sicot 189 & DeltaEmerald (resistance) and Siokra 1-4 (susceptible)], the *G. sturtianum* parent (Gos-5275), the *G. hirsutum* parent (CPI-138969) and their hexaploid hybrid were tested for Fusarium wilt response. Sampling for *Fov* incidence data was based on vascular browning (VB, ranking 0-5). Mean disease incidence and its standard error was calculated for VB, and used to determine a coefficient of variation (CV) with a P= 0.05.

[14] Brubaker, C. L. and A. H. D. Brown. 2003. The use of multiple alien chromosome addition aneuploids facilitates genetic linkage mapping of the *Gossypium* G genome. *Genome* 46**:** 774-791.

The Canberra and Indooroopilly trials indicate that the *G. hirsutum* X *G. sturtianum* hybrid does have improved Fusarium wilt resistance relative to its *G. hirsutum* parent and this enhanced tolerance originates from its *G. sturtianum* parent. On the strength these results, we are pursuing an extensive analysis of the advance backcross progeny focusing our efforts on trying to identify the genomic location of this resistance and identify hybrids in which this resistance has been transferred to *G. hirsutum*.

Table 2. Mean vascular browning for a *G. hirsutum* x *G. sturtianum* hybrid, its parents (*G. hirsutum* CPI138969 and *G. sturtianum* G5275), and three industry standards (Siokra 1-4, Sicot189, Delta Emerald). In all three trials G5271 exhibited levels of Fusarium wilt resistance equivalent to or superior to the industry standards. The symbols "*" and "§" denote statistically equivalent means within trials.

Vascular Browning Index (% 0s & 1s)									
Trial	N	CPI 138969	Gos-5271	Gos-5275	Siokra 1-4	Sicot 189	Delta Emerald	Trial mean
1	20		0.03* (100)		2.1 (48)	0.33* (88)	0.28* (95)	0.89 (75)
2	28	0.48 (89)	0 (100)	0.23 (98)	0.47 (88)	0.06 (98)	0.09 (98)	0.46 (88)
3	40	3.65* (3)	1.64 (52)	0.42 (95)	3.7* (0)	2.9§ (10)	2.8§ (18)	2.5 (30)

<u>New objective 2</u>: Develop chromosome-specific markers for *G. sturtianum*.

The AFLP genotypes of the 11 *G. sturtianum* addition lines revealed using 24 *Eco*RI/*Mse*I and 26 *Pst*I/*Mse*I primer combinations are based on 2036 *G. sturtianum*-specific AFLP loci. Of these AFLPs, 892 (44%) segregated among the 11 *G. sturtianum* addition lines.

Marker inheritance was additive in the hexaploid (Gos-5271) and in the pentaploid (Hyb 629). In seven primer pairs (PAA-MGTC; PAC-MGAG; PAC-MGAT; PAG-MGTC; PAT-MGTC; PAGT-MGAC; PATC-MGAC) at least one AFLP fragment was clearly absent from both parents and appeared in the hexaploid (Gos-5271). Of these primer pairs, two (PAC-MGAT and PAGT-MGAC) showed AFLP fragments that was transmitted to the pentaploid (Hyb 629) and segregated among the first generation aneuploids. While the loss of parental AFLP fragments and gain of *de novo* bands in the aneuploid segregating lines was rare, some of these events may be indicating *de novo* evolutionary sequence changes. However, these rare events are most likely to be caused by heterozygous loci present in the diploid parent and consistent with the overall stability of synthetic allopolyploids in *Gossypium*.

Of the 892 AFLPs observed among the *G. sturtianum* addition lines, 796 (89%) were putatively assigned to 17 distinct suites or linkage groups (linkage groups) of cosegregating AFLPs, designated A to N (Table 3). Of these AFLPs, 572 (70%) were produced by *Eco*RI/*Mse*I and 224 (27%) by *Pst*I/*Mse*I. Thus, the number of polymorphic fragments produced by *Eco*RI/*Mse*I was two and a half time larger than those produced by *Pst*I/*Mse*I. Ninety-six AFLPs (11%) had confounded or eccentric patterns. The number of AFLP loci (796) assigned to each *G. sturtianum* linkage group were not evenly distributed, ranging from six (0.8%) for linkage group-Q to 79 (9.9%) for linkage group-A. Four of these linkage groups were small (≤19 AFLP loci), while 13 larger linkage groups comprised 33 to 79 AFLP loci. Considering that the *G. sturtianum* chromosome would be inherited largely unrecombined in the *G. hirsutum* background the 13 larger linkage groups should correspond to the 13 *G. sturtianum* chromosomes. The presence of the four smaller linkage groups suggests that at least some *G. sturtianum* chromosome restructuring has occurred. The location and extent of these events was determined from comparison of the aneuploid AFLP distributions to a preliminary C genome F_2 linkage analysis.

Table 3: Frequency and occurrence of non-recombinant suites of *G. sturtianum*-AFLP alleles among 11 *G. sturtianum* x *G. hirsutum* first generation aneuploids.

	G. sturtianum DNA transmission (BC₁ Progeny)																	
		4n *G. hirsutum* x 2n *G. sturtianum* BC1 progeny													C genome-specific AFLP loci			
	Linkage groups	645	651	685	686	687	710	711	712	734	735	736	Total No.	Total %	No. of EcoRI-AXX/MseI-CXX	No. of PstI-AX(X)/MseI-GXX	Total No.	Total %
1	A												0	0	48	31	79	9.9
2	B					X							1	9	3	7	10	1.3
3	C					X		X	X		X	X	5	45	28	13	41	5.2
4	D				X								1	9	27	15	42	5.3
5	E			X				X		X	X		4	36	51	14	65	8.2
6	F			X	X				X				3	27	33	8	41	5.2
7	G		X			X	X	X	X		X	X	7	64	35	19	54	6.8
8	H		X	X	X		X				X	X	6	55	55	17	72	9.0
9	I	X			X	X	X	X			X	X	7	64	53	16	69	8.7
10	J	X	X		X	X	X	X	X		X	X	9	82	51	27	78	9.8
	[Q]	X	X	X	X	X	X	X	X		X	X	10	91	6	0	6	0.8
	K	X	X	X		X				X			5	45	48	15	63	7.9
11	[P]	X	X	X	X	X				X			6	55	7	0	7	0.9
	O	X	X	X	X	X			X	X			7	64	47	7	54	6.8
12	M	X	X	X	X			X					5	45	22	11	33	4.1
	[L]	X	X	X				X					4	36	14	5	19	2.4
13	N	X	X	X	X		X	X	X	X	X	X	10	91	44	19	63	7.9
	Total No.	9	10	10	10	9	6	9	7	5	8	7	90		572	224	769	
	Total %	52.9	58.8	58.8	58.8	52.9	35.3	52.9	41.2	29.4	47.4	42.1		48.1	69.6	27.3		

The preliminary linkage analysis of the C genome chromosome-specific AFLP markers in an intraspecific *G. sturtianum* F_2 family showed that the congruence between AFLP cosegregation in the aneuploids and the linkages inferred in the F_2 family was consistent. Thirteen largest F_2 linkage groups could be associated with the 13 largest aneuploid linkage groups, and three of the four small aneuploid linkage groups, Q, P, and L could be associated with the larger aneuploid linkage groups J, K, and M, respectively, based on their joint association with the F_2 linkage groups. Only, the identity of one small aneuploid linkage group (Linkage group-B) remained undetermined.

One hundred BC_2 aneuploid plants from ten of the 11 derived BC_1 aneuploid lines were analysed to estimate the frequency and the fidelity of *G. sturtianum* chromosome transmission. Selection of these 100 plants was made on the basis of germination and seedling establishment. Thus, the selection pressure was solely driven by the genetic stability of the alien chromosomes in the *G. hirsutum* background, and conclusions regarding chromosome transmission from the BC_1 to the BC_2 are subject only to this bias. Twenty-four *Eco*RI/*Mse*I and 26 *Pst*I/*Mse*I PCs were employed and 796 *G. sturtianum*-specific molecular markers were screened (Table 4). Segregation analysis suggests that each of these BC_2 families contain a subset of the *G. sturtianum* chromosomes in their respective BC_1 parent.

Table 4: Frequency of C genome chromosome transmission from the BC_1 to the BC_2 population

Linkage groups	No. AFLP loci[c]	*G. sturtianum* x *G. hirsutum* BC_1 families[a,b]											Transmission BC_2	
		645	651	685	686	687	710	711	712	734	735	736	No.	%
A	79												0	0
B	10					2/9							2	22
C	39(2)					1/9		0/2	2/10 |97 [1]|		1/7	0/4	4	13
D	42				0/1								0	0
E	61(4)			1/1				0/2		3/12 |46 [1]|	1/7 |82 [1]|	5	23
F	40(1)			1/1	1/1				3/10				5	42
G	52(2)		-			4/9	25/51 |94-98 [7]|	0/2	6/10 |98 [1]|		1/7	0/4	36	44
H	64(8)		-	1/1	0/1		18/51 |48-98 [4]|				3/7 |95-98 [2]|	3/4	25	40
I	67(2)	0/3			0/1	2/9	24/51 |58-87 [4]|	0/2			1/7	3/4	27	36
J	74(4)	1/3	-		0/1	3/9	17/51 |77-99 [4]|	0/2	1/10		0/7	1/4	23	27
Q	5(1)	1/3	-	1/1	0/1	3/9	17/51 |80 [1]|	2/2 |40 [2]|	1/10		0/7	1/4	26	30
K	60(3)	0/3	-	1/1		4/9 |3.3 [1]|				9/12			14	56
P	6(1)	0/3	-	1/1	0/1	3/9				9/12			13	50
O	54	1/3 |98 [1]|	-	1/1	0/1	3/9			4/10	3/12			12	33
M	32(1)	0/3	-	1/1	0/1			2/2					3	43
L	18(1)	0/3	-	1/1				2/2					3	50
N	56(7)	0/3	-	0/1	0/1		9/51	2/2 |96 [1]|	2/10	3/12	1/7	1/4	18	20
No. LGs: BC_2 <average>		3 <1>	-	9 <9>	1 <1>	9 <2.4>	6 <2.1>	4 <4>	7 <1.9>	5 <2.25>	6 <1.1>	5 <2.25>	2.16	0-56 <32.4>

a No. LGs / No. BC_2

b values in brackets are percentage of total loci different from 100% and in parentheses number of individuals

c in parentheses missing data

The mean of number linkage groups in the BC_1 plants from which the 100 plants of the BC_2 progeny were analysed was 8.2, while the mean number of linkage groups among the BC_2 progeny was 2.2. Of the 796 diagnostic C genome chromosome AFLP markers, 759 were used to characterise *G. sturtianum* chromosome transmission rate in the second generation C genome alien chromosome addition lines. Only one linkage group (D) of the Hyb-686-BC_1 was not recovered in the BC_2 generation. In total the transmission of 15 linkage groups was followed. Overall, the C genome linkage group transmission was 32.4% for the linkage groups tested. Individual linkage group transmission ranged from 13% for linkage group-C to 56% for the linkage group-K. The average transmission of C genome linkage groups to the second generation aneuploids ($\overline{X}$ =12.7, s^2=127) was more variable than to the BC_1 ($\overline{X}$ =5.3, s^2 =9). The average linkage group transmission within

BC_2 families varied as well, ranging from one in family 686 to four in family 711, with only one BC_2 family (685) carried on average nine of the ten linkage groups present in their BC_1 progenitor.

C genome chromosome-specific AFLP marker transmission also varied among individuals (28) within BC_2 families (687, 710, 711, 712, 734 and 735). The percentage of C genome chromosome-specific AFLP marker transmission ranged from 3.3% for linkage group-K in family 687 to 99% for linkage group-J in family 710 (Table 4). In other words, in these BC_2 individuals between 1% and 96.7% of the C genome chromosome-specific AFLP markers were not transmitted, indicating that some genomic changes may have occurred after the two divergent genomes (C and AD) merged in the hexaploid (*i.e.*, recombination). Chromosome loss was not the only alteration observed in the AFLP DNA-profiling: (i) the appearance of twelve *de novo* AFLP bands (EC: *Pst*I/*Mse*I) in the synthetic hexaploid, and six AFLP bands in the BC_2 hybrids and (ii) the segregation of some these bands (3 AFLP loci) in the BC_1 and BC_2 individuals indicated that *de novo* DNA methylation modifications could be responsible for any gain, or loss, of restriction sites in the AFLPs and, consequently, for the lack of parental bands an the appearance of new ones.

<u>New objective 4</u>: Genetic analysis of *G. sturtianum* parental stocks for F_2 families.

Thirty-six *G. sturtianum* accessions were evaluated using 24 *Eco*RI/*Mse*I and 26 *Pst*I/*Mse*I primer combinations, resolving 2000+ *G. sturtianum* alleles. These data were used to determine the genetic relationships among *G. sturtianum* populations from the entire indigenous range. On the basis of this analysis *G. sturtianum* accessions Gos-5131 and Gos-5050 were selected to generate an F_1 hybrid (Hyb-799-4) from which an F_2 genetic linkage mapping population is being developed. Early in 2003 120+ F_2 progeny have been grown in the glasshouse and leaf tissue of 114 individuals has been collected for DNA extraction to develop a C genome map. Currently, 114 F_2 progeny have been genotype using 24 *Eco*RI/*Mse*I and 26 *Pst*I/*Mse*I primer combinations from which a preliminary linkage analysis has been done. A final linkage analysis will be completed soon.

<u>New objective 5</u>: Glasshouse and evaluation of *G. hirsutum* x *G. sturtianum* aneuploid BC families for Fov resistance.

The results in <u>new objective 1</u> confirmed that Fusarium wilt resistance in *G. sturtianum* is expressed in *G. hirsutum*. In Nov-2002/Jan-2003, 23 *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 at CSIRO-Canberra in collaboration with Helen McFadden. Eight of these lines were particularly promising, exhibiting disease levels significantly better than their *G. hirsutum* parent (Figure 3). In Nov-2002/Jan-2003 and Mar-2003/May-2003, 24 *G. sturtianum* x *G. hirsutum* (5271) BC_2F_3 aneuploid families carrying the remaining untested *G. sturtianum* chromosomes were evaluated for *Fov* resistance at CSIRO-Canberra. One of the Lines (L10) tested in Nov-2002/Jan-2003 and four of the lines (L3, L4, L6 and L10) tested in Mar-2003/May-2003 (Figure 3) exhibited levels of disease response significantly better than their parent (Control). A positive effect of C genome in the *G. hirsutum* background in the third generation aneuploid was confirmed in this study. The VB mean scores were significantly lower in 13 of the 47 C genome addition lines tested, indicating that the C genome chromosomes represented in these lines may contain the genes involve in *Fov* resistant response. Based on this result, we focused our efforts on

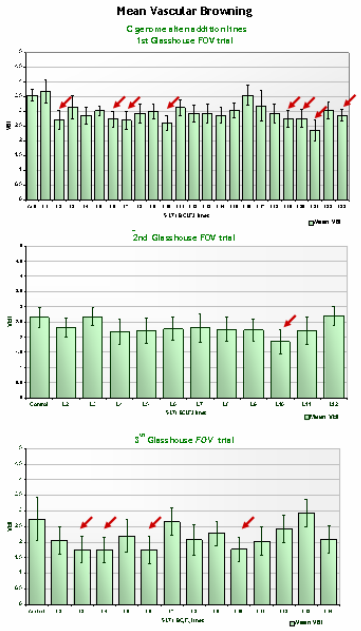

Figure 3: Mean vascular browning (VB) for 47 BC_3 C genome chromosome addition lines. The vertical bars indicated the confidence interval. The arrows show significant differences between the control (CPI 138969) and the addition lines to Fov resistance at $_{90\%}$CI

identifying the C genome chromosomes that were significantly associated with the enhanced *Fov*-resistance in the C genome aneuploid addition lines.

New objective 6: Field evaluation of 30 *G. hirsutum* x *G. sturtianum* aneuploid BC_2F_2 families for *Fov* resistance.

Based on the results of the abstracted above, we have undertaken field evaluations of the *G. hirsutum* x *G. sturtianum* aneuploid stocks. 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 will be planted in the Queensland *Fusarium* field nurseries.

New objective 7: Genetic analysis of *G. hirsutum* x K genome BC_1 individuals.

In order to assess the frequency and fidelity of the K-genome chromosome transferring *G. hirsutum*, DNA material has been obtained from 32 BC_1 to develop a K genome BC_1 aneuploid map using 24 *Psf*I-*Mse*I primer combinations. Twenty-four *Psf*I-*Mse*I primer pairs have already been tested and the construction of the BC_1 aneuploid map progeny to evaluate chromosome transmission frequency in the *G. hirsutum* x K genome hybrids is under way. Twenty-four *PstI-MseI* primer combinations have been tested and F_2 mapping is under way. Also, we crossed these BC_1 individuals back to the *G. hirsutum* cv. Sicala V2 (approx 650 crosses) for developing a BC_2 progeny to evaluate the fidelity and frequency of alien chromosome transmission in the *G. hirsutum* x K genome hybrids.

New objective 8: Generate a K genome map.

We generated 86 F_2 progeny from a cross between *G. populifolium* x *G. enthyle* (Hyb-638) for analysis of chromosome recombination in *G. hirsutum* x K genome aneuploid hybrids. These progeny have been genotype using 24 *Psf*I/*Mse*I primer combinations. The analysis is currently in progress.

New objective 9: AFLP genotyping of the 47 *G. hirsutum* x *G. sturtianum* aneuploid BC families.

Table 5: Logistic regression analysis on 47 C-genome chromosome addition lines. Solid bars show the C-genome LGs that had a significant effect on *Fov* disease resistance response at 95% confidence level.

Likelihood Ratio Tests

Dependent variable: VBI

Effect	-2 Log Likelihood of Reduced Model	Chi-Square	df	Sig.
CHRA	181.657	.000	0	.
CHRB	182.360	.703	1	.402
CHRC	196.955	15.298	1	.000**
CHRD	181.657	.000	0	.
CHRE	183.586	1.929	1	.165
CHRF	187.601	5.944	1	.015**
CHRG	184.138	2.482	1	.115
CHRH	181.801	.144	1	.704
CHRI	183.567	1.910	1	.167
CHRJ	185.455	3.798	1	.051
CHRK	189.917	8.260	1	.004**
CHRL	189.406	7.749	1	.005**
CHRM	191.175	9.518	1	.002**
CHRN	184.087	2.430	1	.119
CHRO	192.525	10.868	1	.001**
CHRP	190.455	8.798	1	.003**
CHRQ	188.984	7.327	1	.007**
CHRR	181.762	.105	1	.746
CHRS	183.234	1.577	1	.209
LINE	257.035	75.378	8	.000**

*Significant at the 0.10 level
**Significant at the 0.05 level

Linkage groups have a significant effect on resistance

Based the results abstracted in new objective 5, 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*. Thus, a suite of 181 *G. sturtianum* chromosome-specific molecular markers were selected to assess the relationship between *G. sturtianum* chromosome transmission and *Fov* resistance. Using these markers, we have currently evaluated 47 aneuploid backcross lines in the hope of identifying specific chromosomes carrying genes that improve Fusarium wilt resistance. Analyses to date have identified five putative *G. sturtianum* chromosomes (8 *G. sturtianum* linkage groups) that improve resistance to Fusarium wilt. Linkage groups "C", "F", "K-P-O", "L-M" and "Q" have showed a significant association with *Fov* resistance in the ADC synthetic hybrids. This result constitutes evidence that the incorporation of several genomic regions of the C genome into the cultivated cotton significantly improve the levels of *Fov* disease-resistance response observed in the recipient cultivated cotton genotype.

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

We have looked to wild related cotton species (Australian C-, G- and K-genomes) for novel sources of Fusarium wilt resistance. Among the 17 Australian *Gossypium* species, *G. sturtianum* is the most promising source of resistance to the Australian *Fov* strains. The data obtained from this study clearly indicated that *G. sturtianum* possesses genes that confer good levels of resistance against the two Australia strains of *Fov*. Knowing that *G. sturtianum* carry genes for *Fov* resistance, we assessed the *Fov* disease response in Gos-5271, a *G. hirsutum* x *G. sturtianum* synthetic hybrid. The results obtained from this study indicated that the *G. hirsutum* x *G. sturtianum* hybrid does have improved Fusarium wilt resistance relative to its *G. hirsutum* parent (CPI138969) and that this enhanced tolerance originated from its *G. sturtianum* parent (Gos-5275). This result is particularly important because it confirmed that the resistance carried by *G. sturtianum* (Australian *Gossypium* C-genome) can be expressed in the cultivated cotton (*G. hirsutum*) background. Furthermore, with the apparent lack of immunity in the *G. hirsutum* gene pool, this novel germplasm could be an important source of *Fov* resistance to the Australian cotton breeders if the *G. sturtianum* genes can be transferred to the *G. hirsutum* genome.

With the confirmation that the *G. sturtianum* genetic material can be expressed in the cultivated cotton background, we focused our attention on testing advanced *G. hirsutum* x *G. sturtianum* backcross (BC_2F_3) aneuploid families (47 aneuploid addition lines) carrying different *G. sturtianum* chromosomes for *Fov* resistance. A positive effect of the C-genome genetic material in the *G. hirsutum* background was confirmed in 13 third-generation aneuploid families, indicating that the C-genome chromosomes represented in these lines contained the genes involved in the *Fov* resistant response. Based on this result, we focused our efforts on identifying the specific C-genome chromosomes that were significantly associated with the enhance *Fov*-resistance in the C-genome aneuploid addition lines. *Gossypium sturtianum* chromosome-specific molecular markers were selected to assess the relationship between *G. sturtianum* chromosome transmission and *Fov* resistance. Analyses of this material identified five putative *G. sturtianum* chromosomes that significantly improve *G. hirsutum* resistance to Fusarium wilt. This result constitutes evidence that the incorporation of several genomic regions of the C-genome into the cultivated cotton could improve the levels of *Fov* disease-resistance response observed in the recipient cultivated cotton genotype, suggesting that more than one gene is involved in this resistance response. This result highlights the need to broaden our genetic understanding of Fusarium wilt resistance, which, in turn, will greatly facilitate our ability to effectively transfer genes from this novel plant material. Genetic analysis of the BC families suggests, however, that genetic recombination between the *G. sturtianum* and the *G. hirsutum* chromosomes is infrequent, and because the data indicate the multiple genes are involved in *Fov* resistance, transferring the *G. sturtianum* genes using bridging populations will be difficult. Nonetheless, advanced backcross families are being evaluated in the field.

The important outcomes of this project are summarized below.

- *G. sturtianum* has been identified has a possible source of novel *Fov* resistance genes and this resistance is expressed in the *G. hirsutum* background.
- Five genomic regions in *G. sturtianum* that may contain genes conferring *Fov* resistance have been identified, confirming the operating hypothesis that *Fov* resistance in cotton is multigenic.
- Transferring *G. sturtianum* genes to *G. hirsutum* directly will be very difficult. The observed level of recombination between *G. sturtianum* and *G. hirsutum* chromosomes is very low.
- The *G. sturtianum* genome has been mapped so that once the specific genes conferring resistance have been identified; molecular markers are already available for future work.
- Better experimental populations for *Fov* resistance gene discovery have been developed.

The primary conclusion obtaining from this grant is that we need a much better understanding of the genetics of Fov *resistance in cotton if we are to most effectively deploy the resistance sources available (this work is now ongoing in CSP159C), and once specific resistance genes have been identified, we can search more widely for new sources of genetic resistance.*

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

Fusarium wilt resistance is a significant cause of crop yield losses in nearly all New South Wales and Queensland cotton districts, significantly reducing the profitability and sustainability of cotton production. This research identified Australian native *Gossypium* species (*G. sturtianum* accessions) with superior levels of *Fov* disease resistance when compared against the two industry standards (Sicot 189 and DeltaEmerald). *Fov* disease levels were evaluated on advance backcross C-genome addition lines establishing the potential for this novel resistance to express in the cultivated cotton background. Under this grant, five possible *G. sturtianum* genetic regions were implicated as carrying genes involved in the *Fov* disease response, stressing the need for an explicit genetic understanding of Fusarium wilt resistance under the Australian *Fov* pathogenic regime. Thus, genetic studies based on the C-genome germplasm and alien-specific molecular markers developed under this grant will produce a number of tangible benefits to the industry. In the short term, the novel Australian wild germplasm provide a genetic system for inferring the number of genes responsible for the *Fov* resistance response, while the molecular markers will provide surrogates for the genetic regions carrying the resistant genes. Cotton breeders will be able to use these molecular markers to improve the efficiency of breeding for Fusarium wilt resistance in cotton eliminating costly biological disease assays.

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.)

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?

Not applicable

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

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

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

(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.

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.

See point A

9. List the publications arising from the research project and/or a publication plan.

In preparation

1.- Frequency and fidelity of alien chromosome transmission in hexaploid bridging populations.
2.- Genetic linkage mapping of the *Gossypium* C genome.
5.- Genome diversity in *G. sturtianum*.

Anticipated

3.- Genetic linkage mapping of the *Gossypium* K genome
4.- C genome chromosome introgression for Fusarium wilt resistance in cultivated cotton

10. 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.

Because the indigenous Australian *Gossypium* species evolved on the Australian continent, they are likely to possess adaptations to the Australian environment and natural resistance to indigenous microbial or insect pests. That some of the C genome species are resistant to the two pathotypes of *Fusarium* found in Australia but more importantly that this resistance can be transferred and expressed in the cultivated lines constitute a good example of the importance of this research. Continuing evaluation of the wild Australian *Gossypium* species is likely to lead to a better understanding of the genetics of this vital trait. Under this grant we have concentrated efforts in developing the germplasm necessary to understand the mechanism of Fusarium wilt resistance in cotton and to facilitate the transfer of traits from the wild Australian *Gossypium* species into cultivated cotton.

Part 4 – Final Report Executive Summary

Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *vasinfectum* (*Fov*), is a serious disease of cotton in Australia responsible for substantial yield reductions. Since its detection on the Darling Downs in 1993, *Fov* has spread to all major eastern cotton growing districts except the lower Namoi. The significant crop losses that have already occurred and the increasing incidence and severity of Fusarium wilt make Fusarium wilt the most significant challenge to long term sustainable cotton production in Australia.

Improved farm management strategies can reduce yield losses and disease spread, but developing resistant cultivars is by far the most effective long-term means of combating fungal diseases of agricultural plants. Australian cotton breeders have significantly improved the Fusarium wilt resistance of their cultivars, and new selections with even greater resistance are nearing commercial release. Despite the admirable progress that has been made, however, the current assessment is that new sources of Fusarium wilt resistance are needed.

With the realization that the best the *G. hirsutum* gene pool has to offer may not be good enough, We have looked to related Australian *Gossypium* species for novel sources of Fusarium wilt resistance, identifying one possible source of Fusarium wilt resistance in *G. sturtianum*. Although some of the *G. sturtianum* accessions tested are susceptible to fusarium wilt, many of the accessions are more resistant to fusarium wilt than the industry standards, and this resistance is expressed in the *G. hirsutum* background. Genetic analysis of *G. hirsutum* x *G. sturtianum* hybrids, however, suggests transferring the *G. sturtianum* genes to *G. hirsutum* will be difficult. Nonetheless, breeding lines are currently in the Fusarium field nurseries and this selection process will continue.

The other important outcome of this project was the development of new experimental populations and molecular markers that will, in ongoing research, provide a much better understanding the genetic control of fusarium wilt resistance in cotton. Under this grant, five chromosomes of the *G. sturtianum* genome have been identified as carrying genes that may contribute fusarium wilt resistance. The experimental populations and molecular markers will contribute to a more explicit genetic understanding of Fusarium wilt resistance that will facilitate our ability to effectively transfer genes from *G. sturtianum* as well as other novel resistance sources. With the apparent lack of immunity in the *G. hirsutum* gene pool, these novel germplasm resources will become increasingly important to cotton breeders.