

Final Report

On Farm Series | Cotton Research & Development Corporation

***If you are participating in the presentations this year, please provide a written report and a copy of your final report presentation by 31 October.
If not, please provide a written report by 30 September.***

Part 1 - Summary Details

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

CRDC Project Number: DAQ0002

Project Title: Tobacco streak virus (TSV) in cotton

Project Commencement Date: 01/07/2008 **Project Completion Date:** 30/06/2011

CRDC Program: Value Chain

Part 2 – Contact Details

Administrator:	Ms Helen Kamel, Principal Coordinator (External Funding)
Organisation:	Dept of Employment, Economic Development and Innovation
Postal Address:	Queensland Department of Employment, Economic Development and Innovation, PO Box 102, Toowoomba, Qld 4350
Ph: 07 4688 1286	Fax: 07 4688 1190 E-mail: Helen.Kamel@deedi.qld.gov.au
Principal Researcher:	Mr Murray Sharman, Senior Plant Pathologist (Virology)
Organisation:	Dept of Employment, Economic Development and Innovation
Postal Address:	DEEDI, Level 2C-West, Ecosciences Precinct, GPO Box 267, Brisbane, 4001
Ph: 07 3255 4339	Fax: 07 3846 2387 E-mail: Murray.Sharman@deedi.qld.gov.au
Supervisor:	Dr John Thomas, Principal Research Fellow
Organisation:	University of Qld, Queensland Alliance for Agriculture & Food Innovation
Postal Address:	Level 2C-West, Ecosciences Precinct, GPO Box 267, Brisbane, 4001
Ph: 07 3255 4393	Fax: 07 3846 2387 E-mail: John.Thomas@deedi.qld.gov.au

Signature of Research Provider Representative: _____

Part 3 – Final Report Guide (due 31 October 2008)

(The points below are to be used as a guideline when completing your final report.)

Background

1. Outline the background to the project.

Tobacco streak virus (TSV) has a worldwide distribution. It is transmitted by thrips and has a wide host range including species in more than 30 plant families. Although TSV has been known from Australia from at least the 1970s, the virus has not previously been recorded as causing disease in any crops in the grain and cotton growing regions of Australia.

In 2006, TSV was identified as the causal agent of the devastating sunflower necrosis disease in central Queensland (CQ), and subsequently in 2007 as the cause of major losses in mungbeans in the same area. It has been a major factor in the recent downturn in the sunflower industry in CQ. The diseases caused by TSV appear to have been present for a several years prior in these and other crops. Our studies have shown that parthenium is a major weed host of TSV in CQ and we have demonstrated thrips transmission. Surveys in 2007/2008 (as part of the one year scoping study 03DAQ005) found TSV in cotton in CQ. The symptoms were mostly confined to the feeding sites of the thrips and appeared as reddish spots and rings, but occasionally the plants were systemically infected and showed a chlorotic mosaic and leaf deformation.

TSV has also recently been recorded on cotton from India (Bhat *et al* 2002) and Pakistan (Ahmed *et al* 2003), but where described, the symptoms differ in being usually a chlorotic mosaic, and genome sequence comparisons indicate that the Australian strain of the virus is distinct.

The 2007-2008 growing season (during the scoping study) was abnormally wet in CQ, and we needed to know how the disease may develop in cotton in more normal seasons. The factors inducing systemic infection of cotton and the potential yield affects were also crucial questions that needed to be addressed.

Many aspects of the epidemiology of TSV in CQ were unknown prior to this project (DAQ0002) and the related GRDC project (DAQ00130), including the role of many potential weed hosts and possible thrips vector species. These knowledge gaps were jointly addressed in the complementary GRDC project and the linking of these two projects has provided synergies to maximise efficiencies in this research.

Objectives

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

Objective 1. Surveys to assess the incidence, distribution and impact of TSV

- This objective has been achieved. Disease surveys were conducted at multiple times during each growing season from 2008 to 2011 in central Queensland. Cotton samples were also tested from southern Qld and northern NSW cotton crops in most seasons.

Objective 2. Determine the thrips vector species associated with cotton

- This objective has been achieved. Thrips species were collected and identified from multiple seasons and locations in cotton crops affected by TSV in central Qld.

Objective 3. Examination of factors that lead to systemic infection of cotton

- This objective was partly achieved. Multiple attempts to experimentally induce systemic infection in cotton were only rarely successful. However, these attempts, along with field observations, do provide evidence about possible factors that lead to systemic infection of cotton.

Objective 4. Determine yield effects of TSV on cotton.

- This objective was partly achieved. Due to the inconsistent nature of being able to systemically infect cotton under experimental conditions, effect of yield could not be assessed under controlled conditions as originally planned. However, field surveys over multiple seasons do provide evidence to the likely impact of TSV on yields in cotton.

Objective 5. Dissemination of disease management data based on research objectives.

- This objective has been achieved. Several pieces of extension material and publications have been released to industry and the scientific community.

Methods

3. Detail the methodology and justify the methodology used. Include any discoveries in methods that may benefit other related research.

Objective 1. Surveys to assess the incidence, distribution and impact of TSV.

Where possible, a standardised disease count method was used in each block inspected by starting within the margins of the crop, approximately 100 m in from the edge. Plants were then visually inspected for typical TSV symptoms in approximately 10 m sections (or the equivalent of 100 plants) back towards the edge of the crop. This was done in a staggered format where a 10 m section of row was inspected, then moved across 10 rows then inspected the next 10 m section and repeated until the inspection finished at the edge of the crop. This approximated a diagonal transect across a number of rows from the interior to the edge of the block being inspected and resulted in approximately 1000 plants being visually inspected for symptoms of TSV. This was done to try to avoid any significant edge or single row effects which may result in disease counts that are not representative of the majority of the block being inspected. For example, it was common to find much higher incidences of TSV symptoms in edge rows of a block which were close to the source of infection, parthenium weed. Plants were also inspected outside of the transect counts (e.g. while moving into the crop and between rows) to record the presence of TSV. Crops were inspected across several regions and seasons to assess the distribution of TSV in cotton and to determine whether seasonal differences in disease incidence occur.

Representative samples with typical TSV symptoms were collected during each survey and tested for the presence of TSV by enzyme-linked immunosorbent assay (ELISA), essentially as per the manufacturer's instructions (AGDIA ELISA reagent set, Cat. No.SRA25500/0500) to confirm that the visual assessments were accurate. A limited number of ELISA-positive samples were also tested by TSV-specific PCR to confirm which strain of TSV was present.

Cotton samples with apparently systemic TSV infection were collected during disease surveys and different parts of the plants were tested by ELISA to determine the extent of detectable systemic infection.

During the 2008/09 season, the in-crop distribution of TSV infected plants was determined in a typical block with apparently scattered TSV infections. A quadrat about 100 m in from the crop edge (to avoid edge effects) of 21 rows across by 40m long was marked (Fig.1). Every plant within the quadrat (approximately 8400 plants) was carefully inspected for any local lesion symptoms of TSV and the position of the plant recorded on a grid map. The same plants were inspected about 1 month later to see if any further symptoms had developed. This was done to determine the spatial distribution of TSV infected plants within a crop (i.e. are they clumped or randomly distributed) and also to track if any plants with local lesion symptoms developed systemic symptoms.

During the 2010/11 season, the incidence of Cotton bunchy top (CBT) symptoms greatly increased compared to previous seasons and appeared to be frequently associated with systemic TSV symptoms which were very rare in the absence CBT in previous seasons. During that season, disease counts were also done for CBT and TSV symptoms. To investigate this further, and to confirm the mixed infections of CBT and TSV, a PCR assay was developed to screen samples for the presence of CBT. This was also done as a pre-emptive start to the closely related CRDC project (11-12FRP0062) which was due to begin in July 2011. Partial Cotton bunchy top virus (CBTV) genome sequence (unpublished), isolated from a CBT sample from Narrabri, NSW was kindly provided by Dr Marc Ellis (CSIRO). In order to provide the best chance of detecting any possible sequence variations in CBTV strains, a sequence alignment of different polerovirus species (related to CBTV) was done and PCR primers were designed to conserved regions of the coat protein gene. The polerovirus species used for the primer design included Cotton leafroll dwarf virus (CLRDV; cotton blue disease) isolates from Argentina and Brazil (Genbank accessions GU167940 and GQ379224), Cucurbit aphid borne yellow virus (CABYV; EU636992) and Chickpea chlorotic stunt virus (CpCSV; AY956384). The resulting PCR assay was used to screen a number of cotton samples with CBT symptoms collected as part of this project and was also utilised by Dr Cherie Gambley as part of project DAQ0001 to confirm visual assessments of CBT symptoms from samples collected and stored during disease surveys from 2008/9 to 2010/11.

Objective 2. Determine the thrips vector species associated with cotton.

Thrips were collected from 17 cotton crops over the 2008/09 and 2009/10 seasons. No collections of thrips were made in the 2010/11 season due to very low thrips populations probably resulting from the very regular, large volume rainfall events experienced during that season. Thrips were also collected from a number of weeds surrounding crops which were known to be alternative hosts of TSV. This was done to try to associate the likely thrips species involved in the movement of TSV from the surrounding alternative hosts into cotton crops. The collected thrips were initially mounted by the Principal Researcher and species identifications were confirmed by Department of Employment and Economic Development (DEEDI) Senior Entomologist, Desley Tree. Tomato thrips (*Frankliniella schultzei*) is a known TSV vector species and was commonly associated with cotton and surrounding weeds. For this reason, *F. schultzei* was cultured for use in transmission tests described in Objective 3.

Objective 3. Examination of factors that lead to systemic infection of cotton.

TSV is transmitted in pollen and requires thrips feeding to facilitate transmission. Under glasshouse conditions, cotton cv. Sicot 71 (only treated with Dynasty fungicide) of different ages was exposed to thrips and TSV-infected pollen for different lengths of time (up to several weeks) to hopefully simulate different levels of disease pressure. Test plants were observed for typical local lesions of TSV on inoculated leaves, indicating transmission of TSV, and were also grown for up to several months to determine if systemic symptoms developed.

TSV-infected pollen was collected from *Parthenium hysterophorus* (parthenium weed) known to be infected with the parthenium-TSV strain (see Results of Objective 1 for further details of TSV strains) and either used fresh in transmission tests or stored at 5°C for later use. *Frankliniella schultzei* (tomato thrips) are an efficient vector species for TSV and were commonly found on field crops of cotton. They were cultured for use in transmission tests. Typically, TSV-infected pollen was mixed with tomato thrips and both were sprinkled onto cotton test plants and also several plants of a known susceptible host such as mung beans or sunflower to determine the efficiency of the inoculation process. Any plants with suspected systemic infection were tested by TSV ELISA.

Objective 4. Determine yield effects of TSV on cotton.

This objective was not achieved under controlled conditions due to the significant difficulties in being able to consistently achieve systemic infection in cotton using controlled inoculation methods detailed in Objective 3.

The same cultivar of cotton (Sicot 71) was also planted in small plots at two trial sites in the Clermont region over 3 seasons from 2009 to 2011 in areas known to have a history of high TSV disease pressure. These trial sites were conducted primarily for screening TSV tolerance in sunflower cultivars as part of the GRDC project but provided a good opportunity to also test cotton under high TSV pressure. Infection relied on natural transmission by thrips from surrounding TSV-infected parthenium. It was hoped that the close proximity of the test cotton to high density stands of TSV-infected parthenium may simulate a worse case scenario and provide the most likely conditions for systemic infection to occur and possibly affect yields.

Conclusions about likely effect of TSV on cotton yields were made based on rarely observed natural systemic infections in field crops.

Objective 5. Dissemination of disease management data based on research objectives.

Several pieces of extension material, based on the research data obtained, were prepared in collaboration with other workers (i.e. industry development officers) and also communicated directly to growers in the TSV affected region of CQ.

Results

4. Detail and discuss the results for each objective including the statistical analysis of results.

Objective 1. Surveys to assess the incidence, distribution and impact of TSV.

Over 78,000 plants were visually inspected for typical TSV symptoms across three seasons (2008/9 to 2010/11) and several regions in central and southern Qld (Table 1). Within the transect counts, only 107 plants out of the 78,000 were seen with TSV symptoms (approximately 0.1%) although edge effects were observed when close to parthenium where up to 10% of plants had local lesion symptoms (Table 3). Cotton planted in the dryland field trials near Clermont were subjected to relatively high TSV disease pressure and 21% to 26% of the plants developed typical TSV local lesions in 2009 and 2010 respectively. However, none of these plants developed systemic symptoms. No TSV-like symptoms were detected in cotton from regions outside of CQ. The TSV symptoms seen in cotton crops were usually mild and generally consisted of less than a few diffuse dark purple lesions probably indicative of inoculation sites where thrips have fed. Systemic TSV symptoms was very rarely observed in field crops until the 2010/11 season when mixed infections with CBTV were observed in several locations as discussed further in results for Objective 3.

Cotton samples with and without typical TSV symptoms were collected from disease surveys across three seasons from 2008/09 to 2010/11 and a total of 458 cotton samples were tested by ELISA. Of the 125 samples with typical TSV symptoms, 124 were positive by ELISA, indicating that visual assessment of TSV symptoms is reliable but confirmation of representative samples is recommended for confirmation, particularly while the disease inspector becomes familiar with typical TSV symptoms.

TSV infected cotton (usually only local lesion symptoms) was more commonly found in the 2008/9 season than in the 2009/10 season (Table 1) which tended to follow the same level of infection observed in other susceptible crops such as sunflower and mungbeans from the same region. However, incidence of TSV infection in cotton appeared to increase in the 2010/11 season (still usually less than 0.5%) even though there appeared to be much lower TSV disease pressure compared to previous years as indicated by the very low levels of TSV infection in commercial sunflower crops.

The spatial mapping of TSV-infected plants (Figure 1) shows 87 TSV-infected plants out of about 8400 plants (1%) and suggests a relatively random distribution of infected plants within the crop with several small clumps of infected plants. Given the absence of pollen producing TSV host plants within the crop (the cotton was pre-flowering), TSV is entering the crop from outside, either as wind blown TSV-infected pollen (and thrips present in the crop are then facilitating transmission), or thrips carrying TSV-infected pollen are entering the crop, or a combination of both. The spatial distribution may indicate some thrips entering the crop are carrying TSV-infected pollen and then moving between neighbouring plants, causing the small clumps of infected plants. None of the 87 plants with local lesions within the quadrat area developed systemic symptoms when checked several weeks later and in most cases plants appeared to be symptomless.

Work by the Principle Researcher conducted in conjunction with a GRDC project (DAQ00130) has identified two distinct strains of TSV occurring in central Qld (CQ). These strains differ both genetically and in their respective host ranges and symptoms on some hosts. One strain appears to be most commonly found in crownbeard, *Verbesina encelioides* (Crownbeard-TSV) while the other is most commonly found in parthenium (parthenium-TSV). A strain-specific multiplex PCR has been developed and used to screen a selection of cotton TSV isolates from various locations (Table 2). To date, almost all the TSV infections

found in cotton have been the parthenium-TSV strain. The crownbeard-TSV strain has been found in cotton near Theodore and Arcturus (Emerald region). Both TSV strains appear to cause similar symptoms in cotton. Crownbeard occurs outside of central Qld but it is still unclear if the crownbeard-TSV strain also occurs outside of CQ in other growing regions. The geographical distribution of TSV disease in cotton (and other susceptible hosts) appears to be closely related to the distribution of the major alternative host, parthenium weed which is mostly restricted to the Central Highlands region of CQ.

Severe CBTV disease was observed in the 2010/11 season at several locations with up to 70%, 76% and 96% incidence observed in crops at Jimbour, Mondure and Emerald respectively. Disease counts at the affected crops at Jimbour and Mondure were done with Cherie Gambley and these are reported in more detail in the Final Report for DAQ0001. The severely affected crop to the NE of Emerald was under pivot irrigation and disease counts were done across the pivot area in May 2011. CBT disease incidence ranged from 96% (48/50) at 20m from the eastern edge, 48% (24/50) at 200m, 42% (21/50) at 400m, to 15% (9/61) at about 600m from the eastern edge (close to the western edge). This decreasing disease incidence from East to West across the pivot area may indicate that infective aphids were entering the crop from an upwind source as the prevailing winds are NE through to SE.

CBTV infection was confirmed in several samples from Mondure, Jimbour and Emerald by PCR using the polerovirus primers described in the methods. Preliminary sequencing results suggest that the CBTV strain found at Mondure and Emerald is genetically distinct from a CBTV strain from Narrabri previously described (unpublished) by CSIRO workers. It appears that significant further work may be required to establish the diversity and geographical distribution of CBTV strains causing CBT and whether these may also display biological differences such as alternative hosts.

In localised patches along the edge of a CBTV-affected crop in Emerald, up to 70% of CBTV-affected plants also had apparently systemic TSV infection suggesting there may be some kind of synergistic relationship between CBTV infection possibly enabling systemic TSV infection to occur more readily than would normally occur with TSV alone.

In contrast to the extensive damage observed in sunflower and mungbean crops from the same region, Tobacco streak virus (TSV) has caused negligible damage in commercial cotton crops surveyed in CQ over the last few seasons. Systemically infected plants are rarely seen in commercial crops and have also been rarely produced by controlled inoculation tests. It appears that systemic infection may be transient with mild symptoms being produced intermittently. With current cultivars and conditions, it appears likely that TSV will continue to cause only minor levels of mild local lesions with negligible or no impact on yield in the Emerald irrigation area.

Table 1. Details of TSV survey sites in cotton and results obtained.

Nearest locality	Lat° / Longitude (ddd.ddddd°) of surveyed block	TSV infected plants / total plants counted in disease count	Survey month / year
Emerald	-23.46717, 148.09299	< 1 / 1000	Jan 2009
Emerald	-23.49057, 148.06358	11 / 1350	Nov 2008
Emerald	“	3 / 800	Dec 2008
Emerald	“	3 / 800	Jan 2009
Emerald	-23.44923, 148.11517	8 / 400	Nov 2008



Emerald	“	0 / 1000	Dec 2008
Emerald	“	1 / 1000	Jan 2009
Emerald	-23.48323, 148.32755	15 / 2500	Nov 2008
Emerald	“	1 / 1000	Dec 2008
Emerald	-23.48594, 148.07425	3 / 350	Nov 2008
Emerald	-23.43470, 148.38672	0 / 1500	Nov 2008
Emerald	-23.48706, 148.06866	5 / 2500	Nov 2008
Emerald	“	3 / 1000	Dec 2008
Emerald	-23.48024, 148.13860	1 / 800	Nov 2008
Emerald	“	17 / 1800	Dec 2008
Emerald	“	2 / 800	Jan 2009
Emerald	-23.47859, 148.12421	1 / 600	Nov 2008
Emerald	-23.48485, 148.08302	1 / 500	Nov 2008
Emerald	-23.44234, 148.44836	5 / 1500	Nov 2008
Emerald	-23.48671, 148.33417	<1 / 1000	Dec 2008
Emerald	-23.46604, 148.09209	9 / 2300	Dec 2008
Emerald	“	1 / 2000	Jan 2009
Emerald	-23.47436, 148.12743	1/1000	Nov 2009
Emerald	-23.48042, 148.13916	0/1000	Nov 2009
Emerald	-23.47813, 148.11963	0/1000	Nov 2009
Emerald	“	0/1000	Nov 2009
Emerald	-23.45409, 148.11087	0/1000	Nov 2009
Emerald	-23.44180, 148.11913	0/1000	Nov 2009
Emerald	-23.45647, 148.08199	1/1000	Nov 2009
Emerald	-23.46719, 148.09299	0/1000	Nov 2009
Emerald	-23.48485, 148.08302	0/1000	Nov 2009
Emerald	-23.48706, 148.06866	4/1000	Nov 2009
Emerald	-23.50864, 148.09703	0/1000	Nov 2009
Emerald	-23.51571, 148.17882	1/1000	Nov 2009
Emerald	-23.50626, 148.26410	0/1000	Nov 2009
Emerald	-23.48323, 148.32755	0/1000	Nov 2009
Emerald	-23.45648, 148.42960	0/900	Nov 2009
Emerald	“	0/1000	Nov 2009
Arcturus	-24.00651, 148.49419	1/1000	Nov 2009
Arcturus	-24.01223, 148.48190	0/1000	Nov 2009
Comet	-23.52426, 148.50995	0/700	Nov 2009
Comet	-23.51545, 148.50479	1/1000	Nov 2009
Baralaba	-24.31468, 149.82312	0/1000	Nov 2009
Moura	-24.65956, 149.95790	0/1000	Nov 2009
Moura	“	0/1000	Nov 2009
Theodore	-25.07361, 150.14842	0/1000	Nov 2009
Theodore	-25.06951, 150.13605	0/700	Nov 2009
Theodore	-24.98759, 150.07486	0/1000	Nov 2009
Theodore	-24.96918, 150.07539	0/1000	Nov 2009
Theodore	-24.95270, 150.08937	0/1000	Nov 2009
Theodore	-24.94148, 150.09113	0/800	Nov 2009
Theodore	-24.94263, 149.97980	0/1000	Nov 2009
Theodore	-24.93358, 150.00467	1/1000	Nov 2009
Warra	-26.87080, 150.91725	0/1000	Dec 2009
Warra	-26.87080, 150.91725	0/1000	Dec 2009
Macalister	-27.05750, 151.07010	0/1000	Dec 2009
Dalby	-27.25818, 151.14732	0/1000	Dec 2009
Dalby	-27.24514, 151.14420	0/1000	Dec 2009
Dalby	-27.31640, 151.27673	0/1000	Dec 2009



Cecil Plains	-27.58784, 151.26729	0/1000	Dec 2009
Cecil Plains	-27.69028, 151.34448	0/500	Dec 2009
Cecil Plains	-27.68066, 151.33305	0/1000	Dec 2009
Cecil Plains	-27.52365, 151.38870	0/1000	Dec 2009
Emerald	-23.47436, 148.12743	0/1000	Dec 2009
Emerald	-23.47376, 148.12587	0/200	Dec 2009
Emerald	-23.48042, 148.13916	0/500	Dec 2009
Emerald	-23.45647, 148.08199	0/200	Dec 2009
Emerald	-23.46719, 148.09299	0/900	Dec 2009
Emerald	-23.48706, 148.06866	2/1000	Dec 2009
Emerald	-23.49057, 148.06358	0/1000	Dec 2009
Arcturus	-24.00651, 158.49419	0/500	Dec 2009
Arcturus	-24.01550, 148.48021	0/700	Dec 2009
Arcturus	-23.99645, 148.50032	0/1000	Dec 2009
Emerald	-23.46070, 148.10936	4/1000	Dec 2010
Emerald	-23.49057, 148.06358	4/1000	Dec 2010
Emerald	-23.48454, 148.06027	8/1000	Dec 2010
Emerald	-23.48042, 148.13916	3/1000	Dec 2010
Emerald	-23.47376, 148.12587	1/1000	Dec 2010
Emerald	-23.47436, 148.12743	3/1000	Dec 2010
Emerald	-23.48042, 148.13916	2/1000	Jan 2011
Clermont	-22.65219, 147.78154	1/1000	Jan 2011
Clermont	-22.65151, 147.78986	0/1000	Jan 2011
Clermont	-22.68649, 147.80304	0/500	Jan 2011
Clermont	-22.67659, 147.79970	3/1000	Jan 2011
Clermont	-22.66915, 147.77313	0/1000	Jan 2011

Table 2. Identity of TSV strains infecting cotton from various locations.

Isolate number ^A	Collection Date	Collection location	TSV strain ^B
TSV-2117	Nov 2007	Foley Rd, Emerald	Parthenium-TSV
TSV-2119	Nov 2007	Wills Rd, Emerald	Parthenium-TSV
TSV-2120	Nov 2007	Gregory HWY, Emerald	Parthenium-TSV
TSV-2144	Jan 2008	Codenwarra Rd, Emerald	Parthenium-TSV
TSV-2285	Nov 2008	Maloney's Rd, Moura	Parthenium-TSV
TSV-2399	June 2009	Arcturus, Springsure	Crownbeard-TSV
TSV-2509	Nov 2009	Bertles Rd, Emerald	Parthenium-TSV
TSV-2510	Nov 2009	Theodore-west	Crownbeard-TSV
TSV-2515	Nov 2009	Arcturus, Springsure	Parthenium-TSV
TSV-2719	Dec 2010	Wills Rd, Emerald	Parthenium-TSV
TSV-2734	Jan 2011	Wills Rd, Emerald	Parthenium-TSV
TSV-2735	Jan 2011	Wills Rd, Emerald	Parthenium-TSV
TSV-2803	Jan 2011	Donahue Rd, Emerald	Parthenium-TSV
TSV-2807	Jan 2011	Donahue Rd, Emerald	Parthenium-TSV

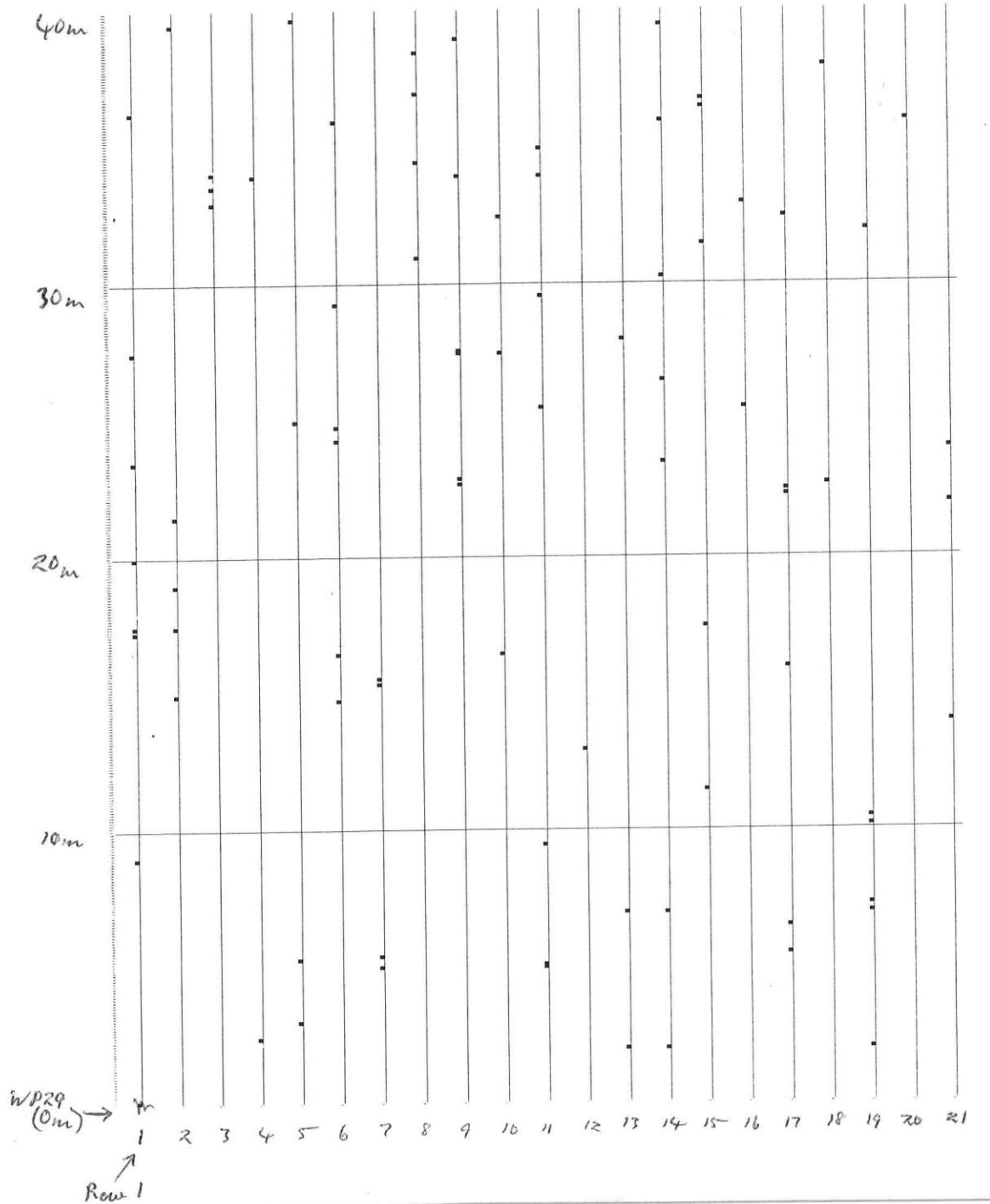
^A Virus isolate stored in QDEEDI Plant Virus collection.

^B TSV strain determined by either sequencing or strain-specific PCR.

Table 3. TSV disease gradient perpendicular to rows at an Emerald site. Row 2 was closest to TSV infected parthenium which was upwind of crop.

Row #	Number of TSV plants per 250 plants
2	23
4	20
6	16
8	14
10	6
12	0
14	7
16	3
18	1
20	5
22	2
28	0
34	1
40	0
60	1

Figure 1. Spatial map of TSV-infected cotton plants within a crop. The count area was 21 rows across by 40 m and was centrally located within a cotton block. Black dots represent plants with typical TSV local lesion symptoms.



Objective 2. Determine the thrips vector species associated with cotton

Twelve thrips species were identified from the 17 cotton samples (Table 4) and results indicate that onion thrips (*Thrips tabaci*) and tomato thrips (*Frankliniella schultzei*) are two of the more commonly found species on cotton. Both these species are known vectors of TSV. Only 1 out of 13 crownbeard and parthenium samples had onion thrips whereas tomato thrips were on 6 samples of the weeds. This may indicate that tomato thrips could be the most likely vector species moving TSV-infected pollen from nearby TSV hosts into cotton crops. *Microcephalothrips abdominalis* is also a known TSV vector species and was found in 11 of the 13 nearby crownbeard and parthenium samples but only from 2 of the 17 cotton samples

Table 4. Thrips identified from cotton and nearby weeds.

Collection Date	Host common name	Collector	Collection location	Thrips species (number of individuals)
4/11/2008	Cotton	M. Sharman	Bertles Rd, Emerald	<i>Thrips tabaci</i> (11) <i>Caliothrips striatopterus</i> (2) <i>Arorathrips mexicanus</i> (1)
5/11/2008	Cotton	M. Sharman	Wills Rd, Emerald	<i>Frankliniella williamsi</i> (1) <i>Tenothrips frici</i> (1) <i>Desmothrips propinquus</i> (1)
5/11/2008	Cotton	M. Sharman	Foley Rd, Emerald	<i>Thrips tabaci</i> (8) <i>Pseudanaphothrips achaetus</i> (1)
5/11/2008	Cotton	M. Sharman	Wills Rd, Emerald	<i>Thrips tabaci</i> (3) <i>Desmothrips propinquus</i> (1) <i>Caliothrips striatopterus</i> (1)
5/11/2008	Cotton	M. Sharman	Foley Rd, Emerald	<i>Thrips tabaci</i> (1) <i>Frankliniella schultzei</i> (1) <i>Pseudanaphothrips achaetus</i> (1)
5/11/2008	Cotton	M. Sharman	Bertles Rd, Emerald	<i>Thrips tabaci</i> (1) <i>Microcephalothrips abdominalis</i> (1) <i>Tenothrips frici</i> (1) <i>Desmothrips propinquus</i> (1)
11/12/2008	Cotton	M. Sharman	Bertles Rd, Emerald	<i>Thrips tabaci</i> (1) <i>Haplothrips sp</i> (1) <i>Microcephalothrips abdominalis</i> (1) <i>Desmothrips propinquus</i> (1)
8/01/2009	Cotton	M. Sharman	Wills Rd, Emerald	<i>Frankliniella schultzei</i> (11) <i>Bolacothrips sp</i> (1) <i>Caliothrips striatopterus</i> (1)
3/11/2009	Cotton	M. Sharman	Arcturus Downs, Arcturus	<i>Thrips tabaci</i> (4) <i>Tenothrips frici</i> (1) <i>Haplothrips sp</i> (1)
4/11/2009	Cotton	M. Sharman	Wills Rd, Emerald	<i>Thrips tabaci</i> (3)
4/11/2009	Cotton	M. Sharman	Wills Rd, Emerald	<i>Thrips tabaci</i> (1) <i>Anaphothrips sp</i> (1)
1/12/2009	Cotton	M. Sharman	Dalby Cecil Plains Rd, Dalby	<i>Thrips tabaci</i> (5) <i>Frankliniella schultzei</i> (3) <i>Haplothrips sp</i> (2) <i>Desmothrips tenuicornis</i> (1)
1/12/2009	Cotton	M. Sharman	Moonie HWY, Dalby	<i>Thrips tabaci</i> (6) <i>Frankliniella schultzei</i> (2) <i>Haplothrips</i> (1)
1/12/2009	Cotton	M. Sharman	Brookstead Norwin Rd, Pittsworth	<i>Thrips tabaci</i> (8) <i>Pseudanaphothrips achaetus</i> (2)
15/12/2009	Cotton	M. Sharman	Wills Rd, Emerald	<i>Thrips tabaci</i> (3)

16/12/2009	Cotton	M. Sharman	Wills Rd, Emerald	<i>Desmothrips tenuicornis</i> (2) <i>Thrips tabaci</i> (8) <i>Frankliniella schultzei</i> (1) <i>Desmothrips sp</i> (1)
16/12/2009	Cotton	M. Sharman	Wills Rd, Emerald	<i>Thrips tabaci</i> (12) <i>Frankliniella schultzei</i> (2)
5/11/2008	Crownbeard	M. Sharman	D. Walker, Wills Rd, Emerald	<i>Microcephalothrips abdominalis</i> (11) <i>Haplothrips sp.</i> (2) <i>Tenothrips frici</i> (1)
6/11/2008	Crownbeard	C. Gambley	Theodore	<i>Pseudanaphothrips achaetus</i> (3) <i>Microcephalothrips abdominalis</i> (2)
8/01/2009	Crownbeard	M. Sharman	Wills Rd, Emerald	<i>Frankliniella schultzei</i> (4) <i>Microcephalothrips abdominalis</i> (2)
27/03/2009	Crownbeard	M. Sharman	Darling Downs	<i>Frankliniella occidentalis</i> (13)
3/06/2009	Crownbeard	M. Sharman	Arcturas Downs	<i>Microcephalothrips abdominalis</i> (36)
6/09/2009	Crownbeard	M. Sharman	Moree	<i>Frankliniella occidentalis</i> (30) <i>Thrips tabaci</i> (11)
16/04/2010	Crownbeard	M. Sharman	Arcturus	<i>Frankliniella schultzei</i> (14) <i>Microcephalothrips abdominalis</i> (1)
7/05/2010	Crownbeard	M. Sharman	Arcturus	<i>Microcephalothrips abdominalis</i> (37)
3/03/2009	Parthenium	M. Sharman	Kenlogan Rd, Clermont	<i>Frankliniella schultzei</i> (24) <i>Microcephalothrips abdominalis</i> (11) <i>Thrips sp.</i> (4)
1/04/2009	Parthenium	M. Sharman	Kenlogan Rd, Clermont	<i>Microcephalothrips abdominalis</i> (21) <i>Frankliniella schultzei</i> (17) <i>Caliothrips striatopterus</i> (2)
2/11/2009	Parthenium	M. Sharman	Wills Rd, Emerald	<i>Microcephalothrips abdominalis</i> (4) <i>Frankliniella schultzei</i> (4) <i>Thrips imaginis</i> (3)
16/12/2009	Parthenium	M. Sharman	Fernlees, Gindie	<i>Microcephalothrips abdominalis</i> (5) <i>Haplothrips froggotti</i> (5)
7/05/2010	Parthenium	M. Sharman	Fernlees, Gindie	<i>Microcephalothrips abdominalis</i> (16) <i>Frankliniella schultzei</i> (4)

Objective 3. Examination of factors that lead to systemic infection of cotton.

At least 33 cotton plants cv. Sicot 71 (only treated with Dynasty fungicide) were inoculated using tomato thrips (*F. schultzei*) and TSV pollen from parthenium (parthenium-TSV strain) under controlled conditions. Plants were of different ages and exposed to TSV inoculum for varying lengths of time (Table 5). About 80% these test plants displayed local lesions typical of TSV several days after inoculations started and all susceptible control plants (mungbeans or sunflowers) developed typical severe systemic symptoms. All plants were grown for several months post inoculation. Only one cotton test plant did develop systemic symptoms typical of TSV after 4 months, with necrotic ring spots on the upper-most leaves and was positive by TSV-specific ELISA and PCR. These systemic symptoms appeared to have been transient as subsequent growth was symptomless on the same plant. This is not unlike what has been observed in field infected plants that appear to be displaying systemic symptoms and subsequent growth is symptomless. This suggests that cotton may have infrequent and incomplete systemic infection and symptoms can be displayed in a transient manner perhaps induced by an unknown change in environmental conditions.

In early 2011, TSV disease incidences were very low in commercial sunflower crops and field trials of sunflower and mung beans conducted as part of GRDC project DAQ00130.

This was in agreement with very low thrips populations and limited amounts of flowering parthenium being present in many parts of central Queensland. It was expected that similarly very low incidences of TSV local lesion symptoms would be observed in cotton crops, and even fewer systemically infected plants. However, there were several locations in the Emerald region where many plants appeared to have systemic TSV symptoms in mixed infections with Cotton bunchy top virus. Several of these mixed infections were confirmed by TSV ELISA and CBTV PCR. One possible explanation is that when TSV occurs alone in cotton, the plant defence mechanisms almost completely limit the movement of TSV, resulting in only local lesions and infrequent, transient systemic symptoms. However, perhaps CBTV is able to silence the plant defence mechanisms (hence the systemic infections observed with CBTV) and in doing so, enable TSV to more readily systemically infect the cotton plants.

TSV was detected in different plant parts from field collected plants that had symptoms indicating systemic infection. TSV was detected by ELISA in symptomatic parts of the leaf but not from non-symptomatic parts of the same leaf. TSV was detected in petioles of symptomatic leaves, in the main stem between leaves that had symptoms (but not in other parts of the stem), and also in the pollen of plants with apparently systemic symptoms. These results suggest that infection of cotton by TSV can spread to different parts of the plant but is perhaps only partially systemic.

Table 5. Details of plant ages and periods of exposure to TSV inoculum in transmission tests using Sicot 71.

Number of test plants	Age post planting at start (days)	Period of exposure to TSV (days)	Number of inoculation events	ELISA results (number positive / total plants)	Systemic symptoms / total plants
5	14	23	5	0/5 ^A	0/5
5	8	23	5	1/5 ^{A,B}	1/5
10	15	49	2	n/t ^C	0/10
4	37	49	2	n/t	0/4
5	14	9	1	0/5	0/5
4	14	18	2	0/5	0/4

^A Tested twice by ELISA at 1 month and 4 months post inoculation.

^B One test plant was negative at 1 month, then positive (with typical TSV symptoms) at 4 months. It was also positive by TSV PCR.

^C Not tested by ELISA

Objective 4. Determine yield effects of TSV on cotton.

As detailed in results for Objective 3, out of at least 33 test plants in controlled inoculations, only one developed systemic symptoms of a transient nature that only appeared on a couple of upper leaves 4 months post inoculation. This made it unfeasible to measure any affect of systemic infection on yield under controlled conditions.

The incidence of cotton displaying systemic-like symptoms was very low (<0.05%) during TSV disease surveys in 2008/09 and 2009/10 and often plants that were marked would be

symptomless in subsequent inspections several weeks later. In 2010/11, the incidence of systemic-like TSV symptoms was higher but limited to small patches, almost always in mixed infections with CBT symptoms which were far more severe than the TSV symptoms alone.

Higher rates of infection were observed in dry-land cotton trial plots grown in the Clermont region compared to commercial crops in the Emerald irrigation area. This is probably due to the higher density of the alternative host and higher levels of inoculum (parthenium pollen) in these dry-land areas. High TSV disease pressure was experienced at one of the two trial sites in 2009 and 2010 with rates of severe TSV infection in a susceptible sunflower cultivar, planted at the same site, ranging from 65% to 40% in 2009 and 2010 respectively. Even though up to 26% of cotton plants in the trial plots displayed TSV local lesions, none developed systemic symptoms and most plants grew out of the local lesions and became symptomless.

Systemically infected cotton plants are rarely seen in commercial crops and have also been rarely produced by controlled inoculation tests. It appears that systemic infection may be transient with mild symptoms being produced intermittently. With current cultivars and conditions, it appears likely that TSV will continue to cause only minor levels of mild local lesions and no likely impact on yield in the Emerald irrigation area.

Objective 5. Dissemination of disease management data based on research objectives.

Several pieces of extension material, based on the research data obtained, were prepared in collaboration with other workers, including:

- A 3 page TSV section in the Integrated Disease Management (IDM) guide for cotton
- Presentation of project outputs at the FUSCOM meeting in 2009 and 2011
- Communication of project aims and outcomes with growers from Emerald, Springsure, Moura, Theodore and the southern Downs during annual surveys.
- Update article in Cotton Tales newsletter number 9, 2008/09
- Online resource for TSV identification on Cotton CRC website (http://web.cotton.crc.org.au/content/Industry/Publications/DiseaseMicrobiology/Tobacco_streak_virus_in_cotton_in_Central_QLD.aspx)

Outcomes

5. Describe how the project's outputs will contribute to the planned outcomes identified in the project application. Describe the planned outcomes achieved to date.

The annual surveys for TSV in different regions has provided definitive records for the presence of TSV in cotton from central Qld and also provided significant surveillance evidence that TSV is not currently present in cotton growing regions outside of CQ. The outputs of the project have enabled the industry to recognise the cropping districts in which TSV is likely to be found and the very unlikely potential for yield losses.

The identification of thrips species from cotton and surrounding TSV weed hosts has demonstrated that some of the known TSV vector species occur in both the cotton crops and surrounding weeds and suggest that the tomato thrips (*Frankliniella schultzei*) may be the most important vector of TSV into cotton but other species such as *Thrips tabaci* may also play a role. Controlled transmission tests demonstrated that *F. schultzei* is an efficient vector

of the parthenium-TSV strain, causing high incidences of local lesion symptoms on cotton test plants. This project (along with outputs from the related GRDC project DAQ00130) have demonstrated that while TSV has a wide host range, parthenium weed (*Parthenium hysterophorous*) appears to always be the major source of the virus in CQ which leads to TSV disease outbreaks in neighbouring crops. The control of parthenium weed around crops, particularly upwind of crops, is seen as the most effective way to prevent TSV disease outbreaks in susceptible crops.

While the initial plans for inducing systemic TSV infection were mostly unsuccessful, these attempts, along with field observations, clearly demonstrated that systemic infection of TSV alone in cotton is a very rare event, often with transient symptoms which often disappear with new growth. This indicates that the factors likely to promote systemic TSV infection rarely occur in nature. It appears that even though local lesion symptoms can occur at high incidence with high disease pressure, systemic infection rarely follows and even more rarely persists in the plant. Any yield loss from the very rare occurrence of systemic TSV infection is expected to be negligible and almost certainly compensated for in the crop. While the mixed infections with CBTV did appear to increase the incidence of systemic TSV infection, the relative impact of TSV in these mixed infections appeared to be negligible compared to the severe disease symptoms of CBTV. It is unlikely that any control strategies are required to limit the systemic infection of cotton by TSV. The control of CBTV is a far more important priority in commercial cotton. However, general farm hygiene to minimise the presence of the major alternative host, parthenium weed is advised and may be of vital importance if TSV susceptible rotational crops such as mung beans are grown.

6. Please describe any:-

- a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
- b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
- c) required changes to the Intellectual Property register.

The CTAB extraction method developed and used for isolating virus genomic material for use in PCR diagnostics may be useful for other viral pathogens of cotton and will probably be used in the CRDC project (11-12FRP00062) to investigate alternative hosts of CBTV. The poliovirus PCR primers developed will be further refined and also used in the project 11-12FRP00062.

Conclusion

7. Provide an assessment of the likely impact of the results and conclusions of the research project for the cotton industry. What are the take home messages?

In contrast to the extensive damage observed in sunflower and mungbean crops from the same region, Tobacco streak virus (TSV) has caused no measurable damage in commercial cotton crops surveyed in Central Qld over the seasons 2008/9 to 2010/11. No TSV infected cotton was found in regions outside of Central Qld and the geographical distribution of TSV disease in cotton (and other susceptible hosts) appears to be closely related to the distribution of the major alternative host, parthenium weed. Systemically infected plants are rarely seen in commercial crops and have also been rarely produced by controlled inoculation tests. It appears that systemic infection may be transient with only mild symptoms being produced

intermittently. With current cultivars and conditions, it appears likely that TSV will continue to cause only minor levels of mild local lesions with no impact on yield. It appears that no specific control strategies are required to limit the impact of TSV in cotton. As demonstrated by recent outbreaks, the control of CBTV is of far greater importance for the cotton industry. However, general farm hygiene to minimise the presence of the major alternative host of TSV, parthenium weed, is advised and may be of vital importance if TSV susceptible rotational crops such as mung beans are grown.

Extension Opportunities

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 extraction methods and PCR primers for detection of CBTV (and other poleroviruses) developed in this project will be used and further developed in CRDC project 11-12FRP00062 to investigate various aspects of the epidemiology and genetic diversity of CBTV.
 - (b) for the future presentation and dissemination of the project outcomes.
 - > Continued contact with cotton growers and industry members through CRDC project 11-12FRP00062 will enable further dissemination of project outcomes.
 - (c) for future research.
 - > Further studies (not currently anticipated) may be useful to test the hypothesis that systemic TSV infection in cotton is greatly enhanced in plants already infected with CBTV. If this is true it may be possible to determine the mechanism by which this occurs which could be a form of gene silencing induced by CBTV which then enables TSV to more readily move through the cotton plant.
 - > Preliminary results suggest that there is more than one strain of CBTV causing CBT disease in cotton. Many things are unknown, such as: the complete genome diversity of each of the strains (and possibly others), the likelihood of genetic recombination between strains, the impact that genetic variation between strains may have on the effectiveness of any future CBTV-resistant cotton lines that may be developed, the geographic distribution of these CBTV strains, the relative incidence and importance of these strains and any biological differences such as host range. Some of these unknowns will be investigated as part of the new project 11-12FRP00062 (such as host range) but other aspects are outside the objectives of this project and resource and time limitations would mean that further investment would be required to investigate these aspects adequately.

8. A. List the publications arising from the research project and/or a publication plan.
(NB: Where possible, please provide a copy of any publication/s)

Sharman M, Thomas JE, Persley DM (2008) First report of *Tobacco streak virus* in sunflower (*Helianthus annuus*), cotton (*Gossypium hirsutum*), chickpea (*Cicer arietinum*) and mung bean (*Vigna radiata*) in Australia. *Australasian Plant Disease Notes* **3**, 27-29.

Sharman M, Persley DM, Thomas JE (2009) Distribution in Australia and seed transmission of Tobacco streak virus in *Parthenium hysterophorus*. *Plant Disease* **93**, 708-712.

Planned journal publications in conjunction with the outputs of GRDC project DAQ00130 include: a paper describing the genetic diversity and detection of TSV strains in Australia, and a paper describing further aspects of the epidemiology, thrips and seed transmission, and natural host range of TSV strains found in Australia.

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

- Online resource for TSV identification on Cotton CRC website ([http://web.cotton.crc.org.au/content/Industry/Publications/DiseaseMicrobiology/Tobacco streak virus in cotton in Central QLD.aspx](http://web.cotton.crc.org.au/content/Industry/Publications/DiseaseMicrobiology/Tobacco%20streak%20virus%20in%20cotton%20in%20Central%20QLD.aspx))
- I contributed content for the Crop Consultants Australia online survey to determine importance of Cotton bunchy top: <http://cropconsultants.com.au/cbt>

Part 4 – Final Report Executive Summary

Provide a one page Summary of your research that is not commercial in confidence, and that can be published on the World Wide Web. Explain the main outcomes of the research and provide contact details for more information. It is important that the Executive Summary highlights concisely the key outputs from the project and, when they are adopted, what this will mean to the cotton industry.

In 2006, Tobacco streak virus (TSV) was identified as the causal agent of the devastating sunflower necrosis disease in central Queensland (CQ), and subsequently in 2007 as the cause of major losses in mungbeans in the same area. It has been a major factor in the recent downturn in the sunflower industry in CQ. Surveys in 2007/2008 as part of a one year scoping study (project 03DAQ005) found TSV in cotton in CQ. The symptoms were mostly confined to the feeding sites of the thrips and appeared as reddish spots and rings, but only occasionally the plants were systemically infected and showed a chlorotic mosaic and leaf deformation.

The major objectives of this project (DAQ0002) were to determine: the incidence and distribution of TSV in cotton and its likely effect on yield; the thrips vector species associated with TSV infections in cotton; and the factors that may lead to systemic infections.

In contrast to the extensive damage observed in sunflower and mungbean crops from the same region, TSV has caused no measurable damage in commercial cotton crops surveyed in CQ over the seasons 2008/9 to 2010/11. No TSV infected cotton was found in regions outside of CQ and the geographical distribution of TSV disease in cotton (and other susceptible hosts) appears to be closely related to the distribution of the major alternative host, parthenium weed. The most likely thrips species responsible for transmission of TSV into cotton is the tomato thrips (*Frankliniella schultzei*) and onion thrips (*Thrips tabaci*). Systemically infected plants are rarely seen in commercial crops and have also been rarely produced in controlled tests. It appears that systemic infection may be transient with only mild symptoms being produced intermittently. With current cultivars and conditions, it

appears likely that TSV will continue to cause only minor levels of mild local lesions with no impact on yield in cotton crops.

It appears that no specific control strategies are required to limit the impact of TSV in cotton. However, general farm hygiene to minimise the presence of the major alternative host of TSV, parthenium weed, is advised and may be of vital importance if TSV susceptible rotational crops such as mung beans are grown.

For further information, contact:

Murray Sharman

Plant Pathologist (Virology)

Department of Employment, Economic Development and Innovation

Address:

Department of Employment, Economic Development and Innovation

Level 2C-West

Ecosciences Precinct

GPO Box 267

Brisbane

Queensland, 4001

Australia

Telephone: + 61 7 3255 4339 **Facsimile:** + 61 7 3846 2387

Mobile: 0467 721 400

Email: murray.sharman@deedi.qld.gov.au