Australian Government
Cotton Research and Development Corporation

FINAL REPORT 2007

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

Part 1 - Summary Details

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

CRDC Project Number: **DAQ130C**

Project Title: Management of Fusarium wilt of cotton

Project Commencement Date: 01/07/2004 **Project Completion Date:** 30/06/2007

CRDC Program: 3 Crop Protection

Part 2 – Contact Details

Administrator: Ms Helen Kamel, Senior Planning Officer
Organisation: Queensland Department of Primary Industries and Fisheries
Postal Address: Queensland Department of Primary Industries and Fisheries, PO Box 241 DARLING HEIGHTS Qld 4350
Ph: 07 4631 5380 **Fax:** 07 4631 5378 **E-mail:** Helen.Kamel@dpi.qld.gov.au

Principal Researcher: Dr Linda Smith, Senior Plant Pathologist
Organisation: Queensland Department of Primary Industries and Fisheries
Postal Address: Plant Pathology Bldg, 80 Meiers Rd, Indooroopilly Qld 4068
Ph: 07 3896 9538 **Fax:** 07 3896 9533 **E-mail:** Linda.Smith@dpi.qld.gov.au

Supervisor: Dr Linda Smith, Senior Plant Pathologist
Organisation: Queensland Department of Primary Industries and Fisheries
Postal Address: Plant Pathology Bldg, 80 Meiers Rd, Indooroopilly Qld 4068
Ph: 07 3896 9538 **Fax:** 07 3896 9533 **E-mail:** Linda.Smith@dpi.qld.gov.au

Signature of Research Provider Representative: _____

Part 3 – Final Report Guide (due 31 October 2007)

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

Background

1. Outline the background to the project.

Diseases have become more of a problem for cotton growers during the last decade. Seedling diseases, Fusarium wilt, Black root rot and Verticillium wilt have appeared or become prevalent in many cotton-growing areas. Root knot nematodes (preliminary identification) were also found in one small area of cotton, growing on lighter soil in Central Queensland. Hence there is a need to continue annual disease surveys of commercial cotton crops in all areas of eastern Australia for the presence or absence of disease, including continuation of the diagnostics laboratory at Indooroopilly, as they provide information on disease incidence and severity, the possible occurrence of new strains, location and farming practices.

There is also a need to conduct research into control strategies for specific diseases such as Fusarium wilt. Fusarium wilt of cotton caused by the fungus *Fusarium oxysporum* f. sp. *vasinfectum (Fov)*, is considered by many growers, ginners, consultants and other industry personnel as the most important constraint to sustainable cotton production to have developed in recent years. Wilt was first identified in Australia in 1993. Since then, this destructive disease of cotton has continued to spread and is now found in most cotton growing districts in Queensland and New South Wales, with the exception of Hillston, Tandou and Emerald, but has not been found in the Northern Territory or Western Australia. The disease has caused significant yield losses, mainly in Queensland (estimated $57 million on the Downs during the 1999-2000 season), but continues to be recorded in new areas in New South Wales. The disease is proving difficult to manage, with relatively low levels of resistance identified in varieties and germplasm to date. In addition, the levels of resistance in some varieties do not appear to be consistent from season to season. A contributing factor may be that dry season's can mask how some varieties perform. If we experience some wet summers in the future, Fusarium wilt will be a problem and we believe production will be severely affected. Despite this, resistant varieties are still the cornerstone of any strategies to manage this disease but they will need to be used in conjunction with other agricultural practices to provide sufficient control to allow sustainable cotton production.

Other agricultural practices to be investigated are alternative rotation crops, biological control and silicon fertilisation. Growers in production districts of known high disease incidence have been seeking effective crop rotation options to assist in the management of Fusarium wilt. Other studies have indicated that non pathogenic *Fusarium oxysporum* (npFo) isolates may be used to control Fusarium wilt in various crops of economic importance hence the biological control potential of npFo commenced in project DAQ107C and will continue in this project. Silicon fertilisation has been reported to improve plant resistance to disease and pathogenic fungal attack in numerous crops. The potential of silicon amendment to reduce severity of Fusarium wilt of cotton will be investigated.

This project will continue on from project DAQ107C in evaluating experimental and new varieties for reaction to the disease (particularly important as the introduction of new GM cotton varieties accelerates) and further investigate the effect of agricultural practices on pathogen survival and disease development. This will allow better management of the disease and the continued sustainable production of cotton.

Objectives

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

Objective 1. Monitor cotton diseases in Queensland as well as monitoring the diversity and distribution of strains of *Fov* in cotton-growing areas in Australia. Conduct disease surveys during early stage and adult growth. This objective has been achieved.

Objective 2. Identify, using glasshouse and field trials, sources of resistance to *Fov* and investigate the heritability and genetics of these traits. Select resistant germplasm and develop segregating populations for heritability studies. This objective has been achieved.

Objective 3. Assess early stage varietal development germplasm, in collaboration with plant breeders, to ensure that new varieties have the highest resistance to *Fov* available, prior to their release. This objective has been achieved.

Objective 4. Investigate the role of crop rotation on the ecology of *Fov* and on subsequent disease development (including the continued validation of the molecular diagnostic test). This objective has been mostly achieved; however the molecular diagnostic test was not validated due to cross-specificity with other Fusaria.

Objective 5. Investigate non-pathogenic *Fusarium oxysporum* populations in soil to determine if they can modify the ecology of the pathogenic populations and subsequent disease development in cotton. This has been achieved.

Objective 6. Investigate the potential of silicon applied to the soil to reduce severity of Fusarium wilt. This has been achieved.

Objective 7. Develop and extend new information packages for disease management. This has been achieved.

Methods

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

Objective 1

Annual disease surveys

Annual disease surveys are conducted in commercial Queensland cotton crops during the seedling stage and mature plant stage. Data on diseases present, their severity and incidence together with other information such as paddock history, ground preparation, planting dates, rates and cotton varieties are noted. These surveys, conducted in collaboration with CSD staff and local consultants, provide valuable information on seasonal effects on disease and keeping check on any new diseases or strains of *Fov* which may arise.

Diagnostics

A diagnostic service is provided for growers, consultants and researchers from all growing districts. The genetic diversity and geographical distribution of *Fov* in Australia is monitored by direct isolation of the fungus from suspect specimen plants followed by Vegetative Compatibility Group analysis and DNA fingerprinting analyses. The importance of this service should not be underestimated as it is a means to determine not only the spread of known strains of the pathogen, but enables the detection of new evolved strains and introduction of exotic strains.

Database

A database of all *Fov* isolates recovered from diseased cotton samples received at the Indooroopilly laboratories is continually maintained. The database is searchable under several fields such as VCG, cotton variety, state, district or year.

Objectives 2 & 3

A large irrigated Fusarium field trial site was located on Graham Clapham's property 'Cowan'. The small plot trials, comprising some 7000 plots, enabled continuing assessment of varietal reaction to *Fov*, screening of early stage varietal development germplasm and the identification of sources of resistance to *Fov*.

Objective 4

Field Rotation Trial

A three year irrigated field trial at 'Cowan' investigated the influence of different rotation crops and their residues on subsequent disease development in cotton grown across the entire trial during the final year. Soil bioassays in the glasshouse were also used to monitor the relative level of *Fov* in the soil during each season by planting a susceptible cotton variety into seedling flats. The proportion of seedlings surviving at 6 weeks and their disease ratings provided an indication of the soil pathogen population.

The influence of sorghum residues on disease

A small replicated field trial at 'Cowan' investigated the level of Fusarium disease in cotton following a sorghum crop where the sorghum residues were either burnt or incorporated. Similar trials on other fields and farms investigating the effect of maize residues on Fusarium were unable to be run due to drought conditions.

Glasshouse pot experiments

Small pot trials in the glasshouse, using naturally infected field soil monitored different rotation options including green manure crops. Pathogen population in the soil was assayed as described above.

Molecular diagnostic

The molecular diagnostic test, developed for the pathogen in 2.2.12AC and DAQ107C, was not utilised to differentiate between pathogenic and non-pathogenic isolates of *Fusarium oxysporum* in crop rotation and ecology studies as planned. This molecular tool was not specific enough due to cross-specificity with other soil Fusaria.

Objective 5

Investigate non-pathogenic *Fusarium oxysporum (*npFo*)* populations in soil to determine if they can modify the ecology of the pathogenic populations and subsequent disease development in cotton.

Development of a glasshouse bioassay

The initial glasshouse bioassay developed in DAQ107C did not enable biocontrol potential to be successfully evaluated as infection of *Fov* was often too low and inconsistent. In addition, using pasteurised potting mix caused nutritional concerns after 4 to 5 weeks as mycorrhizae and naturally diverse populations of microorganisms were not present. Hence in DAQ130C a second bioassay, using field soil naturally infested with *Fov*, was developed and evaluated as described below.

Non-pathogenic Fo isolates

Non-pathogenic *Fo* strains recovered from wild cotton (*Gossypium sturtianum*, *G. australe*, *G. nelsonii* and *G. bickii*) growing in Mt Isa, Qld, Alice Springs, NT and Flinders Ranges, SA have been obtained from Dr. Bo Wang (CSIRO, Canberra) for assessment (reported in DAQ107). Non-pathogenic *Fo* isolates originating from the Darling Downs (collected by Dr. Joe Kochman) were isolated from soil and roots of cotton plants which had not exhibited symptoms of Fusarium wilt.

Soil mix

Soil that was naturally infested with *Fov* was collected from Graham Clapham's property 'Cowan' at Cecil Plains and mixed 50:50 with sand before use. *Gossypium hirsutum* cotton cultivar Siokra V17 was used for all experiments, this cultivar having a mid-range level of resistance against Fusarium wilt.

Preparation of millet

Dry millet (*Pennisetum glaucum*) seed was soaked overnight in distilled water in a 10 L stainless steel pot. The millet was rinsed to remove excess carbohydrate. Excess water was drained from the seed before being steamed for 30 minutes. Approximately 500 mL of millet was transferred into 2 L Erlenmayer flasks which were then plugged and autoclaved for 30 minutes at 120°C.

Preparation of npFo inoculum
Strains of npFo were subcultured on to quarter strength potato dextrose agar amended with streptomycin (¼PDA/S) and incubated at 27°C for four days. Large squares of colonised agar were cut from the plates and under sterile conditions, were used to inoculate the flasks of millet. The flasks were then placed on a bench at room temperature until the grain became colonised (7-10 days). Flasks were shaken regularly to evenly distribute growth. One flask was prepared for each of the isolates, while for the control treatment a flask containing un-colonised millet was used.

Inoculation of cotton with npFo
Seed was first planted into disease-free potting mix plus npFo. This process allowed seedling roots to become colonised by npFo before coming in contact with the pathogen. A layer of paper was placed on the bottom of each seedling flat (34 x 28.5 cm) to permit ease of handling, while not restricting the drainage of water from the trays. Millet (140 mL) colonised by npFo was scattered in three rows over 2 L of pasteurised M mix placed in the seedling flats. Three rows of 10 cotton seeds were then planted on top of the inoculated millet grain, and covered with 1 L of pasteurised M mix. Vermiculite was used to cover the potting mix to protect it from over-drying throughout the experiment. Prior to transplanting, root samples were surface sterilised and plated onto growth media to confirm root-colonisation by npFo.

Transplanting of seedlings into naturally infested soil mix
Three weeks after planting, seedlings were transplanted into field soil naturally infested with *Fov* mixed 50:50 with sand. Pots (10 cm) were half filled with soil mix and five plants were transplanted into each pot, then pots were filled with soil mix. The surface of the soil mix was covered with a layer of vermiculite. Pots were placed in the glasshouse in a randomised block design and watered.

Harvest and disease rating
Plants were grown in infested soil mix until external disease symptoms were obvious in the control treatments. Plants were then harvested and examined internally for disease severity using a rating for vascular discoloration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to cotyledons, 3 = infection above cotyledons, 4 = infection up to top node and 5 = dead plant.

Evaluation of npFo for biocontrol potential against *Fov* using the glasshouse bioassay
Selected isolates of npFo were evaluated for potential to modify the ecology of the pathogenic populations and subsequent disease development in cotton.

Experiment 1
Five npFo isolates (M1-4, NF6-4, NFP4-2, NFP5-5, M3-4) recovered from cotton field soils were evaluated for their biocontrol potential using the bioassay described above.

Experiment 2
Six npFo isolates (NFP5-2, M1-7, M3-14, NF5-1, NF3-11, and NFP5-5) recovered from cotton field soils were examined for their biocontrol potential. In this experiment only 70 mL of millet inoculum was applied to the trays due to concerns that the higher rate of inoculum may have slowed germination of the cotton seedlings. A rate trial was incorporated into experiment 2 using a repeat isolate from experiment 1 – NFP5-5. Using millet inoculated with this isolate, seeds were planted as outlined previously using three rates of inoculum – 30 mL, 70 mL and 140 mL.

Experiment 3
An experiment was conducted to determine if the severity of Fusarium wilt in cotton is decreased by non-pathogenic strains of *Fo* isolated from soil in which native cotton plants displayed no symptoms of Fusarium wilt. Isolates evaluated were provided by Dr Bo Wang (CSIRO, Canberra) - isolate 1512 from Mt Isa, QLD, *G. australe* and isolate 1528 Mt Isa, QLD, *G. australe*. In this experiment some modifications were made to experimental procedure. These were that seeds were planted directly into Fusarium-infested field soil mix rather than growing seedlings in pasteurised M mix then transplanting. A volume of 70 mL of npFo colonised millet /un-colonised was applied.

Experiment 4
Five isolates obtained from Bo Wang (CSIRO) (BW 5, 6, 11, 14 and 16) were evaluated. NpFo colonised millet inoculum was applied at two rates, 50 mL and 100 mL per pot. The control was treated with either 50 or 100 mL of sterile millet.

Objective 6

Investigate the potential of silicon applied to the soil to reduce severity of Fusarium wilt.

Although silicon has not been established as an essential nutrient, it is considered an important constituent of plants. Multiple benefits have been observed where silicon is available to growing plants, with reported improvements in plant resistance to disease and pathogenic fungal attack where silicon has been applied to numerous crops. Mechanisms suggested for the improvement in disease resistance include a mechanical action whereby silicon accumulated in the cell walls acts as a barrier to penetrating fungi. Recent research has also suggested that the production and accumulation of antifungal phenolic compounds and the activation of defence related enzymes may also have a role in this plant defence mechanism. The aims of these trials were to examine the effect of soil amendment of various sources of silicon on severity of fusarium wilt of cotton.

***In-vitro* plate test**

The aim of this experiment was to determine if silicon amendment affects *Fov* spores directly. To examine the effect of potassium silicate on spore germination and growth of *Fov*, a spore suspension of *Fov* was mixed with various rates of potassium silicate and spores were spread onto solid growth media. After 5 days the number of germinated spores and diameter of colonies was determined.

Glasshouse bioassays

Experiment 1
A glasshouse bioassay to evaluate the effect of silicon (and potassium) on disease severity was set up basically as described previously for npFo trials, with some variations described here. Potassium silicate was applied weekly as a soil drench at 0, 0.25, 2, 5 and 10 mL/L. To determine whether potassium has an effect on disease severity, a solution of potassium sulphate (5 g/L) was also applied weekly as a soil drench. Seed was sown directly into 10 cm pots containing naturally infested field soil mix.

Experiment 2
Other forms of silicon were also investigated such as Silvine (magnesium silicate), and acidified wollastonite (acidified calcium silicate). These powder formulations were applied pre-plant and mixed uniformly into the soil prior to sowing. Potassium silicate (5 mL/L) was also applied weekly as a soil drench. Plant material was collected at harvest, dried and analysed for content of silicon and other key nutrients (BSES Indooroopilly).

Experiment 3
Research conducted at the University of New England indicated that soil application of phosphorus reduces the availability of silicon in soil solution, hence silicon available for plant uptake. Previous research has shown that increasing phosphorus increases Fusarium wilt severity (Dick and Tisdale (1937, Tharp and Wadleigh (1939)). These findings are of concern as phosphorus is essential for plant growth. It has an important function in cell division; and therefore it is especially important in young, rapidly growing plant tissue, and for cotton production is commonly applied pre-plant. These findings prompted the question 'Is there a relationship between silicon, phosphorus and disease severity?' Glasshouse trials were conducted to investigate the effect of silicon fertilisation and high phosphorus application on severity of Fusarium wilt.

Field trial evaluation

Three field trials were conducted at 'Cowan' to investigate the effect of various silicon sources applied to soil and leaves on silicon uptake measured using the 5th terminal leaf, disease severity, seed cotton yield and fibre quality.

Trial 1 (2004-2005)
This experiment consisted of 4 rows, each row containing 6 treatment plots that were 10 m in length. All plots were separated by buffers of the same dimensions. Treatments applied were 1= Silvine granules (magnesium silicate) 150 kg/ha; 2= Silvine granules 75 kg/ha + Silvine powder 3.5 g/L; 3= potassium silicate powder 150 kg/ha + potassium silicate liquid 10 mL/L + foliar application potassium silicate liquid 2.5 mL/L fortnightly for 8 weeks; 4= As for treatment 3 + potassium silicate liquid foliar application applied monthly until bolls formed; 5= untreated control; 6= potassium sulphate 8 g/L. Silvine granules and potassium silicate powder were applied by hand to a furrow 10 cm deep in the centre of the raised bed. Silvine powder suspended in water, and potassium silicate and potassium sulphate solutions were applied to the furrow using a watering can (1 L/m row). Hills were then reformed. Seeds were sown (cv Sicot F-1) and after germination, potassium silicate liquid was applied to foliage using a watering can. The experiment was irrigated and managed commercially. The fifth terminal leaf was collected for nutrient analysis prior to harvest. After harvest, stems were cut and rated for vascular discolouration.

Trial 2 (2005-2006)
This experiment was conducted as described for Trial 1 with some differences outlined here. All silicon treatments were applied pre-plant into the furrow. Two varieties were sown, Sicot F-1 and Sicot 189 (more susceptible to FW than Sicot F-1). Treatments included: Magnesium silicate granules 450 kg/ha; Magnesium silicate granules 150 kg/ha; Potassium silicate powder 150 kg/ha; Acidified calcium silicate powder 150 kg/ha; and an untreated control.

Trial 3 (2006-2007)
The aims of this field trial were to determine if silicon fertilisation reduces disease severity and improves yield, and to determine if phosphorus application at rates greater than plant requirement significantly increase severity of Fusarium wilt. This experiment was conducted as described for Trial 1 with some differences outlined here. Silicon was applied as magnesium silicate powder 100 kg/ha; Calcium silicate 200 kg/ha; Potassium silicate liquid applied to the furrow pre-plant then foliar application 2.5 mL/L fortnightly until 8 weeks old then monthly until boll formation; and an untreated control. The source of phosphate used was triple superphosphate applied at 70 kg/ha into the furrow pre-plant. In this trial the availability of nitrogen and potassium was not taken into account which will possibly influence the effect of phosphorus on disease severity. Two varieties were grown, Sicot F-1 and Sicot 189.

Objective 7
New information on Fusarium wilt management, obtained during this project, will be extended to the Cotton Industry in a number of formats and forums. These include presentations by staff at: field days, grower meetings, cotton consultant meetings, Industry Development Officer meetings, seed company meetings, national and international conferences. Project staff are members of the Fusarium Management Committee (FUSCOM) and assist with the development of the Integrated Disease Management Guidelines. Papers and brochures will be provided to growers through various avenues.

Results

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

Objective 1
Disease surveys
Disease surveys have been conducted in all cotton growing regions in Queensland since 1990, during seedling and adult plant stages. There was close collaboration between QDPI&F, Cotton Seed Distributors (CSD) and NSWDPI in these surveys. Data was collected for presence and incidence of all diseases. These surveys allow close monitoring of distribution and incidence of Fusarium wilt and provide isolates for pathogen race identification. The incidence of Fusarium wilt has increased in all areas except Emerald and remains one of the most important diseases of cotton in Queensland.

Over the three year period of this project, even though it has been dry, diseased samples were still sent for analysis, with a total of 252 samples received from Qld and NSW. Of these 49% were positive for *Fov*, and of the positive specimens, 121 belonged to VCG 01111 and two belonged to VCG 01112.

There were some new recordings of *Fov* however the rate of reporting of new cases of Fusarium wilt has declined. In 04-05 season some new fields were detected in most growing regions and new farms with *Fov* were detected at St George, and in the Macintyre, Gwydir and Barwon valleys. In 05/-06 season a new record was detected at Theodore. In 06-07 season one new farm was detected with *Fov* at St George and two new cases of Fusarium wilt were reported in NSW; one in the Macquarie Valley and one in the Gwydir Valley.

The slower rate of reporting in recent years may reflect a combination of farm hygiene measures, decreased cropping area due to drought, and increased use of less-susceptible varieties.

New strain
One isolate from the Macintyre valley, which gave negative results for VCG analysis in 2005, was shown to be pathogenic to cotton in a glasshouse pathogenicity test on Siokra 1-4. To confirm if the isolate belonged to VCG 01111 or 01112, or was a new strain, the isolate was characterised using two molecular techniques. Firstly the internal transcribed spacer (IGS) region of the rDNA was sequenced and compared with IGS sequence information for representatives of VCG 01111 and VCG 01112. The aligned sequence data showed that the unknown *F. oxysporum* isolate was genetically different from Australian representatives of VCG 01111 and VCG 01112 (S. Van Brunschot).

This isolate was also characterised using randomly amplified polymorphic DNA (RAPD) PCR with four different primers to compare the unknown *F. oxysporum* isolate to selected overseas strains of *Fov* representing various races (2, 3, 4, 5, 7, 8 and A) of the pathogen and Australian VCGs 01111 and 01112. The unknown *F. oxysporum* isolate was clearly different to all of the overseas isolates tested and was not identical to Australian VCGs 01111 or 01112 (L. Forsyth).

Sampling from the area has not yielded further isolates of this type, indicating that this new isolate, which probably arose from a spontaneous mutation, has not spread. A new VCG will not be designated to this new strain until a larger number of representative isolates are identified.

Objectives 2&3

Disease presence as indicated by observed 0's and 1's was consistent across the trial area but disease pressure as measured by the benchmark, Sicot 189, was much lower in season 06/07 than previous seasons, probably due to very few cool wet events (Figure 1).

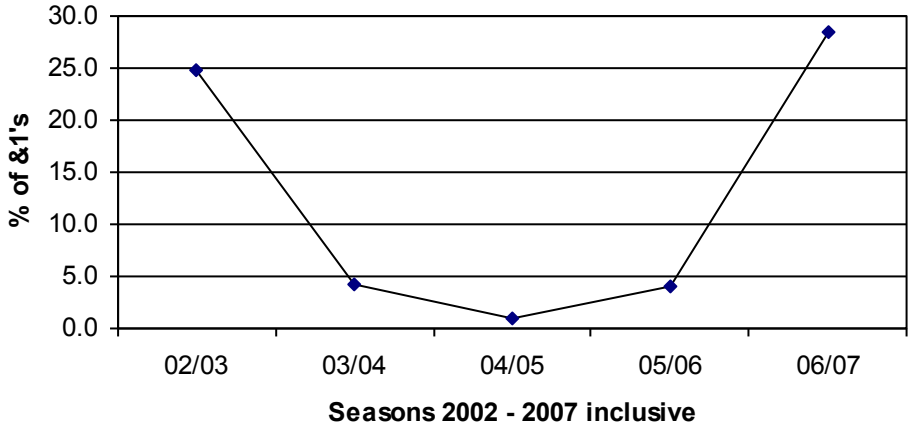


Figure 1. The percentage of plants rating zero and 1 in the standard Sicot 189 (for F rank status) at 'Cowan' during five seasons from 2002 to 2007

Variety Trials
Forty five lines were planted in 4 row plots replicated 4 times. Twenty two lines each from CSD/CSIRO and Deltapine. The remaining line was the benchmark variety for 'F Rank' performance, Sicot 189. Each season data collected included, plant population counts at emergence, establishment and picking as well as disease assessment ratings, using the cut stem technique. Yield, analyses of plants through to maturity and fibre characteristics were also conducted.

The trial design was a row-column design so that any one treatment (variety) did not appear twice in the same row or position. Nearest neighbour analysis was conducted which takes into account any treatment effect. Guard rows were included in the design to minimise any treatment effects.

Variety Trial Summary 04-05
In Table 1, % 0's and 1's and seed cotton yield of cotton varieties in the Fusarium assessment trial conducted during the 2004-05 season are summarised. Variety Sicot F-1 had the highest level of resistance to *Fov* assessed by percentage of plants rated 0 and 1, with all other varieties having significantly lower percentages, hence significantly more disease. Sicot F-1 also produced the highest seed cotton yield. Four other varieties yielded well, but had significantly lower levels of resistance than Sicot F-1 (Table 1). The disease pressure in the 04-05 season was particularly high (Figure 1) hence this season was a good test for a varieties resistance against *Fov*.

Variety Trial Summary 05-06
A total of 16 varieties yielded well, however resistance to *Fov* varied significantly. Variety Sicot F-1 had a significantly higher level of disease resistance than all other varieties evaluated, hence the highest F rank (Table 2). The new F rank, with a maximum of 200 indicating immunity, has greater meaning when comparing resistance of varieties against one another than the old F rank.

Variety Trial Summary 06-07
In Table 3, % 0's and 1's, F rank, yield, gin turnout and lint yield of cotton varieties in the Fusarium assessment trial conducted during the 2006-07 season are summarised.

Disease pressure was not as high this season as for the previous two seasons. In the last two seasons, variety Sicot F-1 had the highest level of resistance to *Fov*, with a significantly higher % 0's and 1's compared to all other varieties tested. This season there were 11 varieties with F ranks ranging from 115 to 128, in which there were no significant differences between % 0's and 1's. There was however great variability in yields between these varieties (Table 3).

Analysed yields were very low, 2.4 - 4.6 bales/ha (Table 3). Yield spread was relatively narrow compared to previous season's results (0.92 - 6.54 bales/ha, 05-06 and 1.96 - 6.59 bales/ha, 04-05). This was due to the extremely dry conditions, and low irrigated water availability, in crop. Only one pre-water and one in-crop irrigation were possible. These dry conditions also impacted on Gin Lint turnout %, which ranged from 39-51%. Smaller seeded seed-cotton samples gave a higher lint turnout. Ranked yields calculated in Bales of Lint /ha differed markedly from yields measured as seed-cotton in kg/ha.

Table 1. Percentage 0's and 1's, and seed cotton yield of varieties in the Fusarium assessment trial, conducted at 'Cowan', Norwin during season 2004-05.

Variety	% 0's and 1's	Seed cotton yield Kg/ha
Sicot F-1	**37.9 a**	**3565 a**
Sicala 45	27.5	**3340 a**
Sicot 14B	20.9	2980
Sicot 71BR	14.2	2989
04Q035	13.8	**3340 a**
04Q032	13.1	**3456 a**
05Q311DR	11.0	3021
Sicala 60BR	10.2	2578
Sicot 80	10.1	2802
03Q301DR	10.1	2794
03Q085	9.2	2834
DP 570 BGII	9.1	**3180 a**
EMERALD	7.7	2923
Sicala V-2RR	7.6	2475
Sicot 289RR	7.5	2730
Sicala 40BR	7.3	2476
Siokra V-18	7.2	2961
06Q516D	7.1	2400
Sicala 40B	7.1	2282
Siokra 24	6.7	2707
Sicala V-3BR	6.5	2408
DP 510 RR	6.1	3021
DP 579 BGII	5.9	3049
06Q517D	5.5	2645
Sicot 60RR	5.3	2562
Siokra V-18B	5.0	2581
Sicot 80B	4.8	2819
Sicot 289BR	4.8	2231
05Q282D	4.8	2716
Sicot 73	4.5	2610
DP 502 RR	4.3	2256
Sicot 289B	3.9	2581
DP 546 BGIIRR	3.9	2035
03Q066	3.5	2444
DP 556 BGIIRR	3.1	2343
03Q208D	2.9	2710
DP 560 BGII	2.3	2506
DP 576 BGII	1.6	1155
Sicot 189 (Standard)	**1.0**	**2394**
Sicot 71	0.4	1716
LSD 5%	7.3	418
LSD 1%	9.6	552

Values followed by the same letter are not significantly different from one another within that column

Table 2. Percentage 0's and 1's, F rank (new and old), seed cotton yield of cotton varieties in the Fusarium assessment trial, conducted at 'Cowan', Norwin during season 2005-06

Variety	% 0's and 1's	New F rank Max 200	Old F rank	Seed cotton yield kg/ha
Sicot F-1	**33.4 a**	131	836	**3174 a**
DPX 05Q408	18.6	115	467	**3120 a**
DPX 04Q035	14.7	111	368	**2810 a**
DPX 06Q154	14.5	111	365	2360
Sicot 14B	11.0	107	276	2571
DP 611 BGII/RR	10.8	107	270	**2694 a**
DPX 05Q414	10.7	107	269	**3113 a**
DP 570 BGII	10.7	107	268	**3221 a**
DPX 05Q010	10.6	107	267	**2656 a**
Sicala 45	9.7	106	244	**2963 a**
DP 510 RR	9.6	106	241	**2898 a**
Sicot 71RR	9.5	106	238	**2688 a**
DPX 08Q566D	9.3	106	234	2573
DPX 05Q103	8.9	105	224	2306
DP 408 BGII	8.4	105	211	**2922 a**
Sicala 350B	8.1	104	204	**3174 a**
DPX 05Q091	7.9	104	198	**2852 a**
Sicot 71B	7.8	104	195	**2625 a**
DPX 05Q233	7.5	104	187	2074
Siokra 24	6.8	103	170	1597
Sicot 43BR	6.4	103	161	2063
Sicot 71	6.3	102	158	**2762 a**
Sicot 289B	6.1	102	152	2459
DPX 04Q032	6.0	102	150	**2644 a**
Sicot 80	5.9	102	147	1827
Sicot 73	5.8	102	145	**2632 a**
DPX 08Q666DR	5.5	102	138	1884
Sicot 43RR	5.3	101	132	1948
Sicot 80RR	5.3	101	132	2564
Sicot 80B	4.8	101	121	**2741 a**
Sicot 289BR	4.6	101	115	1468
Siokra V-18	4.5	101	113	2159
Siokra V-18B	4.5	101	113	2507
Sicot 43B	4.3	100	107	1672
Sicot 189 (Standard)	**4.0**	**100**	**100**	2163
DPX 03Q066	3.7	92	92	2314
Sicala 60BR	3.7	92	92	1482
DPX 05Q153	3.5	88	88	1457
Sicot 289RR	3.3	84	84	1644
DP 546 BGII/RR	3.2	80	80	1154
DPX 06Q246	3.0	75	75	1435
Sicot 71BR	2.7	68	68	2079
DPX 08Q766R	2.3	59	59	1232
DPX 05Q168	1.7	43	43	499
DP 556 BGII/RR	0.5	12	12	731
Average SED:	2.3			310
Average LSD (5%):	4.7			620
CV:	42%			19%

Values followed by the same letter are not significantly different from one another within that column

Table 3. Percentage 0's and 1's, F rank, seed cotton yield, gin turnout and lint yield of cotton varieties in the Fusarium assessment trial, conducted at 'Cowan', Norwin during season 2006-07

Variety	% 0's & 1's	New F rank Max 200	Seed cotton kg/ha	Mean Gin Turnout %	Lint Yield Bales/ha
05Q414	**48.3 a**	128	**2039.1 a**	**51.3 a**	**4.57 a**
Sicot F-1	**47.8 a**	127	**1966.0 a**	45.1	**3.95 a**
Sicala 45	**46.6 a**	125	**1870.5 a**	42.3	3.56
04Q032	**45.1 a**	123	**2107.6 a**	46.8	**4.29 a**
06Q527D	**42.9 a**	120	**2185.6 a**	43.6	**4.30 a**
08Q532D	**42.2 a**	119	**2152.6 a**	43.4	**4.12 a**
06Q154	**41.8 a**	119	**1843.7 a**	42.5	3.41
Sicot 14B	**41.5 a**	118	1750.3	40.5	3.08
06Q038	**40.4 a**	117	1708.4	49.3	3.71
Sicot 350B	**40.2 a**	117	**2172.3 a**	40.7	3.87
05Q010	**39.0 a**	115	1749.0	49.0	3.62
05Q233	37.4	113	1763.9	47.1	3.56
Siokra V-18	37.2	112	**1873.1 a**	48.4	3.93
Sicot 81	34.3	108	1746.8	44.9	3.44
Siokra 24B	34.1	108	1726.1	43.4	3.35
09Q891F	34.0	108	1736.3	45.5	3.52
Sicot 71RR	33.6	107	1739.9	49.0	3.99
Sicot 71B	33.5	107	**1819.9 a**	45.9	3.65
07Q602DR	33.3	107	**1941.6 a**	44.0	3.83
Sicot 71	32.2	107	1626.0	47.0	3.35
Sicot 80BRF	33.1	107	**1829.7 a**	43.2	3.45
Sicot 73	31.6	104	**2168.7 a**	46.8	**4.53 a**
Sicot 80B	31.5	104	**1883.7 a**	43.5	3.59
Siokra V-18B	30.8	103	**1965.7 a**	43.0	3.75
Sicot 289B	29.6	102	**2044.4 a**	44.6	**4.10 a**
09Q901DF	29.0	101	**1944.8 a**	41.9	3.57
Sicot 189 (Standard)	**28.4**	**100**	**1556.8**	**46.7**	**3.17**
Siokra 24	28.4	100	1341.2	47.3	2.78
06Q246	28.2	100	1132.4	49.0	2.48
Sicot 71BR	27.4	99	1738.3	46.6	3.56
03Q066	26.7	98	**1770.0 a**	48.9	3.73
09Q903DF	24.8	95	1617.2	44.8	3.25
08Q712R	24.3	94	1414.4	49.4	3.09
09Q902DF	23.6	93	1765.8	43.5	3.33
Sicot 43RF	23.0	93	1522.6	46.1	3.11
Sicot 80RF	22.7	92	1553.2	43.5	3.00
08Q706R	22.3	92	1449.6	47.5	3.05
Sicot 289BR	22.0	91	1584.6	44.2	3.05
08Q912DF	21.7	91	1558.1	41.5	2.88
08Q818F	21.4	90	1671.5	43.1	2.95
09Q904DF	18.3	86	1592.2	37.8	2.64
08Q711R	18.1	86	1085.1	49.0	2.41
Sicala 60BRF	16.7	84	1455.3	44.7	2.83
Sicot 43BRF	14.1	80	1304.9	45.3	2.66
06Q037	13.9	80	1119.3	**49.7 a**	2.98
Average SED:	4.92		208.6	0.89	0.42
Average LSD (5%):	9.84		417.2	1.78	0.85
CV:	9.80%		1%	2.76%	18.30%

Values followed by the same letter are not significantly different from one another within that column

Objective 4

Field Rotation Trial: 'Cowan'

Rotations including maize yielded highly and had mid-range disease levels as noted by the percentage of plants surviving to maturity and the proportion of plants rating 0 and 1 at the end of the season. The different residue management strategies imposed on the maize crop were not significantly different (analysis not included). The percentage of plants surviving until maturity and the number of 0's and 1's were greater where the residues were retained than where they were burnt or incorporated (Table 4).

The benefit of a fallow immediately prior to cotton was highlighted by comparing maize-fallow-cotton to fallow-maize (incorporated)-cotton, with a 17-18% significant increase in both the number of plants surviving through to maturity and the number of 0's and 1's, despite yields being similar.

There were no significant differences in retaining wheat residues on the surface or incorporating them (Table 4). Compared to the other rotations, both wheat treatments had a high % of plants surviving and the % of 0's and 1's was greatest. However, the yield of the wheat rotations was lower than a lot of other treatments.

Table 4. Assessment of plant survival, disease severity and yield of cotton following different rotations

Treatment	% 0s & 1s	% Survival	Yield (bales/ha)
Fallow- sorghum (retained)- cotton	34	61	4.66
Maize- fallow- cotton	55	76	4.42
Fallow- maize (incorporated)-cotton	38	59	4.30
Fallow- maize (burnt)- cotton	36	58	4.29
Fallow- maize (retained)- cotton	51	72	4.14
Cotton- cotton- cotton	42	66	3.93
Fallow- wheat (incorporated)- cotton	61	76	3.69
Fallow- sorghum (spray, retained)- cotton	22	55	3.68
Fallow- sorghum (burnt)- cotton	19	45	3.60
Fallow- wheat (retained)- cotton	65	79	3.49
Cotton- fallow- cotton	59	81	3.42

(statistical analysis not included)

Retaining sorghum residues on the soil surface was better for disease levels than the other two residue treatments examined in all areas measured. This treatment had the greatest yield of all rotations (Table 4).

A cotton-fallow-cotton or maize-fallow-cotton rotation had significantly less disease (% 0's and 1's) and more plants surviving until maturity than three years of continuous cotton (Table 4).

Although yield benefits do not appear to be related to lower disease, the area of inoculum carryover still needs consideration. Further studies are needed to quantify the actual pathogen population changes in the soil to fully understand what is happening.

The influence of sorghum residues on Fusarium in subsequent cotton crops

The effect of managing sorghum residues differently on Fusarium disease in cotton was investigated in a small replicated field trial at 'Cowan' during the 2006/07 season. As shown in Figure 2 there were no statistical differences in the incidence or severity of Fusarium wilt in cotton (as measured by the % of plants surviving to maturity and the % of plants rating 0 and 1) where sorghum residues of the previous crop had been either burnt or incorporated.

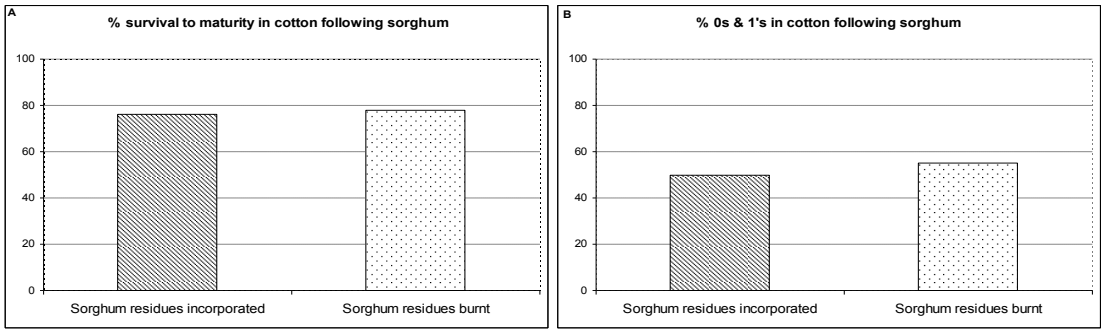
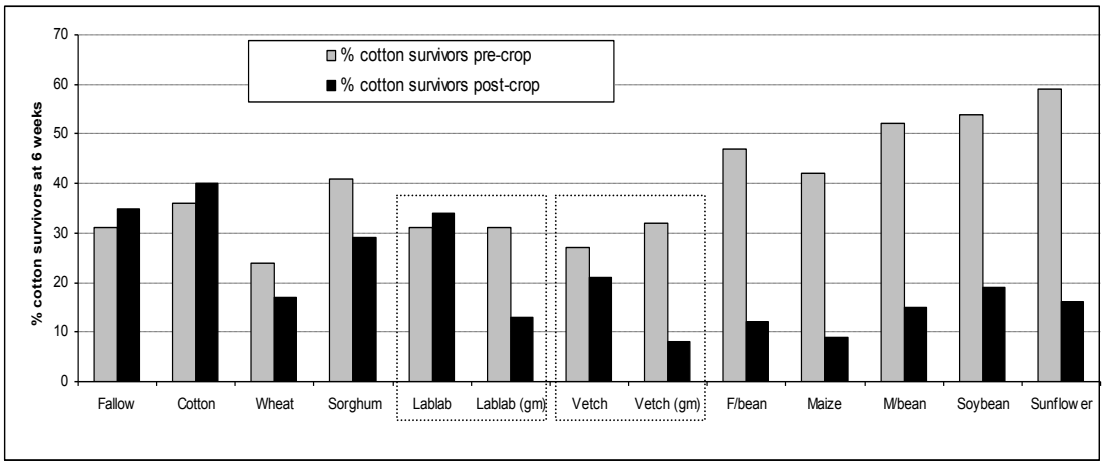


Figure 2. A) The percentage of cotton plants surviving until maturity and B) the percentage of plants rating zero and one where sorghum residues of the previous crop had either been incorporated or burnt

Glasshouse experiments: seedling and green manure study

In a glasshouse study cotton was grown before and after a series of different crops as shown in Figure 3. A large reduction in the number of cotton survivors occurred following fababean, mungbean, maize, soybean and sunflower. A comparison of vetch and lablab was made by either growing and

removing the plants totally, or incorporating the green plant material back into the soil as a green manure crop. Green manuring of these two crops resulted in greater cotton deaths compared to simply removing the plant material. These studies confirm what has been found previously when other crops were incorporated in the presence of Fusarium, highlighting the importance of organic matter in increasing the severity of this pathogen.



(Lablab (gm) = lablab green manure crop; Vetch (gm) = vetch green manure crop)

Figure 3. The percentage of cotton seedlings surviving before and after different crops were grown or green manured (incorporated)

As with previous experiments, continuous bare fallow resulted in the lowest disease in glasshouse pot trials (Table 5). Any crop material going into the soil is maintaining or increasing the fusarium wilt pathogen. Continuous lucerne over 4 cycles had the greatest disease. A cotton-fallow-maize-fallow rotation had significantly more disease than a cotton-fallow-sorghum-fallow or cotton-fallow-cotton-fallow rotation. Bearing in mind the constraints of pot studies, further work needs to continue especially in the monitoring and quantifying of changes in the soil pathogen population.

Table 5. Mean Disease Index (MDI) and the percentage cotton survivors following different rotation sequences

Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	MDI	% survivors
fallow	fallow	fallow	fallow	cotton	1.30 a	74
cotton	fallow	sorghum	fallow	cotton	2.16 ab	67
cotton	fallow	cotton	fallow	cotton	2.24 ab	62
soybean	wheat	fallow	fallow	cotton	2.54 abcd	74
sorghum	chickpea	maize	wheat	cotton	2.61 abcd	58
fallow	barley	soybean	wheat	cotton	2.64 abcd	62
cotton	wheat	fallow	wheat	cotton	2.91 abcd	60
fallow	chickpea	fallow	wheat	cotton	3.08 abcd	56
soybean	wheat	cotton	fallow	cotton	3.17 abcd	62
cotton	fallow	sorghum	chickpea	cotton	3.18 abcd	28
cotton	wheat	fallow	fallow	cotton	3.18 abcd	42
cotton	wheat	sorghum	fallow	cotton	3.23 bcd	50
cotton	wheat	cotton	wheat	cotton	3.27 bcd	56
cotton	wheat	soybean	fallow	cotton	3.31 bcd	39
fallow	wheat	sunflower	fallow	cotton	3.50 bcd	44
cotton	wheat	fallow	chickpea	cotton	3.70 bcd	33
soybean	barley	maize	fallow	cotton	3.92 bcd	34
cotton	fallow	maize	fallow	cotton	4.17 d	26
lucerne	lucerne	lucerne	lucerne	cotton	4.38 d	12

Objective 5

Development of a glasshouse bioassay

Cotton growth was healthy in non-infected pots indicating adequate nutrition and growing conditions. Inoculated isolates of npFo were successfully recovered from seedlings. Not all seedlings became infected with the pathogen, however overall sufficient seedlings were diseased to determine differences between the control and treated seedlings. A glasshouse bioassay was successfully

developed yielding healthy cotton seedling growth and adequate infection of *Fov* in the control treatment.

Evaluation of npFo for biocontrol potential against *Fov* using the glasshouse bioassay

Experiment 1

Visual observations of seedling growth

A difference in seedling growth was evident throughout the earlier stages of this experiment. Noted primarily were differences in height, seedling survival and general seedling health and appearance. Reductions in the germination and growth of inoculated seedlings were evident in comparison to seedlings of the control treatment.

Colonisation of seedling roots

Growth occurred in each of the plated out root samples, indicating that the seedling roots had been colonised by the npFo isolates. However root growth was extremely poor, indicating that npFo isolates were potential root pathogens. No vascular discolouration was observed indicating the isolates were not vascular wilt pathogens.

Vascular discolouration rating

Plants that survived treatment with isolates of npFo were evaluated for their biocontrol potential against *Fov*. None of the isolates tested significantly reduced the mean rating for vascular discolouration (P=0.405) (Table 6).

Table 6. The effect of soil application of *Fusarium oxysporum* at sowing on infection of *Fusarium oxysporum* f. sp. *vasinfectum* of cultivar Siokra V17

Treatment	MRVD[A]
Control	1.04
M1-4	1.13
M3-4	1.53
NF6-4	0.57
NFP4-2	1.3
NFP5-5	0.98
Wald test (P=0.405)	NS

[A] Mean rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant. NS = Not significant

Statistical analysis

This data was analysed using REML in GenStat for Windows (Seventh Edition 2004, Lawes Agricultural Trust, Rothamsted Experimental Station, Herts AL5 2JQ, England). A mixed model was used with the random effects being reps/pots i.e. Reps + pots within Reps (residual) and the fixed effects being Treatment. Significance of the fixed effect Treatment was assessed using a Wald test.

Experiment 2

Seedling germination

There was a significant effect of the application rate of isolate NFP5-5 on seed germination (Table 7). When applied at 70 and 140 mL/tray, significantly fewer seeds germinated. There was no significant effect of NFP5-5 applied at 30 mL however fewer seedlings emerged than in the control treatment.

Table 7. Rate effect of non-pathogenic *Fo* isolate NFP5-5 on seed germination of cultivar Siokra V17

Treatment	No. seedlings
Control	22
140 mL NFP5-5	3
70 mL NFP5-5	4
30 mL NFP5-5	14
LSD (P=0.05)	9

All isolates examined severely reduced germination of Siokra V17 (Table 8), indicating these isolates were potential root pathogens. No further assessment was therefore conducted on these seedlings.

Table 8. The effect of various non-pathogenic *Fo* isolates on seed germination of cultivar Siokra V17

Treatment	No. seedlings
Control	22
M1-7	1
M3-14	2
NF5-1	3
NFP3-11	2
NFP5-2	2
LSD (P=0.05)	5

Statistical analysis
Variances (ANOVA) for replicate and treatment effects were determined using GenStat for Windows (Seventh Edition 2004, Lawes Agricultural Trust, Rothamsted Experimental Station, Herts AL5 2JQ, England).

Experiment 3
Non-pathogenic *Fo* isolates evaluated had no significant effect on disease severity (P=0.685) of cultivar Siokra V17 (Table 9).

Table 9. The effect of non-pathogenic strains of *Fusarium oxysporum* on infection of *Fusarium oxysporum* f. sp. *vasinfectum* on cultivar Siokra V17 when applied as a pre-plant soil application to field soil naturally infested with *Fov*

Treatment	MRVD[A] ± SE
Control	2.58 ± 1.28
FO2	2.19 ± 0.96
FO3	1.37 ± 1.01
Wald test (P=0.05)	NS

[A] Mean rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant. NS = Not significant

Statistical analysis
Data were analysed using a mixed model in REML in GenStat. Random effects were Rep/Plot/Plant, i.e. Rep + Rep*Plot + Rep*Plot*Plant (residual), and fixed effect of Treatment. The full model was fitted initially, then negative and zero variance components removed to arrive at the final random model. Significance of the fixed effect was assessed using a Wald test. If the Wald test indicated a significant Treatment effect, approximate pair-wise comparisons between means were made. For each trial, treatment means, their standard errors and differences between them were calculated.

Experiment 4
When 50 ml of npFo inoculum was applied only 5% of seedlings survived. It was determined that poor emergence and seedling survival was due to *Pythium* spp. rather than *Fusarium*. When 100 mL of inoculum was applied a greater percentage (39%) of seedlings survived. In addition, when 100 mL of inoculum was applied, there was a significant effect of npFo isolates on plant survival. Isolates 5,

a

6, 14 and 16 significantly increased the percentage of plants surviving (Figure 4) however there was no significant effect of these isolates on vascular discolouration caused by *Fov*.

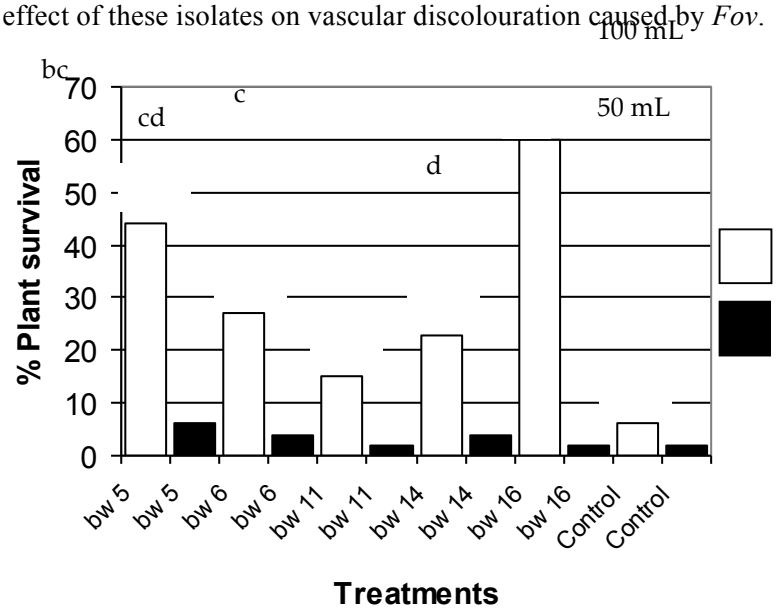


Figure 4. Rate effect of non-pathogenic *Fusarium oxysporum* on survival of 11 week old seedlings (Siokra V17) when seed was sown into field soil naturally infested with *Fusarium oxysporum* f. sp. *vasinfectum* and *Pythium* mixed 50:50 with sand
Data columns with different letters above are significantly different from one another

Conclusions
While other studies have indicated that npFo isolates may be used to control Fusarium wilt in various crops of economic importance, the results from experiments conducted and reported here did not identify any potential npFo isolates for the control of Fusarium wilt in cotton seedlings. No significant reductions in disease severity were found when seedlings were colonised by npFo isolates prior to exposure to *Fusarium oxysporum*. It may be concluded that none of the npFo isolates screened within these experiments cross-protect cotton against Fusarium wilt.

Some isolates were detrimental to germination and seedling growth, and as such, are suggested to be root pathogens, forming part of what is referred to as a cotton-seedling-complex (pers. comm. J. Kochman). Where the rate of inoculum was varied, it was shown that such inhibitory effects increased with the rate applied. Interestingly four isolates significantly increased seedling emergence and survival of cotton plants grown in field soil naturally infested with *Pythium* spp. and may have potential to form part of an integrated management program for this pathogen.

Objective 6

In-vitro plate test
Germination of *Fov* spores was reduced when mixed with potassium silicate at 10 and 20 mL/L. This response is most likely a pH affect as potassium silicate is highly alkaline at pH 11.3. There was a significant increase in growth of colonies (colony diameter) when conidia were treated with potassium silicate at 0.1, 0.25, 1.0 and 5 mL/L (Table 10).

Table 10. Effect of potassium silicate on spore germination and growth of *Fov* on ¼PDA/S

Potassium silicate (mL/L)	No. Colonies	Colony Diameter (mm)
0	13.7	19.33
0.1	15.0	21.60
0.25	15.0	23.27
1	11.0	22.23
5	13.0	21.37
10	6.3	19.6
20	6.3	19.0
LSD (P<0.05)	4.3	1.9

A growth response to silicon has been reported previously (Al-Falih-Abdullah-M (2000)) in which silicic acid was shown to stimulate the growth of various Fusarium species, including Fusarium oxysporum when added to Czapek Dox liquid medium. The biomass of *Fusarium oxysporum* was five-fold higher than the control.

It appears from our study that unless very high concentrations of potassium silicate are used in glasshouse bioassays it is unlikely that potassium silicate will have a direct effect on germination and growth of *Fov*.

Statistical analysis
Variances (ANOVA) for replicate and treatment effects were determined using GenStat for Windows Sixth Edition 2002 (Lawes Agricultural Trust, Rothamsted Experimental Station, Herts AL5 2JQ, England).

Glasshouse bioassays
Experiment 1
Results suggest that silicon (and potassium) may play a role in suppression of Fusarium wilt of cotton, but only when more resistant cultivars are grown (Figure 5). When a highly susceptible cultivar was grown, such as Siokra 1-4, there was no disease suppression (data not shown).

Potassium silicate applied at 5 mL/L significantly reduced the mean rating of vascular discolouration (Figure 5). The mean rating of vascular discolouration was also significantly reduced when 10 mL/L of potassium silicate was applied; however at this rate potassium silicate was also phytotoxic. Phytotoxicity is most likely due to the high pH of solution.

Potassium sulphate also significantly reduced disease severity (Figure 5). There are reports that the application of potassium to potassium deficient soils significantly reduces wilt severity (Rast 1922, Armstrong & Alber 1941, Walker 1957 and others).

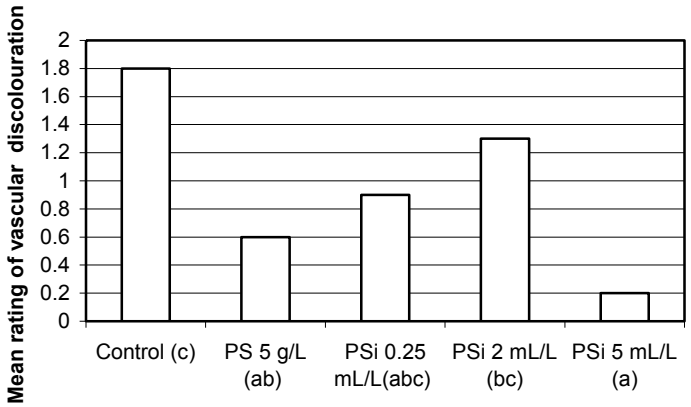


Figure 5. The effect of weekly application of potassium silicate and potassium sulphate solution on mean rating of vascular discolouration when cotton seedlings cv Siokra V17 were grown in soil naturally infested with *Fusarium oxysporum* f. sp. *vasinfectum* mixed 50:50 with sand
Where PS = potassium sulphate (0.45 g K /seedling flat) and PSi = Potassium silicate solution (maximum K applied = 0.1 g/seedling flat)
Rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant.
Treatments followed by different letters are significantly different from one another (P=0.024) (n=3, 40 seeds per tray).

Figure 6 also describes data from experiment 1 however it has been displayed differently from that in Figure 5 to show how different treatments effect the distribution or percentage of plants rated 0 to 5 for vascular discolouration. This display allows for a better understanding of how the treatments are affecting disease severity. For the untreated control treatment approximately 50% of plants displayed no vascular discolouration. When potassium silicate (at 5 mL/L) and potassium sulphate were applied weekly, there was an increase in the percentage of plants that were not diseased, in which approximately 87% of plants were rated 0 for no discolouration for both these treatments. In the

control treatment nearly 20% of plants died (rated 5) however there were no plant deaths when plants were treated with potassium silicate at 5 mL/L and with potassium sulphate.

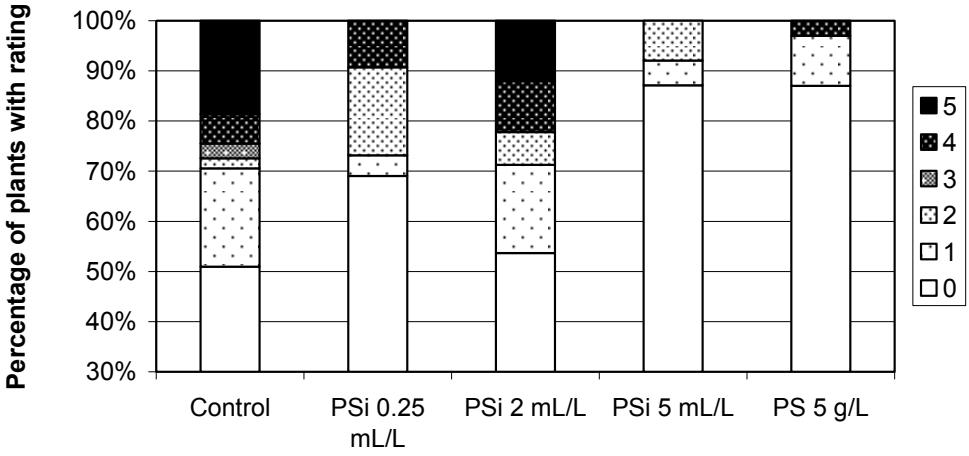


Figure 6. The effect of weekly application of potassium silicate and potassium phosphate on percentage of cotton plants rated 0 to 5 for vascular discolouration when seed was sown into soil naturally infested with *Fusarium oxysporum* f. sp. *vasinfectum* mixed 50:50 with sand
Where PSi = Potassium silicate solution (maximum K applied = 0.1 g/seedling flat). PS = Potassium sulphate (0.45 g K /seedling flat) n=3, 40 seeds per tray.
Rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant.

Statistical analysis
Variances (ANOVA) for replicate and treatment effects were determined using GenStat for Windows Sixth Edition 2002 (Lawes Agricultural Trust, Rothamsted Experimental Station, Herts AL5 2JQ, England).

Experiment 2
In addition to potassium silicate, both magnesium silicate and acidified wollastonite, when applied pre-plant and mixed uniformly into the soil mix, significantly reduced disease severity (P=0.027) confirming that disease reduction may be due to silicon amendment as well as potassium (Table 11).

Looking at the distribution of plants rated 0 to 5 (Figure 7) there was a significant increase in the percentage of plants that were rated 0 for no vascular discolouration when silicon was applied. There were also no plant deaths in silicon treated plants. The percentage of plants rated 0 has significance as this has the potential to translate to an increase in the number of plants surviving to maturity thereby increasing yield.

In these trials, where a significant reduction in disease occurred the pathogen inoculum was not high. The mean rating for discolouration was below 2 meaning that vascular discolouration was below the cotyledons and 50% or more plants were not showing vascular discolouration.

Table 11. The effect of silicon on infection of *Fusarium oxysporum* f. sp. *vasinfectum* on cultivar Siokra V17 following weekly soil application of Potassium Silicate solution and single pre-plant application of powdered silicon sources mixed uniformly into naturally infested field soil prior to sowing

Treatment	MRVD[A] ± SE
Control	1.64 ± 0.38 a
Silvine (0.7 g/L soil)	0.50 ± 0.32 b
Treated Wollastonite (1 g/L soil)	0.40 ± 0.40 b
Potassium Silicate solution (5 mL/L tap water)	0.15 ± 0.35 b

Means followed by the same letter are not significantly different at the P=0.05 level
[A] Mean of the rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant.

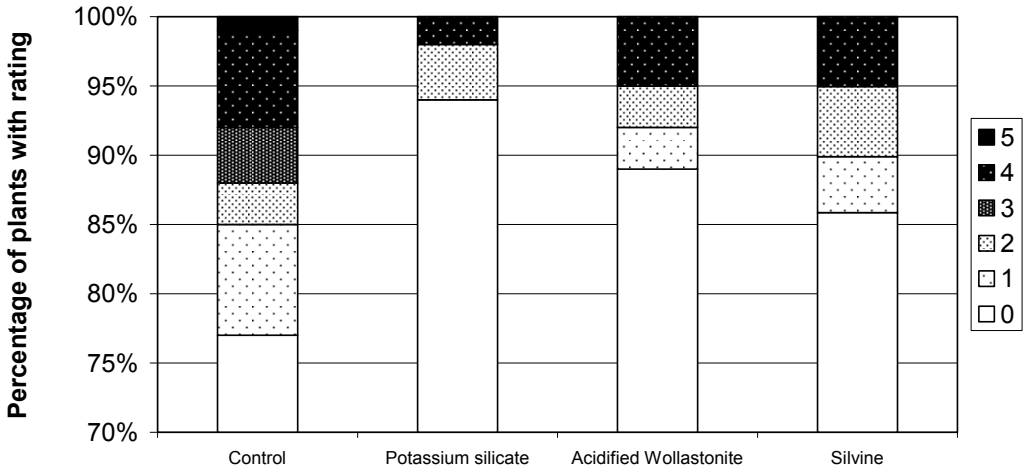


Figure 7. The effect of silicon on infection and disease severity of *Fusarium oxysporum* f. sp. *vasinfectum* on cultivar Siokra V17 following weekly soil application of potassium silicate solution and single pre-plant application of powdered silicon sources mixed uniformly into naturally infested field soil mixed 50:50 with sand prior to sowing
Rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant.

Statistical analysis
Data were analysed using a mixed model in REML in GenStat. Random effects were Rep/Plot/Plant, i.e. Rep + Rep*Plot + Rep*Plot*Plant (residual), and fixed effect of Treatment. The full model was fitted initially, then negative and zero variance components removed to arrive at the final random model. Significance of the fixed effect was assessed using a Wald test. If the Wald test indicated a significant Treatment effect, approximate pair-wise comparisons between means were made. Treatment means, their standard errors and differences between them were calculated.

Silicon analysis
Although there was a significant reduction in disease, there was no significant difference in silicon uptake into the leaves (Table 12). It is possible that the leaves were not the ideal plant part for assessment of silicon in these bioassay experiments which run for approximately 12 weeks. Silicon has been shown to be deposited first into the roots then other parts of plants, and as such root tissue may provide a more accurate reading of silicon uptake. However silicon analysis of the roots is difficult as all soil must be removed otherwise silicon present in soil particles attached to the roots contaminates the results, hence this was not performed. To accurately determine silicon uptake into plant roots a hydroponic system is required.

As nutrient analysis is expensive, replicate plants for each treatment were pooled in this trial. Separating plants according to rating for vascular discolouration for analysis of nutrient content may provide more accurate information on the relationship between nutrient uptake and disease severity, rather than pooling plants with and without disease for each treatment, which may have diluted differences in silicon uptake.

Fertiliser application influenced uptake of other elements. Calcium, magnesium, sulphur and phosphorus were significantly decreased following application of potassium silicate liquid weekly. Sulphur was significantly increased following application of Silvine and Treated Wollastonite. Phosphorus was significantly decreased following application of Treated Wollastonite (Table 12).

Table 12. Effect of silicon fertiliser on nutrient uptake measured by leaf analysis when silicon as a powder was mixed into the soil prior to sowing and liquid form was applied as a soil drench

Treatment	Ca	Mg	S	K	P	Si
Control	3.964	1.221	0.836	2.15	0.4402	0.277
Potassium silicate liquid	3.166↓	1.002↓	0.492↓	2.531	0.345↓	0.362
Silvine	4.095	1.26	1.086↑	2.323	0.4228	0.238
Treated Wollastonite	4.184	1.254	1.025↑	2.227	0.365↓	0.238
LSD	0.5475	0.1706	0.1275	NS	0.04793	NS

Experiment 3

There was no effect of silicon on disease at a higher inoculum level of the pathogen (Figure 8), and there was no interactive effect of phosphorus and silicon on disease in this experiment (data not shown). What was interesting was that there was an effect of phosphorus on disease severity. When phosphorus was applied at a high rate of 42 kg/ha, (phosphorus is more commonly applied at 20 kg/ha) there was a significant increase in disease severity (Figure 9). There were fewer plants without disease and more plants died following phosphorus application.

Studies conducted by Sharoubeem *et al* in 1967 investigating the influence of potassium, nitrogen and phosphorus in relation to the incidence of cotton Fusarium wilt concluded that the amounts of phosphate should not be elevated above their natural soil level in the soil, while potassium and nitrogen should be raised up to 1000 ppm so as to reduce incidence to the lowest level.

When the disease levels were much higher in the untreated control (Figure 8) with a mean rating of 3.0 where discolouration was above the cotyledon and the % of plants rated 0 was only around 20% the application of silicon had no effect on disease severity. Hence inoculum level of the pathogen is very important in terms of level of control provided by silicon application.

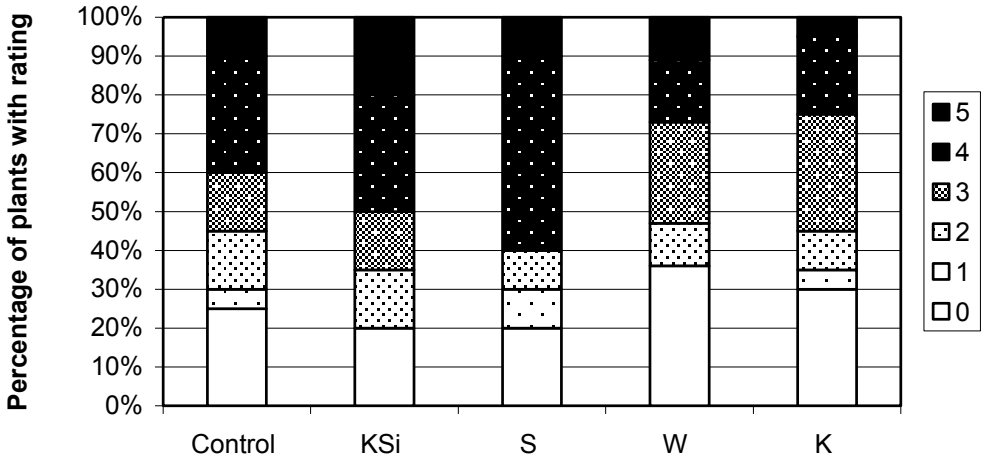


Figure 8. The effect of silicon amendment on disease severity

KSi – Potassium silicate, S – Silvine, W – Wollastonite, K – Potassium sulphate

Rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant.

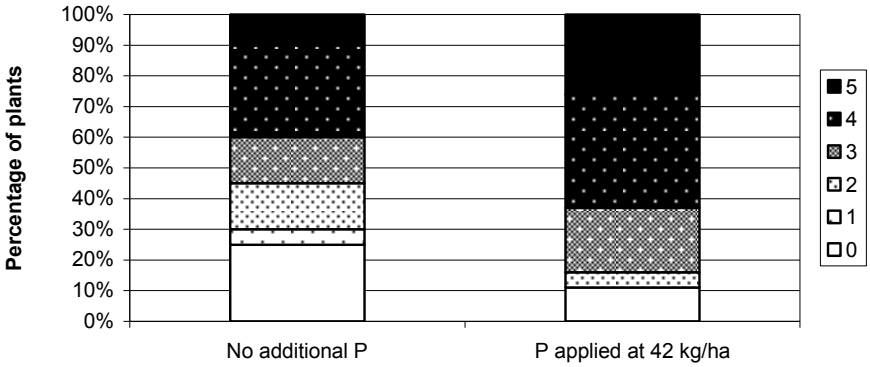


Figure 9. The effect of high phosphorus application on severity of Fusarium wilt of cotton
Rating of vascular discolouration where 0 = no infection, 1 = base of stem infected below soil level, 2 = infection up to node 0, 3 = infection above node 0, 4 = infection up to top node and 5 = dead plant (n=20).

Field trial evaluation

Trial 1 (2004-2005)

There was no significant effect of silicon treatments on emergence, establishment or 3rd count (data not shown). The plot below (Figure 10) shows the pattern of percentage plants present over time (measured as days since the first observation) for each of the treatments. These plots indicate considerable variability within each treatment i.e. between replicate plots. Hence it is very difficult to determine statistically, differences among treatments with this level of variability.

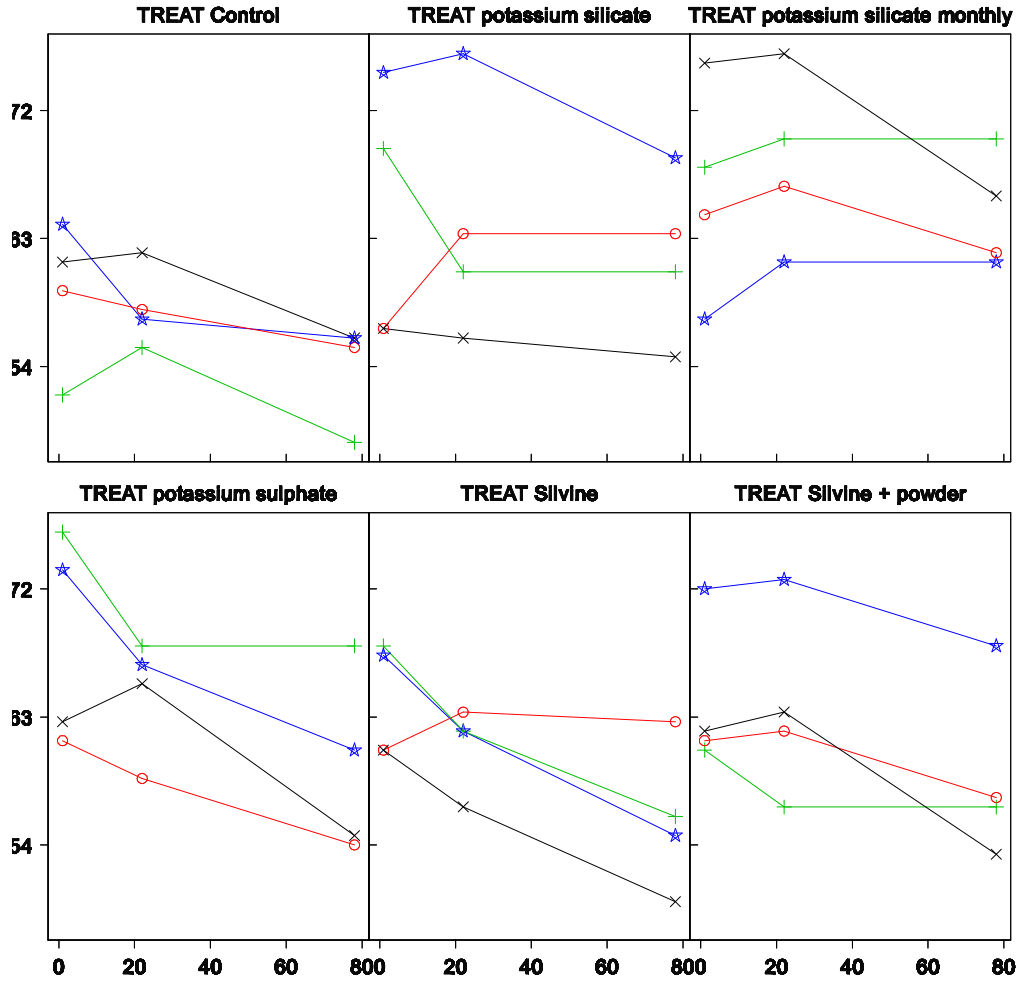


Figure 10. Pattern of percentage plants present over time for each replicate per treatment

Table 13. The effect of silicon based products and potassium sulphate on nutrient content of the 5th terminal leaf, disease severity assessed as % 0 and 1's, and seed cotton yield of cotton plants grown at 'Cowan', Cecil Plains in soil naturally infested with *Fusarium oxysporum* f. sp. *vasinfectum*

Treatment[A]	Nutrient analysis of the fifth leaf				% 0 and 1's	Yield kg/10m plot
	Si % dm[B]	P % dm	K % dm	Mg % dm		
1	0.0635 (0.025)	0.040	0.123	0.124	65.9 (0.95)	4.47
2	0.071 (0.027)	0.041	0.124	0.120	72.7 (1.022)	4.29
3	0.0652 (0.026)	0.041	0.132	0.122	67.5 (0.965)	4.13
4	0.066 (0.026)	0.042	0.130	0.120	68.9 (0.995)	4.00
5	0.0385 (0.020)	0.043	0.129	0.121	67.1 (0.96)	3.85
6	0.0362 (0.020)	0.042	0.125	0.120	67.5 (0.96)	4.38
LSD (P=0.05)	(0.002)	ns	ns	ns	ns	ns

[A] = Treatments applied where 1 = Silvine granules (Magnesium silicate) 150 kg/ha; 2 = Silvine granules 75 kg/ha plus Silvine powder 3.5 g/L water; 3 = Potassium silicate powder 150 kg/ha plus Potassium silicate liquid 10 mL/L plus foliar application potassium silicate liquid 2.5 mL/L fortnightly for 8 weeks; 4 = As for treatment 3 however Potassium silicate liquid foliar application also applied monthly until bolls formed; 5 = Untreated control; 6 = Potassium phosphate 8 g/L per metre row.
[B] = Data in parentheses are transformed percentage data (ASIN(SQRT(X/100)).

An important result of this trial was that silicon uptake was significantly increased following silicon soil amendment in an alkaline soil (Table 13). However, despite an increase in silicon uptake there was no effect of silicon on disease severity (Table 13). The threshold level of silicon required in cotton for disease control is not known and may not have been met in this trial. There was also no significant effect of silicon treatments on yield (Table 13 and Figure 11), even though the untreated control treatment had the lowest yield and magnesium silicate the highest. There was however a lot of variation between replicate plots in this trial, as discussed, a common problem encountered in field trial assessment.

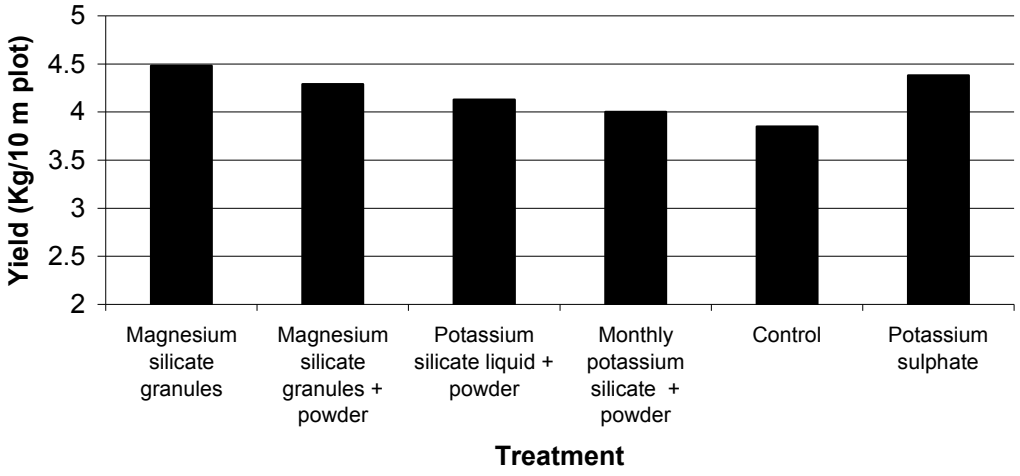


Figure 11. The effect of silicon amendment on seed cotton yield of cultivar Sicot F-1

There were some significant effects of silicon on fibre quality. The upper half mean length (UHML) was significantly reduced when potassium silicate solution was applied to the leaves until boll formation (Table 14). This result is interesting as there was no difference in silicon (and potassium) uptake between treatments 3 and 4 in which potassium silicate was applied to foliage fortnightly for 8 weeks for treatment 3 and until boll formation for treatment 4, but the longer application of silicon solution influenced fibre length. More research is needed to understand the impact of foliar application of silicon on fibre development.

Table 14. The effect of silicon fertilisation on aspects of cotton fibre quality

Treatment [A]	Mic	SFI	Uniformity	Strength	UHML
1	4.525	4.95	85.2	33.32	1.160
2	4.753	5.43	85.2	33.73	1.156
3	4.313	4.95	85.4	34.28	1.187
4	4.457	5.93	84.8	32.96	**1.146**
5	4.575	5.27	85.2	33.92	**1.172**
6	4.547	4.55	85.4	33.6	1.174
LSD (P<0.05)	NS	0.81	NS	NS	0.023

A B

NS = not significant

[A] = Treatments applied where 1 = Silvine granules (Magnesium silicate) 150 kg/ha; 2 = Silvine granules 75 kg/ha plus Silvine powder 3.5 g/L water; 3 = Potassium silicate powder 150 kg/ha plus Potassium silicate liquid 10 mL/L plus foliar application potassium silicate liquid 2.5 mL/L fortnightly for 8 weeks; 4 = As for treatment 3 however Potassium silicate liquid foliar application also applied monthly until bolls formed; 5 = Untreated control; 6 = Potassium phosphate 8 g/L per metre row.

MIC = Micronaire, SFI = Short fibre index, UHML = Upper half mean length.

Trial 2 (2005-2006)

In this trial silicon uptake into the plant was not increased by Si amendment of the soil pre-plant (Table 15), and there was no effect of Si amendment on establishment, disease severity, seed cotton yield (Figure 12) of fibre quality. There was however a significant effect of variety on disease severity as expected, with less disease in Sicot F-1 as determined by percentage of plants with less than 5% vascular discolouration (% 0 and 1's).

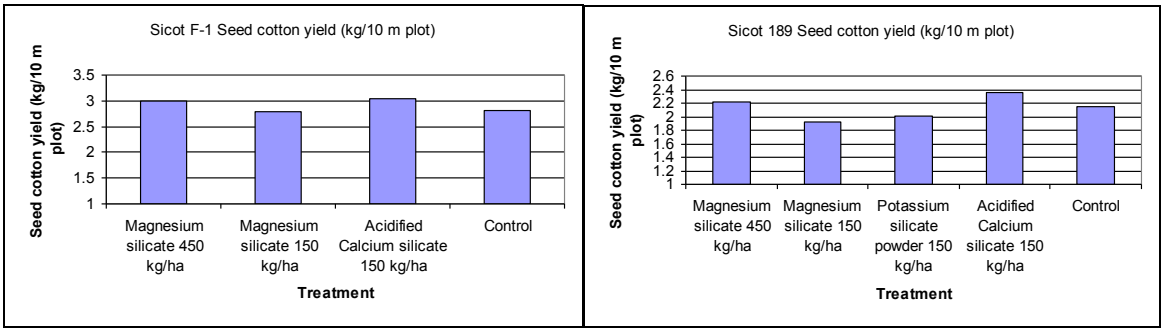


Figure 12. The effect of silicon amendments on seed cotton yield of Sicot F-1 (A) and Sicot 189 (B)

Although there was no treatment effect on silicon uptake, there was however a varietal effect. There was a significantly higher level of silicon in the leaves of the more susceptible variety Sicot 189 than the more tolerant variety Sicot F-1 (Table 15). Other nutrient differences in the leaves were determined between varieties including aluminium, iron and manganese which were significantly lower in Sicot F-1 than Sicot 189 (Table 15). Nutrient difference may not be important from an agronomic sense when in sufficient concentrations above what is considered deficient, however it is not known if they are important for disease resistance/susceptibility?

Trial 3 (2006-2007)

There was a varietal effect on emergence and establishment, with a higher number of Sicot F-1 plants establishing compared to Sicot 189. However there was no treatment effect of silicon or phosphorus, on emergence or establishment. There was also no treatment effect (Si or P) on the percentage of plants rated 0&1 for disease or fibre quality.

Silicon amendment had no significant effect on seed cotton yield (P=0.547), however for phosphorus, although differences were not significant at P=0.052, the application of phosphorus to Sicot 189 increased yield in the untreated control and foliar potassium silicate treatment (Figure 13).

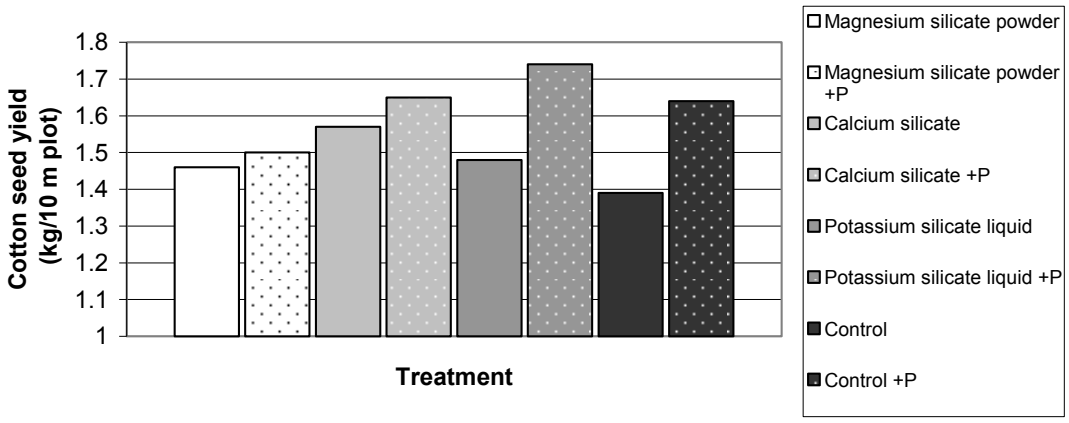


Figure 13. The effect of silicon and phosphorus on seed cotton yield of Sicot 189

Conclusions

Results from the silicon trials supported prior research that silicon may be important in reducing the severity of Fusarium wilt in cotton seedlings. Significant reductions in disease severity were obtained following the application of silicon-containing fertilisers in glasshouse trials but were not effective under field conditions. Results of glasshouse trials suggest that inoculum level influences effectiveness of silicon amendment. The trial site at 'Cowan' which was used for all experiments is known for its high inoculum load, and for this reason has been used routinely for screening germplasm and new lines for wilt resistance. It is possible that inoculum load was too high for an effect of silicon to be realized. Increasing nutrient availability in an alkaline soil also can be difficult and may have contributed to poor uptake. However the same soil was used in both the glasshouse bioassays (although it was diluted with sand) and field trial assessment, therefore a response in the field was expected. Another consideration is that placement of fertiliser, which dictates availability of silicon for uptake, may not have been optimal as it was applied to a 10 cm trench dug into the raised bed. Interesting findings were published by Singh *et. al.* in 2005 regarding deep placement of phosphorus and the effect on various crops including cotton grown at Kununurra in Western Australia. Significant increases in the yield of seed cotton occurred when 50 kg/ha was applied at depth (10-15 and 25-30 cm), compared with the conventional placement at 7-10 cm. The response to deep phosphorus in these layers was attributed to the rapid drying of the soil surface layers, reducing the availability of soil or phosphorus fertiliser in these layers. The deep phosphorus remained available during the growing season and alleviated the phosphorus deficiency that appeared to be a feature of these soils when the surface layers became dry. This may very well be the case for silicon. Had the silicon fertiliser been applied deeper in the bed, or mixed uniformly throughout the soil profile, different results may have been observed.

So why does high or excessive phosphorus (P) increase disease severity? Unfortunately the influence of soil P on *Fusarium* is poorly understood. Possible reasons for increased severity of Fusarium wilt include given in the literature are: 1) Excessive P reduces mycorrhizal colonisation which influences phenol metabolism and therefore lignification. A decrease in phenols may increase wilt susceptibility; 2) P-mediated increase in shoot growth makes plants more succulent and therefore more susceptible; 3) An increase in transpiration in high-P plants may increase there susceptibility; 4) High P may create an imbalance in the plants nutrient uptake, thereby influencing disease susceptibility; and 5) Plants fertilised with excessive P have less Si available in soil solution, hence reduced uptake of Si resulting in lowered biochemical defenses and resistance to disease. Further study is required to investigate the effect of P and interaction of nitrogen and potassium on disease severity.