BSES Limited

FINAL REPORT - SRDC PROJECT BSS255

IMPROVING THE PLANT BREEDING SELECTION SYSTEM FOR FIJI DISEASE RESISTANCE

by

BJ CROFT, AW RIDLEY, K DHILEEPAN and AP JAMES

SD05014

Contact:
Barry Croft
Principal Research Officer
BSES Limited
90 Old Cove Rd
Woodford Q 4514
Telephone: 07 54963357
Facsimile: 07 54963266
Email: bcroft@bses.org.au

BSES is not a partner, joint venturer, employee or agent of SRDC and has no authority to legally bind SRDC, in any publication of substantive details or results of this Project.

BSES Limited Publication
SRDC Project Final Report SD05014 **August 2005**

Copyright © 2005 by BSES Limited

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior permission of BSES Limited.

Warning: Our tests, inspections and recommendations should not be relied on without further, independent inquiries. They may not be accurate, complete or applicable for your particular needs for many reasons, including (for example) BSES Limited being unaware of other matters relevant to individual crops, the analysis of unrepresentative samples or the influence of environmental, managerial or other factors on production.

Disclaimer: Except as required by law and only to the extent so required, none of BSES Limited, its directors, officers or agents makes any representation or warranty, express or implied, as to, or shall in any way be liable (including liability in negligence) directly or indirectly for any loss, damages, costs, expenses or reliance arising out of or in connection with, the accuracy, currency, completeness or balance of (or otherwise), or any errors in or omissions from, any test results, recommendations statements or other information provided to you.

CONTENTS

Page No

SUMMARY i

1.0 BACKGROUND 1

2.0 OBJECTIVES 2

3.0 OBJECTIVES 1 AND 2 – DEVELOPMENT OF A SCREENING ASSAY AND DETERMINE THE OPTIMUM TIME FOR INSPECTION OF TRIALS, RELIABILITY IN DIFFERENT SEASONS AND THE ROLE OF PLANTHOPPER PREFERENCE IN DETERMINING RESISTANCE .. 4

3.1 Introduction 4

3.2 Materials and methods 6

3.2.1 Review of field trials 1974-2001 6

3.2.2 Technique 1. Poly-tunnel 7

3.2.3 Technique 2. Greenhouse-field 9

3.2.4 Technique 3. Field 10

3.2.5 Effect of clone on acquisition of FDV 12

3.3 Results 12

3.3.1 Review of field trials 1974-2001 12

3.3.2 Technique 1. Poly-tunnel 13

3.3.3 Technique 2. Greenhouse-field 21

3.3.4 Technique 3. Field trials 30

3.3.5 Correlation of disease severity rating from greenhouse-field, poly-tunnel and field trials 35

3.3.6 Effect of clone on acquisition of FDV 36

3.3.7 Variation in FDV incidence in different sexes and life stages of the planthopper 36

3.4 Discussion 36

4.0 OBJECTIVE 3 – INTEGRATION OF THE NEW TESTING PROCEDURES INTO THE BSES-CSIRO VARIETY IMPROVEMENT PROGRAM SO THAT NEW FLG-RESISTANT CULTIVARS ARE DELIVERED TO INDUSTRY 40

5.0 OUTPUTS 41

6.0 OUTCOMES 42

7.0 RECOMMENDATIONS 43

8.0 PUBLICATIONS 43

9.0 REFERENCES 43

APPENDIX 1 - Arcsin disease severity and rating for clones in trials 5-2 and 5-3. .. 45

APPENDIX 2 - Ratings for clones in trials 7-1 to 7-8 47

APPENDIX 3 – *BSES Bulletin* article 55

APPENDIX 4 – ASSCT paper 57

APPENDIX 5 - EXTENSION MESSAGE 68

APPENDIX 6 - MANUAL……… 69

SUMMARY

Fiji leaf gall (FLG) has caused major epidemics in the Australian sugar industry in the past. All new cultivars of sugarcane released in areas affected by FLG should be resistant to the disease. New cultivars have been tested for resistance in field trials in the past but the field trials are unreliable with only a third of trials in the last 28 years giving reliable ratings. The aim of this project was to develop a method of rating clones for resistance to FLG that would be accurate, reliable and practical to implement in the BSES-CSIRO variety improvement program.

A new greenhouse-field method gave excellent results in three separate years. The clones to be tested were grown on outside benches for 3-4 months, transferred to the greenhouse and planthoppers bred on FLG-infected plants were released into the greenhouse. After 2 weeks, the plants were transplanted into the field and 6 months later they were inspected. Plants were rated on the incidence and severity of disease. The correlation of the disease severity rating and the long-term rating of a set of 10 standard clones was highly significant in all but 1 of 12 trials with this method. The ratings of 35 clones tested in two separate seasons were also highly significantly correlated.

The greenhouse-field method was extensively tested for implementation into the BSES-CSIRO variety improvement program. It successfully provided ratings for 639 clones under conditions that will be practical for implementation in the routine breeding program. The method requires only minor increases in labour compared to the traditional field method and provides ratings in less than 12 months, compared to 18 months with the field method. A plan was developed for implementing the new method in the BSES-CSIRO variety improvement program that will significantly improve the efficiency of the breeding program. With the new method, clones can be screened in the year that they are being propagated for planting into the final assessment trials (FATs) and results are obtained in time to eliminate susceptible clones before the trials are planted. This will either reduce the cost of the FATs or allow more FLG-resistant clones to be tested.

A second method, where the plants were grown in the ground under a poly-tunnel and planthoppers bred on FLG-infected plants were released into the poly-tunnel, gave variable results. Planthopper survival was poor in the poly-tunnels in some years. This method was also labour intensive and was not suitable for mass screening.

Variations on the traditional field screening trials were tested, but failed to give sufficiently high disease levels to provide reliable ratings. A windbreak was constructed around one trial and greenhouse-bred planthoppers were released onto the cane. The planthopper population in this trial failed to develop and low levels of disease were recorded. A field trial planted in 2003 and rated in 2004 developed moderate disease levels and the ratings were highly significantly related to the ratings obtained in the greenhouse-field and poly-tunnel trials. No disease was recorded in intermediate to resistant standards in this field trial, so the trial could not distinguish between intermediate and resistant clones.

A manual for conducting the greenhouse-field method of screening clones for resistance to FLG was prepared and the method has been fully implemented into the BSES-CSIRO variety improvement program. The results of the trials conducted in the project have been

included in applications to Queensland Department of Primary Industries and Fisheries for approval of the cultivars Q189[Φ], Q197[Φ], Q205[Φ], Q207[Φ], Q208[Φ], Q209[Φ], Q221[Φ] and Q222[Φ]. Without the ratings obtained in this project, these cultivars would not have met the requirements for approval established by QDPI&F. The project removed a block that may have delayed the release of these new cultivars. Delaying the release of a new cultivar that may have a higher yield potential can cost the industry many millions of dollars. All ratings from the project have been entered into the BSES-CSIRO variety-improvement database, SPIDNet, and are available to plant-breeding and research staff. Research staff have already used the ratings in research into DNA markers for FLG resistance.

The project found no relationship between planthopper preference and resistance to FLG. This finding eliminates one potential mechanism of resistance to FLG. Future research can focus on other potential resistance mechanisms, such as planthopper feeding behaviour on different clones and resistance within the plant to multiplication or movement of the virus.

In this research, acquisition of FDV by the planthoppers was strongly influenced by clone. This supports similar findings in the associated CRC-Tropical Plant Protection project that found a strong relationship between acquisition of the virus and varietal resistance to the disease. Further research on understanding the factors that control acquisition of FDV by planthoppers from different clones is warranted. The ability of the planthopper to acquire the virus from a clone could have a large influence on the development of epidemics of the disease.

1.0 BACKGROUND

Fiji leaf gall (FLG, formerly known as Fiji disease) is a major disease of sugarcane in Australia. It has caused serious epidemics in Southern and Central Queensland and New South Wales. FLG is caused by the *Fiji disease virus* (FDV) and is transmitted by planthoppers in the genus *Perkinsiella.* In eastern Australia, the only species present is *P. saccharicida* Kirkaldy. The disease can cause total crop loss, and in the 1970s threatened the viability of the sugar industry in the Bundaberg region.

Resistance to FLG is a prerequisite for cultivars grown in areas from Mackay south. BSES screens clones for resistance at BSES Woodford, which is isolated from commercial fields to prevent spread of disease within commercial crops. Obtaining consistent and reliable ratings for FLG between epidemics has been an on-going problem. The virus cannot be transmitted mechanically. Field trials to screen clones for resistance to FLG suffer from fluctuating vector populations and low vector populations in recent years have resulted in poor results. Release of new cultivars without a reliable FLG resistance rating could leave the industry vulnerable to another epidemic of FLG, if a highly susceptible clone is released.

BSES has conducted extensive research on rapid glasshouse methods for screening clones for resistance to FLG. The ratings obtained from the glasshouse experiments were poorly correlated with field reaction and BSES abandoned the glasshouse methods. The techniques that were investigated in the current project tried to combine the benefits of the glasshouse, namely standard and consistent inoculum pressure, with plants hardened by growth outside the glasshouse.

The CRC-Tropical Plant Protection (CRC-TPP) has funded a project to study the role of the insect vector in resistance to FLG. Initial results suggested that vector preference for clones may play an important role in determining resistance to FLG. This project is continuing to study the interaction of the vector with the virus and the plant. Newly developed PCR diagnostic techniques are being used to follow acquisition, transmission and development of disease. This current SRDC project was conducted in close consultation with the CRC-TPP project, so that the information gained from the CRC-TPP project was incorporated in the design of methods to screen clones for resistance to FLG.

SRDC has also funded a project to identify DNA markers for resistance to FLG (CTA049). The trials to characterize the mapping population for resistance to FLG using traditional field techniques have failed in two seasons. New screening methods developed in this project were used to assist research into DNA markers for FLG resistance.

2.0 OBJECTIVES

The project aimed to deliver FLG-resistant elite new cultivars to the industry by developing a practical, consistent resistance-screening assay for FLG based on understanding the biological basis of resistance to FLG in sugarcane.

The specific objectives were:

1. Adapt outputs from the current CRC-Tropical Plant Protection-supported project to develop a practical screening assay. The assay will involve delivering a standard inoculum pressure to plants grown under simulated field conditions, thus combining the benefits of greenhouse and field trials.
2. Determine the optimum time for inspection of trials, reliability in different seasons and the role of planthopper preference in determining resistance.
3. Integrate new testing procedures into the BSES Plant Improvement program so that new FLG-resistant cultivars can be delivered to industry.

Each of these objectives was realised as summarised below.

Objective 1 - Adapt outputs from the current CRC-Tropical Plant Protection-supported project to develop a practical screening assay. The assay will involve delivering a standard inoculum pressure to plants grown under simulated field conditions, thus combining the benefits of greenhouse and field trials.

Objective 2 - Determine the optimum time for inspection of trials, reliability in different seasons and the role of planthopper preference in determining resistance.

A new greenhouse-field method gave excellent results in three separate years. The clones to be tested were grown on outside benches for 3-4 months, transferred to the greenhouse and planthoppers bred on FLG-infected plants were released into the greenhouse. After 2 weeks, the plants were transplanted into the field and 6 months later they were inspected. Plants were rated on the incidence and severity of disease. The correlation of the disease severity rating and the long-term rating of a set of 10 standard clones was highly significant in all but 1 of 12 trials with this method. The ratings of 35 clones tested in two separate seasons were also highly significantly correlated.

A second method, where the plants were grown in the ground under a poly-tunnel and planthoppers bred on FLG-infected plants were released into the poly-tunnel, gave variable results. Planthopper survival was poor in the poly-tunnels in some years. This method was also labour intensive and was not suitable for mass screening.

Variations on the traditional field screening trials were tested, but failed to give sufficiently high disease levels to provide reliable ratings. A windbreak was constructed around one trial and greenhouse-bred planthoppers were released onto the cane. The planthopper population in this trial failed to develop and low levels of disease were recorded. A field trial planted in 2003 and rated in 2004 developed moderate disease levels and the ratings were highly significantly related to the ratings obtained in the greenhouse-field and poly-tunnel trials. No disease was recorded in intermediate to

resistant standards in this field trial, so the trial could not distinguish between intermediate and resistant clones.

The project found no relationship between planthopper preference and resistance to FLG. This finding eliminates one potential mechanism of resistance to FLG. Future research can focus on other potential resistance mechanisms, such as planthopper feeding behaviour on different clones and resistance within the plant to multiplication or movement of the virus.

In this research, acquisition of FDV by the planthoppers was strongly influenced by clone. This supports similar findings in the associated CRC-Tropical Plant Protection project that found a strong relationship between acquisition of the virus and varietal resistance to the disease. Further research on understanding the factors that control acquisition of FDV by planthoppers from different clones is warranted. The ability of the planthopper to acquire the virus from a clone could have a large influence on the development of epidemics of the disease.

Objective 3 - Integrate new testing procedures into the BSES Plant Improvement program so that new FLG-resistant cultivars can be delivered to industry.

The greenhouse-field method was extensively tested for implementation into the BSES-CSIRO variety improvement program. It successfully provided ratings for 639 clones under conditions that will be practical for implementation in the routine breeding program. The method requires only minor increases in labour compared to the traditional field method and provides ratings in less than 12 months, compared to 18 months with the field method. A plan was developed for implementing the new method in the BSES-CSIRO variety improvement program that will significantly improve the efficiency of the breeding program. With the new method, clones can be screened in the year that they are being propagated for planting into the final assessment trials (FATs) and results are obtained in time to eliminate susceptible clones before the trials are planted. This will either reduce the cost of the FATs or allow more FLG-resistant clones to be tested.

A manual for conducting the greenhouse-field method of screening clones for resistance to FLG was prepared (Appendix 6) and the method has been fully implemented into the BSES-CSIRO breeding program. The results of the trials conducted in the project have been included in applications to Queensland Department of Primary Industries and Fisheries for approval of the cultivars Q189[Φ], Q197[Φ], Q205[Φ], Q207[Φ], Q208[Φ], Q209[Φ], Q221[Φ] and Q222[Φ]. Without the ratings obtained in this project, these cultivars would not have met the requirements for approval established by QDPI&F. The project removed a block that may have delayed the release of these new cultivars. Delaying the release of a new cultivar that may have a higher yield potential can cost the industry many millions of dollars. All ratings from the project have been entered into the BSES-CSIRO variety-improvement database, SPIDNet, and are available to plant-breeding and research staff. Research staff have already used the ratings in research into DNA markers for FLG resistance.

3.0 OBJECTIVES 1 AND 2 – DEVELOPMENT OF A SCREENING ASSAY AND DETERMINE THE OPTIMUM TIME FOR INSPECTION OF TRIALS, RELIABILITY IN DIFFERENT SEASONS AND THE ROLE OF PLANTHOPPER PREFERENCE IN DETERMINING RESISTANCE

3.1 Introduction

Fiji leaf gall (FLG) has caused major epidemics in southern and central Queensland and New South Wales. It is caused by the *Fiji disease virus* (FDV), which is a double-stranded RNA virus with an icosahedral particle shape. The symptoms of FLG are severe stunting, distortion of the leaves and galls on the underside of the leaves (Figure 1). FLG is spread by planthoppers in the genus *Perkinsiella* and, in eastern Australia, the only species in this genus is *Perkinsiella saccharicida* Kirkaldy (Ridley *et al.* 2006) (Figure 2). *P. saccharicida* has five nymphal stages, and each nymphal stage lasts for 4-5 days at 28-30°C. Adults come in two wing types, long-wing and short-wing. Short-wing males have been reported, but are rare. The planthoppers acquire FDV as young nymphs (first and second instars), the virus replicates in the insect, and the insect remains infective for its entire life. The virus cannot be transmitted artificially.

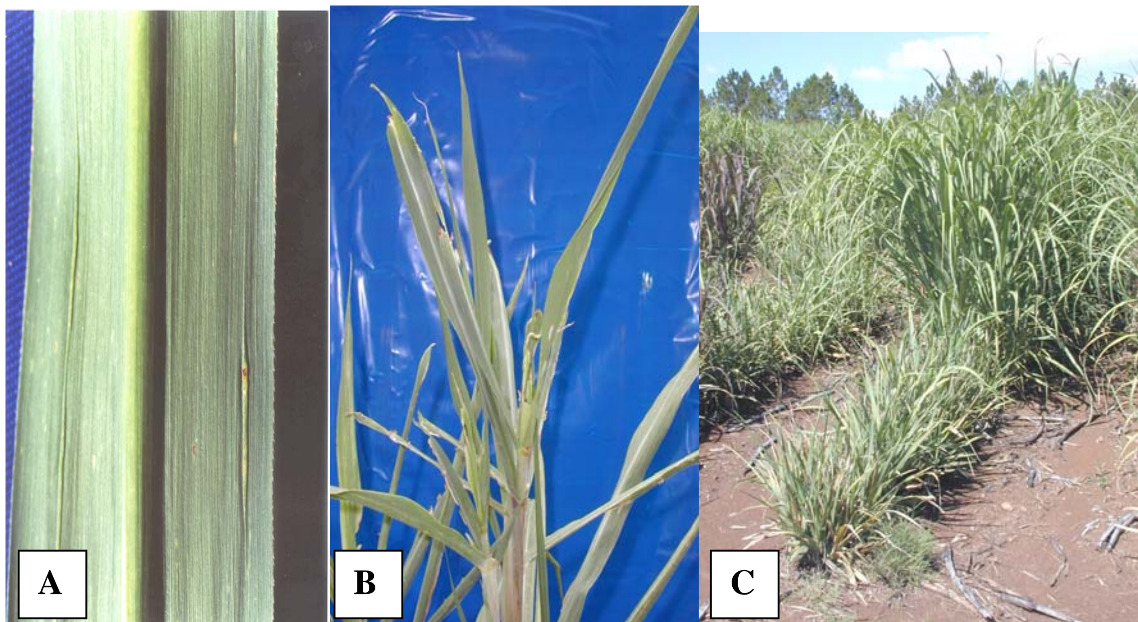

Figure 1 Symptoms of Fiji leaf gall. A, Galls on underside of leaf; B, Distorted leaves; C, Severe stunting

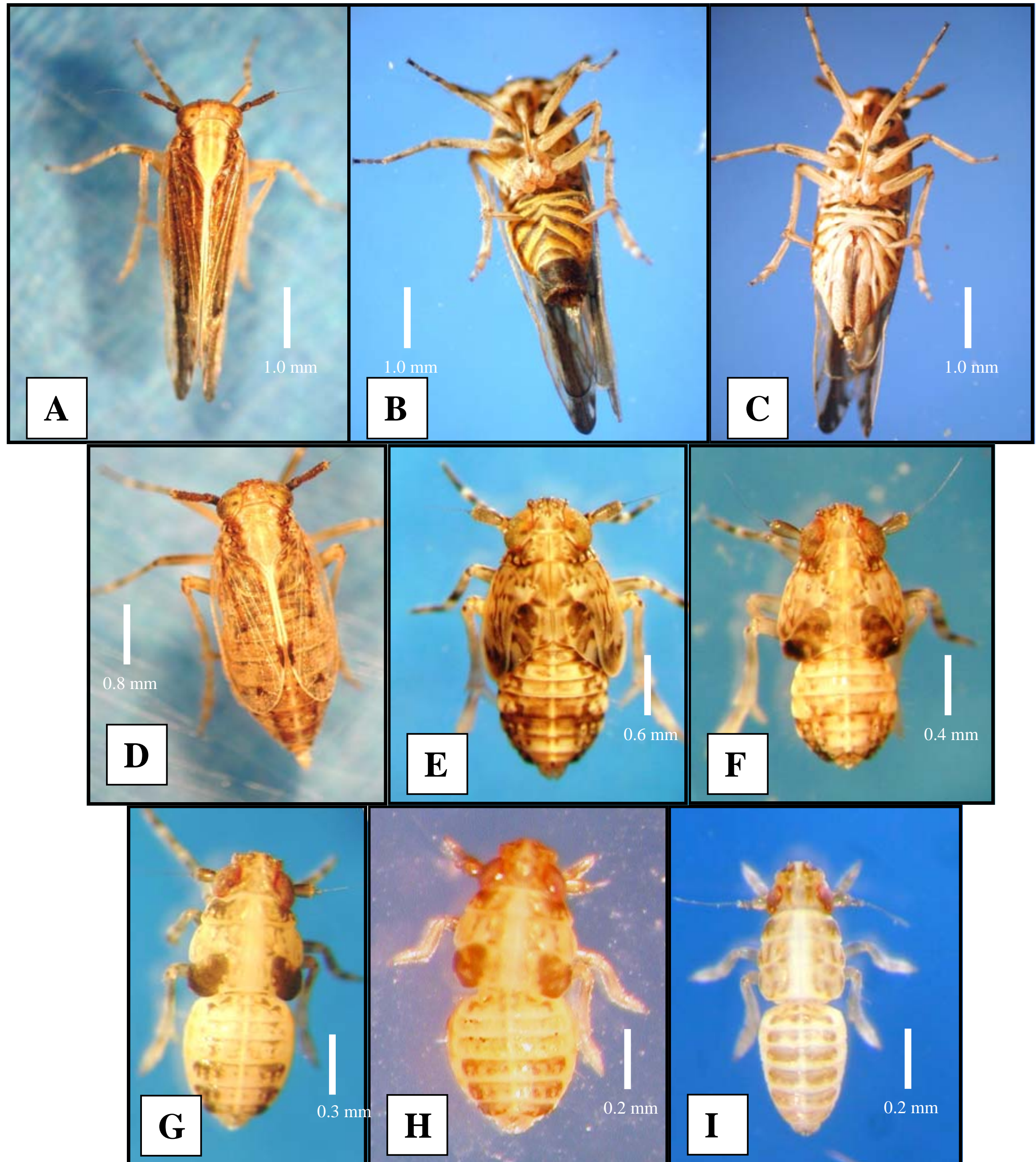

Figure 2 ***Perkinsiella saccharicida.*** **A, Adult long wing form; B, Adult male; C, Adult female; D, Adult short-wing female; E, Fifth-instar nymph; F, Fourth-instar nymph; G, Third-instar nymph; H, Second-instar nymph; I, First-instar nymph**

FLG is managed by planting resistant cultivars and by disease-free planting material. Resistance to FLG is a prerequisite for all cultivars of sugarcane released in central and southern Queensland and New South Wales. During epidemics, clones can be rated for resistance in selection trials in commercial fields (Leverington *et al*. 1977), but when infection pressure in commercial fields is low, clones can only be tested in artificial screening experiments. The method used in the past by BSES is described in Steindl *et al.* (1977). This field-based method involves planting small plots of the test clones between rows of FLG-infected cane. Natural populations of the planthoppers are relied on to transmit the disease. Clones are rated in terms of the proportion of plants to develop FLG.

Daniels *et al.* (1969) devised a glasshouse-insectary technique for screening for FLG resistance. The method rated clones on the numbers of days for plants to develop symptoms, but Ledger and Ryan (1977) reported that this technique did not always correlate well with field reaction. The method is labour intensive, with clones being inspected up to five times a week for 3-4 months to determine the number of days for symptom development.

We first reviewed the results of field-based screening for FLG resistance over the last 28 years to examine the reliability of trials at different infection pressures. This review identified the optimum infection levels in standard clones to give reliable ratings for test clones.

We then investigated three techniques to improve the screening for FLG resistance:

- The poly-tunnel technique involved breeding planthoppers in the greenhouse on FLG-infected plants and then releasing them onto test plants that were planted in the ground under a poly-tunnel.
- The greenhouse-field technique involved growing the test plants in pots on outside benches for 3-4 months and then transferring the plants into a greenhouse chamber where they were exposed to planthoppers bred on FLG-infected plants. After a 2-week infection period, the plants were transplanted into the field.
- Variations on the traditional field technique were also tested. Greenhouse-bred insects were released into the field trials to supplement the natural planthopper population.

The greenhouse-field technique was the most successful and this technique was further tested and adapted for use in the routine BSES-CSIRO breeding program.

3.2 Materials and methods

3.2.1 Review of field trials 1974-2001

The percent FLG-infected plants for eight standard clones that were included in the majority of field based screening trials conducted from 1974-2001 was collated from BSES records. There were three to five trials conducted in most years. Trials from 1974-1999 were conducted at the BSES Eight Mile Plains, and the trials from 2000-2001 were conducted at BSES Woodford. There were 93 trials for which data were available, but

only trials with more than 20% infection in NCo310 were included in the analyses. The results are reported for the year in which the trial was rated.

During this period, the trials were conducted using similar methods (Steindl *et al.* 1977). Single or dual rows of small plots of the test clones were planted between rows of FLG-infected cane. The plots were 3-5 m in length and usually contained 8-10 plants. Trials were planted in a randomised complete-block design with three or five replicates. Standard clones were included in all trials. The eight standards that were included in the majority of the trials during the period and their resistance ratings are shown in Table 1. Trials were rated at 4-6 months of age in the first-ratoon crop or in a few cases in the second-ratoon crop. The total number of plants and the number of infected plants were recorded and the percent-infected plants calculated.

The long-term ratings have been assigned based on extensive field experience during FLG epidemics and/or repeated testing in FLG resistance trials. The International Society of Sugar Cane Technologists' recommended rating system was used. Rating 1 is highly resistant and 9 highly susceptible.

Table 1 Standard clones and their resistance ratings

Standard clone	Rating
Q110	1
Q57	2
Q87	2
Q90	4
Pindar	5
Q117[a]	6
Q124[a]	6
NCo310	8
Q102	9
Q71	9

[a]Not included in analysis of historical data because they were only included as standards at the end of the period.

Trials were grouped into four classes based on the percentage of infected plants in NCo310. The classes were 20-40%, 40-60%, 60-80% and 80-100% infected plants in NCo310. A regression analysis was performed on the average percent infection for each standard for all trials in that class versus the long-term ratings.

3.2.2 Technique 1. Poly-tunnel

The poly-tunnel trials were planted in beds in the field at BSES Woodford with four plants across each bed at 250 mm centres and 40 plants down the beds at 250 mm centres. Each plot of one clone consisted of eight plants (4x2 plants across the bed). There were four replicates arranged in randomised complete blocks. The details of the number of clones included, inoculation, planting and inspection dates for the three trials conducted

using the poly-tunnel technique are shown in Table 2. Each trial contained the 10 standard clones listed in Table 1.

Table 2 Number of clones and inoculation, planting and inspection dates for three poly-tunnel trials

Trial No.	BSES Trial code	No. clones	Number of replicates of clones (standards)	Date			
				Planted	Inoculation	Final inspection first-ratoon crop	Final inspection second-ratoon crop
1-1[a]	FDPT1-01	20	4(4)	15/2/02	9/5/02	17/11/02	16/1/03
1-2	FDPT1-02	39	4(4)	8/10/02	9/12/02 and 17/12/02	12/6/03	8/12/03
4	FDPT1-03	40	4(4)	23/7/03	23/10/03	10/5/04	18/2/05
Total		99					

[a] This trial commenced 1 year before the SRDC project was funded.

The height of the plants to the top visible dewlap was measured at the time of release of the planthoppers and the number of planthoppers on each plant was recorded after release of the insects. Samples of the planthoppers were screened for FDV by reverse transcription-polymerase chain reaction (RT-PCR) assay using the methods described by Ridley *et al.* (2006).

The plants in the poly-tunnels grew quickly and they had to be ratooned at 3-4 month intervals when the plants had reached the roof of the tunnels. The plants were inspected for percent infected plants and disease severity. Each infected plant was given a severity rating from the following four categories:

1. G = Galls and/or mild breakdown, no apparent stunting;
2. M = Moderate stunting and breakdown;
3. S = Severe stunting and breakdown, stalks approximately 50% shorter than healthy stalks;
4. V = Very severe stunting and death, stalks more than 50% shorter than healthy stalks.

The disease severity (DS) was calculated as

$$DS = ((1*G)+(2*M)+(3*S)+(4*V))/(4*\text{total plants})*100$$

In all trials, ratings were calculated from the regression equation of the relationship between arcsin disease severity or arcsin percent infection and long-term rating of the 10 standard clones (Table 1).

3.2.3 Technique 2. Greenhouse-field

Plants for the greenhouse-field trials were established by planting one-eye setts in 90 mm peat pots filled with sand-peat potting mix. The pots initially were placed in a greenhouse or poly-tunnel to aid germination and establishment and were moved to outside benches after 1-2 months. The plants were trimmed to reduce the variation in plant size at this time. Approximately 3-4 months after planting, the plants were transferred to a greenhouse chamber. There were four replicates of each clone arranged in randomised complete blocks with eight plants of a clone in each plot.

Planthopper colonies were established on FLG-infected NCo310 for the greenhouse-field trials. The FLG-infected NCo310 plants were grown in 300 mm plastic pots for 6-12 weeks, covered with voile cages and 50 adult planthoppers were added to each cage. After 6-8 weeks, the number of planthoppers in the cages was estimated by slowly immersing a sample of the cages in large bins filled with water, so that the planthoppers retreated to the voile cage. When the plant was fully submerged, the cage, which contained the planthoppers, was carefully removed, sealed and placed in a freezer to kill the planthoppers. The average number of planthoppers from three cages was 3900, with 70.3% first and second instars, 26.5% third to fifth instars and 3.2% adults. This estimate of the planthoppers in a cage was used to determine the number of cages required to achieve the target population in the trials.

Although high numbers of planthoppers were achieved on the NCo310 plants, the plants at the end of the breeding period were in poor condition due to the FLG infection, the reduced light in the voile cages and the high planthopper population. Some plants were affected by mites, which added to the stress on the plants. At these high populations, few planthoppers progressed through to the adult stage. There was excellent survival of the nymphs once they were transferred to the test plants in the greenhouse-field trials.

Planthoppers bred on the FLG-infected plants were released onto the test plants in the greenhouse chambers. The height of the plants to the top visible dewlap was measured at the time of release of the planthoppers in experiments 2 and 5-1. The number of planthoppers on two plants in every plot was counted on three occasions at 3-4 day intervals in trials 2 and 5-1 and on a random sample of 50 plots in the other trials. A sample of the planthoppers was collected from each trial and was screened for FDV by RT-PCR.

After 2 weeks, the chamber was sprayed with insecticide and the plants were transplanted into the field. In experiments 2 and 5-1 the plants were inspected for FLG on three occasions in the plant crop and once in the ratoon crop. In the other trials plants were inspected once in the plant crop approximately 5 months after planting. The number of FLG-infected plants and the total number of plants was recorded for each plot. At the final inspection in the plant crop and in the ratoon crop, infected plants were given a disease severity rating (see above).

The details of the number of clones included, inoculation, planting and inspection dates for the 12 trials conducted using the greenhouse-field technique are shown in Table 3. Each trial contained the 10 standard clones listed in Table 1.

Table 3 Number of clones and inoculation, planting and inspection dates for 12 greenhouse-field trials

Trial No.	BSES Trial code	No. clones	Replicates (standards)	Date			
				Inoculation	Planted-field	Final inspection plant crop	Final inspection ratoon crop
2	FDGH1-02	36	4(4)	26/11/02	13/12/02	15/8/03	28/1/04
5-1	FDGH1-03	40	4(4)	3/9/03	24/9/03	16/2/04	16/2/05
5-2	FDGH2-03	42	4(4)	7/10/03	20/10/03	17/2/04	-
5-3	FDGH3-03	42	4(4)	22/9/03	6/10/03	17/2/04	-
7-1	FDGH1-04	57	3(3)	19/4/04	4/5/04	10/1/05	-
7-2	FDGH2-04	56	3(3)	19/4/04	5/5/04	11/1/05	-
7-3	FDGH3-04	72	3(3)	30/8/04	14/9/04	3/2/04	-
7-4	FDGH4-04	120	2(4)	30/8/04	14/9/04	7/2/04	-
7-5	FDGH5-04	120	2(4)	30/8/04	14/9/04	4/2/04	-
7-6	FDGH6-04	120	2(4)	20/9/04	4/10/04	9/2/04	-
7-7	FDGH7-04	120	2(4)	20/9/04	4/10/04	9/2/04	-
7-8	FDGH8-04	104	2(4)	20/9/04	4/10/04	9/2/04	-
Total		929					

A detailed manual for conducting the greenhouse-field technique was written and is attached as Appendix 6.

3.2.4 Technique 3. Field

Trial 3 Windbreak

In an attempt to improve the environment for planthopper survival early in the season, BSES funded the construction of a 2.6 m high windbreak enclosure. Sheltered environments with highly fertile soils favour planthopper populations. The enclosure is 108 by 25m in area and is surrounded by Sarlon Ultra Wind fabric on 2.7 m treated pine posts. The area can be irrigated by overhead sprinklers to help maintain humidity during periods of dry weather.

The clones for the trial were planted into this area with a randomised complete-block design on the 22 October 2002. To maximise the number of clones that could be planted into this enclosure, they were planted in beds with 1.5 m spacing between beds and three rows at 0.4 m spacing within the beds. The two outside rows were the test clones and the centre row was 20% FLG-infected NCo310 and 80% FLG-infected WD1. To improve the soil structure in the area and to maximise the growth of the cane, the area was treated with 100 t/ha mill mud. The cane made excellent growth, and canopy closure was achieved 2 months after planting.

Planthoppers to infest the windbreak trial were bred on FLG-infected cultivar WD1 that was planted into 2.5 L plastic planter bags with 14 planter bags placed in a 23 L plastic tray. The plants were grown for 6-8 weeks and then the whole tray was covered with a voile cage and 100 adult planthoppers were released into the cage. The planthoppers in one caged tray were counted in the same way as described above. A total of 4789 planthoppers was counted, with 26.4% first to second instars, 44.6% third to fifth instars and 29% adults. Planthoppers were released into the enclosure on 19 December 2002. One planter bag was placed at the junction of each set of four plots in the trial.

The number of planthoppers was monitored on plots of NCo310 throughout the windbreak enclosure and compared to NCo310 in a conventional field trial. We planned to count planthoppers on all clones in the experiment when planthopper numbers peaked in the NCo310, but this was impossible because of severe lodging.

The trial was inspected for FLG infection in the first-ratoon crop 6 moths after ratooning.

Trial 6 Field

Field trial 6 was planted with a similar design to traditional field trials in September 2003. Test plots were planted between rows of FLG-infected cane. The infection rows consisted of alternating plots of four plants derived from systemically infected NCo310 and four plants of NCo310 infected in the greenhouse just prior to planting.

The test clones were planted in dual rows with each plot consisting of one row of eight plants. No planthoppers were released into the trial area, but the trial was planted beside the three greenhouse-field trials planted in 2003 that retained some planthoppers and had many egg punctures that would have hatched when the plants were in the field. Mill mud was applied to the site where the trial was planted to encourage lush growth of the cane to make it more suitable for planthopper breeding.

Planthoppers were counted on 11 occasions in the plant crop. The number of adults, nymphs and egg punctures were counted on one stalk in each stool in plots of the 10 standard clones. Samples of planthoppers were collected from the infection rows and the test plant rows on the 4 February 2004 and were screened for FDV by RT-PCR.

The trial was inspected for FLG infection in the first-ratoon crop 6 moths after ratooning.

3.2.5 Effect of clone on acquisition of FDV

An experiment was conducted to determine whether the clone used for breeding FDV-infected planthoppers has an influence on the proportion of insects that carry the virus. Planthoppers were bred for one generation on 10 plants of FLG-infected NCo310 and WD1 in voile cages in the greenhouse. A sample of 10-12 planthoppers was collected from each plant and the planthoppers were screened for FDV by RT-PCR.

3.3 Results

3.3.1 Review of field trials 1974-2001

In 11 of the 28 years from 1974-2001, the field FLG resistance trials failed (Figure 3). In nine years, the trials failed because the infection was less than 20% in the susceptible standard NCo310, and in two years the trials failed because the infection in all clones was so high that it was impossible to discriminate among resistant and susceptible clones.

The results were available for 68 trials with infection levels greater than 20% in NCo310 (Figure 3). The average infection level in trials in the period 1977-1982 was high. This period corresponded to the epidemic in commercial crops in southern Queensland. During 1983 to 1997, the infection level in NCo310 was generally between 20-40%, with two years when trials were abandoned because of low infection. From 1998 to 2001, trials at both Eight Mile Plains and at Woodford failed because of low infection levels.

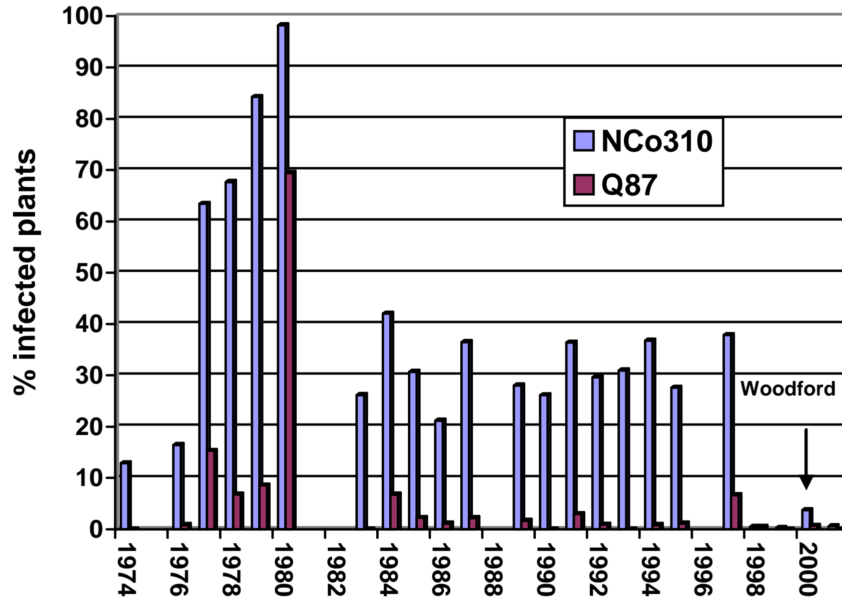

Figure 3 **Average percent infected plants for NCo310 and Q87 for trials conducted in each year between 1974 and 2001 (listed by date of inspection)**

The 68 trials with infection levels in NCo310 above 20% were divided into four groups and the average infection in the standards in each group of trials was calculated. The regression of the percent infection versus the standard rating for each group of trials is presented in Figure 4. In the ranges 20-40, 40-60 and 60-80% infection, the regressions have a similar intercept on the *x*-axis, which means that the highly resistant standards have low levels of disease. The slope of the regression lines increased as disease levels increased. The discrimination between ratings is better when the slope of the regression line is greatest, which occurred in the 60-80% range of infection in NCo310. The 80-100% group had a slope intermediate between the 40-60 and the 60-80% ranges but the line has a parallel shift upwards. In years of high disease pressure, resistant clones such as Q87 develop higher levels of disease (see Figure 3 - 1980). In 1981-1982, resistant clones such as Q87 approached 100% infection and the trials were abandoned.

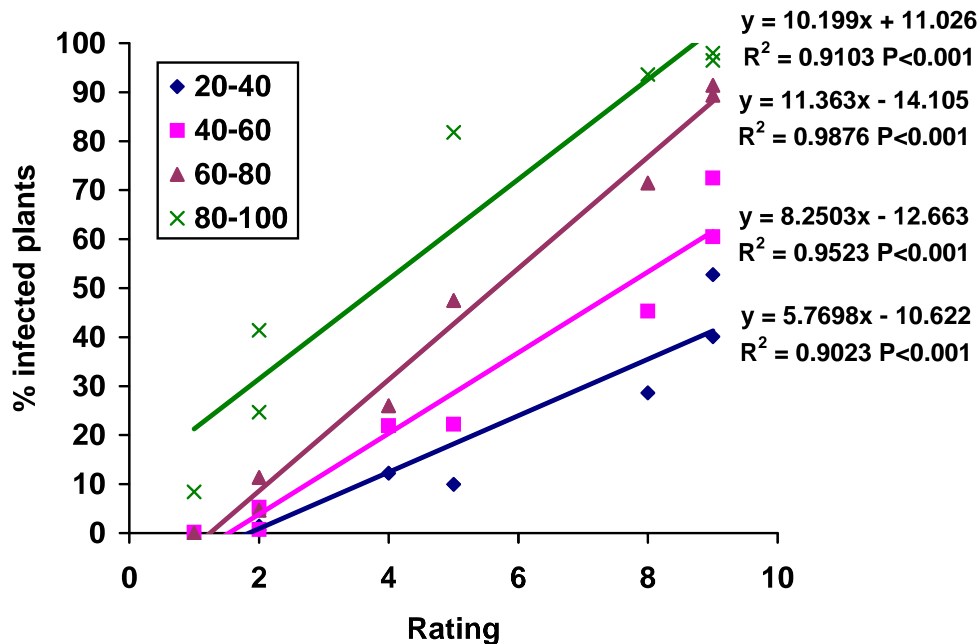

Figure 4 **Regression of percent infection of eight standard clones and the standard rating in field resistance trials conducted between 1974 and 2001. Trials were separated into groups based on 20-40 (33 trials), 40-60 (11 trials), 60-80 (12 trials) and 80-100% (12 trials) infection in NCo310**

3.3.2 Technique 1. Poly-tunnel

Trial 1-1

Poly-tunnel trial 1-1 was planted in February 2002, before the commencement of the SRDC funded project, inoculated in May 2002 and inspected in November 2002. A sample of the planthoppers was screened for FDV infection with the RT-PCR assay and 60% of planthoppers were positive for FDV (Table 4). This was the highest incidence of FDV in a planthopper population recorded during this project. The planthoppers for this

experiment were bred on FLG-infected NCo310 in the greenhouse with no cages on the plants. This allowed the plants to stay in better condition, but it was difficult to move the planthoppers to the poly-tunnel because the insects jumped off the plants when they were disturbed. Planthopper survival was low in this experiment and the average number of planthoppers per plant was 3.9 (Table 5).

Table 4 Incidence of FDV infection in samples of planthoppers collected from all trials.

Sample	LWF[a]		SWF[a]		LWM[a]		Adults[a]		Nymphs		Total	
	% pos.	No.	% pos.	No.	% pos.	No.	% pos.	No.	% pos.	No.	% pos.	No.
Bred on NCo310												
1-1	70.8	24	41.7	24	83.3	24	61.8	72	45.8	24	60.4	96
1-2	NA	NA	NA	NA	NA	NA	26	50	NA	NA	26	50
2-1	NA	NA	NA	NA	NA	NA	33.3	36	10.0	40	21.1	76
4-1	27.9	43	46.2	13	34.1	41	33.0	97	NA	NA	33.0	97
5-1	30	10	0	7	21.2	33	20.0	50	16.0	50	18.0	100
5-2	6.9	29	0	5	31.3	16	14.0	50	26.5	49	20.2	99
5-3	37.0	27	33.3	12	4.5	22	24.6	61	18.2	77	21.0	138
7-3	10	10	0	17	0	11	2.6	38	0	12	2.0	50
7-4	0	5	15.4	13	27.8	18	19.4	36	28.6	14	22.0	50
7-5	0	4	0	10	0	19	0	33	0	17	0	50
7-6 to 7-8	NA	NA	NA	NA	NA	NA	6.5	92	NA	NA	6.5	92
Mean/Total	22.8	152	17.1	101	25.3	184	21.9	615	18.1	283	20.9	898
Bred on WD1												
3-1	NA	NA	NA	NA	NA	NA	0	50	NA	NA	0	50
Collected from field												
6-1 Infect.[b]	0	26	0	6	3.4	29	1.6	61	0	2	1.6	63
6-1 Test[b]	0	31	0	1	0	24	0	56	0	1	0	57

[a] LWF, long-wing females; SWF, short-wing females; LWF, long-wing males; Adults is the combined results for LWF, SWF and LWM.

[b] Infect., planthoppers collected from the infection row; Test, planthoppers collected from the test row.

Table 5 Arcsin % FLG-infected plants and the calculated resistance rating for clones inoculated in poly-tunnel trial 1-1. The average number of planthoppers on each clone during the inoculation period is also shown

Clone	Arcsin % FLG	Rating	Adult planthoppers/plant
NCo310*	54.4	8	3.6
Pindar*	25.0	4	3.5
Q102*	54.2	8	2.5
Q110*	0	1	3.9
Q117*	45.4	7	1.7
Q124*	44.5	6	2.1
Q57*	10.8	2	3.6
Q71*	72.7	9	4.8
Q87*	16.8	3	3.3
Q90*	19.3	3	2.7
89A570	52.6	8	3.7
89C748	11.3	2	3.8
90N6006	78.8	9	5.3
92C300	72.3	9	4.2
92C768	50.7	7	5.3
92S2423	0	1	3.1
92S250	55.3	8	5.9
Q189$^{\phi}$	6.0	1	4.4
Q205$^{\phi}$	79.7	9	5.6
Q222$^{\phi}$	52.9	8	4.9
LSD P<0.05	26.3		1.4
Mean	40.1	6	3.9

*standard clones

The regression of arcsin percent FLG-infected plants and long term rating of the standard clones in the first-ratoon crop was highly significant ($R^2 = 0.90$, $P<0.01$ Figure 5). This trial was inspected before the disease severity rating technique was developed. There were significant differences in the number of planthoppers per plant for clones but these differences were not related to the percentage of FLG in the clones. The regression of adult planthopper numbers and plant height was significant ($R^2 = 0.61$, $P<0.05$, Figure 6).

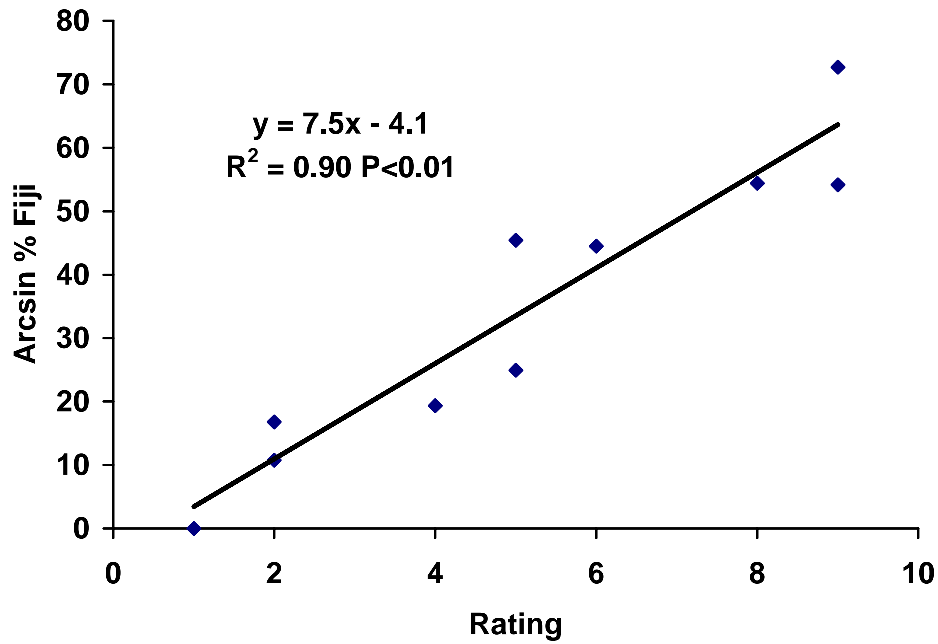

Figure 5 **Regression of the percent FLG-infected plants and the long-term rating for the 10 standard clones in poly-tunnel trial 1-1**

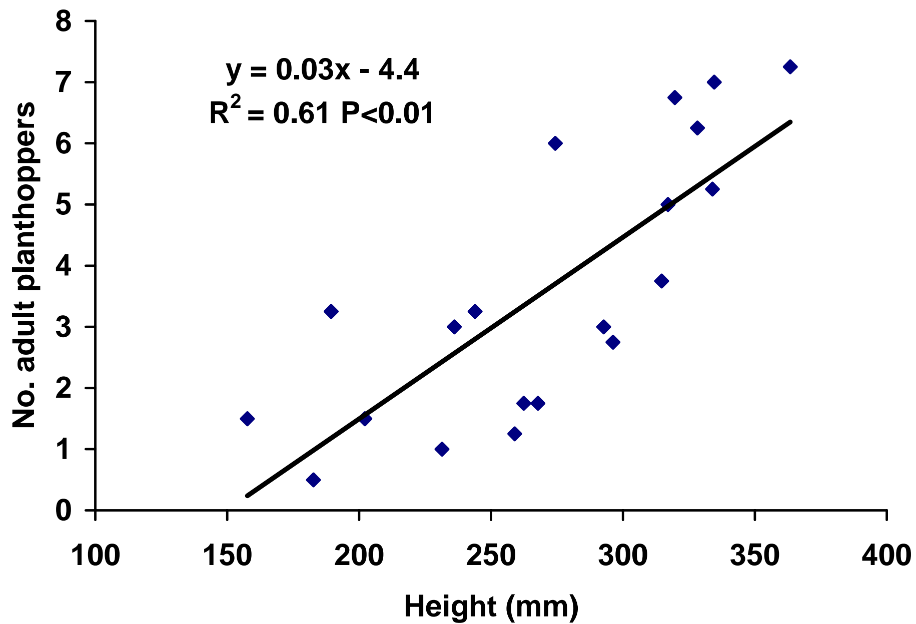

Figure 6 **Relationship between the number of adult planthoppers and the plant height of 20 clones in poly-tunnel trial 1-1**

Trial 1-2

Poly-tunnel trial 1-2 was planted on 8 October 2002 and the planthoppers were released into the poly-tunnel in December 2002. Extra planthoppers had to be added to the poly-tunnels because of poor survival of the initial batch of planthoppers (Figure 7). Monitoring showed that there was poor survival of the planthoppers, with a maximum population of only 3.5 planthoppers per plant. 26% of the planthoppers tested positive for FDV by RT-PCR (Table 4).

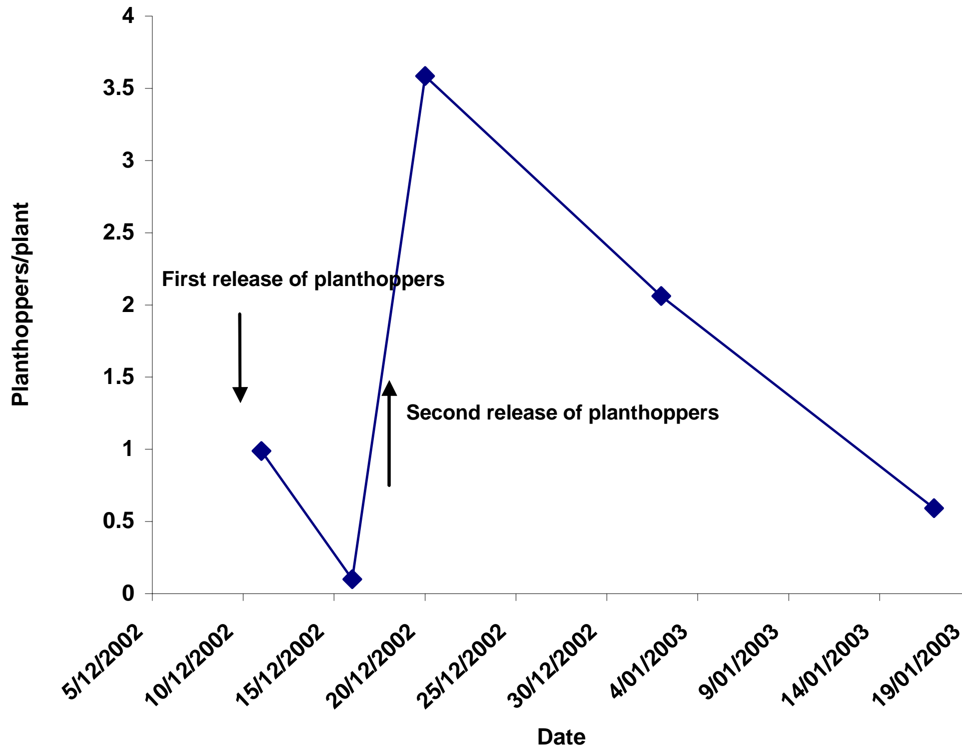

Figure 7 Average number of planthoppers/plant in poly-tunnel trial 1-2

Trial 1-2 was inspected on three occasions and after each inspection the plants were ratooned. The level of disease in the experiment was low, with an average for all clones of 4.6 % infected plants. The regression of the arcsin % FLG-infected plants versus the long-term rating for the 10 standard clones was significant, but was low compared to most of the greenhouse-field trials (R^2 = 0.50, Figure 8). The ratings for all clones in the trial are shown in Table 7.

Table 7 Arcsin percent FLG-infected plants and rating for clones in poly-tunnel trial 1-2

Clone	Arcsin % FLG	Rating
NCo310*	8.3	8
Pindar*	8.6	9
Q102*	8.0	8
Q110*	0.0	2
Q117*	0.0	2
Q124*	0.0	2
Q57*	0.0	2
Q71*	15.6	9
Q87*	0.0	2
Q90*	3.6	5
89W60	5.2	5
90S7069	10.2	9
91S7266	0.0	2
91S7347	0.0	2
92S206	0.0	2
92S2423	0.0	2
92S250	15.9	9
92S330	22.7	9
BN82-2384	26.6	9
BN86-3012	5.2	5
BN87-3009	0.0	2
BN88-3347	0.0	2
CP51-21	5.2	5
Q133	6.0	6
Q141	0.0	2
Q155	0.0	2
Q174[ϕ]	0.0	2
Q177[ϕ]	0.0	2
Q189[ϕ]	0.0	2
Q205[ϕ]	9.3	8
Q207[ϕ]	0.0	2
Q208[ϕ]	5.6	5
Q209[ϕ]	0.0	2
Q210[ϕ]	0.0	2
Q212[ϕ]	0.0	2
Q214[ϕ]	5.6	5
Q221[ϕ]	10.7	9
Q222[ϕ]	14.2	9
Q63	0.0	2
Mean	4.6	

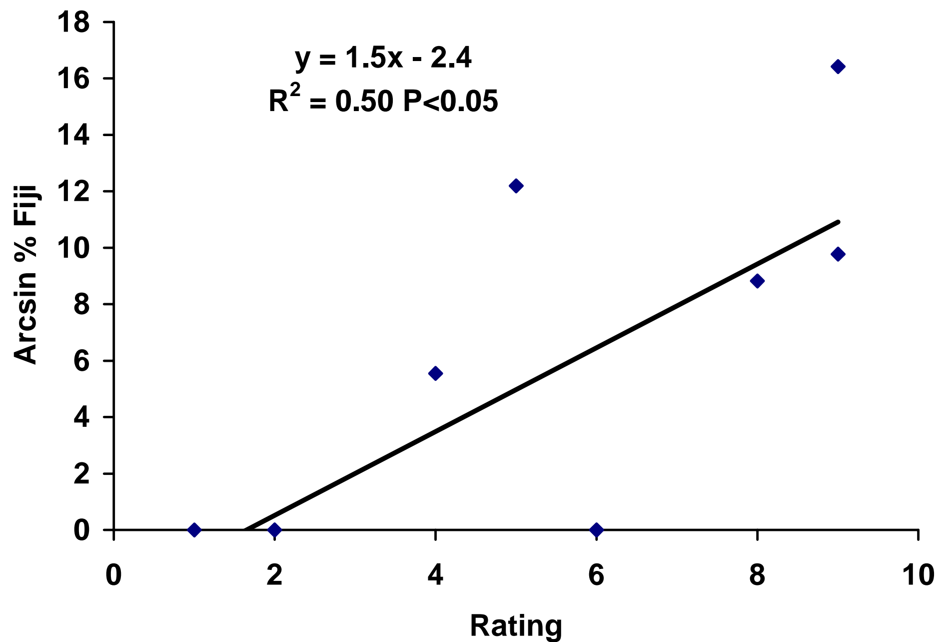

Figure 8 **Regression of arcsin percent FLG-infected plants and long-term rating for the 10 standard clones in poly-tunnel trial 1-2**

Trial 4

Poly-tunnel trial 4 was planted in August 2003 and the planthoppers were introduced into tunnel 1 in October and into tunnel 2 in November 2003. There were two replicates in each tunnel. The plant crop grew rapidly and was ratooned in early 2004. Three inspections were made in the first-ratoon crop and the experiment was ratooned again in April/May 2004. Two inspections were made in the second-ratoon crop in November 2004 and February 2005.

Moderate to high levels of disease developed in the clones in the trial (Table 8). There was a significant regression between disease severity and long term ratings of the 10 standard clones in both the first- and second-ratoon crops (first ratoon $R^2 = 0.64$, $P<0.01$; second ratoon $R^2 = 0.65$, $P<0.05$, Figure 9). Some clones were deleted from the analysis in the second-ratoon crop because of poor ratooning. There was a marked difference in disease levels in the two tunnels (Table 9). Initial survival of the planthoppers was higher in tunnel 2, but tunnel 1 had a high population of planthoppers develop in the young first-ratoon crop.

Table 8 **Arcsin disease severity (DS) for clones in the first and second ratoon crops in poly-tunnel trial 4 and the calculated ratings for the clones**

Clone	First-ratoon crop		Second-ratoon crop	
	Arcsin DS	Rating	Arcsin DS	Rating
NCo310*	19.0	6	35.0	7
Pindar*	14.1	4		
Q102*	33.0	9		
Q110*	0.0	1	0.0	1
Q117*	18.6	8	41.3	8
Q124*	14.8	4	29.7	6
Q57*	0.0	1		
Q71*	24.1	8	40.5	8
Q87*	15.5	6	26.0	5
Q90*	19.1	7	25.4	4
89W60	26.5	9	46.8	9
90S7069	29.2	8	42.1	9
91S7266	17.7	7	26.9	5
91S7347	9.4	3	38.4	8
92S206	25.3	9	39.4	8
92S2423	8.9	2	27.9	5
92S250	25.7	9	36.0	7
92S330	19.6	7	31.9	6
BN82-2384	32.8	9	56.4	9
BN86-3012	17.4	7	34.8	7
BN87-3009	14.5	5	26.5	5
BN88-3347	13.7	4	36.3	7
CP51-21	13.9	4	33.5	6
Q133	25.6	9	55.0	9
Q141	13.3	5	21.9	4
Q155	11.1	4	16.8	2
Q174[Φ]	8.8	2	12.2	1
Q177[Φ]	14.2	4	32.2	6
Q189[Φ]	22.4	6	31.8	6
Q197[Φ]	16.0	5	28.8	5
Q205[Φ]	18.6	7	34.5	7
Q207[Φ]	32.5	9	53.5	9
Q208[Φ]	21.4	7	48.5	9
Q209[Φ]	28.2	8	37.1	7
Q210[Φ]	7.7	2	13.8	1
Q212[Φ]	0.0	1	2.7	1
Q214[Φ]	20.1	9	43.3	9
Q221[Φ]	13.9	5	28.8	5
Q222[Φ]	16.5	6	33.5	6
Q63	10.6	3	24.8	4
Mean	17.3	5.7	33.0	6.0
LSD P<0.05	12.3		16.4	

* = standard clone.

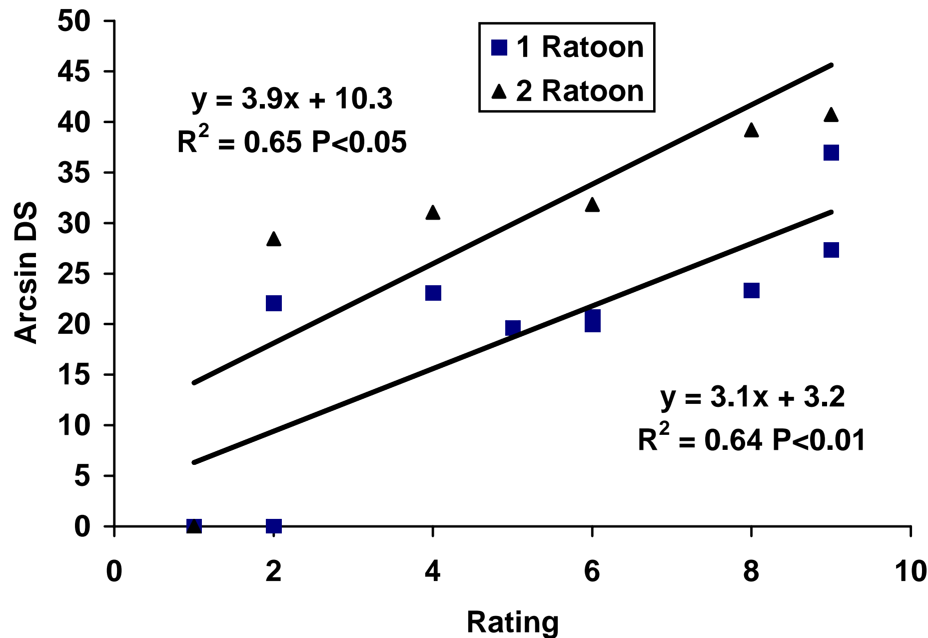

Figure 9 **Regression of arcsin disease severity and long-term rating for the 10 standard clones in poly-tunnel trial 4**

Table 9 **Percent FLG-infected plants for each replicate in the first ratoon crop of poly-tunnel trial 4**

Tunnel	Replicate	Arcsin DS
1	1	27.6
1	2	26.6
2	3	8.0
2	4	7.1
LSD P<0.05		3.9

3.3.3 Technique 2. Greenhouse-field

Trial 2

The average number of planthoppers per plant in trial 2 was 26 (Figure 10) and the majority of insects were nymphs throughout the inoculation period. FDV was detected in 33% of adults and 10% of nymphs by RT-PCR (Table 4).

There were no significant relationships between plant height or percent infection and planthopper numbers. Plants in this experiment were trimmed to reduce the variation in plant height.

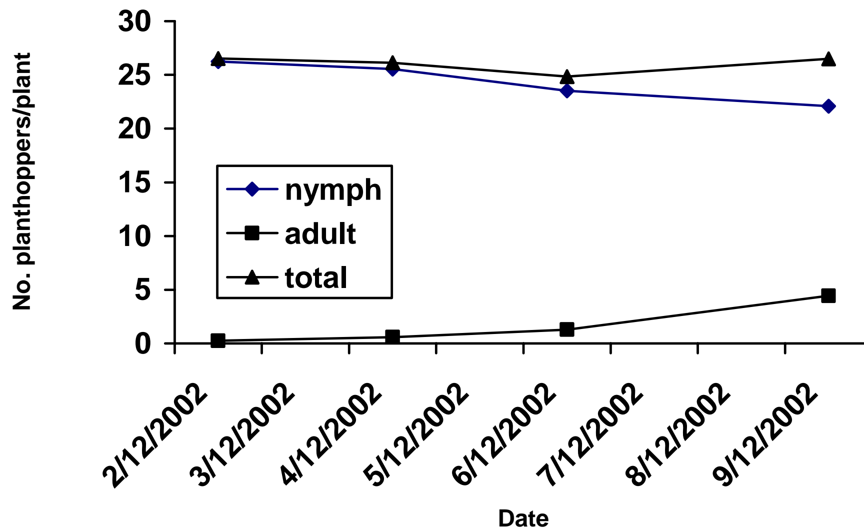

Figure 10 Planthopper numbers recorded in greenhouse-field trial 2

Arcsin percent infection in the standard clones in trial 2 was highly significantly related to long-term rating of the clones at all three inspection times in the plant and ratoon crops (Figure 11, only the final inspection of the plant and ratoon crops are shown). All of the standards rated 6 or above had levels of infection greater than 88% in both crops.

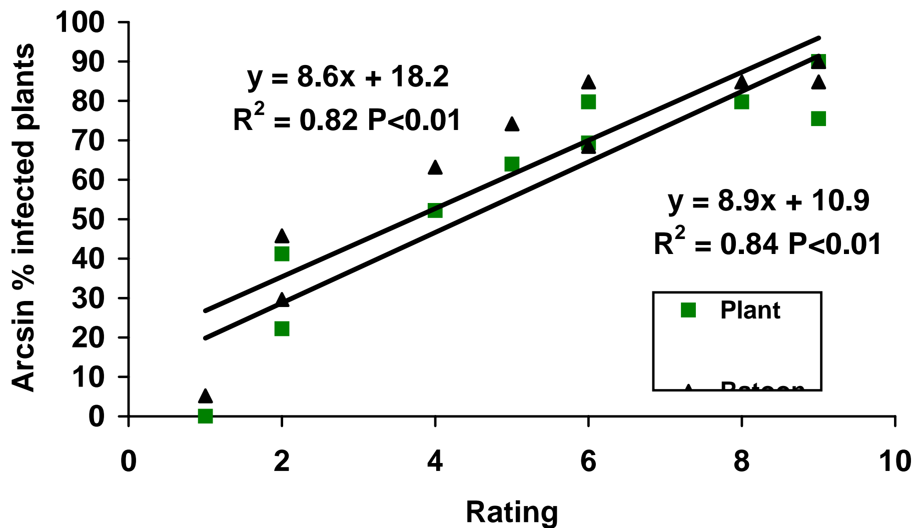

Figure 11 Regression of arcsin percent infection and long-term rating for the 10 standard clones in trial 2 in the plant and ratoon crops

The disease severity in the standard clones was also highly significantly related to long-term rating of the clones in the plant and ratoon crops (Figure 12). The intermediate rated standards (ratings 5-6) had less severe symptoms than the highly susceptible standards, even though the percent infection was high in these clones. Using this rating system there was greater discrimination among the intermediate and susceptible clones.

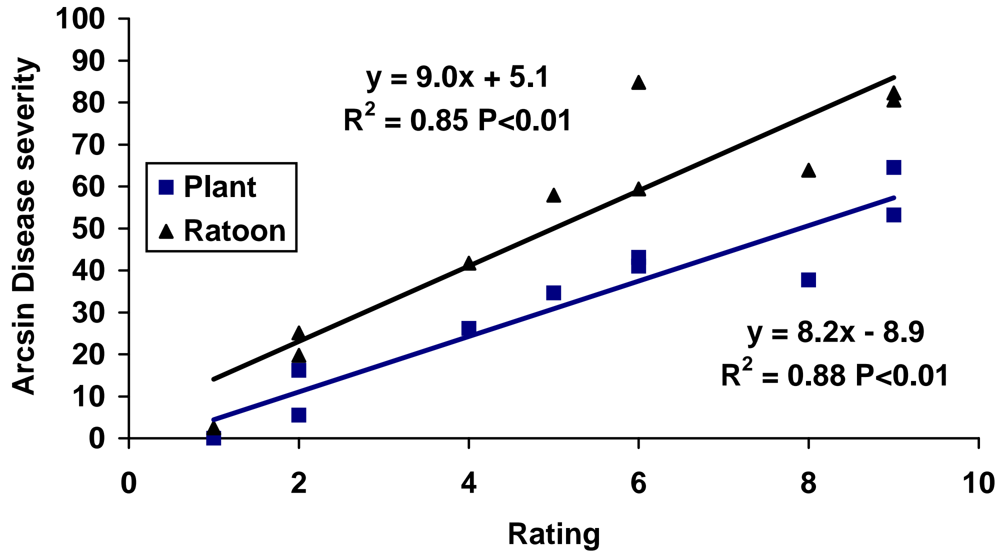

Figure 12 **Regression of disease severity and long-term rating for the 10 standard clones in trial 2**

The arcsin disease severity and calculated ratings in the plant and ratoon crops for the clones in trial 2 are shown in Table 10.

There were highly significant correlations between the arcsin percent infection and the arcsin disease severity in the plant and ratoon crops for all clones ($r = 0.91$, $P<0.01$ and $r = 0.89$, $P<0.01$, respectively, $n = 36$).

Table 10 Arcsin disease severity (DS) for clones in the plant and ratoon crops in trial 2 and the calculated ratings for the clones

Clone	First-ratoon crop		Second-ratoon crop	
	Arcsin DS	Rating	Arcsin DS	Rating
NCo310*	37.7	6	63.9	7
Pindar*	34.6	6	57.9	6
Q102*	53.2	8	80.6	8
Q110*	0.0	1	2.5	1
Q117*	41.0	7	59.4	6
Q124*	43.1	7	84.8	9
Q57*	5.5	1	19.8	2
Q71*	64.5	9	82.3	9
Q87*	16.2	3	25.1	2
Q90*	26.2	4	41.7	4
89W60	33.5	5	57.8	6
90S7069	43.1	7	83.8	9
91S7266	32.9	5	48.2	5
91S7347	26.5	4	40.0	4
92S2423	25.5	4	40.5	4
92S250	38.6	6	59.0	6
92S330	40.4	6	72.2	7
BN82-2384	28.2	5	87.0	9
BN87-3009	27.3	4	39.0	4
BN88-3347	19.6	3	40.0	4
CP51-21	42.5	7	51.5	5
Q124	47.7	8	81.2	8
Q133	47.0	7	70.7	7
Q141	19.3	3	32.4	3
Q151	32.4	5	41.5	4
Q155	28.9	5	41.5	4
Q174[Φ]	2.6	1	6.2	1
Q177[Φ]	39.1	6	68.4	7
Q189[Φ]	32.1	5	46.2	5
Q205[Φ]	32.7	5	61.6	6
Q207[Φ]	40.4	6	53.6	5
Q209[Φ]	28.7	5	47.7	5
Q212[Φ]	0	1	0	1
Q214[Φ]	37.5	6	56.0	6
Q221[Φ]	27.1	4	32.6	3
Q222[Φ]	36.6	6	54.8	6
Q63	36.5	6	52.5	5
Q71	43.2	7	67.8	7
Mean	31.1		50.0	
LSD P<0.05	13.0		16.8	

* = standard clone.

Trial 5

There were approximately 26 planthoppers per plant in trial 5, which was similar to the number of planthoppers in greenhouse-field trial 2. 20% of the adult planthoppers and 16% of the nymphs were infected with FDV as measured by RT-PCR (Table 4). The majority of the planthoppers were nymphs at the start of the trial, but the numbers of adults and nymphs were similar at the end of the inoculation period (Figure 13).

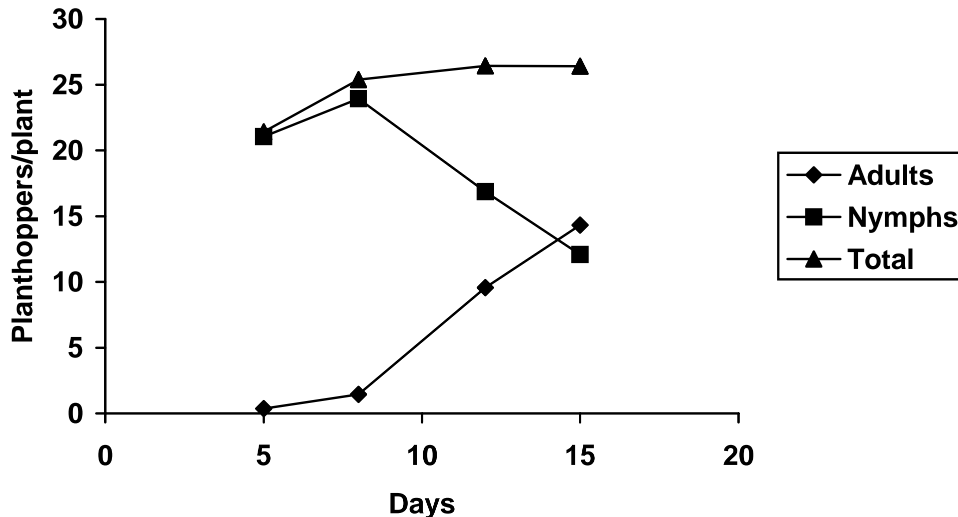

Figure 13 Number of planthoppers recorded in greenhouse-field trial 5

The number of adult planthoppers per plant was significantly related to the height of the plants, but there was no relationship between the resistance of the standard clones and number of planthoppers per plant.

The arcsin disease severity and ratings obtained from the plant and ratoon crops for all clones are shown in Table 11.

The regressions of arcsin percent infection and arcsin disease severity against long-term rating for the standard clones in the plant and ratoon crops were both highly significant (Figures 14 and 15). The regression coefficients were higher for both arcsin percent infection and arcsin disease severity in the plant crop compared to the ratoon crop. Infection and disease severity in the resistant and intermediate standards in the ratoon crop were higher than would be predicted by their long-term ratings. There was a highly significant relationship between the arcsin disease severity obtained for the 35 clones common to both trials 2 and 5-1 in the plant crops (Figure 16).

Table 11 **Arcsin disease severity (DS) for clones in the plant and first-ratoon crops of trial 5 and the calculated ratings for the clones**

Clone	First-ratoon crop		Second-ratoon crop	
	Arcsin DS	Rating	Arcsin DS	Rating
NCo310*	41.4	6	82.5	7
Pindar*	30.6	4	76.1	7
Q102*	62.9	9	90.0	8
Q110*	0.0	1	2.5	1
Q117*	41.4	6	80.4	7
Q124*	45.5	7	90.0	8
Q57*	10.9	2	25.8	1
Q71*	67.7	9	90.0	8
Q87*	30.0	4	66.5	5
Q90*	22.8	3	38.5	2
89W60	45.0	7	90.0	8
90S7069	50.0	7	90.0	8
91S7266	11.6	2	26.0	1
91S7347	39.5	6	67.5	6
92S206	36.6	5	78.5	7
92S2423	25.6	4	34.2	2
92S250	43.2	6	82.0	7
92S330	46.8	7	90.0	8
BN82-2384	58.7	9	90.0	8
BN86-3012	19.9	3	33.5	2
BN87-3009	26.3	4	41.8	3
BN88-3347	21.7	3	41.3	3
CP51-21	32.9	5	64.6	5
Q133	54.7	8	90.0	8
Q141	23.8	3	29.6	1
Q155	28.9	4	39.8	2
Q174[φ]	2.5	1	5.1	1
Q177[φ]	32.4	5	90.0	8
Q189[φ]	27.8	4	43.1	3
Q197[φ]	23.8	3	44.3	3
Q205[φ]	44.1	7	81.5	7
Q207[φ]	46.8	7	76.0	7
Q208[φ]	45.4	7	90.0	8
Q209[φ]	36.3	5	78.4	7
Q210[φ]	33.4	5	50.0	4
Q212[φ]	0.0	1	0.0	1
Q214[φ]	50.2	7	90.0	8
Q221[φ]	39.6	6	76.7	7
Q222[φ]	42.3	6	72.1	6
Q63	31.4	5	50.3	4
Mean	34.4	5.1	62.0	5.2
LSD $P<0.05$	7.5		14.3	

* = standard clone.

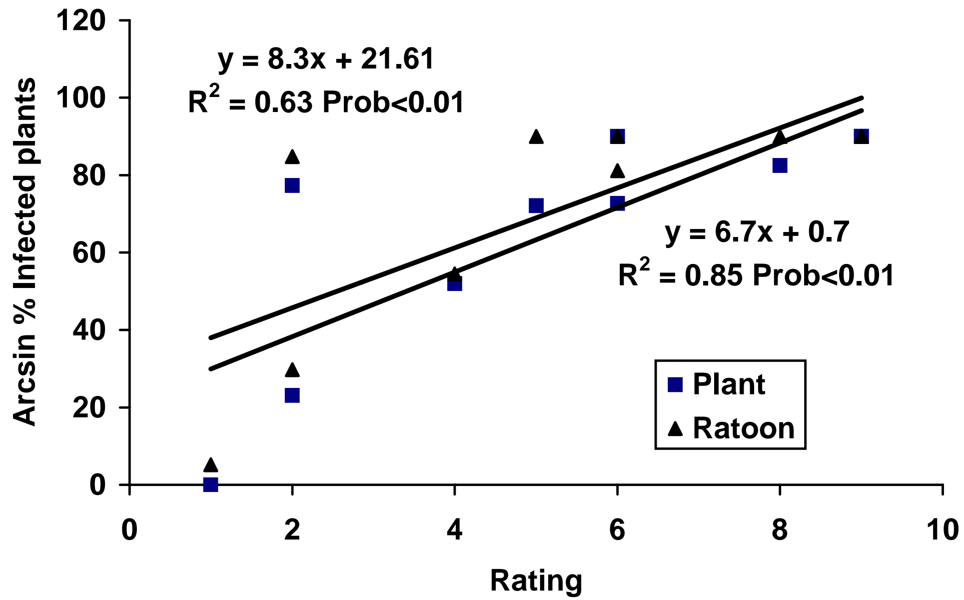

Figure 14 Regression of arcsin percent infection and long-term rating for the 10 standard clones in the plant and ratoon crops of trial 5

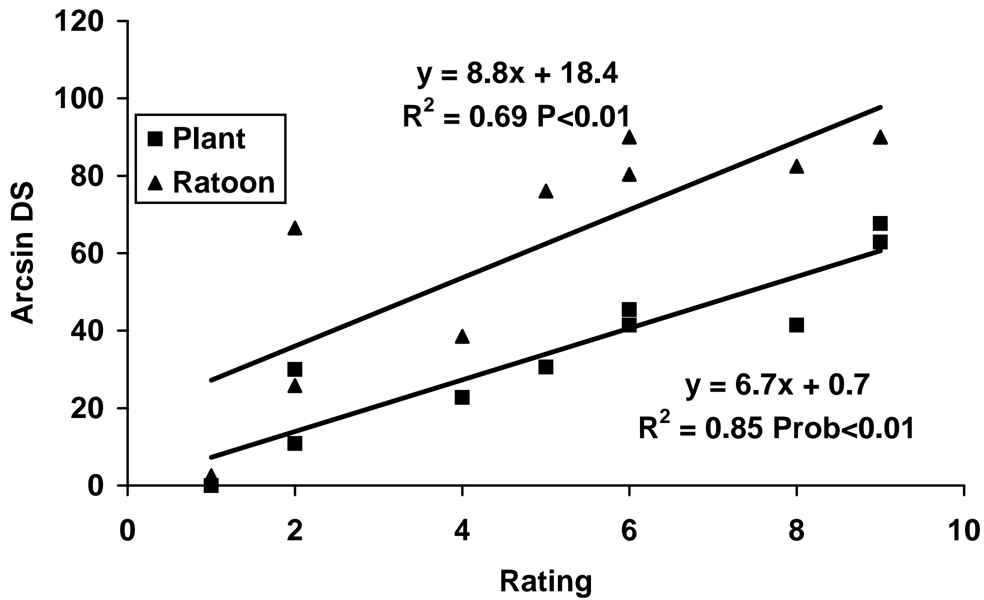

Figure 15 Regression of arcsin disease severity and long-term rating for the 10 standard clones in the plant and ratoon crops of trial 5

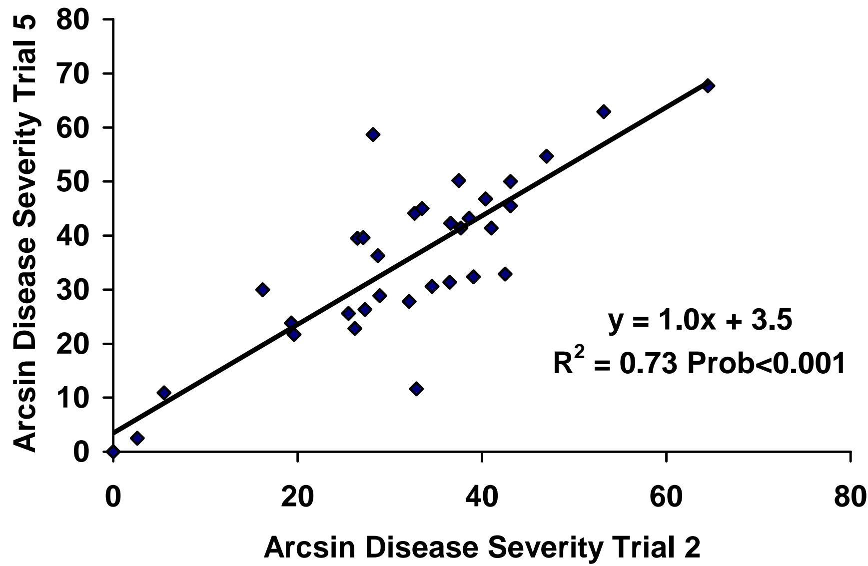

Figure 16 Comparison of the disease severity of 35 clones in the plant crops of trial 2 and trial 5-1

Trials 5-2 and 5-3

In addition to the greenhouse-field trials specified in the SRDC project, two greenhouse-field trials were established with 58 advanced clones from the BSES southern and central selection programs (trials 5-2 and 5-3). Both trials had highly significant relationships for the arcsin disease severity and long term ratings for the standard clones (Figure 17). The disease severity and ratings for the 58 clones are shown in Appendix 1.

Trial 5-2 grew rapidly in the greenhouse and was ratooned immediately after the infection period to make it easier to plant into the field. This trial developed more severe symptoms than the other trials that were not ratooned before planting.

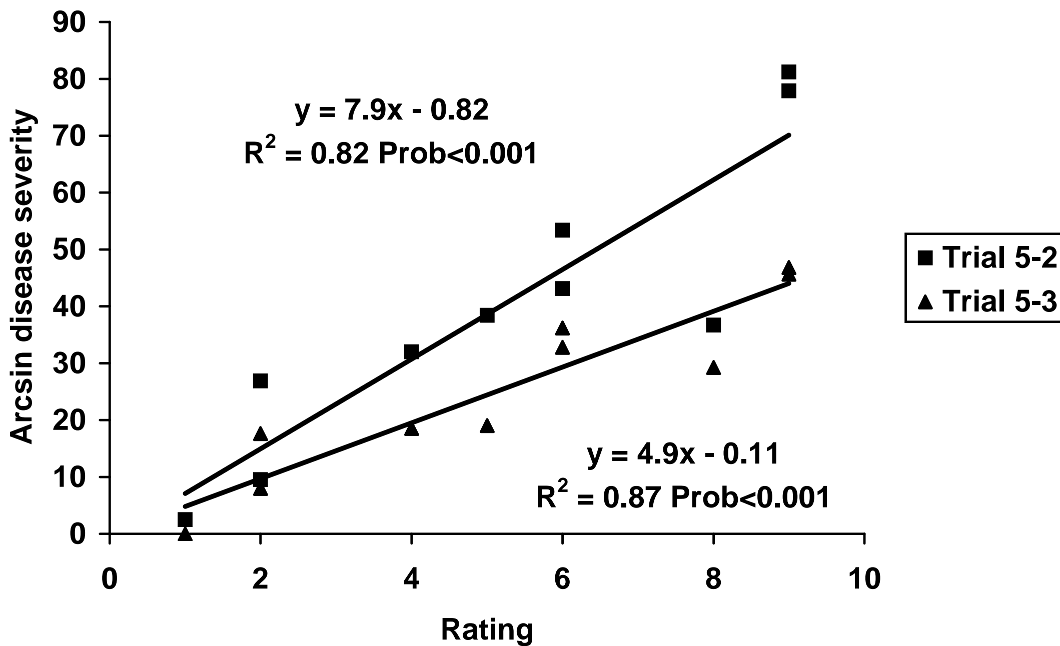

Figure 17 Regression of arcsin disease severity and long term rating for the 10 standard clones in greenhouse-field trials 5-2 and 5-3

Trials 7-1 to 7-8

Trials 7-1 to 7-8 aimed to test the best of the new screening methods for full-scale implementation in the BSES-CSIRO variety improvement program. The greenhouse-field method was the best of the new methods and it was extensively tested in trials 7-1 and 7-2 planted in autumn and 7-3 to 7-8 planted in spring. The total number of clones tested in these trials was 639 (Table 12).

The greenhouse-field technique has the potential to be conducted in both autumn and spring planting seasons, which would increase the utilisation of greenhouse space. To test the method in the autumn planting season, two trials containing a total of 95 test clones plus standards were planted in February 2004, inoculated in the greenhouse in April/May, and planted into the field in May (trials 7-1 and 7-2). The test clones in the trials came from the mapping population (Q1) that has been used to screen for quality trait loci (QTLs) as part of SRDC project CTA049. The DNA markers developed in CTA049 have been screened for associations with FLG resistance using the data provided by these trials.

Five two-replicate trials containing 482 clones (trials 7-4-to 7-8) and one three-replicate trial (trial 7-3) containing 62 clones were planted in 2004. These clones came from BSES-CSIRO selection programs and parent collections. The plants were pre-germinated in May 2004 at each of the BSES centres, transported to Woodford in August, inoculated in the greenhouse in September/October, and planted into the field. The program went as planned with no major problems. The number of planthoppers in all trials was in the range 10-15 insects per plant. The percentage of planthoppers infected with FDV was

lower than in previous years (Table 4). This resulted in lower disease levels in some trials. The clones from Mackay were infested with a moth borer that spread in the greenhouse and killed the growing point in some plants. This resulted in gaps in the trials that included clones from Mackay. Death of the primary shoot may also have interfered with FLG infection.

The autumn-planted trials were inspected in early January 2005 and the spring-planted trials were inspected in February 2005. Results of the trials are summarised in Table 12 and ratings for all clones are given in Appendix 2. The trials with the lower disease levels had the lowest correlation for the standards. The lower infection levels in trials 7-3 and 7-5 compared to trial 7-4 corresponds with the lower incidence of FDV in the batches of planthoppers used to inoculate these trials (Table 4). The correlations for the standards were highly significant ($P < 0.01$) for all but trial 7-3, where the correlation was significant only at $P<0.05$. Trial 7-3 had the most severe damage from the moth borers.

Table 12 Summary of results from eight greenhouse-field trials planted in 2004

Trial code	No. clones	No. replicates (standards)	Arcsin DS mean for standard clones	Correlation with long-term rating standards	Probability
FDGH1-04	47	3(3)	9.6	0.78	< 0.01
FDGH2-04	46	3(3)	13.6	0.82	< 0.01
FDGH3-04	62	3(3)	7.2	0.78	< 0.05
FDGH4-04	100	2(4)	14.8	0.90	< 0.01
FDGH5-04	100	2(4)	8.5	0.81	< 0.01
FDGH6-04	100	2(4)	26.0	0.88	< 0.01
FDGH7-04	100	2(4)	29.2	0.83	< 0.01
FDGH8-04	84	2(4)	33.5	0.87	< 0.01
Total	639				

This series of trials included all tentative selections for final assessment trials (FATs) in Central and Southern regions and the clones that were rated susceptible in the trials were discarded before the FAT trials were planted.

3.3.4 Technique 3. Field trials

Trial 3 Windbreak

The windbreak trial 3 was planted on 22 October 2002 and planthoppers bred in the greenhouse were released into the enclosure on the 19 December 2002. The number of planthoppers was monitored on NCo310 throughout the windbreak enclosure and compared to NCo310 in a conventional field trial. We had planned to count planthoppers on all clones in the experiment when planthopper numbers peaked in the NCo310, but this was impossible because of severe lodging.

The numbers of planthoppers in the windbreak and field experiments are shown in Figure 18. It should be noted that the counts in these trials are per stalk not per plant as in the greenhouse and poly-tunnel experiments. Also shown is the number of planthopper egg punctures per stalk.

The numbers of planthoppers were higher in the windbreak enclosure up until the 8 February compared to the conventional field trial, but numbers were similar on the 10 March. The number of planthoppers in the windbreak (0.72/stalk) was low relative to the greenhouse-field experiments. The reason for the drop in the number of planthoppers in the windbreak in March is difficult to explain. Good rainfall, high humidity and warm temperatures in February should have been ideal for planthopper survival. Egg punctures per stalk increased rapidly after the 10 February. This indicates that there was significant planthopper activity during February.

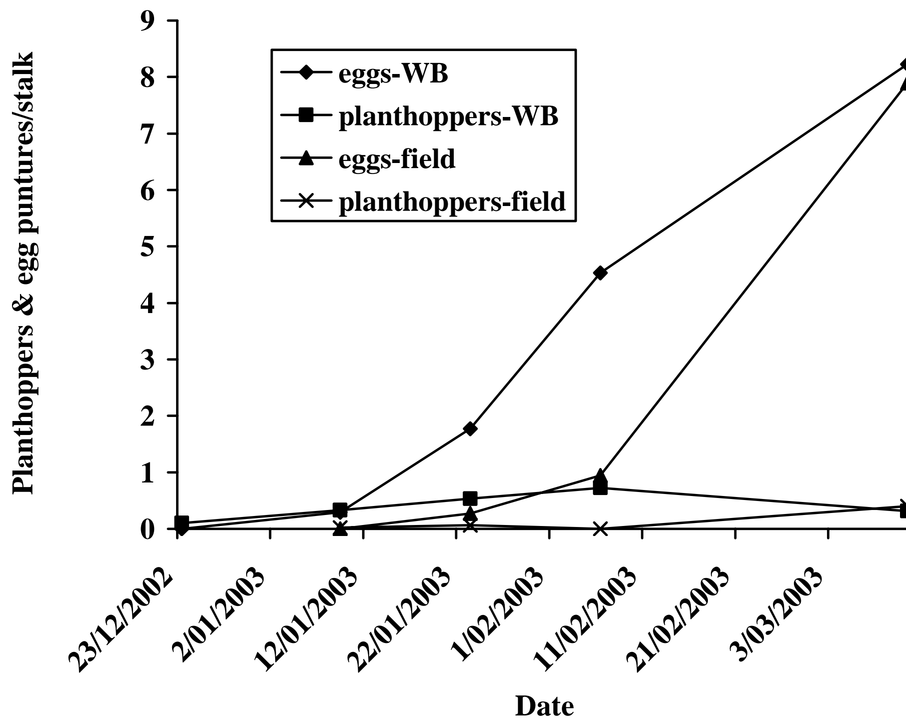

Figure 18 **Planthopper numbers and planthopper egg punctures recorded in windbreak trial 3**

The trial was ratooned in October 2003. No inspections were possible in the plant crop because of severe lodging. Three inspections in the first-ratoon crop were made, but only low levels of disease were found. T he clones that had some infection are shown in Table 13. No disease was found in the highly susceptible standard clones Q102 and Q71. All of the clones with some infection were rated intermediate to susceptible in the greenhouse-field trial. The trial was abandoned because of the low infection.

Table 13 Clones that showed some Fiji infection in the windbreak trial 3

Clone	Infected plants
89W60	1
90S7069	1
92S330	1
BN82-2384	1
BN87-3009	1
Pindar	2
Q124	1
Q177[(b]	1
Q205[(b]	1
Q207[(b]	1
Q209[(b]	1

Trial 6 Field

Field trial 6 was planted with a similar design to the traditional field trials in September 2003. Test plots were planted between rows of FLG-infected cane and the infection rows consisted of alternating plots of four plants derived from systemically infected NCo310 and four plants of NCo310 infected in the greenhouse just prior to planting. Planthoppers were counted on one stalk on every plant in each plot of the 10 standard clones on seven occasions. There were no significant differences between clones for number of planthoppers per stalk on any sampling date or when the results from all sampling dates were combined.

The population of planthoppers increased up until April and then declined (Figure 19). The number of insects per stalk recorded in 2004 was 10 times higher than in previous years at Woodford.

Samples of planthoppers were collected from the infection rows and the test plant rows and were screened for FDV by RT-PCR. Only 1.6% of the insects collected from the infection row were infected and none of the insects collected from the test plant row were infected (Table 4).

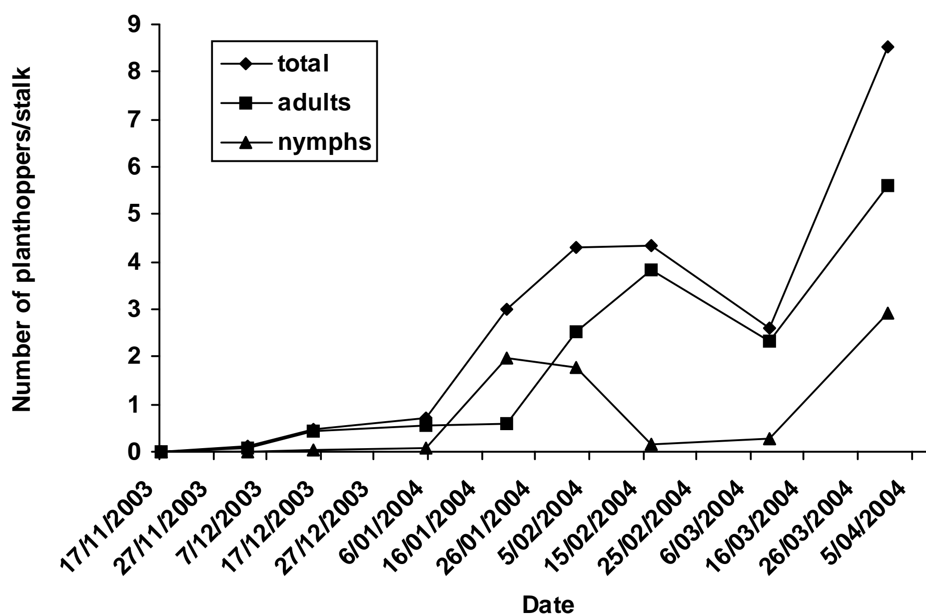

Figure 19 Planthopper numbers recorded in field trial 6

The first-ratoon crop of trial 6 was inspected in February 2005 and low levels of disease were recorded (Table 14). No disease was recorded in standard clones rated 5 and below, and the susceptible standards had low to moderate levels of disease. There was a significant regression between disease severity and the long term ratings of the 10 standard clones ($R^2 = 0.71$ $P<0.01$, Figure 20). A regression analysis was also conducted for only standards with a long-term rating of greater than 4. The regression for the standards rated greater than 4 better fitted the data for these clones ($R^2 = 0.79$ $P<0.05$). The ratings given in Table 3 were calculated from the regression for standards rated greater than 4 and all clones with no infection were rated as 3 (2.5), the mid point of the range for standard clones that had no infection. The ratings in this trial must be considered unreliable because of the low level of infection.

Table 14 Arcsin disease severity (DS) for clones in the first-ratoon crop of trial 6 and the calculated ratings for the clones

Clone	Arcsin DS[a]	Rating
NCo310*	7.5	5
Pindar*	0.0	3
Q102*	26.1	9
Q110*	0.0	3
Q117*	5.4	5
Q124*	9.4	6
Q57*	0.0	3
Q71*	22.7	9
Q87*	0.0	3
Q90*	0.0	3
89W60	6.4	5
90S7069	13.3	7
91S7266	0.0	3
91S7347	0.9	3
92S206	5.8	5
92S2423	0.0	3
92S250	10.6	6
92S330	12.7	7
BN82-2384	20.6	9
BN86-3012	6.3	5
BN87-3009	8.0	6
BN88-3347	3.6	4
CP51-21	0.0	3
Q133	29.4	9
Q141	2.5	4
Q155	0.0	3
Q174[Φ]	0.0	3
Q177[Φ]	9.1	6
Q189[Φ]	3.6	4
Q197[Φ]	0.0	3
Q205[Φ]	14.0	8
Q207[Φ]	3.9	4
Q208[Φ]	8.1	6
Q209[Φ]	3.6	4
Q210[Φ]	0.0	3
Q212[Φ]	0.0	3
Q214[Φ]	5.9	5
Q221[Φ]	2.9	4
Q222[Φ]	6.0	5
Q63	5.2	5
Mean	6.3	4.9
LSD Prob.<0.05	13.6	

[*] = standard clone.

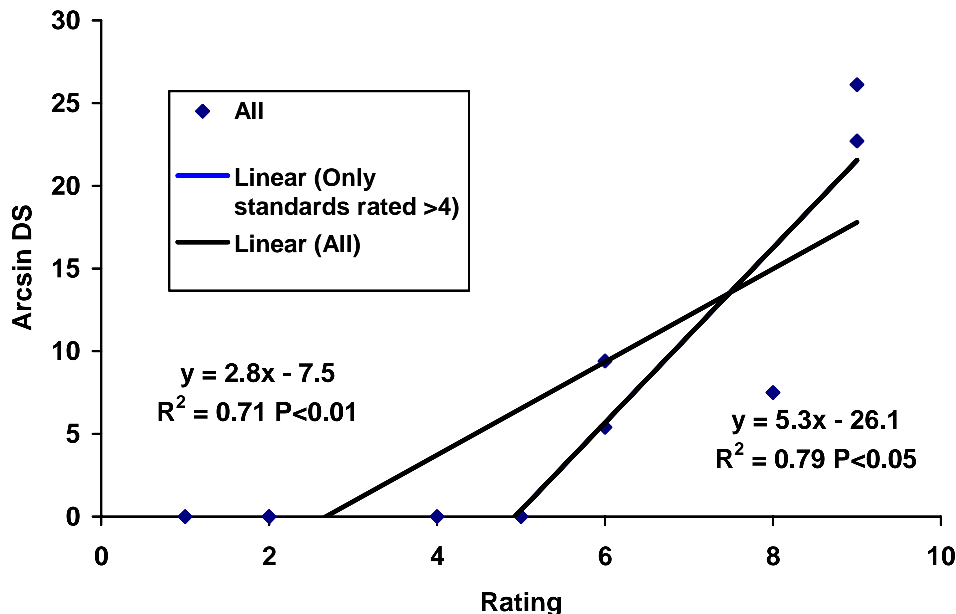

Figure 20 Regression of arcsin disease severity and long-term rating for the 10 standard clones in field trial 6

3.3.5 Correlation of disease severity rating from greenhouse-field, poly-tunnel and field trials

The correlations between the disease severity for clones that were common to trials 2 greenhouse-field, 4 poly-tunnel, 5-1 greenhouse-field and 6 field were all highly significant (Table 15).

Table 15 Correlations among disease severity of clones tested in trials 2 greenhouse-field, 4 poly-tunnel, 5-1 greenhouse-field and 6 field (number of clones included in the comparison is shown in brackets)

	Trial 2 Greenhouse-field	Trial 4 Poly-tunnel	Trial 5-1 Greenhouse-field
Trial 4 Poly-tunnel	0.7045 $P < 0.0001$ (32)		
Trial 5-1 Greenhouse-field	0.8548 $P < 0.0001$ (35)	0.8034 $P < 0.0001$ (37)	
Trial 6 Field	0.6418 $P < 0.0001$ (35)	0.6148 $P < 0.0001$ (37)	0.749 $P < 0.0001$ (40)

3.3.6 Effect of clone on acquisition of FDV

Planthoppers were bred for one generation on 10 plants of FLG-infected NCo310 and WD1. The clone NCo310 is highly susceptible to FLG and, when it is infected, it is severely stunted and can die, particularly when plants are stressed or ratooned. The clone WD1 is resistant to FLG and, when it becomes infected, is highly tolerant showing galls but no stunting. WD1 has been used in the past as the infection rows in FLG resistance field trials because it is easy to maintain a source of infected plants. It is difficult to maintain a source of FLG-infected NCo310 because plants do not produce stalks suitable for propagation and plants often die.

Samples of the planthoppers were collected from each plant and assayed for FDV by RT-PCR. The percentage of planthoppers infected with FDV was significantly higher (paired t-test, $P<0.01$) when they were bred on NCo310 (21.1% positive, total of 102 planthoppers) compared to WD1 (7.6% positive, total of 120 planthoppers). This result confirmed similar findings from research conducted in the CRC-Tropical Plant Protection project.

3.3.7 Variation in FDV incidence in different sexes and life stages of the planthopper

Eleven samples of planthoppers bred on FLG-infected NCo310 were screened for FDV and the average incidence of FDV infection was 21% (Table 4, range 0-60%). The different sexes, wing forms and life stages did not significantly differ in their incidence of FDV (paired t-tests).

3.4 Discussion

The ideal infection level in FLG resistance trials would give the maximum difference between resistant and susceptible standards. In the review of the field FLG resistance trials conducted over the last 28 years, the optimum infection level was when NCo310 had between 60-80% disease. Only 12 of 68 successful trials (18%) conducted during the period fell within this range. A further 11 (16%) trials had 40-60% and 12 trials (18%) 80-100% infection in NCo310, which were considered to give acceptable discrimination between clones. This analysis does not include the trials that were abandoned because of either low or high infection.

During the period under review, trials in 11 of the 28 years were abandoned because of either low infection (9 years) or high infection (2 years). This means that more than 66% of all trials failed or gave suboptimum results. The low probability of obtaining successful FLG resistance ratings from field trials could leave the industry vulnerable to a future outbreak of the disease because insufficient information is available about the resistance of clones. Breeding for resistance to the disease is also affected, because decisions on crosses to make for districts at risk from FLG are based on poor information about the resistance of parent clones.

The poly-tunnel technique gave variable disease levels. In two trials, the level of disease was high to moderately high, but in one trial there were only low levels of disease, because of poor survival of the planthoppers. In one poly-tunnel trial, two replicates had high levels of disease, while the other two replicates had low levels of disease. The poly-tunnels were exposed to greater fluctuations in temperature and the plants had populations of planthopper predators, including ladybirds and spiders. The poly-tunnel technique is labour intensive, because all operations are performed by hand. The poly-tunnel technique could be used to screen clones for resistance to FLG, but the problems with the technique listed above make it less favourable than the greenhouse-field method.

The greenhouse-field method gave high infection levels in most trials. When trials were rated with the traditional method based on percent infected plants, the regressions for this character and long-term rating of the standards was significant, but the discrimination between intermediate and susceptible clones was poor because of the high levels of disease. A disease severity rating was developed that combined the percentage of infected plants and the severity of disease symptoms. This rating was highly significantly related to the long-term rating in all but one of 12 trials, and the relationship was stronger in the plant crop than the ratoon crop. The disease severity rating gave better discrimination between intermediate and highly susceptible clones. This is important because this is the range where decisions are made on whether to retain or discard clone. Traditional field trials were rated in the first-ratoon or second-ratoon crops.

In field trials, there is no control on when disease infection occurs; therefore, it is difficult to compare disease severity in clones that may have been infected at different times. With the greenhouse-field method, the time of infection is controlled to a 2-week period and it was able to give reliable ratings 6 months after planting the plant crop. This has allowed ratings to be obtained before BSES-CSIRO selection meetings which are held in February-March each year. The ratings obtained from the greenhouse-field trials have already been used in these meetings to make decisions on the future of clones.

The greenhouse-field technique was highly repeatable across years and correlated well with ratings obtained from both the poly-tunnel and field trials for more than 30 clones that were included in the developmental trials. Because there is less variation in the greenhouse-field technique than in traditional field trials, reliable ratings can be obtained with fewer replicates, which will assist with applying the technique earlier in the selection program.

A plan for implementation of the greenhouse-field technique was developed in consultation with BSES-CSIRO variety improvement staff. The plan involves testing clones for regions affected by FLG before they are planted into the final assessment trials (FATs). Susceptible clones will be discarded before they are planted into the FATs. Clones would be tested again when tentative selections are made after the harvest of the plant crop of the FATs. Advanced selections from regions with no FLG, parent clones and clones imported from overseas will also be tested. The testing scheme is described in detail in Appendix 6.

The scheme was thoroughly tested in trials 7-1 to 7-8, which included 639 clones from all selection programs in Queensland and NSW. Approximately 10% of clones propagated for FATs in Central and Southern regions were rated susceptible to FLG in trials 7-4, 7-5

and 7-6. These clones were discarded before the FAT trials were planted, and the trials can either be planted with 10% fewer clones with no effect on the selection of clones to the next stage or clones could be selected to replace the clones that were discarded. In effect, this will either reduce the cost of conducting the FATs by 10% or allow more clones that have potential to be selected to be included in the trials, which should increase the chances of finding higher yielding clones.

Controlling the inoculum pressure is essential for disease screening techniques (Tuite 1969). With both the greenhouse-field and poly-tunnel techniques, the infection pressure can be controlled to some extent by controlling the number of planthoppers added to the chambers and by managing the acquisition of the virus in the greenhouse. Achieving the required number of planthoppers of known virus status is a challenge, but is far more certain than relying on natural field populations that fluctuate wildly between seasons.

A better understanding of the factors that control the acquisition of the virus by the planthopper would assist with controlling inoculum pressure. The proportion of planthoppers carrying the virus after breeding on FLG-infected NCo310 varied from 0% in trial 7-5 to 60% in trial 1-1. Producing large numbers of planthoppers on FLG-infected plants requires careful management. Heavily diseased plants are severely stunted and the voile cages used to contain the planthoppers and high numbers of planthoppers further restrict plant growth. Mites and thrips are also present in the greenhouse and can further damage the plants because the diseased plants cannot grow away from the damage. Pesticides cannot be used to control mites and thrips because they may kill the planthoppers. Coordinating the test plants and the planthoppers to be available at the same time is also challenging. Although breeding the planthoppers will have its challenges, we were able to successfully breed planthoppers in three years and with careful management we see no major problems in supplying planthoppers for future FLG resistance trials.

Planthoppers bred on the FLG-infected tolerant clone WD1 were three time less likely to be infected with FDV than planthoppers bred on FLG-infected NCo310. WD1 is easy to grow, because it shows no stunting from FLG, which is the reason staff specifically selected it for use as infection rows in FLG resistance trials in the past. However, to get the same infection pressure with planthoppers bred on WD1, you would have to use three times the number of planthoppers. For field trials where the natural population of the planthoppers may be low, the use of WD1 as infection rows would exacerbate the problems of low disease transmission.

The techniques used in this project relied on scoring clones with a disease severity rating that combined percent infection and severity of symptoms. Previous research on insectary/glasshouse methods for screening for FLG resistance used the time for symptom development as the character on which clones were rated (Daniels *et al.* 1969). Measuring time to show symptoms is labour intensive, because the plants have to be inspected regularly (daily at the peak of symptom development). After extensive testing in Australia, the insectary/glasshouse method was abandoned because it did not correlate well with field reaction (Ledger and Ryan 1977). Our disease severity rating gave a strong correlation with long-term ratings for the standard clones. We attempted to ‘harden’ the plants by growing them for 3-4 months on an open bench for the greenhouse-field technique before the planthoppers were introduced. We believe that this assisted in

making the reaction of the standards more like their field reaction. The greenhouse-field technique is relatively easy and quick to rate, and our results showed that one rating at 5-6 months in the plant crop is sufficient.

The greenhouse-field technique can be conducted in autumn as well as spring planting seasons. This will increase the utilisation of greenhouse space. Planting material for an autumn planting would be required in January, which does not coincide with availability of propagation material in routine selection programs. Autumn trials may be best used for special projects where planting material can be specifically grown for the trials.

Previous research has suggested that planthopper preference plays a significant role in varietal resistance to FLG (Dhileepan and Croft 2001; Dhileepan *et al.* 2003). None of the greenhouse-field, poly-tunnel or field trials conducted in this project displayed any relationship between planthopper numbers on different clones and resistance to FLG. Bull (1977, 1981) found that NCo310 was highly favourable to planthoppers in the field, but we found no significant differences in planthopper numbers on this or other clones in field trial 6. Four blocks of NCo310 were planted at Woodford in an attempt to increase the natural population of planthoppers but they appear to have had little effect. The windbreak set up for trial 3 also had little effect on increasing the population of planthoppers in the field. Bull (1977, 1981) reported populations in excess of 200 planthoppers per stalk, whereas the highest population recorded at Woodford was 8 planthoppers per stalk. We believe that the factors that combined to allow the planthoppers to reach such high numbers in Bundaberg have not been fully explained.

There was a relationship between plant height and planthopper preference in two trials. Plant height can vary in greenhouse trials, depending on rate of germination of plants and rate of early growth. To reduce the influence of plant height, the plants for the greenhouse-field trials were trimmed once or twice to even out the height of the plants. This does not completely eliminate differences, but should reduce this source of variation.

Our results suggest that planthoppers do not selectively feed on susceptible clones, but it cannot rule out that feeding behaviour is different on susceptible and resistant clones. Research in the CRC Tropical Plant Protection project is looking at feeding behaviour using an electronic penetration graph (EPG) device. EPG uses a gold wire attached to the insect. An electrical current is passed through the plant and when the insect probes the leaf it completes a circuit. The electrical resistance from probing in different tissues is recorded on a computer and the wave forms can be related to feeding behaviour of the insect. This research may show whether insect feeding behaviour is related to resistance to FLG.

4.0 OBJECTIVE 3 – INTEGRATION OF THE NEW TESTING PROCEDURES INTO THE BSES-CSIRO VARIETY IMPROVEMENT PROGRAM SO THAT NEW FLG-RESISTANT CULTIVARS ARE DELIVERED TO INDUSTRY

The BSES-CSIRO Variety Improvement Program has three main stages of selection:

- Stage 1. Progeny Assessment Trials (PAT);
- Stage 2. Clonal Assessment Trials (CAT);
- Stage 3. Final Assessment Trials (FAT).

Between the CAT and the FAT there is a propagation stage with approximately 140 clones that are tentatively selected for planting in FATs. The greenhouse-field technique will allow all 140 tentative selections for FATs for central and southern selection programs to be screened in the year of propagation, so that susceptible clones can be discarded before the FAT is planted.

Advanced selections from all regions will be screened to confirm the ratings obtained in the earlier test for central and southern clones and to obtain ratings for advanced clones from Burdekin, Herbert and Northern programs. A selection of parent clones and foreign introductions will also be screened for resistance. It is important to know the resistance of parent clones, because the ratings are used to restrict crosses to reduce the proportion of susceptible progeny produced. Foreign clones are imported for use as parents. The MOUs for exchange of clones with other countries provides for BSES to screen foreign clones for resistance to FLG in exchange for the other countries rating our clones for smut and other diseases endemic in their country.

The number of clones to be screened at each stage of the selection program is shown in Table 16.

This program of testing was developed in consultation with BSES-CSIRO variety improvement staff. The program was tested in 2004/05 and everything went according to plan. The second year of testing with the new program is currently in progress and all clones have been propagated at the different centres, shipped to Woodford and will be inoculated and planted in coming months. Variety Improvement staff in NSW and Southern and Central Queensland have been particularly supportive of the new program. They have appreciated receiving timely ratings after a long period of receiving no ratings.

Table 16 Proposed number of clones for FLG resistance trials from each stage of selection from the five regions in Queensland.

Selection program	Stage of selection	No. of clones	No. of replicates
Southern (including NSW)	Tentative FATs	140	2
	Advanced selections	15	3
Central	Tentative FATs	140	2
	Advanced selections	10	3
Burdekin	Advanced selections	5	3
Herbert	Advanced selections	5	3
North	Advanced selections	5	3
Foreign introductions (to be sent from Bundaberg)		50-60	2
Parents (to be sent from Meringa and Bundaberg)		60-80	3
Total 2 replicate		330-340	
Total 3 replicate		100-120	

5.0 OUTPUTS

This project has developed a new greenhouse-field technique for rating clones for resistance to FLG that will provide reliable and timely ratings for the BSES-CSIRO variety improvement program. The technique has replaced a field-based test that often failed because of low or high numbers of the planthopper vector of FDV. The new technique has been documented in a procedures manual (Appendix 6) and has been adopted by the BSES-CSIRO variety improvement program as the method for screening FLG resistance.

Research projects in the CRC for Sugarcane Industry Innovation through Biotechnology (CRC-SIIB) have used the ratings for clones from this project and from past BSES trials to investigate association DNA mapping for FLG resistance. Two trials were specifically conducted with clones from the DNA mapping population Q1 and the ratings were used to screen for DNA markers for FLG resistance using this population. Initial results are promising for both DNA marker techniques, and follow up research is planned in the CRC-SIIB.

The project found no relationship between planthopper preference and resistance to FLG. This finding eliminates one potential mechanism of resistance to FLG. Future research can focus on other potential resistance mechanisms, such as planthopper feeding behaviour on different clones and resistance within the plant to multiplication or movement of the virus.

Acquisition of FDV by the planthoppers was strongly influenced by clone in this research. This supports similar findings in the associated CRC-TPP project. The CRC-TPP project

has found a strong relationship between acquisition of the virus and resistance to the disease. Only FDV-infected intermediate to susceptible clones should be used to breed planthoppers for FLG resistance trials. Although it has some problems, the recommend clone for breeding FDV-infected planthoppers is NCo310. Further research on understanding the factors that control acquisition of FDV by planthoppers from different clones is warranted. The ability of the planthopper to acquire the virus from a clone could have a large influence on the development of epidemics of the disease.

6.0 OUTCOMES

The developmental trials in this project included advanced clones from the BSES-CSIRO variety improvement program. The ratings obtained for these clones in the project have been used to make decisions on the future of these clones and the ratings have been included in applications to Queensland Department of Primary Industries and Fisheries for approval of Q189[Φ], Q197[Φ], Q205[Φ], Q207[Φ], Q208[Φ], Q209[Φ], Q221[Φ] and Q222[Φ]. Without the ratings obtained in this project, these cultivars would not have met the requirements for approval established by the QDPI&F. The project removed a block that may have delayed the release of these new cultivars. Delaying the release of a new cultivar that may have a higher yield potential can cost the industry many millions of dollars.

All ratings for clones in the trial have been entered into the BSES-CSIRO database, SPIDNet, and are available to BSES-CSIRO breeding and research staff. SPIDNet is a valuable resource that is used for selection decisions and in making decisions on crosses to be made in the breeding program. A significant benefit of the new technique is that results can be obtained during the propagation stage for the FAT trials and any susceptible clones can be discarded before the FATs are planted. This will improve the efficiency of these trials by eliminating clones that will eventually have to be discarded.

The procedure manual for the new greenhouse-field technique (Appendix 6) has been developed in consultation with the BSES-CSIRO variety improvement and pathology staff to maximise the implementation of the technique. The technique performed well in a full scale trial in 2004/05 and everything is progressing smoothly for establishment of the 2005/06 trial program. Staff at BSES Woodford have been involved in the research into developing the greenhouse-field technique and, therefore, will be able to implement it into the routine screening program.

BSES-CSIRO variety improvement staff, Productivity Service staff and grower groups have visited Woodford to look at the trials and these visits have given them confidence in the new technique. The confidence of research staff and growers is essential for their acceptance of the decisions to discard or proceed with a clone because of its rating for resistance to FLG.

7.0 RECOMMENDATIONS

1. The greenhouse-field technique for rating clones for resistance to FLG should be adopted as the standard method in the BSES-CSIRO variety improvement program.

2. Further research into the mechanisms of resistance to FLG should be conducted to better understand the interactions between the planthopper, the virus and the plant. Identifying the genes responsible for resistance may provide an understanding of the mechanisms of resistance.

8.0 PUBLICATIONS

Croft BJ. 2003. Fiji leaf gall resistant varieties. *BSES Bulletin* Spring 2003: 6-7 (Appendix 3).

Croft BJ, Dhileepan K, Ridley A and Smith G. 2003. Cultivar resistance to *Fiji disease virus* and/or to *Perkinsiella saccharicida,* the planthopper vector of the virus. Proc. International Society of Sugar Cane Technologists Plant Pathology Workshop Baton Rouge, Louisiana.

Croft BJ, James AP, Ridley AW and Smith GR. 2004. Developing methods to screen sugarcane varieties for resistance to Fiji leaf gall. Proc. Aust. Soc. Sugar Cane Technol., 26 (CD-ROM), 11 pp. (Appendix 4.

9.0 REFERENCES

Bull RM. 1977. Leafhopper populations on some sugar-cane varieties. *Proc. Qld Soc. Sugar Cane Technol.* 44: 83-87.

Bull RM. 1981. Population studies on the sugar cane leafhopper (*Perkinsiella saccharicida,* Kirk) in the Bundaberg district. *Proc. Aust. Soc. Sugar Cane Technol.* 3: 293-303.

Daniels J, Husain AA, Hutchinson PB and Wismer CA. 1969. An insectary method for testing sugar cane varieties for resistance to Fiji disease. *Proc. Int. Soc. Sugar Cane Technol.* 13: 1100-1106.

Dhileepan K and Croft BJ. 2001. Resistance to Fiji disease in sugarcane: role of cultivar preference by planthopper vector *Perkinsiella saccharicida* (Hemiptera: Delphacidae). *J. Econ. Entomol.* 96:148-155.

Dhileepan K, Greet A, Ridley A, Croft BJ and Smith GR. 2003. Fiji disease resistance in sugarcane: Relationship to cultivar preference in field populations of the planthopper vector *Perkinsiella saccharicida. Ann. Appl. Biol.* 143: 375-379.

Ledger PE and Ryan CC. 1977. Screening of sugar-cane varieties for resistance to Fiji disease in Queensland - The insectary-glasshouse method. *Proc. Qld Soc. Sugar Cane Technol.* 44: 79-82.

Leverington KC, Egan BT and Hogarth DM. 1977. Breeding for resistance and other possible methods of control of Fiji disease at Bundaberg. *Proc. Qld Soc. Sugar Cane Technol*. 44: 93-95.

Ridley AW, Dhileepan K, Johnson KN, Allsopp PG, Nutt KA, Walter GH and Croft BJ. 2006. Is the distribution of Fiji leaf gall in Australian sugarcane explained by variation in the vector *Perkinsiella saccharicida*? *Australas. Plant Pathol.* (in press)

Smith GR. 2000. Fiji disease. In: Rott P, Bailey RA, Comstock JC, Croft BJ and Saumtally AS (eds). *A Guide to Sugarcane Diseases*. CIRAD and ISSCT, Montpellier.

Steindl DRL, Hughes CG and Hogarth DM. 1977. Developments in Fiji field-trial design. *Proc. Qld Soc. Sugar Cane Technol.* 44: 75-77.

Tuite J. 1969. *Plant Pathological Methods*. Burgess Publishing Co., Minnesota.

APPENDIX 1 - Arcsin disease severity and rating for clones in trials 5-2 and 5-3.

Trial 5-2

Clone	Plant	
	Arcsin DS[a]	rating
NCo310*	36.7	5
Pindar*	38.4	5
Q102*	81.2	9
Q110*	2.5	1
Q117*	53.4	7
Q124*	43.1	6
Q57*	9.5	1
Q71*	77.9	9
Q87*	26.9	4
Q90*	32.0	4
QC90-823	17.3	2
QC91-423	23.6	3
QC92-1187	24.8	3
QC92-1188	55.2	7
QC92-1216	25.6	3
QC92-1287	49.7	6
QC92-1644	28.5	4
QC92-300	40.9	5
QC92-619	44.9	6
QC92-768	30.8	4
QC92-778	36.2	5
QC92-804	33.7	4
QC92-928	67.9	9
QC93-1188	43.4	6
QC93-1210	6.2	1
QC93-1594	34.4	4
QC93-1952	26.3	3
QC93-455	15.0	2
QC93-724	11.5	2
QC93-889	84.2	9
QC93-894	66.1	8
QC93-896	78.9	9
QC94-2174	65.5	8
QC94-2249	30.0	4
QN84-3588	44.2	6
QN84-3672	42.3	5
QN85-1802	15.7	2
QN87-9008	39.9	5
QS90-6001	28.8	4
Mean	38.8	4.9

Trial 5-3

Clone	Plant	
	Arcsin DS[a]	**rating**
NCo310*	29.3	6
Pindar*	19.0	4
Q102*	45.7	9
Q110*	0.0	1
Q117*	32.8	7
Q124*	36.2	7
Q57*	8.0	2
Q71*	46.8	9
Q87*	17.6	4
Q90*	18.5	4
Q183[(b]	0.0	1
QA88-1399	19.8	4
QC86-501	32.9	7
QN88-12	13.9	3
QN90-6006	29.1	6
QS87-7121	8.4	2
QS88-7073	44.6	9
QS88-7205	8.4	2
QS88-9095	0.0	1
QS88-9323	0.0	1
QS89-7124	10.6	2
QS89-8124	26.6	5
QS90-6002	19.3	4
QS90-6006	8.4	2
QS90-7085	2.6	1
QS90-7146	25.6	5
QS90-7187	32.3	7
QS93-2188	25.5	5
QS93-2272	15.2	3
QS93-2613	42.8	9
QS93-286	29.1	6
QS93-298	20.2	4
QS94-18	70.7	9
QS94-2329	23.1	5
QS94-2395	36.3	7
QS94-2614	14.7	3
QS94-2641	30.4	6
QS94-91	8.7	2
QS94-930	34.2	7
Mean	22.8	4.6

APPENDIX 2 - Ratings for clones in trials 7-1 to 7-8

FDGH1-04	Rating	FDGH2-04	Rating	FDGH3-04	Rating
NCo310	5	NCo310	5	NCo310	9
PINDAR	4	PINDAR	3	PINDAR	7
Q102	9	Q102	9	Q110	1
Q110	1	Q110	2	Q117	5
Q117	9	Q117	4	Q124	3
Q124	9	Q124	6	Q57	1
Q57	1	Q57	2	Q71	9
Q71	9	Q71	9	Q87	5
Q87	4	Q87	4	Q90	1
Q90	1	Q90	3	92C1187	1
95N9294	3	74C42	3	93C896	9
95N9262	1	95N9228	7	92C1287	5
95N9381	1	95N9342	2	92C1188	9
95N9314	5	95N9200	3	94C2249	9
95N9280	1	95N9213	5	Q186	5
95N9240	9	95N9313	3	Q187	1
95N9345	6	95N9231	2	Q200	1
95N9373	2	95N9179	2	92N19	1
95N9206	2	95N9268	2	Q195	1
95N9183	4	95N9265	2	Q215	5
95N9296	1	95N9278	2	MIDA	1
95N9270	6	95N9380	2	81S546	4
95N9321	2	95N9267	2	Q208	1
95N9261	7	95N9237	2	91H7055	1
95N9349	2	95N9187	5	92H1548	1
95N9246	1	95N9219	2	ARGOS	8
95N9224	1	95N9230	6	84C621	1
95N9192	2	95N9368	3	Q194	1
95N9301	1	95N9360	4	TELLUS	1
95N9259	1	95N9365	2	89A3305	1
95N9212	5	95N9199	4	93A2795	1
95N9361	6	95N9275	2	93A2179	1
95N9247	4	95N9375	3	Q183	1
95N9225	2	95N9266	3	Q216	5
95N9189	2	95N9323	2	KQ98-673	4
95N9351	1	95N9393	7	Q201	1
95N9305	1	95N9344	2	Q175	4
74C42	1	95N9252	4	92N1234	1
95N9327	1	95N9369	2	92N158	9
95N9285	1	95N9338	2	Q199	9
95N9382	8	95N9322	2	92C928	9
95N9216	8	95N9289	4	92C1644	1
95N9226	9	95N9376	8	93C1210	4
95N9272	1	95N9357	7	91C423	1
95N9271	1	95N9229	6	93C1594	4
95N9198	1	95N9356	3	Q202	4
95N9282	1	95N9317	2	85N1819	3
95N9379	1	95N9250	2	BN88-3291	1
95N9258	1	95N9354	5	BN88-3108	1
95N9249	1	95N9309	4	95S208	9

95N9358	7	95N9205	2	95S2446	1
95N9383	2	95N9274	3	SP79-2313	5
95N9333	1	95N9330	2	95S2176	8
95N9311	1	95N9288	2	88S9322	1
95N9384	6	95N9208	3	BN88-3345	1
95N9207	1	95N9204	8	89C6002	4
95N9197	9	95N9211	2	Q210	5
				95S2474	1
				85S7325	1
				EMPIRE	1
				92W201	1
				79N1170	6
				94S551	1
				Q213	1
				Q203	4
				93S451	5
				90W79	3
				Q211	6
				94S2641	9
				93S2272	9
				94S930	5

FDGH4-04	Rating	FDGH5-04	Rating	FDGH6-04	Rating
NCO-310	8	NCo310	5	NCo310	7
PINDAR	7	PINDAR	5	PINDAR	6
Q102	9	Q102	9	Q102	9
Q110	1	Q110	1	Q110	1
Q117	4	Q117	6	Q117	6
Q124	5	Q124	7	Q124	7
Q57	2	Q57	1	Q57	1
Q71	9	Q71	9	Q71	8
Q87	4	Q87	3	Q87	5
Q90	4	Q90	3	Q90	4
QN96-1352	9	00C6598	1	94H2458	1
QN96-1140	1	00C1848	1	96S2015	1
QN95-599	3	00C6413	1	96S2787	1
QN93-957	1	00C6499	1	96S2817	2
QN93-928	1	00C6010	1	96S2820	2
QN93-3542	1	00C1893	1	96S429	1
QN92-158	1	00C6461	1	96S434	5
QN91-3322	1	00C6688	1	96S457	1
QN90-921	1	00C1876	1	96S6006	3
QN90-1230	9	00C6868	1	96S6176	1
QN89-109	1	00C6630	1	96S6178	5
QN88-410	4	00C1817	1	96S73	2
QA89-3567	1	00C6796	1	96S788	6
97C2509	2	BN88-3291	1	96S850	1
97C2469	4	00C6185	1	96S986	1
97C2462	4	00C6240	1	97S164	1
97C2432	1	00C6135	1	97S2014	2
97C2406	1	00C6522	1	97S2029	1
97C2393	1	00C6783	1	97S2033	1
97C2380	3	00C6588	1	97S2067	1
97C2374		00C1805	1	97S233	1
97C2372	2	00C6278	1	97S36	1
97C2360	7	00C1794	1	99S1038	4
97C2350	1	00C6435	1	99S1059	4
97C2295	1	00C6328	1	99S1077	4
97C2239	1	00C6734	1	99S1081	1
97C2195	1	00C1668	1	99S1214	1
97C2194	4	92W201	1	99S1229	1
97C2193	1	00C6760	1	99S1315	1
97C2108	3	00C6294	1	99S1319	1
97C2090	9	00C6662	1	99S1377	2
97C2066	1	00C6258	1	99S1404	1
97C2059	4	00C1792	1	99S1465	1
97C2045		00C6381	1	99S1471	1
97C1832	1	00C1659	1	99S1484	5
97C1714	1	00C1714	1	99S1495	7
97C1673	9	00C6315	1	99S1537	1
97C1470	1	00C1679	1	99S1550	4
97C1445	1	00C6754	1	99S1567	3
97C1398	4	00C6500	1	99S1572	3
97C1333	1	00C6632	1	99S2007	1

97C1313	1	00C6803	1	99S2014	1
97C1308	3	QN93-1305	1	99S2026	1
97C1270	1	QN93-4779	1	99S2071	2
97C1215	1	QN86-640	1	99S2076	2
97C1194	3	QN93-1392	1	99S2078	2
97C1189	1	00C6534	1	99S2080	6
97C1070	1	00C6050	1	99S2084	4
00C926	1	00C6364	1	99S2086	1
00C921	7	00C6375	1	99S2408	2
00C919	1	00C6140	1	99S368	2
00C876	5	00C1691	1	99S439	3
00C1653	2	00C6012	1	99S482	1
00C1651	9	00C6210	1	99S497	3
00C1644	1	00C6263	1	99S501	5
00C1632	9	00C6426	1	99S562	3
00C1624	1	00C6103	1	99S6139	1
00C1602	5	00C1693	1	99S6141	1
00C1601	4	00C6631	1	99S6151	3
00C1600	1	00C6415	1	99S6202	1
00C1590	6	00C6870	1	99S6213	1
00C1585	6	00C6789	1	99S629	2
00C1578	1	00C1788	1	99S666	4
00C1573	4	00C1765	4	99S668	1
00C1572	3	00C6307	4	99S670	3
00C1570	4	00C6004	4	99S672	2
00C1569	1	00C6230	4	99S675	4
00C1557	1	00C6034	4	99S678	6
00C1538	3	00C1699	4	99S712	1
00C1528	1	00C6382	4	99S714	4
00C1522	3	QN93-1304	4	99S715	1
00C1520	1	QN89-1659	4	99S723	7
00C1515	9	00C6376	4	99S743	7
00C1477	3	00C1716	4	99S759	1
00C1464	1	QN95-1867	4	99S809	2
00C1463	3	00C6663	4	99S826	1
00C1457	1	00C6171	5	99S905	2
00C1455	1	00C6399	5	99S909	1
00C1452	1	00C6686	5	99S983	1
00C1449	1	00C6859	5	CP92-1641	2
00C1436	1	00C6636	5	CP92-1666	1
00C1430	1	00C6611	5	CR74250	1
00C1418		00C6450	5	CR80291	5
00C1417	6	00C6610	5	D84-15	1
00C1415	3	00C6271	5	DB71-60	1
00C1413	1	00C6604	5	DB75-159	2
00C1404	5	QN93-4111	5	DB78-69	2
00C1400	1	00C6074	6	H73-5659	2
00C1374	5	00C6559	6	H75-6104	3
00C1369	1	QN89-917	6	H78-3567	2
00C1353	8	95S2176	7	N31	5
00C1327	4	00C1823	7	R84-408	4
00C1308	7	00C1672	7	R84-472	7
00C1299	5	QN88-2097	8	R84-75	1

00C1281	1	00C1674	8	R85-1102	1
00C1151	4	00C6735	8	R85-252	8
00C1149	1	00C1789	9	R85-348	1
00C1139	6	00C6478	9	R85-449	2
00C1107		00C1835	9	R85-579	5
00C1020	1	89C6002	9	R85-991	4

FDGH7-04	Rating	FDGH8-04	Rating
NCo310	4	NCo310	4
PINDAR	6	PINDAR	5
Q102	9	Q102	9
Q110	1	Q110	1
Q117	8	Q117	8
Q124	7	Q124	7
Q57	1	Q57	3
Q71	8	Q71	9
Q87	4	Q87	3
Q90	4	Q90	4
CP89-2377	3	87S7221	4
94S2682	4	90W8	5
R85-1157	3	94W18	7
B77602	1	99S2098	1
N23	4	99S2110	1
R85-1238	3	99S2111	4
H84-778	1	99S2119	6
93C1328	6	99S2167	3
95A1707	1	99S2173	1
95S932	5	99S2174	1
M96-82	2	99S2178	1
ROC14	3	99S2182	2
90S6006	6	99S2200	2
ROC15	3	99S2238	1
95A1736	1	99S2247	1
92C982	1	99S2254	1
CC8563	1	99S2317	1
M261-78	2	99S2349	2
93C599	6	99S2373	4
BBZ82-57	2	99S2425	1
B78208	2	99S2446	1
H79-7808	1	99S2528	3
BJ7015	2	99S2541	2
CC8568	1	99S2602	5
CP89-2376	1	99S2606	1
M52-78	2	99S2612	3
H80-4551	1	99S2637	1
M1551-80	1	99S2642	1
H83-7206	1	99S2645	1
CP89-2143	1	99S2648	1
KQ95-4502	4	99S2652	1
CP88-1762	1	99S2656	1
95A2313	1	99S2676	1
95S2552	9	99S2687	2
B75466	1	99S2729	1
92N903	1	99S2731	2
M2350-79	1	99S2750	5
BJ7627	1	99S2751	8
L67-771	2	99S6016	7
ROC17	6	99S6018	1
95S762	6	99S6032	3

H79-2583	1
N29	4
BJ7504	1
H78-7750	1
95S2394	5
LCP86-454	4
95S2473	5
ROC16	3
CP86-1633	1
N28	2
CP81-1405	1
85S7341	1
M1658-78	1
H78-3606	1
H87-4094	1
CC87434	1
H73-3775	3
94H2440	3
BJ8226	1
KQ95-3254	4
89N1043	1
H85-7362	1
M1246-84	1
LCP85-384	4
N30	3
92N2053	1
CP94-1340	3
95S2122	7
92N1234	1
95S6019	4
CP88-1409	1
H87-4319	1
CP88-1508	1
N26	2
ROC13	1
R85-1334	3
93C1574	1
BT74209	1
94S2167	3
HCP91-555	1
N24	2
ROC11	1
95S2617	7
ROC12	1
BJ82119	1
N25	2
R85-699	1
93C988	5
92S250	2
92N2219	1
95S520	7
CP88-1540	1
CP94-1607	3

99S6035	1
99S6037	2
99S6074	1
99S6086	2
99S6090	3
99S6092	1
99S6098	1
99S6112	1
99S6126	3
99S6133	4
99S6135	3
NSW02?1	1
NSW02?2	5
NSW02?3	1
Q167	4
QA93-1098	2
QA93-2768	1
QN86-1128	7
QN89-1727	4
QN89-1765	3
QN93-3670	3
SP77-5181	1
SP79-2233	2
SP80-1816	1
SP80-1836	1
SP80-1842	1
SP80-185	2
SP80-3280	1
SP81-1763	1
SP81-3250	2
SP83-2847	1
SP83-5073	2
SP84-1431	1
SP84-2025	3
SP85-3877	1
SP85-5077	1
SP86-155	5
SP87-344	1
SP87-365	7
SP87-396	1
TCP87-3388	1
WD1	1

99S6216	8
M1176-77	1
95S2645	7
CP94-1100	3
HCP85-845	1
88A1399	1

APPENDIX 5 - EXTENSION MESSAGE

New method to screen varieties for resistance to Fiji leaf gall

Barry Croft, BSES Principal Scientist

During the 1970s, an outbreak of Fiji leaf gall (formerly known as Fiji disease) in Southern Queensland in the variety NCo310 caused massive losses and expensive control programs were initiated just to keep the industry alive. The lesson learnt from surviving the trauma of the Southern Queensland Fiji leaf gall epidemic was that new varieties must have adequate resistance to the disease if future epidemics are to be prevented.

A new greenhouse-field technique for rating varieties for resistance to Fiji leaf gall has been developed as part of a SRDC funded project. The new technique uses planthoppers that are bred on Fiji leaf gall infected NCo310 in voile cages in a greenhouse. In each cage, up to 4000 planthoppers can be bred from 30-40 adult planthoppers within six weeks.

The test varieties are brought into the greenhouse and the virus-infected planthoppers are then released onto the test plants. About 20 planthoppers are added for every plant to be tested. After the plants have been exposed to the virus-infected planthoppers, they are planted into the field and inspected for disease symptoms six months after planting.

The new technique has been adopted as the standard method for rating new varieties for resistance to Fiji leaf gall. The rating of varieties is essential to reduce the risk of another epidemic like the one that crippled the Bundaberg district in the 1970s. Ratings obtained from the new technique have been used when approving new varieties such as Q205[Φ], Q208[Φ], Q221[Φ] and Q222[Φ].

A crucial aspect of breeding varieties resistant to Fiji leaf gall is the accurate rating of each new variety's resistance to the disease. In the past, new varieties were rated for Fiji leaf gall resistance through field trials where the test variety was planted beside infected cane. Natural populations of the planthopper (*Perkinsiella saccharicida*) were relied on to spread the disease. As with many insects, the natural populations of this insect fluctuate from season to season depending on climatic conditions and the presence of natural enemies. The planthoppers, originally from PNG, prefer long periods of high humidity, overcast showery weather and high summer temperatures - a rare combination in recent the dry years in Queensland. As a result, the populations of the planthoppers in southern Queensland have been relatively low for some time. This means that the spread of Fiji leaf gall in screening trials has also been low, making it impossible to accurately rate new varieties for resistance. The new greenhouse-field technique developed as part of the SRDC project will overcome the problem of getting reliable Fiji leaf gall resistance ratings for varieties. Growers can be confident that the new varieties they receive will have reliable ratings and highly susceptible varieties will not be released in areas still at threat from Fiji leaf gall.

APPENDIX 6 - Manual

BSES Limited

INSTRUCTION MANUAL FOR CONDUCTING FIJI LEAF GALL GREENHOUSE-FIELD RESISTANCE TRIALS

by

BJ CROFT

MN05003

Contact:
Barry Croft
Principal Research Officer
BSES Limited
90 Old Cove Rd
Woodford Q 4514
Telephone: 07 54963357
Facsimile: 07 54963266
Email: bcroft@bses.org.au

BSES Limited Publication
Manual MN05003 **August 2005**

APPENDIX 6 - Manual

Copyright © 2005 by BSES Limited

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, electronic, mechanical, photocopying, recording, or otherwise, without the prior permission of BSES Limited.

Warning: Our tests, inspections and recommendations should not be relied on without further, independent inquiries. They may not be accurate, complete or applicable for your particular needs for many reasons, including (for example) BSES Limited being unaware of other matters relevant to individual crops, the analysis of unrepresentative samples or the influence of environmental, managerial or other factors on production.

Disclaimer: Except as required by law and only to the extent so required, none of BSES Limited, its directors, officers or agents makes any representation or warranty, express or implied, as to, or shall in any way be liable (including liability in negligence) directly or indirectly for any loss, damages, costs, expenses or reliance arising out of or in connection with, the accuracy, currency, completeness or balance of (or otherwise), or any errors in or omissions from, any test results, recommendations statements or other information provided to you.

APPENDIX 6 - Manual

CONTENTS

Page No

1.0 INTRODUCTION 1

2.0 CLONES TO BE TESTED 1

3.0 STANDARD CLONES 2

4.0 PLANTING TEST CLONES AND STANDARDS 3

5.0 GROWTH OF PLANTS BEFORE INOCULATION 3

6.0 BREEDING PLANTHOPPERS 4

7.0 ESTABLISHMENT AND INOCULATION OF TRIALS 5

8.0 TRANSPLANTING INTO THE FIELD 8

9.0 INSPECTION OF TRIALS 9

10.0 ANALYSIS OF TRIALS AND REPORTING RESULTS 9

11.0 REFERENCES 10

APPENDIX 6 - Manual

1.0 INTRODUCTION

Fiji leaf gall (FLG, previously known as Fiji disease) has caused major epidemics in southern and central Queensland and New South Wales. The symptoms of FLG are severe stunting, distortion of the leaves and galls on the underside of the leaves. FLG is caused by the *Fiji disease virus* (FDV) (Smith 2000). The virus is spread by planthoppers in the genus *Perkinsiella* and, in eastern Australia, the only species in this genus is *Perkinsiella saccharicida* Kirkaldy. The planthoppers acquire the virus as young nymphs, the virus replicates in the insect, and the insect remains infective for its entire life. Fiji disease virus cannot be transmitted artificially.

FLG is controlled by planting resistant cultivars and by disease-free planting material. Resistance to FLG is a prerequisite for all cultivars of sugarcane released in central and southern Queensland and New South Wales. During epidemics, varieties can be rated for resistance in selection trials in commercial fields (Leverington *et al*. 1977), but when infection pressure in commercial fields is low, clones can only be tested in artificial screening experiments. The method used in the past by BSES is described in Steindl *et al.* (1977). This field-based method involves planting small plots of the test clones between rows of FLG-infected cane. Natural populations of the planthoppers are relied on to transmit the disease.

Daniels *et al.* (1969) devised a glasshouse-insectary technique for screening for FLG resistance that rated clones on the numbers of days for plants to develop symptoms. Ledger and Ryan (1977) reported that this technique did not always correlate well with field reaction, and, after extensive testing by BSES, it was no longer used by BSES.

This manual describes a new method for screening sugarcane for resistance to FLG by inoculation in a greenhouse followed by growth of the inoculated plants in the field. It was developed as part of SRDC-funded project BSS255 (Croft *et al.* 2005). The technique attempts to control inoculum pressure to ensure adequate infection and growing the plants before inoculation in the open for 3-5 months to harden the plants and make them more like field grown plants. Plants are rated on the percent plants infected, as well as a disease severity rating that combines percent infection and symptom severity. Initial tests of the method have shown that disease severity is highly correlated with field reaction for 10 standard varieties (Croft *et al.* 2004). The method also has the advantages that it is less variable than conventional field trials so less replicates are required, it is not greatly influenced by different seasons, results are available in less than 12 months, and it is possible to conduct trials two times during the year maximising the use of the greenhouse.

2.0 CLONES TO BE TESTED

The BSES-CSIRO Plant Improvement Program (PIP) has three main stages of selection:

- Stage 1. Progeny Assessment Trials (PAT);
- Stage 2. Clonal Assessment Trials (CAT);
- Stage 3. Final Assessment Trials (FAT).

Between the CAT and the FAT, there is a propagation stage with approximately 140 clones that are tentatively selected for planting in FATs. The FLG greenhouse-field trial will screen all 140 tentative selections for FATs for central and southern selection programs in the year of propagation, so that susceptible clones can be discarded before the FAT is planted.

Advanced selections from all regions will be screened to confirm the ratings obtained in the earlier test for central and southern clones and to obtain ratings for advanced clones from Burdekin, Herbert and Northern programs. A selection of parent clones and foreign introductions will also be screened for resistance. It is important to know the resistance of parent clones because the ratings are used to restrict crosses to reduce the proportion of susceptible progeny produced. Foreign clones are imported for use as parents. The MOUs for exchange of clones with other countries provides for BSES to screen foreign clones for resistance to FLG in exchange for the other countries rating our clones for smut and other diseases endemic in their country.

The number of clones to be screened at each stage of the selection program is shown in Table 1.

Table 1 Proposed number of clones for FLG resistance trials from each stage of selection from the five regions in Queensland.

Selection program	Stage of selection	No. of clones	No. of replicates
Southern (including NSW)	Tentative FATs	140	2
	Advanced selections	15	3
Central	Tentative FATs	140	2
	Advanced selections	10	3
Burdekin	Advanced selections	5	3
Herbert	Advanced selections	5	3
North	Advanced selections	5	3
Foreign introductions (to be sent from Bundaberg)		50-60	2
Parents (to be sent from Meringa and Bundaberg)		60-80	3
Total 2 replicate		330-340	
Total 3 replicate		100-120	

3.0 STANDARD CLONES

Standard clone should be included in all trials. For two replicate trials, four replicates of the standard clones are included and in three replicate trials there are six replicates of the standards. The 10 standards that are currently used in FLG resistance trials and their

standard ratings are shown in Table 2. The standard ratings have been assigned based on extensive field experience during Fiji leaf gall epidemics and/or repeated testing in Fiji resistance trials. The International Society of Sugar Cane Technologists' recommended rating system is used. Rating 1 is highly resistant and 9 highly susceptible.

Table 2 Standard clones and their resistance ratings

Standard clone	Rating
Q110	1
Q57	2
Q87	2
Q90	4
Pindar	5
Q117[a]	6
Q124[a]	6
NCo310	8
Q102	9
Q71	9

4.0 PLANTING TEST CLONES AND STANDARDS

The clones for testing and the standards should be planted in late April – early May. It is important that the test clones are of approximately the same age, because disease reaction can vary with age of plants. The test clones are planted at the Experiment Station of origin and are held at that Station for 2-3 months, after which they are transported to Woodford by a specialist nursery carrier.

The standard clones are planted at Woodford from cane grown at an isolation plot (Kallangur). The isolation plot is needed to ensure that the standards are free of FLG before the commencement of the trial.

The clones and standards are planted as one-eye setts in 90 mm peat pots in potting mixture. The pots should be fertilised to give strong growth. Osmocote Plus is the recommended fertiliser.

5.0 GROWTH OF PLANTS BEFORE INOCULATION

Before inoculation, we aim to grow the plants in the open on spray benches to obtain plants that approximate field grown plants. Plants can be started in a greenhouse to ensure germination and good early growth if necessary (probably more important in southern districts). The last 2-3 months should be on spray benches in the open.

The plants should be grown for 3-5 months depending on season. Once or twice when plants are 2-4 months old they should be lightly trimmed to a level slightly above the top

visible dewlap of the largest plants. This will even up the growth of the different clones and encourages tillering. At about the same time as the plants are trimmed, they should be fertilised with Osmocote.

6.0 BREEDING PLANTHOPPERS

Good colonies of highly infectious planthoppers are an essential requirement for the success of the trials. Coordinating the growth of the test clones and the breeding of the populations of the planthoppers requires careful planning. We aim to have 10-20 planthoppers per test plant. Therefore, for a standard trial with approximately 1800 plants per chamber 20-30,000 planthoppers are required. We have found that in good colonies on 250 mm pots we get 2000-5000 hoppers/pot. Therefore, for each trial we need 12-15 pots. Multiple batches of planthopper colonies should be established to ensure adequate supplies of planthoppers when the plants are ready for inoculation and to give a backup in case of problems.

Research has shown that the infective cultivar on which the planthoppers are bred is important in obtaining the highest possible proportion of planthoppers carrying the FDV virus. We currently recommend FLG-infected NCo310 for breeding planthoppers for the trials. A source of lightly FLG-infected NCo310 should be planted in the year before the trials are to be planted. To obtain lightly infected NCo310, healthy plants should be infected in the greenhouse and transplanted into the field. Propagating from diseased plants gives severely stunted plants from which it is difficult to obtain planting material. Four to five setts of the diseased NCo310 are planted in 250-300 mm pots with sand-peat potting mixture. These are allowed to grow for 2-3 months before they are caged and the planthoppers added. Plants should be checked for FLG galls to ensure all plants are infected.

The plants for breeding planthoppers should be as free as possible of other pests, particularly mites, thrips and aphids. This is difficult to achieve, because no pesticides can be used, as any residues may kill the planthoppers. We recommend that the chamber for growing the plants for breeding the hoppers should be thoroughly cleaned, disinfected and sprayed with insecticide before commencing to grow the plants. No one who has been in contact with plants infested with mites should enter the chamber and definitely no plants from other chambers or outside the greenhouse should be allowed in the chamber. Actively growing, well-fertilised plants appear to be less susceptible to mite infestation. Mites like a dry atmosphere, so maintaining a high humidity in the chamber is recommended. The temperature of the chamber should be maintained as close as possible to 30°C. Populations have bred well at day temperatures up to 40°C, but, if the temperature drops below 10°C, the populations do very poorly.

The planthoppers to initiate the colony can come from the field or from cages set especially to supply the planthoppers. When catching planthoppers from the field it is important to avoid including any predators (*Tytthus* spp., ladybirds, spiders, etc.). We recommend 30-40 adult planthoppers per cage to start a colony.

The best cages we have found are fine voile sleeves that are tied to the pot and suspended from a wire to support the material (Figure 1). These cages increase the humidity, which is important for survival of the hatching nymphs.

Figure 1 Planthopper breeding cages

The recommended schedule for establishing the plants and colonies is shown in relation to other activities in Table 3.

7.0 ESTABLISHMENT AND INOCULATION OF TRIALS

The test clones and standards should be arranged in randomised complete blocks or another appropriate design in the greenhouse chamber in trays on the floor (Figure 2). A four-plant guard should be placed at the end of each row. The trays are filled with water by automatic trickle-irrigation. Sub-irrigation and placing the trays on the floor increases the humidity around the plants, which is important for survival of young nymphs.

Table 3 Schedule of activities for conducting FLG greenhouse-field resistance trials

Year	Date	Activity	No. pots	Comments
1	May	Plant healthy NCo310 in peat pots	200-400 peat pots	To provide next years diseased plants
1	July-August	Inoculate NCo310 with infective planthoppers (20-40/plant)		To provide next years diseased plants
1	September	Transplant inoculated plants into the field		To provide next years diseased plants
2	January	Clean, disinfect and spray a chamber with pesticide		Preparation for commencing populations of planthoppers
2	February-March	Plant pots of healthy NCo310	20-30 x 250-300 mm pots	To establish seed population
2	Late May	Plant 50 pots of FLG-infected NCo310	50 x 250-300 mm pots	For first batch of planthoppers
2	Mid June	Plant pots of FLG-infected NCo310	50 x 250-300 mm pots	For second batch of planthoppers
2	Late June	Plant pots of FLG-infected NCo310	50 x 250-300 mm pots	For third batch of planthoppers
2	Late April/Early May	Plant test clones and standards	Require 16 peat pots of test clones for 2 rep (standards 32) and 24 for 3 rep	Place in greenhouse or germination chamber to maximise germination
2	Mid-May	Cage and add hoppers to seed population plants	20-30 x 250-300 mm pots	30-40 adults per pot
2	Early July	Arrange for transport of test clones to Woodford, trim and move standards onto outside benches		
2	Mid July	Cage and add hoppers to seed population plants	50 x 250-300 mm pots	30-40 adults per pot
2	Late July	Cage and add hoppers to seed population plants	50 x 250-300 mm pots	30-40 adults per pot
2	Early August	Cage and add hoppers to seed population plants	50 x 250-300 mm pots	30-40 adults per pot

2	Early August	Arrange clones in trays as per trial plan		
2	September	Set up and inoculate trials. Inoculation period 2 weeks	8 rows x 9 trays, 3 clones x 8 plants per tray	15 pots of planthoppers/chamber (depending on estimated number of planthoppers per pot)
2	September	Count planthoppers. Add planthoppers if <10 planthoppers per plant	100 plants 2-3 times during inoculation period.	
2	September	Collect sample of planthoppers for FDV RT-PCR screening for infectivity		50 adults and 50 nymphs
2	Sept./Oct.	Transplant plants into the field		Irrigate immediately after planting
3	February	Inspect trials		Rate for % infected plants and disease severity
3	February	Analyse trials and provide results for inclusion in SPIDS		

Figure 2 Plants in trays ready for inoculation period

When the plants are in position, the planthopper cages are brought into the chamber, the cage is removed, and any planthoppers in the cage released. The shoots from the breeding plants are cut at soil level and the shoots are placed on top of the test plants at random. The pots from the caged plants are left between the rows evenly spaced throughout the chamber. Many young nymphs of the planthoppers will be on the pots, as they tend to drop to the ground when disturbed.

An estimate of the number of planthoppers in a cage can be obtained by sampling one cage. To collect all planthoppers in a cage, place the pot and the cage in a large rubbish bin and slowly fill the bin with water. As the water rises, the planthoppers move onto the cage. When the water reaches the top of the bin, the cage can be carefully removed. The cage can then be placed in a freezer to kill the insects. The insects can then be tipped into a funnel above a container, the total weight of insects measured and a subsample taken and weighed. The number of adults and nymphs can be counted in the sample and an estimate calculated for the total weight of insects. We have found that 2000-5000 insects can be bred on one pot in 6-8 weeks, but most of the insects are nymphs. Excellent survival of nymphs has been achieved when they are transferred to the test plants.

It is important to monitor the planthoppers in the trials to ensure that enough planthoppers are present to give good transmission. In routine trials, we recommend that planthoppers be counted on 100 plants two to three times during the trial, with one count 3-4 days after the planthoppers are released. If there are fewer than 10 planthoppers per plant at the first count, then more planthoppers should be added to the chamber.

A few days before the end of the inoculation period, a sample of 50-100 planthoppers should be collected to screen for FDV by RT-PCR. The planthoppers should be immediately frozen after they are collected.

Two weeks after the planthoppers are released the inoculation period should be terminated by either removing the plants and transplanting them into the field or spraying the plants with insecticide (Confidor® or pyrethrum).

8.0 TRANSPLANTING INTO THE FIELD

The plants can be transplanted directly into the field using the seedling planter (Figure. 3). The plants can be lightly trimmed if necessary (cut above the top visible dewlap). If possible, the same arrangement of rows, plots and replicates as used in the greenhouse should be maintained in the field. In the field, plots are eight plants long with approximately 300 mm between plants with a plant of NG51-142 (red marker cane) between plots. A guard row should be planted on the outside of the block and a four-plant guard planted at both ends of the rows. After it is planted, the trial should be irrigated within 1-2 days. The trial should be grown with standard fertiliser rates and irrigation to provide good growth.

Figure 3 Planting cane with seedling planter

9.0 INSPECTION OF TRIALS

Trials can be inspected in February if they are planted in September to October of the previous year.

The numbers of Fiji leaf gall infected plants and the total number of plants are recorded. The infected plants are given a disease severity rating. The four categories used are:

1. G = Galls and/or mild breakdown, no apparent stunting;
2. M = Moderate stunting and breakdown.;
3. S = Severe stunting and breakdown, stalks up to 50% shorter than healthy stalks;
4. V = Very severe stunting and death, stalks more than 50% shorter than healthy stalks.

The disease severity (DS) rating is calculated as:

DS = ((1*G)+(2*M)+(3*S)+(4*V))/(4*total plants)*100

10.0 ANALYSIS OF TRIALS AND REPORTING RESULTS

The disease severity and percent-infected plants are transformed by the arcsin transformation and then analysed by an analysis of variance. A regression of the arcsin transformed disease severity of the standard varieties and their standard rating is performed. The correlation coefficient from this regression is a measure of the reliability of the ratings in the trial. If the correlation coefficient is over 0.9, ratings are considered to be very reliable. The regression equation is used to calculate the ratings for the test clones as follows:

Regression equation for standards

$$ADS = mR + c$$

Where: ADS = arcsin disease severity
R = rating on 1-9 scale
m = regression coefficient
c = intercept

Ratings of tests clones are calculated as follows:

$$R = (ADS - c)/m$$

The calculated ratings should be distributed to Plant Breeders, Variety Officers, Plant Pathologists and the person responsible for maintaining the Sugarcane Plant Improvement Database (SPIDs).

As a general rule, clones with ratings of 7 or greater are not suitable for areas in which FLG is active. In districts where FLG is extremely active, the acceptable rating will drop, but in low risk areas the acceptable rating may be higher then 7.

11.0 REFERENCES

Croft BJ, James AP, Ridley AW and Smith GR. 2004. Developing methods to screen sugarcane varieties for resistance to Fiji leaf gall. Proc. Aust. Soc. Sugar Cane Technol., 26 (CD-ROM), 11 pp.

Croft BJ, Ridley AW, Dhileepan K and James AP. 2005. Final report – SRDC project BSS255 Improving the plant breeding selection system for Fiji disease resistance. BSES Publication SD05014.

Daniels J, Husain AA, Hutchinson PB and Wismer CA. 1969. An insectary method for testing sugar cane varieties for resistance to Fiji disease. *Proc. Int. Soc. Sugar Cane Technol.* 13: 1100-1106.

Ledger PE and Ryan CC. 1977. Screening of sugar-cane varieties for resistance to Fiji disease in Queensland - The insectary-glasshouse method. *Proc. Qld Soc. Sugar Cane Technol.* 44: 79-82.

Leverington KC, Egan BT and Hogarth DM. 1977. Breeding for resistance and other possible methods of control of Fiji disease at Bundaberg. *Proc. Qld Soc. Sugar Cane Technol*. 44: 93-95.

Smith GR. 2000. Fiji disease. In: Rott P, Bailey RA, Comstock JC, Croft BJ and Saumtally AS (eds). *A Guide to Sugarcane Diseases*. CIRAD and ISSCT, Montpellier.

Steindl DRL, Hughes CG and Hogarth DM. 1977. Developments in Fiji field-trial design. *Proc. Qld Soc. Sugar Cane Technol.* 44: 75-77.