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Final Report

CRDC Project US32C

Isolation of pathogenicity genes from Verticillium wilt fungi infecting cotton

July 1996 – June 2000

1. What was the background of the project?

Verticillium wilt is a fungal disease of numerous plant species that is characterised by symptoms of wilting, chlorosis, necrosis and defoliation. The causal agent, *Verticillium dahliae* can inflict significant losses on cotton crops and, if not adequately controlled, could seriously affect the long-term sustainability of the cotton industry.

The best defence against crop losses from such pathogens is to breed resistant crop varieties, and the introduction of Verticillium wilt-tolerant *Gossypium hirsutum* varieties such as Sicala V-1 and its successor Sicala V-2 has significantly reduced the impact of *V. dahliae* on the Australian cotton crop. However, epidemiological surveys that we conducted in the early 1990's revealed that a high proportion of *V. dahliae* strains are capable of causing severe disease even on these improved varieties (Table 1; Ramsay *et al.*, 1996; Zhu, Multani and Lyon, unpublished). Such strains are prevalent in cotton production regions in both NSW and Queensland.

Table 1. Origin, colour on Potato Dextrose Agar (W, white; B, black) and host pathogenicity of eight selected isolates of *V. dahliae* from cotton. Response of named cultivar to infection: R, resistant; M, moderately resistant; S, susceptible. Disease severity index (SI) is presented in parentheses

Isolate	Origin			Colour	Cultivar response									
	Site	State	Year		Pima S-7		Acala R.		Sicala V-1		DP 90		Siokra 1-4	
1008	Merah North	NSW	1984	W/B	M	(0.27)	M	(0.37)	S	(0.51)	M	(0.41)	S	(0.70)
1011	Trangie	NSW	1992	B	R	(0.20)	M	(0.50)	S	(0.52)	S	(0.64)	S	(0.72)
1012	Dalby	Qld	1992	B	R	(0.24)	M	(0.48)	M	(0.33)	S	(0.60)	S	(0.54)
1013	Boggabilla	NSW	1992	B	M	(0.40)	S	(0.60)	S	(0.60)	S	(0.64)	S	(0.76)
1017	Bourke	NSW	1984	W	R	(0.20)	M	(0.30)	R	(0.20)	R	(0.20)	S	(0.56)
1020	Dalby	Qld	1992	W/B	R	(0.25)	M	(0.36)	M	(0.28)	S	(0.60)	S	(0.68)
1028	Bowenville	Qld	1993	B	M	(0.34)	M	(0.40)	S	(0.51)	S	(0.60)	S	(0.70)
1035	Cecil-Plains	Qld	1993	B	R	(0.22)	R	(0.24)	M	(0.32)	S	(0.70)	S	(0.68)

In order to further enhance host plant resistance to Verticillium wilt, cotton breeding programs will need to be able to rapidly incorporate new resistant cotton germplasm which is known to be effective against strains of the *V. dahliae* pathogen that are predominant in the field at any given time.

During the course of our research on fungal pathogens of cotton, we have developed molecular genetic techniques such as RAPD-PCR for the identification of different isolates of the fungal pathogen *V. dahliae*. In order to understand better the epidemiology of Verticillium wilt disease in cotton, we have examined over one hundred independent isolates of this fungus from a number of cotton production regions. Locations include the Gwydir, Macintyre, Maquarie and Namoi valleys and around Bourke in NSW, and the Darling Downs and around St George and Theodore in Queensland. We have allocated these isolates to 15 RAPD groups (RGs) of closely related strains based on the possession of 80% or more DNA markers in common (Zhu, Multani and Lyon, unpublished).

In addition to the significant diversity observed for fungi from different cotton-producing regions, intensive sampling of single fields in two districts revealed a high level of genetic diversity even within localised *V. dahliae* populations. Interestingly, use of cotton cultivars that are generally more tolerant to Verticillium wilt does not seem to cause any decline in this level of genetic diversity. However, we have no information on whether such changes in host cultivar result in the selection of more pathogenic strains from the background population.

Although we have successfully differentiated isolates of *V. dahliae* using RAPD-PCR, at present pathogenicity tests remain the only means of determining the pathogenic classification, or races, of the fungal strains present in diseased cotton plants. We have employed an artificial inoculation technique for the induction of Verticillium wilt disease in a slection of cotton cultivars, and have tested 30 independent *V. dahliae* isolates on five cotton cultivars. These were Siokra 1-4, Deltapine 90, Sicala V-1, Acala Royale and Pima S7. As expected, all but one of the *V. dahliae* isolates was able to cause significant disease on Siokra 1-4. More alarmingly, over half of the isolates (17) were capable of causing significant disease on the wilt-tolerant variety Sicala V-1, and three were able to cause significant disease on the more tolerant Acala Royale variety (Zhu, Multani and Lyon, unpublished).

We have attempted to correlate genetic fingerprints as determined by RAPD-PCR with the pathogenicity characteristics of these isolates, but have yet to observe any clear trend. The results reveal that some highly pathogenic strains are apparently closely related to some less pathogenic isolates, while the highly pathogenic strains appear to be genetically unrelated even though they may exhibit the same host specificity.

As a result of these findings, we turned to the identification and characterisation of potential fungal pathogenicity genes as a means of more directly identifying genetic markers associated with *V. dahliae* pathogenicity. This approach involved the cloning of putative plant and fungal genes that are highly expressed within roots during infection of cotton plants by *V. dahliae*. The research utilised the cDNA library prepared by Ms. Melissa Hill as part of her Ph.D. project funded by the CRC for Sustainable Cotton Production (SU251; Hill *et al.*, 1999).

This research, initiated by Ms. Catherine Keniry during her Honours year in 1995, successfully identified 30 genes for which the DNA sequences were partially determined. The identified genes did not appear to correspond to any previously characterised pathogenicity genes from other fungal species, but they might nonetheless represent unique genes associated with *V. dahliae* pathogenicity. Ms. Keniry successfully completed her Honours degree, finishing in the top 10% of students in the School, and was awarded an Australian Postgraduate Award to continue this research.

As initially conceived, the research project (US32C) was to have been carried out by Ms. Keniry, who had been awarded an Australian Postgraduate Award to continue the research project begun during her Honours year. A part-time salary (one day per week) was requested from the CRDC to raise the value of Ms. Keniry's stipend to the same level as an industry scholarship.

In mid-1996, Ms. Keniry was awarded a scholarship to undertake a Ph.D. at Cambridge University in England, and at the end of September 1996 she discontinued her studies at the University of Sydney, and concluded her involvement in US32C. Completion of the most important facets of the project subsequently required the employment of a research assistant for 2.5 days per week, with a consequent increase in the requested budget. Due to the altered circumstances, proposed attempts to determine the

functional role of putative pathogenicity genes by using RNA hybridisation, or DNA transformation with altered genes, were deemed to be beyond the scope of the project.

The incidence of Verticillium wilt disease in cotton is increasing and has the potential to cost the industry millions of dollars annually in lost yield. Although the CSIRO has been successful in breeding cultivars of cotton with enhanced disease tolerance, the existence of more pathogenic strains of *V. dahliae* threatens to negate their effectiveness. The cloning of *V. dahliae* genes associated with pathogenicity could play an important role in the control of this disease by providing insight into the characteristics that make some fungal strains more pathogenic on particular cotton cultivars. The development of suitable gene probes would enable the rapid identification of pathogenic organisms and could provide information on their field survival, persistence and mode of spread.

2. What were the project objectives and to what extent were these achieved?

(i) Complete characterisation of putative fungal genes isolated from the cDNA library prepared from Sicala V-1 plants infected with V. dahliae.

The first objective of this project was to complete the characterisation of putative fungal genes isolated from a cDNA (gene) library produced from Sicala V-1 plants infected with a strain of *Verticillium dahliae* that causes severe wilt disease in some cotton varieties. A total of 116 cloned genes were selected from the library on the basis that their activity is increased in cotton plants infected with *V. dahliae*. All of the 116 clones were characterised by partial DNA sequencing, and the majority of the clones accordingly appear to be of cotton plant origin. However, 18 candidate genes with putative functions suggestive of fungal genes, or that could not be assigned a putative function at the time, were selected for further characterisation.

The 18 candidate genes have now been characterised by DNA hybridisation, PCR amplification and/or DNA sequence examination and database comparison to determine their likely origin. Eight of the genes have been shown by activity analysis (Northern blotting), or PCR amplification using specific amplification primers, to be of plant origin. The DNA sequences of the remaining ten genes are now recognised as being closely related to gene sequences of plant or higher eukaryote origin in the DNA sequence database. All 18 clones characterised in this study are thus believed to be of plant rather than fungal origin, and are no longer considered as potential candidates for fungal pathogenicity genes.

(ii) Analyse the functional role of potential fungal pathogenicity genes and conduct surveys to identify genetic polymorphisms in other V. dahliae *isolates that might be associated with increased pathogenicity.*

The second project objective could not be achieved as proposed since the research conducted for Objective (i) revealed that none of the candidate genes isolated from the cDNA library prepared from Sicala V-1 plants infected with *V. dahliae* were actually of fungal origin. It is probable that the isolation of putative fungal pathogenicity genes will only be possible following the construction of a new cDNA library from infected cotton plant tissue harvested at a longer time interval after fungal inoculation. However, there was insufficient time or resources available to construct and screen a cDNA library of this kind.

(iii) Conduct survey of V. dahliae *isolates using AFLP fingerprinting to identify genetic polymorphisms that might be associated with increased pathogenicity.*

The AFLP technique can screen for polymorphism more efficiently than RAPDs, and once found, polymorphic markers are more reproducible, and so more reliable as strain-specific markers. This technique may therefore achieve the overall aim of this project, which is to differentiate pathogenic and non-pathogenic 'races' of *V. dahliae*. The DNA of 22 selected *V. dahliae* isolates has been analysed using a set of 16 AFLP primer combinations (EAA, EAC, EAG and EAT with MA, MC, MG and MT). Using these primers, a large number of AFLP bands have been detected and, importantly, a high percentage of the AFLP bands are polymorphic between the *V. dahliae* isolates. This makes it possible to distinguish between the isolates on the basis of their DNA fingerprints.

Analysis of the full AFLP data set has not yet been completed, so it remains unclear if any associations can be found between specific AFLP polymorphisms and strain pathogenicity characteristics. Further work will include phylogenetic analysis to determine genetic relatedness of the different *V. dahliae* isolates, and multi-dimensional analysis to compare genetic and pathogenic attributes.

(iv) Develop a molecular assay for the differentiation of pathogenic strains in field material.

Development of a molecular assay for the detection of specific *V. dahliae* strains is postponed until potentially useful AFLP polymorphisms can be identified that show association with specific pathogenicity traits.

3. What methodology was used, and a justification for the use of this methodology?

The principal methodology employed in this research utilised genes from a cDNA library produced from the roots of cotton seedlings 24 hrs after infection with *V. dahliae*. An assumption was made that the invading fungus would encode a proportion of the genes being expressed in such tissues, and that some of these genes would be essential for the pathogenicity of the organism on the cotton host. The library was initially screened to identify genes that were up-regulated in this tissue (ie. not present in uninfected cotton tissue). Such genes might reasonably be expected to be either fungal or plant genes expressed in response to the pathogen-host interaction.

DNA fingerprinting using the AFLP technique was undertaken to identify correlations between the DNA fingerprints and pathogenicity characteristics of the V. dahliae isolates. The AFLP technique can screen for polymorphism more efficiently than RAPDs, and once found, polymorphic markers are more reproducible, and so more reliable as strain-specific markers.

4. Detailed results including statistical analysis of results?

In earlier research (CRDC project US8C), we attempted to correlate RAPD-PCR fingerprints of isolates of *Verticillium dahliae* with their pathogenicity characteristics, but an obvious trend did not emerge. As a result of these findings, we turned to the characterisation of potential pathogenicity genes as a means of more directly identifying genetic markers associated with *V. dahliae* pathogenicity. This research utilised a cDNA library, prepared by Ms. Melissa Hill as part of the CRCSCP project SU251, which was expected to include cloned fungal and cotton genes that are highly expressed during *V. dahliae* infection of cotton plants.

The initial research, conducted by Ms. Catherine Keniry as an Honours project in 1995, successfully identified 30 genes that were expressed in a manner suggestive of important fungal infection-associated genes. These genes were further characterised by partial DNA sequencing, and although they did not appear to correspond with any previously characterised pathogenicity genes from other fungal species, they may nevertheless represent unique genes associated with *V. dahliae* pathogenicity.

Ms. Keniry continued work on the research program in 1996, concluding the DNA sequencing of the remaining clones and conducting further molecular genetic tests in efforts to identify the 30 candidate fungal genes. C.A. Keniry, M.K. Hill, K.P. Ridgway and B.R. Lyon presented this work at the 8th Australian Cotton Conference, in a poster entitled "Molecular genetic analysis of the cotton-Verticillium interaction".

Four of the candidate clones were initially analysed in detail using Southern and Northern blotting (see Table 2). These results reveal the presence of homologous sequences to clones 6t and 7h in both *V. dahliae* and cotton, and detect DNA homology with clones 7n and 8z in cotton alone. Such results are unexpected, as these four clones were originally selected on the basis that they were expressed in fungal mycelia and were up-regulated following fungal infection of cotton plants.

Only clones 7n and 8z were previously found to exhibit some cross-homology with cotton, a finding that might suggest that these genes confer some kind of 'housekeeping' function in both species. A larger sample of the candidate clones therefore needed to be examined using the technique of Southern blotting to resolve this current difficulty in defining the host source of each of the clones.

Table 2. Expression pattern, homology with published DNA sequences and Southern and Northern hybridisation results of four cDNA clones. Level of hybridisation signal is indicatsd by the number of plus signs. For the Northerns, time of peak gene expression is indicated in hours post infection

Clone	Differential Gene Expression			DNA Sequence Homology	Plant Southern	Fungal Southern	Northern (peak)
	Fungi	Infection	Plant				
6t	++	++++	-	none	++	+	6h, 48h
7h	+	++	-	none	++	+++	-
7n	++	+++	+	none	++	-	12h
8z	+++	++++	++	none	++	-	-

Ms. Keniry also worked towards ensuring that the laboratory's considerable collection of *V. dahliae* cultures was correctly catalogued and safely consigned to long-term storage. This culture collection, comprising over 100 independent isolates of the Verticillium wilt pathogen from all major cotton production regions in NSW and Queensland, is an important resource for current and future research into this disease in Australian cotton crops.

The first objective of the project was to complete the characterisation of putative fungal genes isolated from a gene (cDNA) library produced from Sicala V-1 plants infected with a strain of *V. dahliae* which causes severe wilt disease in some cotton cultivars. A total of 116 cloned genes were selected from the library on the following basis.

(i) Clones that are up-regulated in cotton plants infected with *V. dahliae* and are expressed in fungal mycelia (up-regulated Verticillium), or
(ii) Clones that are up-regulated in cotton plants infected with *V. dahliae* but are not expressed in fungal mycelia or uninfected plants (up-regulated Verticillium or cotton).

All of the 116 clones were characterised by partial DNA sequencing (approximately 85 of the clones were sequenced as part of CRCSCP student projects SU326 and SU327). The majority of the clones appear to be of cotton plant origin, however 18 candidate genes with expression profiles or putative functions which are suggestive of fungal genes were selected for further characterisation (Table 3).

Some of the genes that originally could not be matched with sequences in the DNA database were subsequently found to be closely related to genes of plant origin. As a result of these findings, eight of the 18 clones (6a, 6t, 7d, 7n, 7p, 8z, 9b and 9c) are now presumed to be of plant origin and can no longer be considered as potential candidates for fungal pathogenicity genes (Table 3).

Table 3. Candidate fungal genes selected from the cDNA library, putative function suggested by sequence comparisons, and proposed origin as determined by DNA sequence or DNA hybridisation analyses

Clone	Candidate gene	Putative function	Origin of clone
5q	up-regulated Verticillium or cotton	arabinosidase	Verticillium or cotton
6a	up-regulated Verticillium	metallothionein-like protein	cotton
6q	up-regulated Verticillium	?	?
6t	up-regulated Verticillium	?	cotton
7d	up-regulated Verticillium	regulatory protein	cotton
7e	up-regulated Verticillium	?	?
7h	up-regulated Verticillium	glycine-rich cell wall protein	Verticillium or cotton
7n	up-regulated Verticillium or cotton	pathogenesis-related protein	cotton
7p	up-regulated Verticillium	?	plant
7z	up-regulated Verticillium or cotton	?	?
8c	up-regulated Verticillium or cotton	?	?
8u	up-regulated Verticillium or cotton	?	?
8z	up-regulated Verticillium or cotton	?	cotton
9a	up-regulated Verticillium or cotton	?	?
9b	up-regulated Verticillium	?	plant
9c	up-regulated Verticillium	photosystem II SKD protein	cotton
9r	up-regulated Verticillium or cotton	?	?
9u	up-regulated Verticillium	chitin synthase	?

The fungal or plant origins of the remaining ten clones (5q, 6q, 7e, 7h, 7z, 8c, 8u, 9a, 9r and 9u) were the subjects of further research. These genes have been characterised by DNA hybridisation, PCR amplification and/or DNA sequence examination and database comparison to determine their likely origin. The remaining genes have been shown by activity analysis (Northern blotting), or PCR amplification using specific-designed amplification primers, to be of plant origin.

Clone pVGh101 (5q), which has high sequence similarity with an arabinosidase gene from the bacterium *Bacteroides ovatus*. The known role of arabinosidase (xylanase) enzymes in plant cell wall degradation (breakdown of xylan cell wall macromolecules), suggested that clone pVGh101 might represent a pathogenicity gene derived from *V. dahliae*, as cell wall degradation would be essential for fungal invasion of plant tissue.

This gene has been further characterised in an attempt to confirm the fungal origin of the clone, with specific PCR primers being designed to enable amplification of the gene from genomic DNA. Although DNA fragments were amplified from both fungal and plant DNA, only the fragment from the plant DNA hybridised with the cloned DNA, thereby proving that the gene is derived from the cotton plant and not *V. dahliae*. It is possible that *V. dahliae* also possesses a gene that is closely related to the cotton gene, but this would need to be cloned from the fungal DNA before further characterisation could be carried out.

Our research has therefore determined that all of the 116 clones selected from the Sicala V-1 – *V. dahliae* cDNA library appear to be of plant origin. This unanticipated result

suggests that the presence of fungal gene activity (mRNA expression) in infected cotton plant root tissue, at such a short time (24 hr) after inoculation with *V. dahliae*, is minimal if not non-existent. It was therefore deemed unwise to re-screen the existing gene library for more likely fungal gene candidates.

It is likely that the isolation of putative fungal pathogenicity genes will only be possible following the construction of a new cDNA library from infected cotton plant tissue harvested at a longer time interval after fungal inoculation (48-96 hr). However, there was insufficient time available to construct and screen a cDNA library of this kind.

As an additional aspect of this project, we surveyed a selection of *V. dahliae* isolates using a new DNA fingerprinting technique known as amplified fragment length polymorphism (AFLP). We have used AFLPs with significant success in the genotyping (DNA fingerprinting) of a number of cotton cultivars. Experiments by other researchers indicate that the basic AFLP technique can be employed to obtain DNA fingerprints for the differentiation of fungi.

Previous experience in the use of AFLPs for plant analysis has enabled us to optimise the AFLP technique for use with *V. dahliae*. This required the design and manufacture of unique AFLP primers with fewer selective nucleotides, due to the significantly smaller genome size of the Verticillium fungus. We have determined that the optimum primer pairs for *V. dahliae* DNA comprise *Eco*RI primers with two selective nucleotides and *Mse*I primers with one selective nucleotide.

The DNA of 22 *V. dahliae* isolates has now been analysed using a set of 16 AFLP primer combinations (EAA, EAC, EAG and EAT with MA, MC, MG and MT). Using these primers, a large number of AFLP bands have been detected and, importantly, a high percentage of the AFLP bands are polymorphic between the *V. dahliae* isolates. This makes it possible to distinguish between the isolates on the basis of their DNA fingerprints. Since a large proportion of the genome is being scanned with these AFLP primers (approx. 25% of potential *Eco*RI restriction sites), the association of a DNA marker with strain pathogenicity will more likely be detected.

The DNA fingerprinting results (AFLP and RAPD) remain to be correlated with the cotton cultivar infection data in an attempt to identify whether the possession of particular DNA markers is associated with degree of strain pathogenicity on selected cotton cultivars. Analysis will include phylogenetic analysis to determine genetic relatedness of the different *V. dahliae* isolates, and multi-dimensional analysis to compare genetic and pathogenic attributes.

5. A discussion of the results, including an analysis of research outcomes compared with the objectives

Our research determined that all of the 116 clones selected from the Sicala V-1 – *V. dahliae* cDNA library appear to be of plant origin. This unanticipated result suggests that the presence of fungal gene activity (mRNA expression) in infected cotton plant root tissue, at such a short time (24 hr) after inoculation with *V. dahliae*, is minimal if not non-existent. It was therefore deemed unwise to re-screen the existing gene library for more likely fungal gene candidates. We believe that the isolation of potential fungal pathogenicity genes will only be possible following the construction of a new cDNA library from infected cotton plant tissue harvested at a longer time interval after fungal inoculation. However, there was insufficient time or resources available to construct and screen a cDNA library of this kind.

Our surveys have revealed that some strains of *V. dahliae* prevalent in Australian cotton production regions are capable of causing severe disease even on wilt-resistant *G. hirsutum* cultivars such as Sicala V-1. The aim of this project was to identify DNA markers associated with enhanced or differential host pathogenicity in these fungi. A successful research outcome would enable the rapid identification of pathogenic organisms and could provide information on their field survival, persistence and mode of spread. Such information could be of use during trials of new disease-resistant cultivars and in the design of disease control strategies.

We have maintained informal communication links with cotton plant pathologists such as Stephen Allen and David Nehl at ACRI and Joe Kochman at DPIQ regarding the identification DNA markers that may be associated with increased pathogenicity in *V. dahliae*. We have also communicated research findings as appropriate in industry forums such as the Australian Cotton Conference and The Australian Cottongrower. Detailed results will be published in a prominent international plant pathology journal when the research is completed.

6. An assessment of the likely impact of the results and conclusions of the Research project for the cotton industry and, where possible, a statement of the costs and potential benefits to the Australian cotton industry and future research needs

Surveys of strains of *V. dahliae* prevalent in Australian cotton production regions have revealed that some are capable of causing severe disease even on wilt-resistant *G. hirsutum* cultivars such as Sicala V-1. As an adjunct to this project, additional cotton plants in the Namoi region were sampled in March 1997 in order to obtain examples of *V. dahliae* currently infecting crops in that district. Although the sampled cotton plants exhibited the symptoms of mild Verticillium wilt, fungi cultured from the vascular tissues of these plants were not identified as *V. dahliae*, but instead comprised at least five distinct species, including isolates of *Fusarium, Phomopsis* and *Cladosporium* which are potential pathogens of cotton.

These findings suggest that not all diseased plants in a field suffering wilt symptoms may be infected with *V. dahliae*, and that a range of previously undetected pathogen species may be prevalent in a particular cotton field at a given time. It is clear that the development of molecular diagnostic tools, such as those used in this analysis, will have important implications for a thorough understanding of disease epidemiology in cotton crops.

7. A description of the project technology (e.g commercially significant developments, patents applied for or granted, licences etc)

Not applicable.

8. A technical summary of any other information developed as a part of the Research Project including discoveries in methodology, equipment design, etc

Previous experience in the use of AFLPs for plant analysis has enabled us to optimise the AFLP technique for use with *V. dahliae*. This required the design and manufacture of unique AFLP primers with fewer selective nucleotides, due to the significantly smaller genome size of the Verticillium fungus. We have determined that the optimum primer

pairs for *V. dahliae* DNA comprise *Eco*RI primers with two selective nucleotides and *Mse*I primers with one selective nucleotide.

A total of eight *Eco*RI primers have now been designed and manufactured (EAA, EAC, EAG, EAT, ECA, ECC, ECG and ECT). When used in conjunction with the four MseI primers (MA, MC, MG, MT), they increase the potential for AFLP analysis to a total of 32 primer combinations. These 32 primer combinations can be used on the existing DNA templates to increase the amount of the *V. dahliae* genome surveyed for DNA polymorphism to 50%.

9. Recommendations on the activities or the steps that may be taken to further develop, disseminate or exploit the project technology

Our research has determined that all of the nearly 120 clones selected from the Sicala V-1 – *V. dahliae* cDNA library appear to be of plant origin. This unanticipated result suggests that the presence of fungal gene activity (mRNA expression) in infected cotton plant root tissue, at such a short time (24 hr) after inoculation with *V. dahliae*, is minimal if not non-existent. This is despite our clear evidence that expression patterns of plant genes undergo dramatic qualitative and quantitative changes during this time period.

It is suggested from our results that the isolation of potential fungal pathogenicity genes using similar procedures will only be possible following the construction of a new cDNA library from infected cotton plant tissue harvested at a longer time interval after fungal inoculation. Our evidence suggests that the expression of some cotton genes begins to decline at 48-96 hr, and this may be in response to high growth rates of the infecting fungus. Infected plant tissue sampled at these times might therefore be predicted to contain higher traces of fungal gene expression, leading to the isolation of fungal genes involved in pathogenicity.

10. A list of publications arising from the research project.

Ramsay JR, DS Multani, and BR Lyon (1996). RAPD-PCR identification of *Verticillium dahliae* isolates with differential pathogenicity on cotton. Aust. J. Agric. Res. 47: 681-93.

Lyon BR, Y Zhu, Y-Y Wang, and DS Multani (1996). Verticillium wilt of cotton: Epidemiology of *Verticillium dahliae*. pp. 657-662, Proceedings of the 8th Australian Cotton Conference, Broadbeach, Australia.

Keniry CA, MK Hill, KP Ridgway, and BR Lyon (1996). Molecular genetic analysis of the cotton-verticilliuni interaction. Poster session, 8th Australian Cotton Conference, Broadbeach, Australia.

Hill MK, KJ Lyon, and BR Lyon (1999). Identification of disease response genes expressed in *Gossypium hirsutum* upon infection with the wilt pathogen *Verticillium dahliae*. Plant Mol. Biol. 40: 289-296.

Ho, K, Y Zhu, DS Multani, and BR Lyon. DNA polymorphism and pathogenicity relationships of *Verticillium dahliae* isolates from cotton plants (In preparation).