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Cotton Research and Development Corporation

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Part 1 - Summary Details

CRDC Project Number: **03DAQ005**

Project Title: Tobacco streak virus in cotton – scoping study

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Part 2 – Contact Details

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Background

Tobacco streak virus (TSV) was recently identified by the project team as the cause of a sunflower necrosis disease which has been a major limiting factor to profitable production in central Queensland since at least 2004. The virus also caused significant losses in mungbean crops in central Queensland in 2007. Most grain legumes are susceptible to TSV and, like mungbeans, often develop terminal necrosis, stunting and necrotic lesions which results in the death of plants. The virus is spread by a range of thrips species, able to transmit the virus while feeding on infected pollen situated on leaves of healthy crops plants.

There are several reports of TSV infection of cotton in other countries, sometimes causing yield depression, and the virus is a potential threat to cotton in central Queensland. A major factor in the importance of TSV in central Queensland is the wide distribution and abundance in the region of Parthenium weed (*Parthenium hysterophorus*), a key alternative host of the virus.

There is an urgent need to screen cotton cultivars and selected lines for their reaction to central Queensland isolates of TSV. Cotton crops in the region should be surveyed at appropriate locations and at suitable growth stages to determine the presence and likely impact of TSV infection. If TSV does naturally infect cotton the economic impact may be significant, given the range of symptoms and damage in other hosts.

On average the Emerald Shire produces more than 25% of Queensland's total production, hence economic loss could be substantial if TSV causes severe disease in Australian cotton varieties. The widespread distribution and abundance in central Qld of Parthenium weed, a symptomless host of TSV, is of major concern. We currently have limited information on the potential of TSV to infect Australian cotton varieties or the impact on production if varieties are susceptible.

Objectives

Objective 1. Survey cotton fields in central Queensland for signs of TSV infection.

Objective 2. Determine abundance and infection level of Parthenium weed adjacent to cotton production.

Objective 3. Identify cotton varieties and lines with resistance/susceptibility to TSV.

The aims of this project are to determine if TSV is a threat to cotton production in Queensland and to screen selected varieties for resistance to the virus.

Methods

Surveys for TSV in cotton and parthenium

Disease surveys were conducted within the Emerald irrigation area at three times during the 2007-2008 cotton season to detect TSV infections both in cotton crops and also in surrounding parthenium plants. Surveys were conducted in early November 2007, mid January and mid February 2008. The January and February surveys coincided with significant rainfall events which restricted survey activities. Six cotton crops from five farms were surveyed in November, two crops were surveyed in January and five crops from three farms were surveyed in February.

Cotton crops were visually inspected for virus-like symptoms and where possible sections from several rows were inspected throughout the crop. Surveys in January and February were restricted to outside rows due to deep mud. Where virus-like symptoms were seen, samples were collected and transported to DPI&F laboratories, Indooroopilly, for TSV testing by Enzyme Linked Immuno-Sorbent Assay (ELISA). For representative cotton samples positive by TSV ELISA, confirmatory TSV-specific Polymerase chain reaction

(PCR) was also done. The resulting PCR product was sequenced to confirm the identity of the TSV strain.

Areas with parthenium were noted during these surveys within the cotton production area. TSV infected parthenium plants do not display symptoms, so they were randomly collected from within the Emerald irrigation area, often adjacent to cotton crops or volunteer cotton plants displaying TSV symptoms, and tested for TSV by ELISA.

TSV resistance / tolerance screening for cotton lines

Eleven cotton lines provided by Greg Constable from the CSIRO germplasm collection were screened for TSV resistance. These included several current or past commercial varieties (Sicot 71, Sicot F-1, Siokra 1-4 and Namcala) as well as experimental lines (CSX102, CSX210, CSX180 and CPX42) and introductions (Krishma, BM13H and CIM442). A further 2 cotton lines were also tested (Siokra V17 and Delta Emerald) bringing the total cotton lines tested to 13.

Eight to fourteen plants of each line were grown in a glasshouse and manually inoculated twice at 2 and 3 weeks post planting with a TSV isolate originally obtained from diseased sunflower from central Queensland. The first inoculation was done on the cotyledons only while the second inoculation was done on newly emerged true leaves. Infection status was determined by visual assessment of local and systemic symptoms of TSV about 2 weeks post second inoculation. Confirmatory TSV ELISA tests were done on representative samples.

Results

Objective 1. Survey cotton fields in central Queensland for signs of TSV infection.

November 2007 survey

Six cotton crops on five properties (sites 1, 7, 8, 10 and 12) within the irrigation area of Emerald were inspected for symptoms of virus in November of 2007 (Table 1 and Fig 1). Forty two cotton plants were sampled (30 plants with virus-like symptoms and 12 random samples) and tested for TSV. Of these, 22 of the 30 symptomatic plants were positive for TSV by ELISA while all symptomless plants were negative. A confirmatory TSV-specific PCR was done on five of the ELISA positive plants, all were positive by PCR. The resulting coat protein gene sequence obtained from TSV infected cotton is the same as that found in sunflower and parthenium in the same region (i.e. all these plant species were infected by the same strain of TSV in central Queensland).

The cotton crops were located north-east, west and north of Emerald (Fig 1), were all on irrigation and were all less than 40cm high (about 2 months after planting). Cotton plants displaying virus-like symptoms were seen in four cotton crops and also in volunteer cotton plants growing next to a sorghum crop (site 11). Symptoms included dark necrotic lesions on leaves, sometimes forming numerous diffuse ring spots. On plants with numerous necrotic lesions the upper leaves sometimes also displayed chlorotic mottle and deformed leaves. Symptoms in the young cotton crops were usually mild and generally consisted of single diffuse necrotic lesions. Photos of symptoms can be viewed by following this website link: http://www.cottoncrc.org.au/content/Industry/Publications/DiseaseMicrobiology/Tobacco_streak_virus_in_cotton_in_Central_QLD.aspx.

No symptomatic cotton plant was seen in two of the six crops inspected, both at site 12. In the other four crops, about 1% of the cotton plants were symptomatic. Counts were done of symptomatic cotton plants at site 1 at about 40m, 120m and 250m from the edge of the crop with about 380 plants checked at each location. The proportions of symptomatic plants were 5/380 at 40m and 120m from the edge and 6/380 at 250m from the edge. This relatively even distribution of symptomatic plants across the crop at site 1 was also seen in the other three crops with infected plants (sites 7, 8 and 10).

The volunteer cotton plants seen at site 11 were older (probably slashed and regrowing from last season) and displayed more severe symptoms with numerous necrotic ring spots and chlorotic mottle. They were growing within 5m of a high density of parthenium and 38/53 cotton plants were symptomatic.

January 2008 survey

Two cotton crops were surveyed within the irrigation area at sites 8 and 9 (Table 1 and Fig 1). Symptomatic cotton plants were collected from both crops and 10/11 were positive for TSV by ELISA. As was the case during the November survey, disease levels were generally very low with less than 1% of cotton plants with any symptoms and these were usually mild necrotic isolated lesions on a couple of leaves. About 1-5% of pigeon pea plants next to cotton at site 9 also had TSV symptoms of chlorotic mottle and necrosis and three pigeon pea plants were tested by ELISA, all were positive for TSV.

February 2008 survey

Five cotton crops were surveyed within the Emerald irrigation area at sites 2, 3, 4, 5 and 10 (Table 1 and Fig 1). Cotton at site 10 was displaying significant reddening of leaves associated with natural senescence making visual assessments for TSV symptoms difficult. Where TSV symptoms were seen in cotton, disease levels were less than 1%. Symptomatic cotton plants were collected from sites 2, 3 and 5 and while symptoms were generally mild, all plants collected were positive for TSV by ELISA (Table 1). A couple of cotton plants from the commercial crop at site 3 displayed slightly more severe, apparently systemic symptoms.

Objective 2. Determine abundance and infection level of Parthenium weed adjacent to cotton production.

Parthenium was seen growing at, or close to (within 200m), 10 out of the 12 sites surveyed with abundance ranging from low to high (Table 1). In general, the abundance of parthenium was less in the Emerald irrigation area than that seen in dryland areas north of Emerald towards Clermont and Kilcummin where parthenium was seen at very high densities and TSV was detected in parthenium from numerous locations (data not shown).

Parthenium plants were collected and tested from 4 sites (2, 6, 9 and 11) within the Emerald cotton production area and TSV was detected in parthenium from all four sites (Table 1 and Fig 1). These results indicate that parthenium is a key alternative host for TSV and is likely to be one of the main sources of inoculum near cotton crops in the Emerald cotton production area.

Table 1. Details of survey sites and results obtained. Map of site locations is shown in Fig 1.

Site #	Location	[A] TSV detected in cotton	Parthenium abundance	[A] TSV detected in parthenium	Survey Month/s
1	Wills Rd	Yes (6/8)	Low	n/t	November 2007
2	Wills Rd	Yes (7/7)	Moderate	Yes (7/30)	January, February 2008
3	Wills Rd	Yes (6/6)	Low	n/t	February 2008
4	Bradshaw Rd	No	Low	n/t	February 2008
5	Wills Rd	Yes (3/3)	Moderate	n/t	February 2008
6	Wills Rd	n/a	High	Yes (6/6)	February 2008
7	Gregory HWY	Yes (5/5)	None	n/a	November 2007
8	Emerald Downs Rd	Yes (11/11)	Low	n/t	November 2007, January 2008
9	Codenwarra Rd	Yes (3/4)	High	Yes (1/1)	January 2008
10	Foley Rd	Yes (2/3)	Moderate	n/t	November 2007, February 2008
11	Foley Rd	Yes (6/10)	High	Yes (17/36)	November 2007, February 2008
12	Francis Rd	No (0/12) [B]	None	n/a	November 2007

[A] Samples tested by ELISA. Proportion of symptomatic plants giving positive ELISA reactions shown in parentheses.

[B] Cotton samples tested from site 12 were randomly collected and did not display TSV symptoms.

n/a: not applicable

n/t: not tested

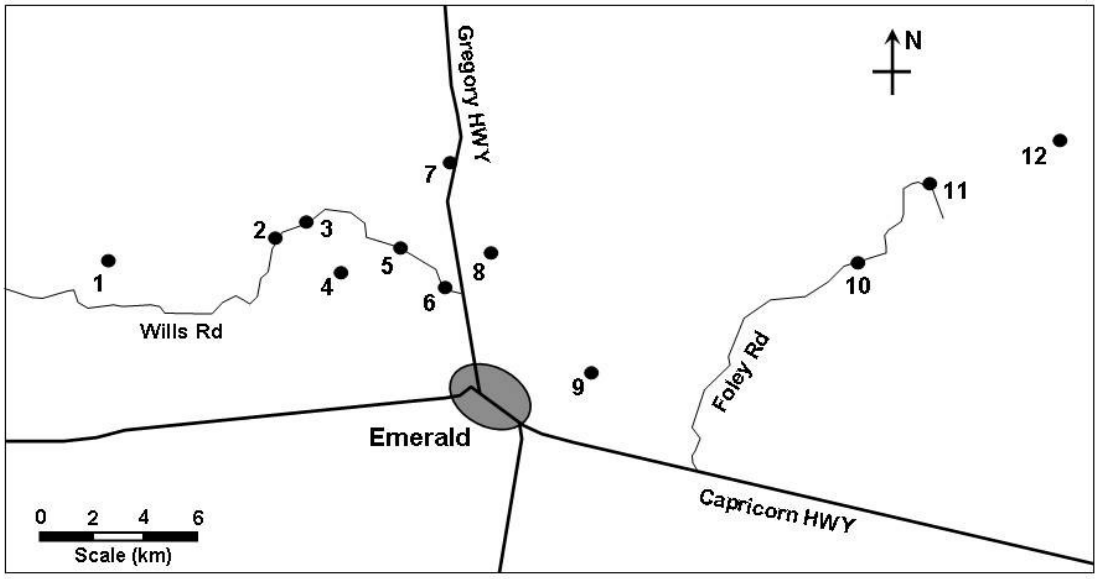

Figure 1. Map of Emerald region with sites (black dots) surveyed for TSV. Details of sites and survey results are listed in Table 1.

Objective 3. Identify cotton varieties and lines with resistance/susceptibility to TSV.

A total of 13 cotton lines have been screened for resistance/susceptibility to TSV. Results indicate no true genetic resistance is present in any of the lines with all lines tested displaying symptoms. These ranged from a few small necrotic local lesions seen on almost all plants on the inoculated leaves, to systemic chlorotic mottle seen on only a few individuals from 3 of the 13 lines tested. There was often necrosis on the petioles of inoculated leaves. Almost all plants of all lines tested had apparently healthy growth after the initial localised symptoms. The more severe disease symptoms seen occasionally on field plants in the Emerald region indicates that under certain conditions (possibly prolonged exposure to inoculum) cotton can develop apparently systemic symptoms over much of the plant. The factors that lead to this are unclear and require further investigation.

Outcomes

Expected Outcome 1:

Science: Knowledge of the potential of cotton to become naturally infected with TSV and the likely impact.

Industry/Applied: Growers will gain knowledge on the likely impacts of TSV on cotton production in Queensland.

Cotton was shown to be susceptible to TSV infection under field conditions in Central Queensland. Surveys were conducted in the irrigated areas around Emerald. The incidence in 2007/2008 was generally low, around 1%, in the commercial crops surveyed. High disease incidence (ca 70%) and more severe symptoms were seen in volunteer cotton growing next to a high density of TSV infected parthenium plants.

While most infected cotton plants showed infection on the inoculated leaves only, a small proportion showed systemic symptoms. The reason for the presence of systemic symptoms is not known, but may be related to higher inoculum pressures caused by proximity to high densities of infected parthenium and high thrips populations. Systemic infection, with its more significant yield impacts, is reported overseas and requires further investigation.

Expected Outcome 2:

Science: Determine if selected Australian cotton varieties and lines are susceptible/resistant to TSV.

Industry/Applied: Potential management strategy for TSV in cotton identified.

All Australian cotton lines tested in glasshouse trials were susceptible to TSV. In most cases only local infection was present, but in 3/13 of the lines at least some of the plants displayed systemic symptoms, suggesting that under high inoculum pressure that systemic infection and yield losses may occur. As with sunflower and grain crops in Central Queensland, it appears that the control of TSV-infected parthenium may be a key factor in limiting the impact of TSV in cotton.

Conclusion

Cotton has been shown to be a field host of TSV in the Emerald cotton production area. Most infections observed were mild and unlikely to cause any losses so these observations suggest there is no current crisis for industry. However, occasional severe systemic infections were noted, and the reason(s) for their development are not known. Also, the results to date are based on observations from a single, unusually wet, season and may not be typical of more "normal" growing seasons. The surveys were conducted in irrigated crops around Emerald, and it is likely that infection rates and severity would be higher when crops were grown in the dryland areas north of Clermont where there is known to be large areas of high density parthenium infected with TSV.

At this stage no effective resistance has been identified from a number of cotton lines so control of alternative hosts in and around crops is likely to be the most effective control for TSV disease. Parthenium is suspected to be the key alternative host of TSV in central Queensland and results from this study have shown that TSV infected parthenium is present at several locations within the Emerald cotton production area.

Crop hygiene with effective control of flowering parthenium in and around crops (particularly when crops are young) is likely to be a key control method to minimise the risk of TSV entering crops. Growers and consultants are encouraged to maintain farm hygiene and be on the look out for the described symptoms.

This project has revealed the need for further work on TSV in cotton, to extend existing work and address some new areas. These include:

- Conduct further surveillance to determine the impact of TSV in "normal" seasons,
- Conduct surveys in NSW and Queensland, including dryland cotton in areas with high levels of parthenium,
- Assess yield-loss in cotton due to TSV, and factors that lead to systemic infection,
- Assess thrips vector species present in cotton,
- Investigate alternative weed and crop hosts,
- Study epidemiological aspects in relation to thrips and weed hosts,
- Provide extension material on the impact and management of TSV in cotton.

Extension Opportunities

A new section about TSV has been prepared and added to the updated Cotton Integrated Disease Management Guide.

A disease note reporting the first record of TSV infecting cotton and other crops in Australia has been published in Australasian Plant Disease Notes as follows:
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.

A disease note for TSV in cotton has been published online as part of the Cotton Catchment Communities CRC website at:
http://www.cottoncrc.org.au/content/Industry/Publications/DiseaseMicrobiology/Tobacco_streak_virus_in_cotton_in_Central_QLD.aspx

Part 4 – Final Report Executive Summary

This project aimed to examine the possible impact of tobacco streak virus (TSV) on the Australian cotton industry. TSV is transmitted by thrips and causes a newly emerging disease which has had a significant impact on grain crops, especially sunflower and mungbean, in Central Queensland since ca. 2004.

This one year scoping study has established that cotton is susceptible to field infection with TSV within the Emerald cotton production area, with infected plants being found at several locations. Results from field surveys indicated that TSV did not cause significant disease or losses in CQ cotton in the 2007/2008 season, with most field crops inspected having less than 1% of plants affected by mild symptoms, often only consisting of single, diffuse necrotic lesions on one leaf of infected plants.

More severe symptoms were occasionally observed in field infected plants which included dark purple necrotic, spreading lesions on leaves, sometimes forming numerous diffuse ring spots. On plants with numerous necrotic lesions the upper leaves sometimes also displayed chlorotic mottle and deformed, down-curled leaves. Higher levels of infection were only observed in volunteer cotton plants near parthenium infestations.

A total of 13 cotton varieties/lines have been screened for resistance/susceptibility to TSV in glasshouse tests. Results indicate that all lines are susceptible to TSV, with all displaying mild symptoms on inoculated leaves. However, almost all lines tested had apparently healthy growth after the initial localised symptoms.

Parthenium is suspected to be the key alternative host of TSV in central Queensland and results from this study have shown that TSV infected parthenium is present at several

locations throughout the Emerald cotton production area. Crop hygiene with effective control of flowering parthenium in and around crops (particularly when crops are young) is likely to be a key control method to minimise the risk of TSV entering crops.

While results from this scoping study indicate that TSV may not cause significant disease or losses in central Queensland cotton crops, the study was conducted during an unseasonally wet cropping cycle and many questions remain unanswered about what factors cause systemic infection, what insect vectors are involved in transmission and the extent of disease damage during a “normal” season.