Cotton RESEARCH & DEVELOPMENT

Annual, Progress and Final Reports

Part 1 - Summary Details **REPORTS**

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

CRDC Project Number: **CSP113C**

Annual Report: ☐ Due 30-Sep-03

Progress Report: ☐ Due 29-Jan-03

Final Report: ☒ Due 31-Mar-04
(or within 3 months of completion of project)

Project Title: Australian native cottons as sources of resistance and new pathotypes of Fusarium wilt

Project Commencement Date: 1 Jan 2001 **Project Completion Date:** 31 Dec 2003

Research Program: Diseases and Weeds

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Part 3.3 – Final Reports

(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 (caused by the fungal pathogen *Fusarium oxysporum* f.sp. *vasinfectum* [*Fov*]) is a major disease of cotton in many parts of the world. It was first recorded in Australia in 1993[1] since when it has become widespread occurring on the Darling Downs and in northern New South Wales. Crop losses associated with the disease range from 10 to 100% with farmers on infected properties on the Darling Downs estimating average yield losses of 25-50%[2].

Eight pathotypes of *Fov* have been identified around the world. However, the two recorded for Australia differ from those detected overseas with respect to vegetative compatibility groups and DNA fingerprints[3]. As a consequence, these two pathotypes are now widely regarded as native ones that have probably evolved in close association with native Australian species of *Gossypium*.

This association of *Fusarium* with native cottons is both a boon and a major potential problem. Extensive work on a wide range of crop plants has repeatedly shown the value of wild germplasm of related species as a rich source of genes for resistance to various pests and diseases[4]. Within Australia, this has been shown to be true with respect to blue mold of tobacco, and rust resistance in flax and soybean. Preliminary screening of eleven native *Gossypium* species, representing each of the three genome groups, indicates that at least two species (*G. bickii* and *G. sturtianum*) are resistant to both *Fusarium* wilt pathotypes in Australia[5]. That some species are susceptible to both *Fusarium* pathotypes is fortunate as hybrids between susceptible and resistant plants provides a possible means of elucidating the genetic control of *Fusarium* resistance in the wild species.

Equally though, while such long-term coevolutionary interactions between plants and pathogens are likely to select for the occurrence of resistance genes, they will also tend to select for the occurrence of a range of different pathotypes of the pathogen. These pathotypes are potential problems for the cotton industry in the future. Assessment of the occurrence of such 'new' pathotypes, their pathogenicity, relationship to the existing known pathotypes, and their geographic distribution will provide very valuable information on which to base future breeding strategies. Furthermore, such new pathotypes would provide the means whereby yet other resistances may be identified.

Here we propose a collaborative research project involving staff in CSIRO – Plant Industry (JJ Burdon, C. Brubaker) and QDPI (Joe Kochman, Natalie Moore, Susie Bentley). Kochman and his colleagues have developed a pot-test for *Fusarium* resistance and are using this in a CRC Tropical Pathology – CRDC program on the 'Ecology and management of *Fusarium* wilt'. Part of that project funding (CSP to Kochman) is to provide *Fusarium* resistance screening facilities for cotton breeders. However, those facilities are fully utilized and the screening needed to both assess a representative range of Australia's 17 native species of *Gossypium* and a range of segregating populations coming out of CSP (grant proposal pending - Brubaker) would need additional technical resources (0.5 technician). The work in this project would therefore be broken down so that the basic resistance screening work would utilize existing skills and expertise at QDPI. Work on crossing and genetic analyses of resistance in *Gossypium*, and the identification of *Fusarium* pathotypes associated with natural stands of wild *Gossypium* species would be conducted under the oversight of Burdon and Brubaker in Canberra. Kochman's group would provide the fingerprint probes that they have already developed for the two known pathotypes of *Fov* for use by the Canberra group. Furthermore we plan at least annual (and preferably more) frequent meetings to review progress and collaboration.

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

- To identify potentially valuable sources of naturally occurring resistance to *Fusarium* wilt by screening a broad range of accessions of native cotton species. These accessions are representative

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[1] Kochman, J. (1995). Fusarium wilt in cotton – a new record in Australia. *Australasian Pathology* 24: 74.

[2] Kochman, J., Moore, N., Obst, N., O'Neill, W., and Bentley, S. (1999). Ecology and management of *Fusarium* wilt.

[3] 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: 1143-1156.

[4] Frankel, O.H., Brown, A.H.D., and Burdon, J.J. (1995). The conservation of plant biodiversity. Cambridge University Press, Cambridge.

[5] Joe Kochman, personal communication.

of native cotton populations from a wide range of geographic locations including eastern areas of Australia already under cotton cultivation as well as areas in the northwest that may well be utilized for cotton production in the near to medium future.

A total of 180 screening trials were conducted at the Queensland DPI laboratories in Indooroopilly, Qld. Seventy-nine accessions representing all five C- and G-genome species were run in replicate trials at the Indooroopilly site. These trials revealed a previously unappreciated range of Fusarium wilt resistance among the Australian *Gossypium* species. In preliminary trials species were designated either as susceptible or resistant. These trials demonstrated that Fusarium wilt resistance within species can vary tremendously (see below).

- To determine the genetic control and heritability of such resistance.

The original intention was to assay existing segregating families for variation in Fusarium wilt resistance. The resistance phenotype was to be assigned based on single plant disease assays. Our experience with the germplasm screening and data coming through CSP120C suggested that this would not provide sufficiently precise estimates of resistance. The germplasm screening results emphasized the need for replicated trials, and this was reinforced by results coming from CSP120C that suggested Fusarium wilt resistance was multigenic (mapping to multiple chromosomes). Collectively this indicated that advanced generation families were necessary for accurate genetic studies. As soon the data were available from the QDPI germplasm assays, we began to develop of these advanced families. They will be available in the coming year.

- To provide early warning of future problems by assessing the possible occurrence of additional pathotypes of *Fusarium* that have yet to be seen in cultivated cotton.

A total of 695 *Fusarium oxysporum* isolates were isolated from soil samples collected from the rhizosphere of 74 (69%) of 108 populations of 4 *Gossypium* species (*G. australe, G. bickii, G. nelsonii, G. sturtianum*) and other native plants in Queensland, the Northern Territory, South Australia, and New South Wales during 2001 and 2002. Five lineages designated A to E were identified among 95% (660) of those isolates, based on the similarity of AFLP fingerprints. Pathogenic isolates (wild *Fov*), accounting for 14% (95) of the total isolates, were found in 45 (42%) of the populations. Wild *Fov* had significantly higher incidence in isolates of lineages A and E (40 and 39%, respectively) than in those of other lineages (2 to 29%). In addition, wild *Fov* isolates in lineage A were more virulent than those in other lineages. The two strains of *Fov* occurring in Australian cotton fields (VCGs 11 and 12) were closely related to lineage A and different from the 8 races of *Fov* found overseas, suggesting that the appearance of Fusarium wilt of cotton in Australia has resulted from recent evolutionary change in the pathogenicity of indigenous, weakly pathogenic *Fusarium oxysporum* types in lineage A. This finding has significant implications for development of new types of *Fov* in Australia.

- To further develop molecular markers for fingerprinting *Fusarium* pathotypes.

A total of 1189 *Fov* isolates were recovered from symptomatic cotton plants collected from 28 fields in Queensland and New South Wales in 2002. AFLP markers were developed based on fingerprints of 472 isolates, of which 155 were genotyped with 4 *Eco*R I and *Mse* I primer combinations and 4 *Taq* I and *Mse* I primer combinations, and 317 with 4 *Taq* I and *Mse* I primer combinations and 1 *Foxy* and *Mse* I primer combination. These AFLP markers revealed VCG-specific fingerprints and also allowed discrimination among isolates from the same VCG.

3. Detail the methodology and justify the methodology used.

- Bulk isolation of *Fusarium oxysporum* from soil.

In order to obtain a large number of *Fusarium oxysporum* isolates for pathogenicity screening tests, a bulk isolation method was used in the study. Soil (approximately 0.5 g) was sprinkled onto the surface of either Peptone-PCNB or Komada agar plates. Colonies of *Fusarium* spp. appeared after the plates were incubated at 25 C for 1 week. Peptone-PCNB seemed to be more effective than Komada medium in preventing the growth of other fungi. With sufficient care and practice *Fusarium oxysporum* could be identified readily based on the morphological features of colonies.

- Testing of pathogenicity of wild *Fusarium oxysporum* isolates on cotton.

Pathogenicity of *Fusarium oxysporum* isolates recovered in the study was tested by challenging plants of susceptible cotton cv. Siokra 1-4. Two-week-old plants were inoculated with their roots dipped in a conidia suspension (1×10^6 spores/ml) for 5 min. and then grown in the glasshouse at 18 to 25 C for 6 weeks. Nine plants were used for each isolate and trials were conducted twice. Isolates causing symptoms of Fusarium wilt in both trials were designated as 'wild *Fov*.' This protocol has proven to be satisfactory in determining pathogenicity of indigenous *Fusarium oxysporum* isolates on cotton.

- Extraction of fungal DNA with DNeasy 96 kit.

Fusarium isolates were grown in 12 ml of PDB in 15-ml centrifuge tubes at 25 C for 3 days. Mycelia were harvested by centrifuging the tubes at 3,500 rpm for 15 min. The supernatants were poured off and the mycelia were transferred onto a Whatman #1 filter paper to get rid of extra moisture. Then the mycelia were put into small tubes provided along with the kit and lyophilized. DNA was extracted according to the Kit's instructions. The quantity and quality of DNA extracts were appropriate for both AFLP genotyping and gene sequencing studies.

- Determination of genetic lineages in *Fusarium oxysporum* isolates from native *Gossypium* populations.

Fusarium oxysporum isolates from native *Gossypium* populations were fingerprinted using AFLPs with an *Eco*R I (AC) and *Mse* I (A) primer combination. Lineages were determined according to similarity of band patterns. Lineages are consistent with the groups established according to UPGMA dendrogram. These results were confirmed by gene sequencing (CSP155)

- Genotyping of *Fov* isolates from cotton fields with AFLPs.

Fov isolates were genotyped using AFLPs with 4 *Eco*R I (AAC, ACC, AGC, AGG) and *Mse* I (A) primer combinations, 4 *Taq* I (ACCA, ACCC, ACCG, ACCC) and Mse I (GAC) primer combinations, and 1 *Foxy* and *Mse* I primer combination. It seems that more variation can be revealed with *Taq* I and *Mse* I primers; however, it is hard to confirm the variation since most of them are related to small DNA fragments (<20 bp). Therefore, *Eco*R I and *Mse* I primers have proven a more reliable marker system in this application.

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

- Incidence of endophytic fungi in native *Gossypium* populations.

281 fungal endophytes were isolated from stem cuttings collected from 55 (70%) of the 79 native *Gossypium* populations sampled. *Phoma*, *Alternaria* and *Fusarium* were dominant, occurring in 47, 39, and 19% of the populations, respectively. *Botryosphaeria*, *Dichomera* and *Phomopsis* were also present in appreciable frequencies (5, 5 and 4%, respectively) (Table 1). The recovery frequency of endophytic fungi varied significantly among *Gossypium* species and regions (Table 2). Among the four *Gossypium* species in Queensland and the Northern Territory, both *Alternaria* and *Fusarium* had their greatest frequency in *G. bickii* stems, *Botryosphaeria* in *G. sturtianum* stems,

while the other three genera had fairly even distributions. The frequencies of the genera, *Phoma* and *Alternaria* were significantly greater in stems from South Australia than in those from Queensland and the Northern Territory. Signs of infection were observed in stems of cotton plants inoculated with representatives of dominant endophytic fungi using the stem puncturing method in glasshouse trials. *Alternaria*, *Fusarium* and *Botryosphaeria* appeared to be more aggressive than *Phoma* and *Scopulariopsis*, but none were able to induce foliar symptoms during the 5-week experimental period, and none were able to invade cotton plants via the roots.

Table 1. Incidence of major fungal endophytes in populations of four *Gossypium* species sampled in Queensland and the Northern Territory (QLD+NT) and South Australia (SA).

Region	*Gossypium* species	Populations sampled	% of populations infected with specific fungal endophytes						
			Total	*Phoma*	*Alternaria*	*Fusarium*	*Botryosphaeria*	*Dichomera*	*Phomopsis*
QLD+NT									
	G. australe	30	67	50	30	10	3	10	3
	G. bickii	13	85	62	77	69	0	0	0
	G. nelsonii	11	55	46	27	27	0	0	0
	G. sturtianum	12	67	17	25	0	25	8	8
	Sub-total	66	68	46	38	23	6	6	3
SA									
	G. sturtianum	13	77	54	46	0	0	0	8
Total		79	70	47	39	19	5	5	4

Table 2. Recovery frequency of major fungal endophytes from stem samples of four *Gossypium* species collected in Queensland and the Northern Territory (QLD+NT) and South Australia (SA).

Region	*Gossypium* species	Stems sampled	% of stems from which specific fungal endophytes were recovered						
			Total	*Phoma*	*Alternaria*	*Fusarium*	*Botryosphaeria*	*Dichomera*	*Phomopsis*
QLD+NT									
	G. australe	382	32.8	23.5	3.4^{b}	0.8^{c}	0.4^{b}	1.0	0.7
	G. bickii	178	35.5	12.3	9.6^{a}	10.9^{a}	0	0	0
	G. nelsonii	113	23.4	13.5	2.9^{bc}	7.1^{b}	0	0	0
	G. sturtianum	173	13.8	4.5	1.6^{c}	0*	2.5^{a}	0	0.4
			0.3198	0.1634	0.0069	0.0011	0.0415	–**	0.9073
SA									
	G. sturtianum	57	63.9	33.1	27.7	0	0	0	3.1
	*P****		0.0004	0.0136	0.0021	–	–	–	0.4594

* Data were not included in statistical analysis.
** Not applicable
*** Comparison was between *G. sturtianum* populations in two regions.

- Incidence of *Fusarium* species in rhizosphere soil of native *Gossypium* populations.

Twenty *Fusarium* species were identified in rhizosphere soil of native *Gossypium* species. *Fusarium solani*, *F. compactum*, *F. oxysporum*, *F. graminearum* and *F. crookwellense* were the 5 most prevalent species, accounting for 90% of the 1627 isolates recovered. Of these *F. solani* was the dominant species (71.4%), while each of the remaining 4 species were isolated at low but appreciable frequencies (2.6–7.6%) (Table 3). The number of *Fusarium* species in the soil differed among *Gossypium* species and geographic regions. Eighteen species were found in *G. australe* samples, 16 in *G. bickii* and *G. sturtianum*, but only 9 in *G. nelsonii*. Nineteen species were found in the samples from Queensland and Northern Territory, but only 14 in those from South Australia. All the 5 most prevalent species as well as *F. chlamydosporum*, *F. semitectum* and *F. eqiseti* were isolated from the soil samples from all *Gossypium* species in both regions. In contrast, *F. dimerum*, *F. scirpi*, *F. tricinctum* and *F. verticillioides* were isolated from only a single *Gossypium* species in a single region.

Table 3. Number and identity of *Fusarium* isolates recovered from rhizosphere soil samples collected from Queensland and Northern Territory (QLD+NT) and South Australia (SA).

Fusarium species	QLD+NT				SA	Total	%
	G. australe	*G. bickii*	*G. nelsonii*	*G. sturtianum*	*G. sturtianum*		
F. solani	229	105	147	269	411	1161	71.4
F. compactum	29	54	21	17	2	123	7.6
F. oxysporum	15	7	8	12	46	88	5.4
F. graminearum	10	13	5	2	16	46	2.8
F. crookwellense	13	10	10	2	8	43	2.6
F. longipes	3	17	1	5		26	1.6
F. chlamydosporum	8	9	1	1	2	21	1.3
F. semitectum	7	4	5	2	1	19	1.2
F. anthophilum	1	1		2	6	10	0.6
F. acuminatum	4	4		1		9	0.6
F. proliferatum	3			4	1	8	0.5
F. subglutinans	1	1		1	5	8	0.5
F. equiseti	2	1	1	1	2	7	0.4
F. babinda	2	4				6	0.4
F. sporotrichioides	1	3			1	5	0.3
F. culmorum	1	1		1	1	4	0.2
F. dimerum	2					2	0.1
F. scirpi	2					2	0.1
F. tricinctum		1				1	0.1
F. verticillioides					1	1	0.1
Unidentified	8	5	4	6	14	37	2.3
Total	341	240	203	326	517	1627	100

Table 4. Number of populations sampled and incidences of *F. oxysporum* in those populations summarised by *Gossypium* species and geographic regions respectively.

Sources of populations	# of populations sampled	# (%) of populations associated with *F. oxysporum*
By *Gossypium* species		
G. australe	33	16 (48)
G. bickii	13	8 (62)
G. nelsonii	11	6 (55)
G. sturtianum	33	30 (91)
Other native plants	18	14 (78)
By geographic regions		
Mount Isa (QLD)	14	8 (57)
Longreach-Theodore (QLD)	12	11 (92)
Alice Springs-Tennant Creek (NT)	51	30 (59)
Flinders Range (SA)	13	11 (85)
Brookstead-Narrabri (QLD-NSW)	18	14 (78)
Total	108	74 (69)

- Isolation of *Fusarium oxysporum* from rhizosphere soil of native *Gossypium* populations.

A total of 695 *F. oxysporum* isolates were recovered from the soil samples collected from 74 of the 108 populations (69%) (Table 4). The incidence of *F. oxysporum* in those populations varied among species and regions. *Fusarium oxysporum* occurred in 91% of *G. sturtianum* populations, 78% of those of other native plants, but only 48 to 62% of those of the other 3 *Gossypium* species. Among the 4 regions where *Gossypium* populations were sampled, *F. oxysporum* occurred at a high incidence in both the Longreach-Theodore region of Queensland (92%) and the Flinders Range region of South Australia (85%), but at a low incidence in both the Alice Spring-Tennant Creek region of the Northern Territory (59%) and the Mount Isa region of Queensland (57%).

- Major lineages of *Fusarium oxysporum* from rhizosphere soil of native *Gossypium* populations.

Five genetic lineages designated A, B, C, D and E were identified, based on the fingerprints revealed by AFLP markers, among 90% (660) of the 695 isolates (Fig. 1 I). Data from 4 representatives of each lineage showed that genetic similarities between any 2 lineages were less than 0.50 in a UPGMA, and bootstrap values for each of the lineages ranged from 95% to 100% in a bootstrap analysis, respectively (Fig 1 II).

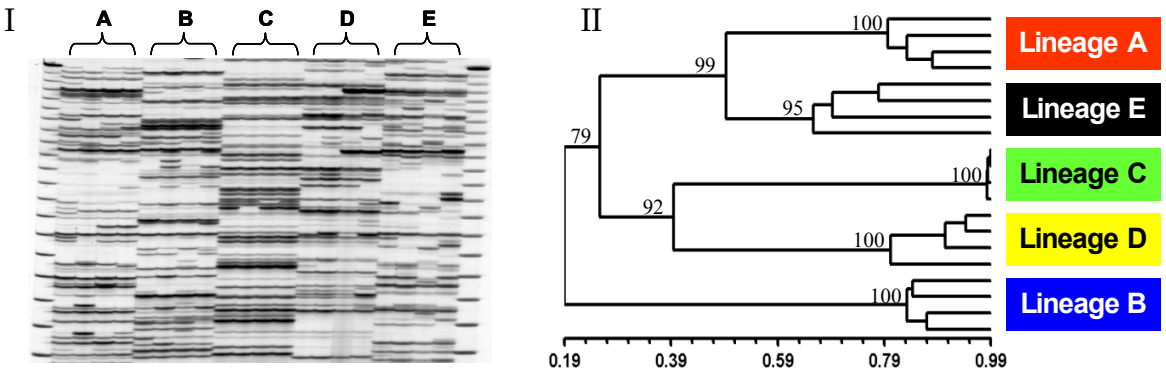
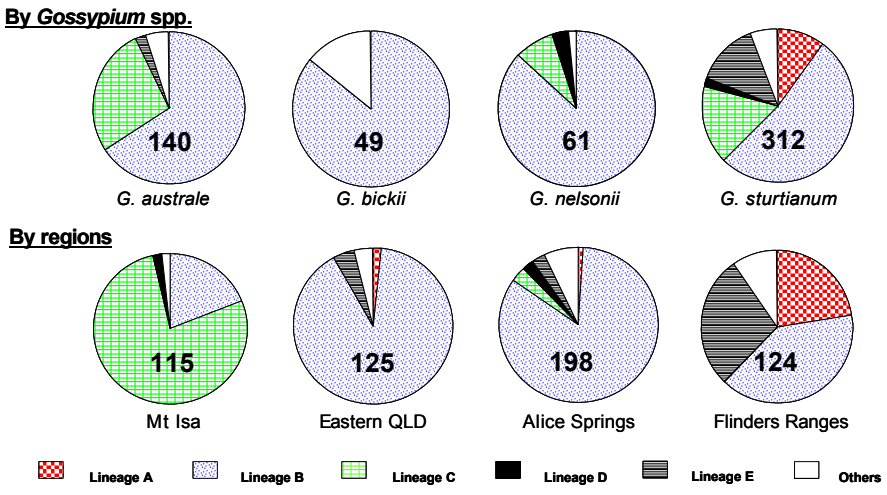


Fig. 1. Representative AFLP gel with 4 representatives of each of 5 major lineages of *Fusarium oxysporum* associated with native *Gossypium* populations (I) and a UPGMA dendrogram showing the genetic relationships among the 5 major lineages (II).

The distribution of lineages varied among species and regions (Fig. 2). Lineage B was the predominant genotype among isolates, irrespective of species, and in 3 of the 4 regions. Lineages A and E were found among isolates from 3 of the 4 regions, but the majority of A and E isolates were recovered from the soils of *G. sturtianum* populations in the Flinders Range region of South Australia. Lineage C appears to be limited in the Mount Isa region of Queensland where it is predominant. Only lineage B was found in the soils from *G. bickii* populations. Lineage D was the rarest of the designated lineages, with only seven isolates.

Fig. 2. Percentage of different lineages in *Fusarium oxysporum* isolates recovered from different *Gossypium* populations and regions.



- Incidence of wild *Fov* in *F. oxysporum* isolates from native *Gossypium* populations

Of the 695 *F. oxysporum* isolates, 95 (14%) were designated "wild *Fov*." These isolates caused mild, but typical Fusarium wilt symptoms, *e.g.*, stunting, foliar necrosis, and vascular discoloration, on plants of cotton cultivar Siokra 1-4. However, no isolate was able to kill any inoculated plants during the 6-week experimental period, and it is probably most accurate to consider these as latent pathogens.

The incidence of wild *Fov* varied among *Gossypium* species and regions (Table 5). Among the four *Gossypium* species, the greatest incidence was found among isolates derived from *G. sturtianum* populations (18%), followed by declining incidences in isolates from *G. bickii* (14%), *G. australe* (11%), and *G. nelsonii* (10%) populations. While the incidence of wild *Fov* in isolates from non-*Gossypium* populations (8%) was lower than that in any of the *Gossypium* species, this was not statistically significant. However it does highlight the probability that these wild lineages are not exclusively associated with native *Gossypium* species and could potentially occur in any Australian soil. The incidence of wild *Fov* appeared to show geographic differences with wild *Fov* accounting for 27% of the isolates originating from the Flinders Range region of South Australia, 14% of those from both the Longreach-Theodore region of Queensland and the Alice Springs-Tennant Creek region of the Northern Territory, but only 8% and 5% of those from the Mount Isa region of Queensland and the Brookstead-Narrabri region of Queensland and New South Wales.

The incidence of wild *Fov* in *F. oxysporum* isolates also varied among the 5 lineages (Table 5), ranging from 40% and 39% in lineages A and E, respectively, through 11% in lineage B, to only 2% in lineage C. The incidence of wild *Fov* in lineage D (29%) must be treated with caution as it is based on the occurrence of 2 wild *Fov* isolates in a sample size of just 7. In addition, isolates in lineages A and E were more virulent than those in the other three lineages.

Table 5. Number of *F. oxysporum* isolates, number of wild *Fov* isolates, incidence of wild *Fov* in *F. oxysporum* isolates, and mean number of symptomatic plants caused by the *Fov* isolates in the 2 pathogenicity screening trials (in which 9 plants were used for each isolate) summarised by *Gossypium* species, geographic regions, and genetic lineages. Means with different letters are significantly different ($P<0.05$).

Source of isolates	Number of *F. oxysporum* isolates obtained	Number (%) of wild *Fov* isolates	Mean number of symptomatic plants
By *Gossypium* species			
G. australe	140	1 (11)	1.23
G. bickii	49	7 (14)	1.43
G. nelsonii	61	6 (10)	1.33
G. sturtianum	312	5 (18)	1.67
Other native plants	133	1 (8)	1.18
By geographic regions			
Mount Isa (QLD)	115	6 (5)	1.25
Longreach-Theodore (QLD)	125	1 (14)	1.59
Alice Springs-Tennant Creek (NT)	198	2 (14)	1.41
Flinders Range (SA)	124	3 (27)	1.69
Brookstead-Narrabri (QLD-NSW)	133	1 (8)	1.18
By genetic lineages			
Lineage A	38	1 (40)	2.20 [a]
Lineage B	474	5 (11)	1.25 [c]
Lineage C	95	2 (2)	1.25 [c]
Lineage D	7	2 (29)	1.25 [c]
Lineage E	46	1 (39)	1.67 [b]
Others	35	8 (23)	1.56 [b]
Total	695	9 (14)	1.51

- Genetic relationships among *Fov* from overseas, Australian cotton fields, and native *Gossypium* populations.

  AFLP fingerprinting studies with 4 primer combinations and sequencing data of 4 genes establish that the two VCGs (11&12) of Australian *Fov* are genetically more closely related to the indigenous lineage A than they are to any of the overseas *Fov* races. This confirms that the *Fov* in Australia have arisen within Australia. In addition, some isolates in lineage E are closely related to *Fov* races 3 and 5. It is uncertain if they may pose future threat to the industry (Fig. 3 I&II).

- Variation of *Fov* population in cotton fields

  AFLP fingerprinting of *Fov* isolates from 28 cotton fields indicate that VCG 11 is more genetically diverse than VCG 12. Variants of VCG 11 were identified in Boggabilla and Cecil Plains areas of QLD. These data were recently obtained and are in the process of being validated.

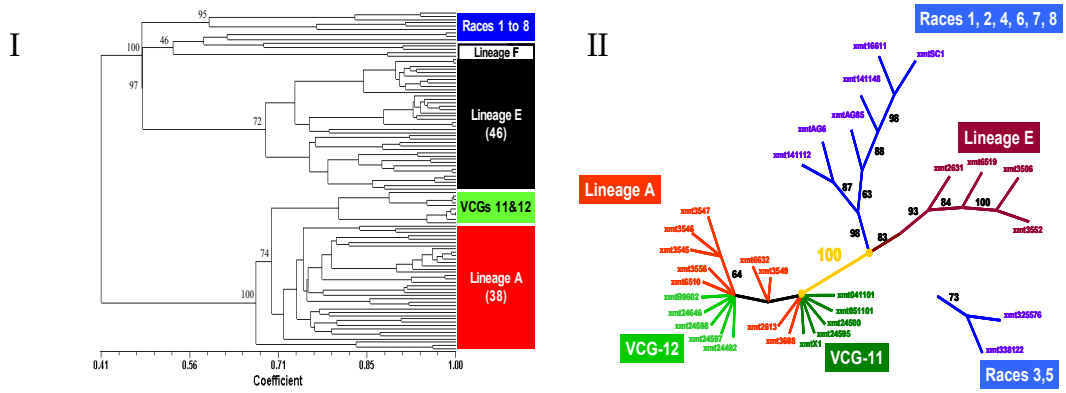


Fig. 3. UPGMA dendrogram showing genetic relationships among overseas *Fov* races 1 to 8, VCGs 11&12 of Australian *Fov* and lineages A and E of wild *F. oxysporum* associated with *Gossypium* populations (I) and parsimony optimized topology of DNA sequence from 4 genes (mtSSU, PP, NR, EF1α) (II)

- Fusarium wilt resistance of Australian *Gossypium* species

A total of 180 screening trials were conducted at the Queensland DPI laboratories in Indooroopilly, Qld. Seventy-nine accessions representing all five C- and G-genome species were run in replicate trials at the Indooroopilly site: 25 *G. australe*; 4 *G. bickii*; 3 *G. nelsonii*; 3 *G. robinsonii*; and 41 *G. sturtianum* accessions (A subset of the data is presented in Table 4). *Gossypium australe*, *G. nelsonii*, and *G. robinsonii* accessions are notably susceptible to Fusarium wilt with no accessions producing vascular browning means (0=immune and 5=susceptible) less than 2 (Fig. 4). Some *Gossypium bickii* performed moderately better with some accession means below 2. The *G. sturtianum* performed best, with 10 accessions consistently rating below 1 (24%). This confirms that *G. sturtianum* is the most promising source of potential resistance genes among the wild *Gossypium* species. The other critical result was the identification of susceptible *G. sturtianum* accessions, which makes this species a suitable model system for elucidating the genetic control of Fusarium wilt resistance in cotton.

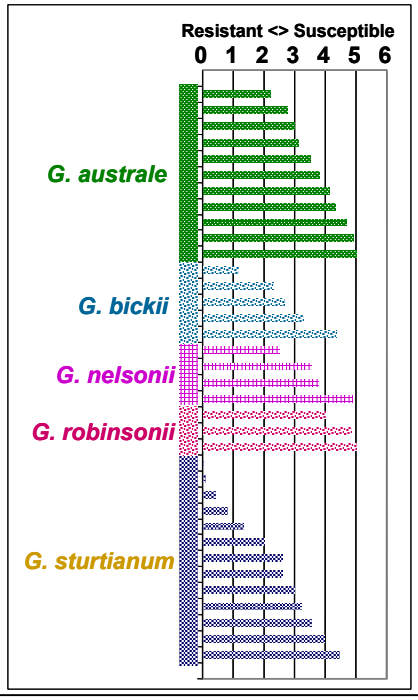


Fig. 4. Fusarium wilt resistance of a sample of Australian *Gossypium* species, based on the extent of vascular browning in glasshouse pot trials.

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

This has been a highly successful project which has provided answers to a number of critical questions regarding the identity, distribution, and evolution of the Fusarium wilt pathogen. The results have also defined the next generation of questions. The data gathered have provided a much clearer indication of how the Australian *Fov* strains arose within Australia and what future risks need to be monitored. The germplasm screening has identified accessions of potential utility, although more work is needed to make full use of these accessions. The key research outcomes are listed below.

- There is a considerable variation of Fusarium wilt resistance among the Australian *Gossypium* species. *Gossypium sturtianum* emerged as the most promising source of novel resistance genes, with the most resistant accessions. At the same time, a number of susceptible *G. sturtianum* accession were identified that can be used to generate segregating populations for genetic analyses.

- Endovascular fungi are commonly found in the stems of Australian *Gossypium* species. *Fusarium* species are found in 19% of the populations, but only in ~3% of stems sampled. Of the 900+ stems sampled, *Fusarium oxysporum* was present in only one.

- *Fusarium oxysporum* is a low frequency but widespread component of the rhizosphere of native *Gossypium* species in Australia.

- *Fusarium oxysporum* in Australian soil is genetically diverse; most isolates fall into one of five distinct lineages, designated A to E.

- Some strains of indigenous *Fusarium oxysporum* are able to cause mild but typical symptoms of Fusarium wilt on cotton (cv. Siokra 1-4), suggesting that they represent latent pathotypes. These isolates have been designated "wild *Fov*".

- Wild *Fov* is more common among isolates of lineages A and E than among those of other lineages.

- Results from both AFLP and gene sequencing demonstrate that the 2 genotypes of *Fov* occurring in cotton fields in Australia (VCGs 11 and 12) are more closely related to *Fusarium oxysporum* isolates in lineage A than to any of the 8 races of *Fov* found in other countries, indicating that they arose *de novo* in Australia.

The fact that Fov developed de novo in Australia coupled with the presence of widespread occurrence of latent pathogens highlights the need to survey the soils in cotton fields and to better understand how virulence evolves in Fusarium oxysporum. This will be the focus on the research for the next three years under CSP156.

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

The increasing incidence of Fusarium wilt across major cotton growing regions in Australia represents a significant and growing problem. Screening Australian *Gossypium* germplasm for Fusarium wilt resistance will provide an insight into the genetics of resistance and may provide a source of resistance genes for a new generation of elite cotton cultivars. Pathogenicity testing and genotyping of indigenous *Fusarium oxysporum* associated with native *Gossypium* populations has clarified the origin of the *Fov* found in cultivated cotton fields. Molecular fingerprinting of *Fusarium* isolates both from cotton-growing regions and from native *Gossypium* populations will provide a broad scale perspective on the scope of the problem. It will provide advance warning if new types of *Fov* exist in cotton fields or native *Gossypium* populations.

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

- **technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc**

  Not applicable.

- **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.

- **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.

Not applicable.

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

The focus over the next six months will be to publish the key results in peer-reviewed journals.

(c) for future research.

Based on the outcomes from this project, a new project titled 'The potential for native *Fusarium* to give rise to new cotton field pathogens' was designed. There are 3 major objectives: (i) assessing the risk of novel *Fov* genotypes emerging from wild *Fusarium oxysporum*; (ii) investigating the evolutionary processes involved in wild *Fov* becoming virulent; and (iii) assessing the competitive abilities of different pathogenic lineages of *Fov*.

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

- Journal papers:

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 Go*ssypium* 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. 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 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. Previous studies identified two distinct genotypes in Australia (VCGs 01111 and 01112) that were morphologically distinct from the eight races of *Fov* found outside Australia, but prior to this study the origin(s) of the *Fov* in Australian cotton fields were unclear.

There are 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, specifically 1) to screen accessions of native *Gossypium* species for *Fov* resistance; 2) to determine if Fusarium wilt pathogens occur in native *Gossypium* populations; and 3) to investigate the genetic relationships between *Fov* causing the disease in Australian cotton fields and indigenous *F. oxysporum* associated with native *Gossypium* populations.

Screening the Australian *Gossypium* species identified a range of accessions that will be useful in the continuing efforts to 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. At the same time, a number of susceptible *G. sturtianum* accession were identified that can be used to generate segregating populations for genetic analyses. Future genetic analysis will assist cotton breeders by providing a clearer picture of how Fusarium resistance is controlled genetically.

Simultaneously, it has become clear that while the native *Gossypium* species are not harbouring cotton field pathogens, there are some of the native soil fungi of potential concern and continuing vigilance would be appropriate.

Fusarium wilt is an endovascular disease, and while endovascular fungi are commonly found in the stems of the wild *Gossypium* species, with only one exception, none of the 600+ isolates tested were related to cotton field pathogens. More importantly, pathogenicity trials of these endovascular fungi established that none of these isolates have the ability to invade cotton through the roots and cause wilt symptoms, and therefore are highly likely to give rise to new cotton field pathogens. It is unlikely that the native Australian *Gossypium* species are harbouring potential new pathogens that will impinge upon the Australian cotton industry in the future.

Surveys of soils from the native cotton populations and native vegetation in the Norwin—Boggabilla region, where Fusarium wilt was first detected, identified a range of diverse *Fusarium oxysporum* genotypes. Broadly speaking, these genotypes fall into one of five distinct lineages, designated A to E. Pathogenicity trials established that 14% of these *Fusarium oxysporum* genotypes could induce mild Fusarium wilt symptoms on cotton. While none of these isolates currently are virulent enough to cause plant death, isolates in lineage A have emerged as the closest known relatives of the cotton field pathogens, and it is now certain that the origin of the cotton field pathogens can be traced to native *Fusarium oxysporum* genotypes. Whether other Australian genotypes have the same potential to develop into new cotton field pathogens will be the focus of ongoing research.

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