Final report - SRDC project BSS243: Chlorotic streak disease of sugarcane

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FINAL REPORT – SRDC PROJECT BSS243
Chlorotic streak disease of sugarcane
by
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SD02014

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# TABLE OF CONTENTS

Page No.

## SUMMARY

1.0 INTRODUCTION ................................................................................................................... 1  
  1.1 The disease .................................................................................................................... 1  
  1.2 Industry incidence ......................................................................................................... 1  
  1.3 Unknowns ...................................................................................................................... 1  
  1.4 Research objectives ..................................................................................................... 2

2.0 METHODS .......................................................................................................................... 2  
  2.1 Resistance screening trials .......................................................................................... 2  
  2.2 Surveys ......................................................................................................................... 3  
  2.3 Yield loss trials ............................................................................................................. 3  
  2.4 Transmission trials ...................................................................................................... 4  
  2.5 PhD project .................................................................................................................. 5

3.0 RESULTS ............................................................................................................................ 6  
  3.1 Resistance screening trials ......................................................................................... 6  
  3.2 Surveys ........................................................................................................................ 6  
    3.2.1 Mill area inspections ......................................................................................... 6  
    3.2.2 Varieties ............................................................................................................. 7  
    3.2.3 Crop class .......................................................................................................... 8  
    3.2.4 Water source .................................................................................................... 9  
    3.2.5 Severity of disease ......................................................................................... 11  
  3.3 Yield loss trials .............................................................................................................. 11  
    3.3.1 Tully .................................................................................................................. 11  
    3.3.2 Herbert ............................................................................................................. 16  
  3.4 Transmission trials ....................................................................................................... 20  
  3.5 PhD project .................................................................................................................. 20

4.0 DISCUSSION ...................................................................................................................... 22

5.0 CONCLUSIONS ............................................................................................................... 24

6.0 ACKNOWLEDGMENTS ................................................................................................... 25

7.0 REFERENCES .................................................................................................................. 25

APPENDIX 1 Resistance ratings of clones included in the Tully and Burdekin resistance screening trials

APPENDIX 2 2002 ASSCT paper 'Chlorotic streak, a disease reducing sugarcane yields in Queensland'
SUMMARY

Chlorotic streak is a disease recognised since 1929 and is widespread through the cane growing countries of the world. Despite considerable research, particularly in the 1940-1970 period, the causal agent of the disease is still unknown. The disease is systemic and affects germination and crop yield. It is widespread through Queensland being favoured by high rainfall and/or poor drainage. The worst affected areas are the high rainfall wet tropics and poorly drained areas in other districts. This three-year project was initiated to gain a better understanding of the effects of the disease on yield; to screen commercial varieties for resistance; to research the distribution in parts of the Burdekin River Irrigation Area (BRIA); and to attempt to identify the causal agent.

Yield loss trials were planted in the Tully and Herbert districts in 2000. Varieties ranging from resistant to susceptible were included to determine the relationship between resistance and yield loss. Plant crop harvests in 2001 revealed losses of up to 60% when crops planted with stalks showing diseased symptoms were compared with crops established with stalks exhibiting no symptoms. Yield effects therefore can be dramatic with the disease. Even some resistant canes suffered significant losses, suggesting farmers should obtain the cleanest planting material they can to establish new crops. One resistant variety (Q162) suffered negligible losses. Germination was slowed, and reduced overall, by chlorotic streak disease (CSD) with the main effect being on tonnes cane/ha rather than ccs (sugar content was largely unaffected by CSD). There was a significant relationship between varietal resistance and yield (r-squared 0.56) in the Herbert trial suggesting there is a correlation between resistance and yield.

Surveys of the Invicta, Pioneer, Kalamia and Inkerman mill areas revealed the presence of a greater level of CSD than known previously. The greatest level of disease was found in Q127 though other varieties were also diseased. There was a link between irrigation water source and the area affected by the disease. Where channel water was used, higher disease levels resulted compared to fields irrigated with bore water. This is not surprising because drainage water from infested fields is known to carry the causal agent and channel water contains some drainage water.

Transmission studies using hydroponics were commenced to provide suitable root material for molecular assay research into the causal agent. However, no disease transmission occurred and it is postulated that a soil-borne vector may be needed to aid transmission.

PhD studies at the University of Adelaide have focused on light and electron microscopy, and molecular methods of pathogen detection. This project finishes in 2003 but so far no agents have been associated with diseased material.
1.0 INTRODUCTION

Chlorotic streak is a disease widely distributed in the Queensland sugar industry affecting tens of thousands of hectares of sugarcane each year. Though known since the late 1920s, the causal agent has still not been elucidated. This has made the normally straightforward task of ensuring plant sources are disease free an uncertain task. Losses caused by the disease have been investigated in part but the relationship between varietal resistance and yield has not. Questions have arisen regarding disease distribution; the relationship between irrigation water sources and crop disease levels; and the resistance of current commercial varieties. All these issues were researched in the SRDC-funded project titled ‘Chlorotic streak disease of sugarcane’, a three-year project funded in the CP2002 program.

1.1 The disease

Considerable research into chlorotic streak disease (CSD) has been undertaken since the disease was recognised. Findings include the following:

- CSD spreads in irrigation water, flood water and planting material;
- favoured by poor drainage;
- no obvious disease agent associated in stalks, leaves or roots;
- resistance is present in the Australian germplasm;
- losses up to 40% may occur in susceptible canes;
- transmission can occur in hydroponic systems;
- causal agent can pass through a 0.5 micron filter;
- low temperature ‘hot water’ stalk treatments can eliminate the agent;
- the disease spread south through Queensland in the 1930–1960 period.

1.2 Industry incidence

The worst affected areas of Queensland are the wet tropics, and poorly-drained areas further south including the Herbert, some parts of the Burdekin, central district and the Rocky Point mill area. The greatest incidence of the disease occurs in high rainfall years; in these years the disease may be found in most blocks of cane in areas such as Tully and Babinda.

1.3 Unknowns

Unknown aspects of the disease include:

- yield losses caused by the disease, particularly the relationship between resistance and yield;
- the identity of the causal agent;
- disease distribution – particularly in new areas in the Burdekin River Irrigation Area (BRIA);
- relationship between irrigation water source and disease levels;
- resistance of current commercial canes.
1.4 Research objectives

The specific objectives of the project were to:

1. determine the yield losses resulting from chlorotic streak in the Burdekin and Herbert district;
2. ensure chlorotic streak resistant varieties are grown in situations where significant yield losses occur;
3. identify the causal agent of chlorotic streak and so enable better controls to be applied at minimal cost to industry, through better knowledge of disease incidence.

The project started in July 1999 and concluded in June 2002. Funding allowed for the appointment of a research assistant (Wendy Neilsen). She was initially located in the Burdekin but transferred to Tully Sugar Experiment Station in January 2001, where the majority of the research work was then occurring.

2.0 METHODS

2.1 Resistance screening trials

Resistance screening trials were initiated in 1999 in two locations to compare ratings between sites and the speed of disease development at each location. Previous BSES research had identified suitable standard varieties, that is a group of commercial canes with known disease reactions ranging from resistant to highly susceptible. These canes were propagated along with a number of promising clones from the BSES breeding program; a list of these clones is included in Appendix 1. Previously, data from three resistance trials, planted in the early days of CSD research in Babinda in the 1930s, were analysed. These trials included such canes as Q2, Q4 and Q12; trial design incorporated infection rows - diseased cane planted every third row with test canes planted between. There was significant disease transmission into the test canes and trial analyses allowed resistance ratings to be assigned. It was apparent the trial design used was effective in screening for resistance. The same design was employed in these trials.

Trial design:

- two replicates;
- one row by 5 metre plots;
- disease inspections made at regular intervals in plant and first ratoon crops on a per cent diseased-stalk basis. The finding of any CSD-specific symptoms was the basis for a disease record.

Trials were planted on the farm of Chris Hesp, Mulgrave (Burdekin) and on Tully Sugar Experiment Station, Tully. The Burdekin trial relied on disease transmission from the diseased infection rows and the possibility of infection from the recycled tail water used in irrigating the crop. The Tully trial also relied on spread from the infection rows but had the added advantage of high disease levels in the site where the trial was planted (soil infection) and transmission from flood waters, the site normally being inundated during the wet season.
2.2 Surveys

The widespread finding of CSD in the promising Q127 in the Burdekin alarmed the Burdekin industry in the late 1990s. The extent of disease occurrence in the Burdekin was an issue requiring investigation – so a detailed survey of the Burdekin was a part of the project. Factors considered included the following:

- **Irrigation sources**: particularly channel (which includes drainage water from irrigated fields) versus bore water.
- **Varieties**: the major commercial canes of the area were included (Q96, Q117, Q124, Q127, Q133, Q165A and several recently released varieties).
- **Mill areas**: 125 farms were to be surveyed in the ratio of 60:40:25, Invicta:Ayr:Inkerman, respectively.

Individual blocks were surveyed by randomly choosing rows and inspecting cane as inspectors walked along the drill. The blocks were chosen by variety (preferably Q127) with a second block of another variety inspected on that farm, and then by crop height (crops up to knee height).

The survey was conducted in October-December 2000. Disease was recorded on a block basis with the severity of the disease in each block assessed subjectively on leaf symptoms. This provided incidence records of the percentage of blocks and the percentage of farms diseased with notes on severity. The local Cane Productivity and Protection Boards assisted with these surveys.

2.3 Yield loss trials

Previous BSES research had investigated CSD-associated yield losses when diseased and healthy plots of only a couple of varieties were planted side by side. Losses of up to 40% were reported by Egan (1962). In other studies, Neilsen, Kaupilla and Roach (1986) investigated the average difference in yield between matched plots of disease-free and diseased cane of the variety Triton in the Herbert District. They estimated losses at 0.24% yield loss for each 1% stalks showing disease symptoms. Their figures therefore provided some basis for estimating the effect of variable disease severity on yield in a single variety.

In the trials reported here, the objective was to examine the relationship between varietal resistance and losses. Accordingly, the trial design included 6-8 varieties of varying resistance to the disease. Nursery material was located (either on farm or in approved seed plots) either showing or not showing disease symptoms. As no assay yet exists for CSD, leaf symptoms are the only basis for determining plant disease status. Individual stalks exhibiting leaf symptoms in diseased plots were tagged and cut for planting in designated diseased plots (for each variety). Symptomless stalks from hot water treated nursery plots were also obtained and used to plant designated disease-free plots.

Yields were assessed only in the plant crop due to anticipated disease re-infection of disease-free plots.
**Herbert**

The Herbert trial was planted on the Herbert Sugar Experiment Station near Ingham. Six varieties were included (Q135, Q157, Q158, Q162, Q179A and Q194A) in a four-replicate randomised complete block design. The trial was planted on 14 July 2000. Disease levels (percentage of stalks showing symptoms) in all plots were monitored regularly and at harvest the yield (tonnes cane/ha; tonnes sugar/ha) and ccs were recorded. A BSES weigh truck was used in conjunction with a commercial harvester to gain yield data.

**Tully**

The Tully trial was located on land owned by Tully Sugar Ltd in the Syndicate district. Eight varieties were included (Q115, Q117, Q120, Q127, Q135, Q138, Q152 and Q172A) in a three-replicate randomised complete block design trial. The trial was planted on 1 September 2000 and harvested using the same methods as for the Herbert trial. Further details for both trials are included in a published ASSCT paper included in Appendix 2.

### 2.4 Transmission trials

Transmission research was investigated to assist with etiology studies conducted during the PhD research project. The aims of the work were to:

- transmit the causal agent from diseased to previously healthy cane; this would provide material for molecular studies guaranteed to be free from potential contaminating DNA/RNA (which would confuse causal agent identification studies);
- provide other types of material (contaminated hydroponic solution etc) which could be probed for the presence of a suspected causal agent;
- provide a regular supply of diseased and healthy root systems for detection research; roots are considered a potential rich source of the causal agent, given the nature of the disease.

To attempt transmission, a pearlite-based hydroponic system was established in a temperature-controlled glasshouse on Tully Sugar Experiment Station. Square plastic containers (approximately one litre volume) were placed on low benches and connected to an air supply to ensure solutions were adequately aerated. Hydroponic solution was added as needed during the growth of test plants and changed as necessary. The pH of the solutions required regular adjustment to avoid mineral deficiencies (particularly iron). Shoots were ratooned when the size of test plants became excessive. The variety Q115 was used throughout the experiments because this cane is susceptible and shows good disease symptoms. Two experiments were undertaken with the following treatments:

**Experiment 1**

- Healthy control
- Disease control
- Healthy plus diseased plants grown side-by-side.
Experiment 2

- Healthy control
- Disease control
- Mixture of diseased and healthy plants
- Mixture of healthy and diseased plants plus soil from diseased field.

2.5 PhD project

When the project began, contact was made with The University of Adelaide and specifically Dr John Randles. Dr Randles has expertise with the identification of unusual pathogens associated with various crop diseases. A PhD student was sought to work on CSD; Kylie Rogers (now Cook) was selected to undertake research on the casual agent of CSD. She began her PhD studies in February 2000. These are not yet complete (due for completion sometime in 2003) and have followed the following aims and lines of activity.

- Aims of the project are to:
  - use nucleic acid detection, isolation, cloning and sequencing techniques to determine the nature of the agent causing CSD of sugarcane in Australia; and
  - develop a diagnostic procedure to detect disease incidence in the field.

- Activities are:
  - visual observation of causal agent: this includes both electron and light microscopy of various diseased and healthy plant sections;
  - molecular detection of potential causal agents including examining material for ds-RNA, an indicator of the presence of viruses.

An initial visit was made to Tully and the Burdekin by both Kylie Cook and Dr Randles to familiarise themselves with the disease and to obtain fresh material for molecular analyses. Transmission research was initiated in Adelaide to provide fresh material for research.

From 9-13 July 2001, Kylie Cook and Dr Randles again visited Tully Sugar Experiment Station to:

- extract total nucleic acid and dsRNA from fresh root tissue and to demonstrate these techniques to BSES collaborators;
- collect tissue suitable for light and electron microscopy;
- review the glasshouse hydroponics transmission experiments and suggest changes to experimental procedure.
3.0 RESULTS

3.1 Resistance screening trials

Inspections made during the growth of the plant and first ratoon crops of the trials showed that the Tully site, with a previous history of CSD screening trials, had significantly higher levels of disease than the Burdekin grower site. Higher infection of test canes at the Tully site resulted from higher levels of soil infestation by the causal agent and increased transmission resulting from several flooding events. Even though the grower site in the Burdekin incorporated the irrigation of recycled tail water collected from infested fields, transmission was at a lower level. The data are illustrated in Figure 1.

![Graph showing % Stalks Infected vs Number of clones for Burdekin and Tully]

Figure 1: A comparison of the disease levels in clones in the Burdekin and Tully resistance screening trials.

A list of the resistance ratings for the varieties is included in Appendix 1.

3.2 Surveys

3.2.1 Mill area inspections

Figure 2 indicates the proportion of the area inspected by mill area – the main focus was the Invicta area because concern was expressed at increased levels of disease in the Burdekin River Irrigation Area (BRIA), which falls largely within this mill district.
It was found that 52% (86/165) of blocks and 57% (60/104) of farms were infested by chlorotic streak in the Burdekin.

**Disease by mill area**

<table>
<thead>
<tr>
<th>Mill area</th>
<th>Farms % infested</th>
<th>Blocks % infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invicta</td>
<td>75</td>
<td>61</td>
</tr>
<tr>
<td>Pioneer/Kalamia</td>
<td>32</td>
<td>27</td>
</tr>
<tr>
<td>Inkerman</td>
<td>25</td>
<td>24</td>
</tr>
</tbody>
</table>

### 3.2.2 Varieties

Figure 3 indicates which varieties were surveyed; because there was a focus on Q127, and because Q117 is widely grown, these two varieties were strongly represented in the survey.
The results of the survey for disease levels by variety are shown in Tables 1 and 2. The other varieties exhibited disease levels of less than 1%.

**Table 1: % area disease by variety**

<table>
<thead>
<tr>
<th>Variety</th>
<th>% area diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q127</td>
<td>24.9</td>
</tr>
<tr>
<td>Q117</td>
<td>9.7</td>
</tr>
<tr>
<td>Q96</td>
<td>4.2</td>
</tr>
<tr>
<td>Q124</td>
<td>3.6</td>
</tr>
<tr>
<td>Q165a</td>
<td>3.6</td>
</tr>
<tr>
<td>Q177a</td>
<td>1.8</td>
</tr>
</tbody>
</table>

**Table 2: Disease by varieties by mill area.**

<table>
<thead>
<tr>
<th>Mill area</th>
<th>Variety</th>
<th>% area infested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invicta</td>
<td>Q127</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>Q117</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Q96</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>Q165a</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Q124</td>
<td>3</td>
</tr>
<tr>
<td>Pioneer/Kalamia</td>
<td>Q127</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>Q124</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Q117</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Q165a</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Q196a</td>
<td>0</td>
</tr>
<tr>
<td>Inkerman</td>
<td>Q127</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Q124</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Q133</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Q96</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>Q117</td>
<td>7</td>
</tr>
</tbody>
</table>

The results suggest Q127 consistently shows more disease than the other varieties - which could be expected from its higher resistance rating (that is, its greater susceptibility to the disease).

### 3.2.3 Crop class

Figure 4 shows the breakdown of ratoon category included in the survey.
Figure 4: Crop class of crops surveyed in the Burdekin CSD survey.

Disease levels in each ratoon category are shown in Table 3.

Table 3: Disease levels in each ratoon category.

<table>
<thead>
<tr>
<th>Ratoon category</th>
<th>% area diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>First</td>
<td>10.9</td>
</tr>
<tr>
<td>Second</td>
<td>13.9</td>
</tr>
<tr>
<td>Third</td>
<td>14.6</td>
</tr>
<tr>
<td>Fourth</td>
<td>7.9</td>
</tr>
<tr>
<td>Fifth</td>
<td>2.4</td>
</tr>
<tr>
<td>Sixth</td>
<td>2.4</td>
</tr>
</tbody>
</table>

As expected, there was an increase in disease level from first to third ratoon; this is likely to result from the increased opportunity for infestation in older crops. The drop off in disease level in older (>3R) ratoons has also been seen with other diseases such as RSD, where farmers select their poorest (diseased) crops for plough-out leaving the non-diseased crops – this results in reduced disease levels in older crops.

3.2.4 Water source

Figure 5 shows where the water was sourced for the blocks surveyed. The majority used channel water, this is expected as much of the survey was conducted in the BRIA.
Figure 5: Water source (% farms in each category) for farms surveyed.

The results from disease inspections indicated the proportion of blocks diseased in each irrigation system (Table 4).

Table 4: Proportion of diseased blocks in each irrigation system.

<table>
<thead>
<tr>
<th>Irrigation method</th>
<th>% blocks diseased</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bore</td>
<td>4.3</td>
</tr>
<tr>
<td>Mixed</td>
<td>15.4</td>
</tr>
<tr>
<td>Channel</td>
<td>33.3</td>
</tr>
</tbody>
</table>

It was an aim of this survey to see if there was a link between the presence of CSD and the type of irrigation water used; open (channel) water (compared to bore water) appears to be linked to higher levels of disease. These results were expected given that channel water includes drainage water from infested cane lands. As the causal agent is known to spread in drainage water, higher levels are a natural result. However, it should be noted that on some properties using only channel water there was no CSD, while on some properties using only bore water the disease was present.
3.2.5 Severity of disease

Figure 6 shows the level of CSD severity in the areas surveyed. Most blocks were only lightly to moderately affected.

CSD was found to be widespread in the BRIA with a relatively high incidence in the Inkerman, Pioneer and Kalamia mill areas. Some disease was found on delta soils but at substantially lower levels than on the duplex, poorer draining soils of the BRIA.

3.3 Yield loss trials

Yield loss trials showed the dramatic effect CSD can have on cane yields. Unfortunately, the Tully trial was somewhat compromised by low yields, probably a result of the wet conditions and low yields generally experienced in the district in the year of harvest. Even so, large losses were recorded at this site. Figures 8-12 show losses in tonnes cane/ha, ccs and tonnes sugar/ha in example varieties at each site. Further detail is provided in Magarey and Neilsen (2002) which includes many more graphs.

3.3.1 Tully

Shoot and stalk counts

Counts for Q115 and Q127 are detailed in Figure 7. Diseased planting material generally resulted in slower germination with lower overall shoot numbers.
Disease incidence

CSD symptoms were seen very soon after germination and, as expected, disease incidence was considerably higher in plots planted with diseased material. It seemed that little transmission into healthy plots occurred. The resistant cane, Q172, and the intermediate variety, Q152, showed lower disease incidence while susceptible varieties exhibited relatively high levels of disease (Figure 8).
Figure 8: Disease levels in plots planted with diseased material (top graph) and healthy (bottom graph) in the Tully yield loss trial.
**Yield losses**

Yield losses caused by CSD were substantial in most varieties. For tonnes sugar/ha, maximum loss was 41% in Q172. Average losses were 15.5%, although these were not significant at the 5% level. However, there was a significant disease status x variety interaction. The varieties of intermediate resistance, Q138 and Q152, unexpectedly had higher yields in diseased compared to healthy plots; this may have resulted from a plant source effect. For tonnes cane/ha, disease status and disease status x variety interaction were both significant at the 5% level. Representative data are presented in Figure 9. Losses were mostly associated with reduced weight rather than ccs - there was no significant effect of CSD on ccs. There was no correlation between resistance rating and yield ($r^2=0.03$) in this trial.
Figure 9: Ccs, cane t/ha and sugar t/ha data for the Tully yield loss trial. LSD (5% level) for comparison of variety means is 1.18 for sugar t/ha.
3.3.2 Herbert

*Shoot counts*

Results were similar to those obtained in Tully with slower germination and reduced shoot numbers in diseased plots (Figure 10).

![Figure 10: Shoot/stalk counts per 40 metre of row for healthy (-----) and diseased (-----) plots of varieties in the Herbert yield loss trial over the period 4 September 2000 to 27 April 2001.](attachment://figure10.png)
Disease incidence

High levels of disease were seen in intermediate and susceptible canes, particularly Q179, with lower incidence in the resistant varieties Q152 and Q157 (Figure 11).

Figure 11: Disease levels in plots planted with diseased material (top graph) and healthy (bottom graph) in the Herbert yield loss trial.
Yield losses

Yield losses were even more substantial in canes in the Herbert trial (Figure 12), with tonnes sugar/ha losses varying from 5 to 62%; the average being 40%. The least affected variety was Q162 while Q135 suffered a yield loss of 62%. The disease status of the cane had a highly significant effect (5% level) on both tonnes cane/ha and tonnes sugar/ha in the Herbert trial, though there was no significant interaction between disease status and variety. Losses were mainly associated with reduced weight of cane per plot; there was no significant effect of CSD on ccs. However, there was a significant correlation between resistance rating and yield ($r^2=0.56$), (Figure 13).
Figure 12: Ccs, cane t/ha and sugar t/ha data for the Herbert yield loss trial. LSD (5% level) for comparison of varieties for sugar t/ha means is 2.64.
3.4 Transmission trials

There has been no evidence of disease transmission in the course of the trials conducted at Tully Sugar Experiment Station, even when soil from a diseased cane field was added to the hydroponic solution.

3.5 PhD project

To fulfill program requirements, a CSD literature review and a research proposal were submitted to Graduate Studies by Kylie Cook.

Transmission experiments

Populations of diseased, heat-treated, and healthy sugarcane, including three different cultivars with varying susceptibilities to CSD, have been established in the Plant Research Centre, Waite Campus. The details are included in Table 5. Numbers in parentheses refer to plants involved in transmission experiments.

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Diseased</th>
<th>Heat-treated</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q115</td>
<td>22 (+4)</td>
<td>21 (+10)</td>
<td></td>
</tr>
<tr>
<td>Q122</td>
<td>16</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Q138</td>
<td>12</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Seedlings</td>
<td></td>
<td></td>
<td>17</td>
</tr>
</tbody>
</table>

Table 5: Number of pots in transmission experiments.
Each pot contains either two or three sugarcane plants. Heat-treated plants differ from the diseased plants only in that the stalk pieces were treated in hot water at 50°C for 30 minutes. This cures the plant of any CSD symptoms but the pathogen may still be present, hence the need for seedlings. The seedlings have had no contact with the disease and are being used as a negative control.

Two small-scale transmission experiments are in progress to verify the transmissibility of the pathogen. Both experiments were commenced in October 2001 and use plants from the susceptible cultivar Q115. In the first experiment, two replicates of one CSD and one heat-treated plant were replanted together in a larger pot, retaining as much of the diseased soil as possible. A heat-treated plant was replanted into a larger pot as a negative control. In the second experiment, two replicates of one CSD and two heat-treated plants were retained in their separate pots but placed in a water-filled tray so that there was a common water source and the roots were able to be in contact. A negative control comprises three heat-treated plants under the same conditions. Both experiments use tap water as the water source and are watered daily. All plants were ratooned in April 2002. No heat-treated plants have as yet displayed symptoms of CSD.

*Nucleic acid analysis*

Total nucleic acid and double-stranded RNA (dsRNA) preparations have been extracted from leaf and root tissue from both diseased and healthy plants. Various extraction buffers have been used to determine optimum isolation techniques. Currently, the dsRNA isolation procedure outlined in Choi and Randles (1997) is being utilised. Nucleic acid extraction is followed by polyacrylamide gel electrophoresis (PAGE) to separate fragments according to size and conformation.

Attempts have been made to isolate virus particles from leaf tissue using high-speed centrifugation. No differences were detected between CSD, heat-treated, and healthy leaf tissue samples.

*Microscopy*

Light microscopy of thin sections of leaf tissue from one CSD and one heat-treated plant showed differences between samples in the stainability of chloroplasts, and also indicated the presence of dark bodies in the CSD sample, potentially verifying the observations made by Abbott and Sass (1945).

Transmission electron microscopy (TEM) of negatively-stained polyethylene glycol (PEG) precipitates of CSD and heat-treated root samples suggested the association of isometric particles of 30 nm in diameter in the CSD samples, but the preparations were too crude to verify whether these were virus particles. TEM of ultra-thin sections of CSD and heat-treated leaf tissue revealed marked differences in the size and number of both bundle-sheath and mesophyll chloroplasts between yellow tissue from a CSD-affected leaf, green tissue from the same leaf, and green tissue from a heat-treated leaf. No potential pathogens were observed.
**Publication**

In September 2001, the student presented a poster of her research at 13th Australasian Plant Pathology Society Conference.

**Plans for research**

The following research goals have been planned for the year 2002.

- Light microscopy of thin sections of leaf and root tissue to verify intracellular changes due to disease.
- TEM of water used in glasshouse transmission experiments to identify a possible pathogen.
- Fractionate and compare nucleic acid profiles from normal and affected sugarcane plants to determine disease association of dsRNA.
- Isolate disease specific bands.
- Use random PCR and other amplification and cloning techniques to amplify unique segments from disease specific nucleic acids.
- Prepare probes.

**4.0 DISCUSSION**

The project research has fulfilled almost all the stated objectives, the one outstanding being the identification of the causal agent, the main focus of the associated PhD project. As the PhD studies are not yet complete, there is still hope that some information on the causal agent may be obtained.

The Tully Sugar Experiment Station site has proved to be the best site for screening for CSD resistance. It is hoped that with time, more rapid disease development may occur so that resistance data can be calculated using plant crop data alone. At the moment there is a need to wait for first ratoon data in order for disease levels to be high enough to assess disease resistance. There is also a need to do further research into the relationship between trial data and field reaction. In this regard, the results from the Herbert yield loss trials provide some confirmation of the value of the trial resistance ratings. A further resistance screening trial has been planted on Tully SES and results will be distributed to industry in due course.

The survey of the Burdekin area provided a good understanding of the extent of CSD in that area. There was more CSD in the Pioneer, Kalamia and Inkerman mill areas than previously known and the survey has highlighted to the local industry the need to put in place further CSD control strategies. It was previously thought that channel water may increase disease levels in irrigated fields and the survey provides the data to confirm this proposition. Recycling of tail water is an ongoing issue for irrigators in the area and care will need to be taken by farmers using this strategy if the disease is to be kept under control. Further research may be needed into how farmers may increase the efficiency of irrigation practices while at the same time achieving satisfactory CSD control.
There is probably little a cane farmer can do to prevent disease incidence in fields irrigated by channel water besides the normal recommendations of maintaining adequate slopes in cane fields, avoiding poorly drained areas, and using disease-free planting material. Further research is required into the optimum slope of cane fields to minimise disease incidence.

Resistance screening data suggest Q127 is susceptible to the disease and the survey showed that more disease was present in this variety in Burdekin fields. The variety exhibits severe disease symptoms early in the life of the crop with symptoms fading to some extent later. This again highlights the need for an assay to determine plant disease status before establishing new crops.

Yield loss studies provide some interesting information on the effect of the disease on shoot numbers during crop development and on various yield components.

The data obtained on the growth of healthy and diseased crops confirm previous research by Egan (1962) that CSD reduces germination speed. The disease reduced stalk number in the mature crop and significantly reduced crop yield (tonnes cane/ha and tonnes sugar/ha). CSD had no significant effect on ccs. This has been seen previously (Egan, 1962).

Large losses in tonnes sugar/ha were recorded and these were higher than those attributed to the disease by Egan (1962) or Neilsen et al. (1986). This was probably due to the increased susceptibility of the varieties used in these experiments or the higher level of CSD in diseased plots.

Losses were not always directly proportional to assigned varietal resistance ratings - though the trend was for highly susceptible varieties to suffer larger losses. This was evidenced in the Herbert experiment where an r-squared value of 0.56 was recorded between assigned rating and percent losses. Losses in resistant and intermediate varieties varied - in some cases they were very high, for instance with Q157 and Q158 (Herbert), though not all the time (Q162). Large losses have also been noted on occasions in commercial crops of relatively resistant varieties. Resistance ratings are based on the transmission of the disease into previously healthy test plots; differences between resistance rating and expected yield losses are determined by varietal tolerance, that is the ability of the variety to yield well when diseased. This will vary with variety and a lack of tolerance to CSD could explain larger losses in some of the more resistant canes. This, coupled with the magnitude of the losses in susceptible varieties, emphasises the need for cane farmers to use best management practices for control of CSD at all times and not to rely solely on varietal resistance.

There were several cases where the diseased cane out-yielded the healthy cane. The reason for this is unknown, but the sourcing of healthy and diseased plants from different localities could have contributed to the growth differences.

The technique used in this study, planting diseased and healthy material of a restricted number of varieties varying in resistance to the disease, was useful. However, the conclusions drawn from the resistance-yield loss relationship were limited because of the small subset of varieties included. The work could be progressed further using the
techniques employed by Magarey et al. (2002) where core trials in the plant improvement program were accessed and yield loss data obtained using more than 80 clones (the studies reported did not include CSD).

These data do provide some relevant up-to-date information for growers on the performance of current varieties affected by chlorotic streak. They also provide the first limited data set on the effect of resistance on yield losses.

The negative result from transmission studies is a cause for concern. Discussions between the PhD student, her supervisor and Tully staff raise the possibility of the need for a soil-borne vector for the disease. It is possible earlier transmission experiments unknowingly incorporated soil-borne vector(s) and an organism could have been involved in the infection of healthy test plants. Further work is needed in this area and this hypothesis does not completely explain why no transmission occurred when some soil was added to hydroponic solutions.

The PhD studies have followed a line that has worked for diseases in sugarcane and other crops. The association of a virus with sugarcane striate mosaic by a student supervised by Dr Randles highlights the ability of the techniques to detect unknown causal agents. The reason for the lack of success so far with CSD is unknown. There is a possibility the causal agent is very unusual and requires other strategies for its elucidation. A report will be written by the student outlining possible strategies for further work at the end of her project.

5.0 CONCLUSIONS

1. More chlorotic streak was found in Pioneer, Kalamia and Inkerman mill areas than known previously. There is a need for the application of appropriate controls in these areas.
2. Resistance data are now available for a number of promising clones in the plant breeding program. Further research relating trial data to the field are needed.
3. Higher disease levels are associated with the use of channel water as compared to bore water. Farmers should take appropriate action to minimise disease buildup when using channel water.
4. Yield losses were high in susceptible canes in trials in Tully and the Burdekin; losses of up to 60% were evident in several canes.
5. There was a relationship between yield losses and resistance in the Herbert yield loss trial, suggesting resistance ratings provide a guide to the losses expected.
6. There was a failure to transmit chlorotic streak in hydroponic experiments; the reason is unclear but could involve the need for a soil-borne vector of the causal agent.
7. PhD research has so far failed to identify a pathogen associated with chlorotic streak.
6.0 ACKNOWLEDGMENTS

The authors would like to thank SRDC for partially funding the research.

7.0 REFERENCES


APPENDIX 1

Resistance ratings of clones included in the Tully and Burdekin resistance screening trials

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<th>Clone</th>
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<td>Q178^</td>
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</table>

*Note: The ratings range from 1 to 9, with higher numbers indicating higher resistance.*
APPENDIX 2

CHLOROTIC STREAK, A DISEASE REDUCING SUGARCANE YIELDS IN QUEENSLAND

By

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