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: **03DAQ003**

Project Title: Cotton Fusarium Wilt Management

Project Commencement Date: 01/07/2007 **Project Completion Date:** 30/06/2010

CRDC Program: 3 Crop Protection

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

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

Background

1. Outline the background to the project.

Since 2002/03 the distribution and importance of diseases of cotton in production areas of Queensland have been monitored by DEEDI pathologists in collaboration with Dr Stephen Allen (CSD) during annual surveys of commercial crops. During this survey information is collected on cropping history, ground preparation, variety, seeding rate, sowing date, carry-over of crop residues, survey date and crop growth stage as well as the incidence and severity of those diseases present. A summary of this data has been published annually by Cotton Seed Distributors Ltd. in their 'CSD Trial Results'.

The information collected during these disease surveys gives direction to cotton disease research and indicates the impact of farming practices on disease incidence and severity. The disease survey data also illustrates the history and success of the cotton breeding effort by identifying significant diseases, quantifying the impact of those diseases and correlating increased use of a resistant variety with declining incidence of a disease.

The diagnostics laboratory at Indooroopilly also plays an important role. Any wilted, stunted or suspicious plants suspected of being infected with *Fusarium oxysporum* f. sp *vasinfectum* (Fov), the causal agent of Fusarium wilt, are collected during annual disease surveys and sent in by cotton consultants and researchers, for analysis using Vegetative Compatibility Group (VCG) analysis to determine the strain of Fov.

Survey data and Fov analysis provides information on disease incidence and severity, location, effect of farming practices and the occurrence of new Fov strains. Hence there is a need to continue annual disease surveys of commercial cotton crops in all areas of eastern Australia for the presence of absence of disease, including continuation of the diagnostic laboratory at Indooroopilly to determine the severity, spread and diversity of this pathogen.

Fusarium wilt of cotton was first identified on the Darling Downs in Queensland in 1993. Since then, this destructive disease of cotton has continued to spread and is now in most cotton growing districts in Queensland and New South Wales, with the exception of Hillston, Tandou and Emerald, but it has not been found in the Northern Territory or Western Australia. The pathogen can infect cotton at all stages of growth and has been shown to cause a significant seedling death at the start of the season, particularly in adverse conditions, often killing the majority of seedlings of very susceptible varieties. It can also cause significant plant deaths during the boll-filling phase. Once a farm is infested with Fov there is no commercially viable way to eliminate the pathogen from the soil.

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 the foundation of any strategies to manage this disease but they will need to be in combination with other agricultural practices to provide sufficient control to allow sustainable cotton production.

Data from DAQ130C highlighted the importance of a fallow field prior to sowing cotton for the management of this disease. Also some rotation crops such as soybean and mungbean potentially could increase inoculum levels of Fov in the soil and therefore increase disease severity in subsequent cotton crops. Residue and organic matter levels may also influence field pathogen survival and disease incidence. This work has given us some understanding of the impact of rotations and organic

matter on Fov survival and consequent disease; however there is a need for further research into rotation options and the role of crop residue, organic matter and green manuring of crops in relation to pathogen survival.

Investigations commenced in DAQ130C on the effect of phosphorus fertilisation on disease severity. The literature shows that high P may increase Fusarium wilt severity, particularly in the absence of adequate N and K (Dick & Tisdale 1937; Young & Tharp 1941). Our results showed that when P was applied at a high rate of 42 kg/ha (P is more commonly applied at 20 kg/ha) there was a significant increase in disease severity. Further glasshouse and small-plot trial evaluations are needed to investigate fertiliser regimes (NPK) that reduce the impact of Fusarium wilt. Hence agricultural practices to be investigated in this project for their influence on Fusarium wilt severity are alternative rotation crops and cotton crop nutrition.

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. This objective has been achieved.

Cotton disease surveys

Commercial cotton crops across Queensland were inspected in November – December (2007, 2008, 2009) and in March – April (2008, 2009, 2010) in collaboration with Stephen Allen (CSD). The incidence and severity of those diseases present were assessed and field history, ground preparation, cotton variety, planting date and seed rate were recorded for each of the 44, 55 and 54 fields surveyed in the 2007/08, 08/09 and 09/10 seasons respectively. The incidence of Fusarium wilt across transects was assessed in each year of the project and trends were identified.

Diagnostic samples and database

Eighty-five (85) diseased cotton samples were received at Indooroopilly for assessment during 1 July 2007 – 30 June 2010. Stem sections with vascular discolouration were plated onto growth media and *Fusarium oxysporum*, if present, was single spored and analysed using Vegetative Compatibility Group (VCG) analysis to determine the strain of the pathogen. The results were entered onto the data base and also communicated back to the sender of the initial sample.

Objective 2.

Investigate the role of crop rotation and crop residue on the ecology of Fov and on subsequent disease development in cotton. This objective has been achieved.

Field rotation trial evaluation

A three year irrigated field trial was conducted at ‘Cowan’, Cecil Plains and the influence of different rotation crops and their residues on subsequent disease development in cotton was assessed in the final year.

Glasshouse trials

A series of glasshouse trials were conducted to investigate the susceptibility of 24 crop species to Fov when artificially inoculated. To confirm if colonisation occurs naturally in these crops when grown in naturally infested field soil, pot trials were conducted to determine the extent of natural infection.

A green manure study was conducted to determine the effect of green manure incorporation on the population of Fov over a 12 month period.

Objective 3.

Investigate the role key nutrients play in host resistance, including the effect on mychorrizal colonisation. Nutrients of importance that require attention for nutrient balance and disease management are nitrogen, phosphorus and potassium. This objective has mostly been achieved; however the effect of NPK on mycorrhizal colonisation was not conducted.

Glasshouse trials

Glasshouse trials were conducted to examine the effects of N, P and K on severity of Fusarium wilt on cotton. Mycorrhizal colonisation was not determined because a piece of equipment used to assist in the clearing and staining of the roots was destroyed by concentrated acid when a colleague mistakenly used concentrated rather than diluted acid. An alternative method will need to be developed as the staining tubes had been made specifically for this task.

Field trials

Two field trials have been completed investigating the influence of N, P and K on disease severity and cotton yield for cultivars ranging in F-rank.

Other trials

A field trial was conducted at 'Atleigh', Cecil Plains to investigate the potential of biological products Natural Nitrogen & C-Cat to reduce the severity of Fusarium wilt and to increase yield.

Objective 4.

Develop and extend new information packages for disease management. Provide new information to industry through the extension network, cotton consultants and other industry forums when data becomes available. This objective has been achieved.

Objective 5.

Training of staff in Amplified Fragment Length Polymorphism PCR, to be used as a tool to characterise *Fusarium oxysporum* f. sp. *vasinfectum* (Fov). This has partly been achieved; training is on-going.

In May 2010 selected isolates were chosen for characterisation using Amplified Fragment Length Polymorphism PCR (AFLP-PCR or just AFLP), a PCR-based tool used in DNA fingerprinting. The AFLP technology has the capability to detect various polymorphisms in different genomic regions simultaneously. It is also highly sensitive and reproducible. As a result, AFLP has become widely used for the identification of genetic variation in strains or closely related species of plants, fungi, animals, and bacteria. Hence AFLP protocols used to characterise selected fungi, including Fov, were obtained from Dr Bo Wang (CSIRO) and Ms Cecilia O'Dwyer (UQ). Based on these methodologies, a protocol is being developed to enable the characterisation of Australian strains of Fov.

Methods

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

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.

Annual disease surveys

The distribution and incidence of diseases of cotton in Qld was determined in surveys conducted of 44, 55 and 54 commercial crops in November – December (2007, 2008, 2009) and in March – April (2008, 2009, 2010). The same fields were generally surveyed; however additional fields were surveyed if diseased plants or unusual symptoms had been observed by the grower, consultant or other researchers.

Field history, ground preparation, planting dates, rates and cotton varieties were recorded for each of the fields surveyed. In each field, the incidence of disease was assessed along at least two diagonal transects (each 200m) by which 100 plants were assessed per transect for various diseases. Within each transect 10 groups of 10 plants spaced 20m apart were physically inspected. A step point method is used to measure distance. GPS coordinates were recorded at each entry point per field to allow for revisits to the same entry point if needed.

Survey methods vary between early and late season surveys. For early disease surveys, seedling mortality is assessed in one metre of row, by comparing an estimate of the number of seeds planted per metre compared to the number of plants established per metre. The comparison produces an estimate of seedling mortality which includes the impact of seedling disease (Rhizoctonia and Pythium etc.) as well as seed viability, the activity of soil insects such as wire worms, physical problems such as fertiliser or herbicide burn and the effects of adverse environmental conditions. Ten plants are also assessed for Fusarium wilt if symptoms of wilting are present. This is repeated at each survey point. On the late season survey, 10 full size plants are chosen at each point. Plants are first assessed for the presence and severity of Phenoxy herbicide damage, Alternaria leaf spot, bunchy top, and the incidence of boll and tight locks. The stem of each plant is then cut and assessed for vascular discolouration, which may be caused by Fusarium, Verticillium or Sudden wilt. Stem samples are collected and examined at Indooroopilly laboratories for confirmation of the presence of *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) or *Verticillium dahliae*, the causal agents of Fusarium wilt and Verticillium wilt of cotton respectively.

These surveys, which are conducted in collaboration with Dr Stephen Allen (CSD) 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 (VCG) analysis. Molecular characterisation has not been conducted at Indooroopilly during this project following the closure of the CRCTTP. However, Dr Bo Wang (CSIRO) based in Canberra has used AFLP analysis to characterise those isolates for which VCG analysis could not provide an answer. Dr. Bo Wang will not be available to assist with the characterisation of Fov in future projects as he is

leaving CSIRO due to lack of funding. Linda Smith is currently training to conduct AFLPs to characterise Fov.

Mungindi isolates – Pathogenicity Test

In 2005, a Fo isolate was recovered from diseased cotton in Wyadrigah, NSW. VCG analysis was conducted but the isolate did not pair with either VCG 01111 or 01112. A pathogenicity test was conducted by Wayne O'Neill in 2006 to confirm that the isolate was pathogenic on a susceptible cotton cultivar under glasshouse conditions.

In 2009, additional isolates were collected from the same field in Wyadrigah, NSW by Chris Anderson. Again VCG analysis did not yield a positive result. Pathogenicity tests were conducted to confirm that isolates were pathogenic on cotton. AFLP analysis was conducted by Dr Bo Wang to determine strain of pathogen.

Pathogenicity test
Siokra 1-4 seeds were sown into a seedling flat containing commercially produced potting mix. After 12 days, roots of treated seedlings were soaked for 5 minutes in a 50 ml suspension of Fov spores containing approximately 4 x 10^6 spores/ml. For the control treatment, seedlings were soaked in 50 ml of water. Seedlings were potted up in UC potting mix in 10 cm black pots and the remaining spore suspension or water only was drenched around roots. Plants were placed in the glasshouse and observed for the development of symptoms of Fusarium wilt. After 9 weeks, plants were harvested and examined internally for vascular discolouration. Vascular tissue from the lower stem was excised and plated onto ¼PDA/S to isolate Fov.

Treatments : Control – water only treatment
24500 VCG 01111 – Fov standard of known VCG
24590 – original Fov isolate from Wyadrigah recovered from diseased cotton in 2005
25358 – isolate recovered from diseased cotton from Wyadrigah in 2009
25359 – isolate recovered from diseased cotton from Wyadrigah in 2009

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.

Objective 2.

Investigate the role of crop rotation and crop residue on the ecology of Fov and on subsequent disease development in cotton. This objective has been achieved.

Field Rotation Trial Evaluation
A three year irrigated field trial at 'Cowan', Cecil Plains 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.

Year 1 (2007/08): 8 m plots, 3 reps of 10 treatments. Sorghum, maize and cotton were planted. Cotton (Sicot 80 BRF, F-rank 115) was planted November 5, 2007. Following harvest the residues of sorghum and maize were either left retained on the surface or incorporated into the soil. Cotton residues were mulched and incorporated.

Year 2 (2008/09): A change in farm machinery being used (8 m equipment replaced by 12 m) resulted in a change to the formation of the rotation trial. The trial became 6 m plots, 4 reps of 10 treatments. Due to the timing of the machinery change the originally planned winter wheat (where the crop

residues were to be managed differently) was unable to be planted. This resulted in 3 of the same treatments (fallow-cotton-cotton); see Table 1. Cotton (Sicot 80 BRF, F-rank 115) was planted November 13, 2008. Soybean was planted December 2008.

Table 1. "Cowan" rotation trial 2007-2010 treatments

2007/08 (8 m plots)	2008/09 (6 m plots)	2009/10 (6 m plots)
cotton	cotton	cotton
cotton	fallow	cotton
fallow (1)	cotton	cotton
fallow (2)	cotton	cotton
fallow (3)	cotton	cotton
fallow	soybean	cotton
sorghum (retained)	fallow	cotton
sorghum (incorporated)	fallow	cotton
maize (retained)	fallow	cotton
maize (incorporated)	fallow	Cotton

Year 3 (2009/2010): All treatments were sown to cotton (Siokra V-18 BRF, F-rank 125) on November 4, 2009.

Plant counts and disease assessment of cotton treatments: Every year when cotton was sown plant stands were determined at emergence (3 weeks after planting), establishment (6-7 weeks after planting) and after picking. Disease assessments were made at the end of the season by cutting the stems close to ground level and assessing the degree of internal vascular discolouration caused by *Fusarium oxysporum* f.sp. *vasinfectum* (Fov). The proportion of plants rated '0' (no vascular discolouration) and 1 (< 5% of the stem cross section showing vascular discolouration) was determined. Yield was also measured.

Soil population counts (soil dilution plate technique and pathogenicity tests): Soil samples were collected from all treatments in August 2008 and August 2009. Soil was plated out on selective medium, using the soil dilution plate technique, to estimate the total Fov/Fo soil population under the different rotation practices. Pathogenicity tests were carried out on a small subset of isolates in 2009 to distinguish pathogenic Fov from non pathogenic Fo. Details of methodology are described below under glasshouse trials.

Glasshouse Trials

1. Artificial inoculation of rotation crops with a spore suspension of Fov using the root-dip technique

Preparation of standard Fov culture

A standard isolate of Fov (VCG 11) was grown on half strength potato dextrose agar plates amended with streptomycin sulfate (½ PDA) for 5 days. A spore suspension was prepared by flooding plates with distilled water, scraping the hyphae off and filtering the suspension through four layers of tissue to separate excess hyphae from spores. The resulting spore concentration was quantified to between 350 000 and 1 500 000 spores/ml (depending on the experiment) with the aid of a haemocytometer. Details of each experiment are summarised below in Table 2.

Root-dip technique for inoculating seedlings

A steam sterilised potting mix, prepared at Indooroopilly, DEEDI was used for all experiments. Seedlings growing in small pots (125 mm diameter) of the soil mix were removed at 3-4 weeks of age and roots were washed in tap water. In initial experiments roots were trimmed severely prior to inoculation. The seedling roots were placed in the prepared spore suspension of Fov for 6 minutes

then replanted into the original pots. Any non-inoculated control seedlings were treated the same except they were dipped in distilled water for 6 minutes. Five pots were used for each crop, containing 3 plants/pot, although there was some variation in seed germination.

Isolation of Fov from plant material

Between 3 and 6 ½ weeks following inoculation (depending on the experiment) plants were removed, washed, examined for symptoms and surface sterilised root and/or stem tissue was plated onto ½ PDA. Any recovered *Fusarium oxysporum* (Fo) isolates after 5-7 days were subjected to pathogenicity tests on cotton.

Pathogenicity Tests on cotton

Isolates of Fo colonies were grown on ½ PDA plates. Small squares of colonised agar (5/plate) were used to inoculate 3 ½ PDA plates per isolate. After 5-7 days incubation at room temperature the hyphae and spores were washed from the 3 plates using approximately 60 ml distilled water, straining the suspension through 4 layers of tissue. Each isolate was used to inoculate four 2 week old cotton seedlings (Sicot 189) growing in sterilised potting mix using the root dip technique. After approximately 5 weeks symptomatic cotton plants were removed and lower surface sterilised stem tissue was plated onto ½ PDA to confirm the presence of Fov.

A total of 5 experiments were completed, including two preliminary experiments to initially trial techniques/methodologies, so there was a variation in spore concentrations used, crops tested, plant tissue plated out and age of plants assessed following inoculation (Table 2).

Table 2. Artificial inoculation experiments: spore concentrations, age of seedlings at inoculation, harvest dates following inoculation and plant tissue plated out for recovery of *Fusarium oxysporum* f. sp. *vasinfectum*

	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Experiment 5
Age of seedlings at inoculation	3 weeks	4 weeks	3 weeks	3 weeks	3 weeks
Harvested (weeks after inoculation)	3 weeks	3 weeks	3 ½ - 4 weeks	6 ½ weeks	7 weeks
Spore Concentration (spores/ml)	350 000	1 215 000	1 208 000	1 500 000	1 526 000
Root tissue plated	Yes	Yes	Yes	Yes	Yes (sunflower only)
Maximum length of stem tissue plated	None	1 cm	2 cm	6 cm	10 cm (3cm for sunflower and panicum)
Non-inoculated controls	Yes	Yes	Yes	No	Yes

2. Natural infection by Fov in crops other than cotton

A preliminary study was set up to determine the extent of natural infection by Fov in 23 crops other than cotton. Naturally infested Fov soil collected from 'Cowan", Cecil Plains was placed into large pots (24 cm diameter) in the glasshouse where the seeds of the different rotation crops were sown. Cotton was sown as a check for infection. Plants (15-20 per crop) were removed 6-7 weeks later and surface sterilised root, crown and lower stem tissue was plated onto ½ PDA media. Fo isolates

recovered from the plant tissue were tested for pathogenicity on cotton seedlings to confirm the presence of Fov (technique described above).

3. Green manure study

Naturally infested field soil collected from "Cowan" was placed into broccolini boxes and seeds of different green manure crops (barley, oats, vetch, soybean, canola, lablab, lupin, Japanese millet and pigeonpea) were sown. A bare fallow treatment was included. After 8 weeks all green plant material was chopped and re-incorporated into the soil. Soil samples (approximately 60-100 g) were collected before the crops were sown (pre-incorporation), on the day of incorporation and then at monthly intervals for 12 months to estimate soil populations of *Fusarium oxysporum* using the soil dilution plate technique. The soil was watered once a month.

Soil Dilution Plate Technique Soil samples were air-dried and ground to pass through a 500 μm sieve. A 1 gram subsample of soil was added to 9 ml distilled water, mixed on a vortex mixer for 1 minute, serially diluted and 0.1 ml of the final soil suspension was spread onto each of 5 plates of Komada media using a sterile stainless steel spreader. Plates were incubated at room temperature for 5-7 days. This was replicated 3 times for each soil sample. Isolates were transferred to ½ PDA plates to confirm morphologically and/or microscopically the presence of *Fusarium oxysporum*. A small representative of Fo isolates were selected from 1 replication at 1, 4, 7 and 11 months to differentiate *Fov* from Fo by testing for pathogenicity on cotton seedlings. Some isolates were originally characterised by Vegetative Compatibility Group (VCG) analysis to separate Fov from Fo but due to the large number of isolates being generated, proved to be too time consuming.

Objective 3.

Investigate the role key nutrients play in host resistance, including the effect on mychorrizal colonisation. Nutrients of importance that require attention for nutrient balance and disease management are nitrogen, phosphorus and potassium.

Disease is the expression of many interacting factors of pathogen, plant environment and time (Huber, 1990) (Figure 1). Nutrients are part of the 'environment' for crop and microbial growth, and interact with various aspects of a pathogen's survival and pathogenesis as well as the response of a plant to infection.

The nutrition of a plant largely determines its resistance or susceptibility to disease, its histological or morphological structure and properties, and the function of tissues to hasten or slow pathogenesis (Huber, 1990). Although the plant's defences to infection are under genetic control, their metabolic expression is regulated by mineral ions. It is thought that mineral elements are directly involved in the mechanisms of defence as integral components of cells, substrates, enzymes, and electron carriers, or as activators, inhibitors, and regulators of metabolism.

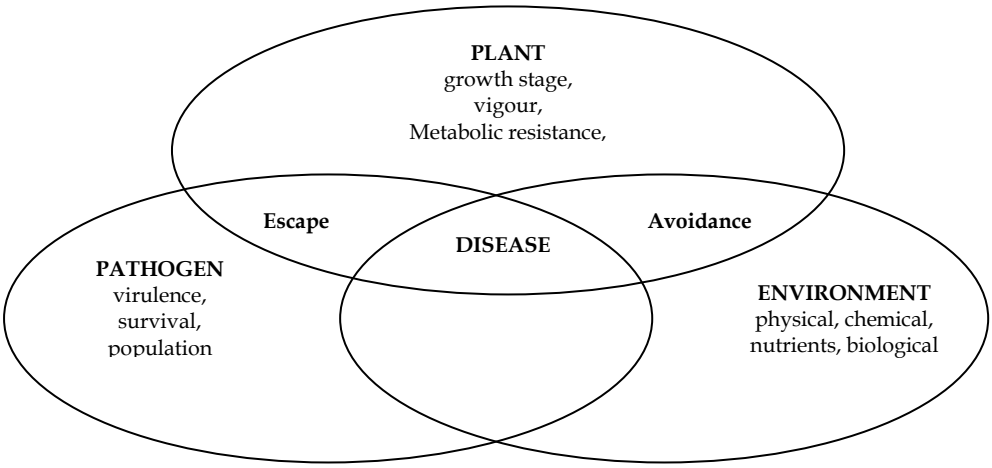


Figure 1. Interactions influencing disease expression and severity

Nutrition and Fusarium wilt management
The most effective way to manage Fusarium wilt is to grow cotton varieties with resistance to the disease; however this needs to be in conjunction with other practices, hence an integrated approach is necessary. Nutrition, although frequently unrecognized, is a primary component of disease control. Nitrogen (N), phosphorus (P) and potassium (K) are essential nutrients for plant growth an in various combinations have been shown to influence severity of Fusarium wilt of cotton in studies conducted overseas.

P 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.
Most Australian cotton soils have sufficient P; however agriculture can further deplete soil fertility, even in soils that initially are high in phosphorus. P is most available for uptake by plants in the pH range 6.5 – 7.5. At pH above 7.0, calcium phosphate is slowly formed. Approximately 40% of area planted to cotton in Australia receives some P. This is probably because under alkaline conditions, like those found in the black cracking clays on the Darling Downs, P availability is low despite the soil having a high P content. P improves water use efficiency, the energy balance and the weight, oil and protein contents of the seed as well as fibre quality.

There are reports that high P increases severity of Fusarium wilt of various crops, including cotton, particularly in the absence of adequate N and K (Dick &Tisdale 1937, Young &.Tharp 1941). Studies conducted by Sharoubeem *et al* in 1967 investigating the influence of N, P and K in relation to the incidence of cotton Fusarium wilt concluded that the amounts of P should not be elevated above their natural soil level in the soil, while K and N should be raised up to 1000ppm so as to reduce incidence to the lowest level.

N has a direct effect on crop development and it is imperative to apply adequate. However oversupply of N encourages rank growth and fruit shedding, reduced lint yield, hampers defoliation, encourages insects and delays maturity. With regard to disease, there are reports that as the N content of many plants are increased beyond sufficient, or when N is out of balance with other nutrients, synthesis of defence related compounds decreases and this can lead to an increase in disease.

The form of N available can also affect disease severity and resistance. For example nitrate–N reduced Fusarium wilt symptoms, whereas ammonium–N increased Fusarium wilt symptoms. The effect of each form appears to be associated with soil pH influences. The uptake and assimilation of nitrate (NO_3) leads to an increase in pH at the root/soil interface, the rhizosphere, whereas with ammonium, (NH_4) nutrition the rhizosphere is acidified. *Fusarium oxysporum* populations have been shown to be lower in soils fertilised with nitrate-N than in soils fertilised with ammonium –N and the

population of Fov was shown to be significantly lower in soil treated with anhydrous ammonia than with urea (Wang 1999). Hence it appears that N has an effect on pathogen populations and this influences disease severity.

K is relatively abundant in most Australian cotton growing soils. With regard to disease, research indicates that K fertilisation reduces the incidence and severity of cotton diseases, including Fusarium wilt, when K is deficient. K plays a critical role in the production and transport of fungus inhibiting phenolic compounds and flavonoids at sites of infection. Hence it has a direct affect on the various stages of pathogen establishment and development in the host. K also improves fibre fineness and strength.

Aims

The aims of these glasshouse trials are:

1. to examine the influence of N, P and K fertilisation on severity of vascular wilt of cotton;
2. determine if increasing rates of P increases Fusarium wilt severity; and
3. to determine the balance of nutrition that provides the lowest level of disease.

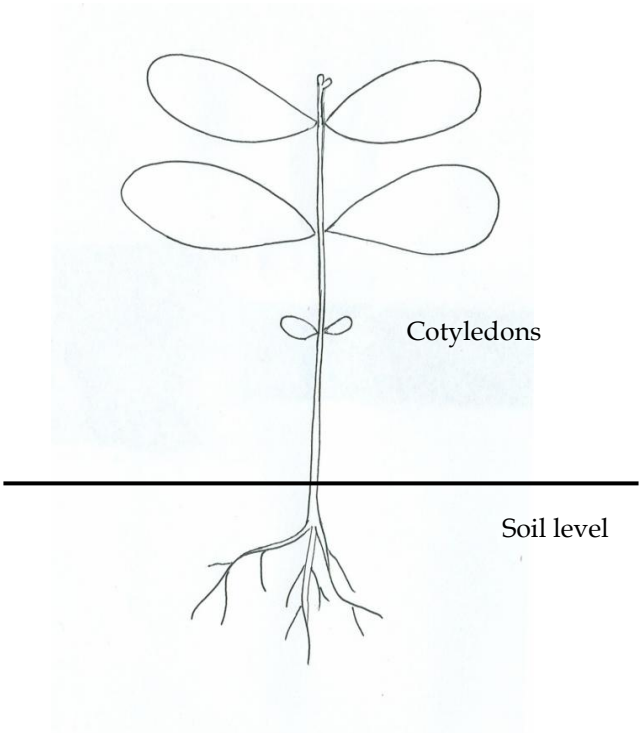
Glasshouse Trial 1

Aim

To examine the effect of P on disease severity in two cotton cultivars in the absence of N and K.

Methods

Seeds (cv Siokra 1-4 (highly susceptible) and Sicot 189 (mid-range resistance)) were sown into pasteurised sand inoculated with the Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *vasinfectum* (Fov) which had been grown on millet. In sand culture nutrients are highly available. P was applied in solution as a drench at 0, 5, 15, 30 and 60 kg/ha, to represent deficient, adequate and excessive levels of P for plant growth, with 15 kg/ha being the recommended rate. P was applied as $CaHPO_4.2H_2O$. Plants were harvested and rated for disease severity using a vascular discolouration index (VDI) (Figure 2).



6. Dead plant
5. Complete vascular discolouration of stem
4. Discolouration of stem above the cotyledons
3. Discolouration up to cotyledons
2. Discolouration above soil level but below the cotyledon
1. Base of stem discoloured below soil level
0. No vascular discolouration

Figure 2. Rating system for vascular discolouration of Fov

Glasshouse Trial 2

Aim
To examine the effect of N, P and K fertilisation on severity of vascular wilt of cotton in two varieties differing in Fusarium wilt resistance.

Methods
Seeds (cv Sicot 189 (mid-range resistance, F-rank 100) and Sicot F-1 (highest level of resistance, F-rank 143)) were sown into a field soil (cracking black clay) and sand mix inoculated with the Fusarium wilt pathogen as described for Glasshouse Trial 1. Fertilisers were applied in a band below seed planting depth. N was applied at 0, 40, 120 and 250 kg/ha as ammonium nitrate; P at 0, 5, 15 and 30 kg/ha as dicalcium phosphate dehydrate; and K at 0, 20, 60 and 100 kg/ha as potassium sulphate. The design was a factorial design, with three plants per pot and three replicate pots per treatment. The soil/sand mix was watered to field capacity as required. Three month old plants were harvested and rated for disease severity using a vascular discolouration index as described in glasshouse trial 1.

NPK Field Trial 1 2008/09

Fertiliser recommendations are developed to optimise nutrient uptake and provide the crop with adequate nutrients for normal growth and yield. Once critical levels of nutrients are met, no response to yield is expected from further nutrient application, but there may be other benefits. In some instances, nutrient applications higher than those needed for optimum growth may result in improved disease resistance. The overall aim of this work is to determine the effect of N, P and K fertilisation on nutrient uptake, plant establishment, disease severity and yield on two cotton varieties (differing in Fusarium wilt resistance) grown in Fusarium infested soil.

Aims
- To examine the influence of nitrogen, phosphorus and potassium fertilisation on severity of vascular wilt of cotton.
- To determine whether increasing rates of phosphorus fertilisation increases Fusarium wilt severity.
- To determine the balance of nutrition that provides the lowest level of disease.

Materials and methods
A field experiment was conducted from November 2008 to May 2009 at "Cowan" near Cecil Plains, QLD, in soil naturally infested with the Fusarium wilt pathogen, *Fusarium oxysporum* f. sp *vasinfectum* (VCG 01111). Soil cores were collected from the field site at three depths, 0-15 cm, 15-60 cm and 60-120 cm then analysed for macro- and micro- nutrients prior to trial commencement to determine fertilisation rates required. Two cotton varieties differing in Fusarium wilt resistance were investigated: Sicala 45 BRF (F-rank 126) and Sicala 60 BRF (F-rank 102). The experimental design was factorial with 16 treatments in randomised blocks, 6 blocks per treatment. Triple Superphosphate was applied at 0, 20, 40 and 80 kg/ha; Urea with Entec at 0 and 150 kg/ha; and Muriate of Potash at 0 and 100 kg/ha. Calcium sulphate (200 kg/ha) was applied to every plot.

Treatments

1. P1 N1 K1	5. P1 N2 K1	9. P1 N1 K2	13. P1 N2 K2
2. P2 N1 K1	6. P2 N2 K1	10. P2 N1 K2	14. P2 N2 K2
3. P3 N1 K1	7. P3 N2 K1	11. P3 N1 K2	15. P3 N2 K2
4. P4 N1 K1	8. P4 N2 K1	12. P4 N1 K2	16. P4 N2 K2

Where P1 = 0, P2 = 20, P3 = 40 and P 4 = 80 kg/ha; N1 = 0 and N2 = 150 kg/ha and K1 = 0 and K2 = 100 kg/ha.

Fertiliser treatments were applied by hand, broadcast to each plot (Figure 3). Hills were reformed following application. Seeds were sown at a depth of 10 cm. The experiment was irrigated and managed commercially.

Figure 3. Preparing field site for fertiliser application – plot boundary was marked, fertiliser treatments were pre-packed and placed at the end of each plot , then applied by hand in a 1m wide band over a 12 m plot.

NPK Field Trial 2 2009/10

Aims:

- To examine the influence of nitrogen, phosphorus and potassium fertilisation on severity of vascular wilt of cotton of three cultivars differing in Fusarium wilt resistance.
- To determine the balance of nutrition that provides the lowest level of disease.

A field experiment was conducted from November 2009 to May 2010 at "Cowan" near Cecil Plains, QLD, in soil naturally infested with the Fusarium wilt pathogen, *Fusarium oxysporum* f. sp *vasinfectum* (VCG 01111). Soil cores were collected from the field site at three depths, 0-15 cm, 15-60 cm and 60-120 cm then analysed for macro- and micro- nutrients prior to trial commencement to determine fertilisation rates required. Three cotton varieties differing in Fusarium wilt resistance were investigated: Siokra V18 BRF (F-rank 125), Sicot 70 BRF (F-rank 115) and Sicala 60 BRF (F-rank 105). The experimental design was factorial with 10 treatments in randomised blocks, 6 blocks per treatment. Urea with Entec was applied at 0 and 150 kg/ha, Superphosphate was applied at 0 and 20 kg/ha, and Muriate of Potash at 0 and 100 kg/ha. Calcium sulphate (200 kg/ha) was applied to every plot.

Treatments

1. N0 P0 K0	4. N0 P1 K1	7.N1 P1 K0	10. N0 P0 K0
2. N0 P0 K1	5. N1 P0 K0	8.N1 P1 K1	
3. N0 P1 K0	6. N1 P0 K1	9.N0 P0 K0	

Where N0 = 0 kg/ha, N1 = 150 kg/ha; P0 = 0 kg/ha, P1 = 20 kg/ha and K0 = 0 kg/ha, K1 = 100 kg/ha.

Fertiliser treatments were applied by hand, broadcast to each plot. Hills were reformed following application. Seeds were sown at a depth of 10 cm, approximately 15 seeds/m of row. The experiment was irrigated and managed commercially. Data collected included: plant emergence and establishment, yield, maturity, disease severity, fibre quality and nutrient uptake.

Other trials
A field trial was conducted at 'Atleigh', Cecil Plains to investigate the potential of biological products Natural Nitrogen & C-Cat to reduce the severity of Fusarium wilt and to increase yield.

Field Trial – Brett Porter, 'Atleigh', Cecil Plains (Ivanhoe Block 2) 08/09

Background
Ian and Marilyn Smith have a property "Medgun" at Mungindi in NSW. Ian has used biological products called Bio-N (now Natural N) and C-Cat on his fields, some of which are infested with the Fusarium wilt pathogen, *Fusarium oxysporum* f. sp. *vasinfectum*. Initially, the 'bad' field consisted of Fusarium in a patch about ½ acre in size. In the first year of treatment, Fusarium was still noticeable; however there was an obvious difference between rows. The Fusarium was confined to the row and had not spread. Less disease was observed in first year. In the second year, Fusarium infected plants were not observed. Six years later Fusarium cannot be found. Based on these observations Brett Porter was willing for a field trial to be conducted on his property to investigate the potential of Natural Nitrogen & C-Cat to reduce disease severity caused by the Fusarium wilt pathogen and to increase yield.

History of paddock for intended trial
A couple of years ago variety Sicot 14B (F-rank 141(7)) was planted in this field. At this time varieties with an F-rank less than this couldn't be planted, indicating a substantial level of Fov in the soil. Last season (07/08) the field was planted to soy bean which is likely to have increased Fusarium levels for this season.

Methods
The design of this experiment is a strip plot design, in which each furrow is 650m in length and each treatment strip consists of 24 rows x 11 m wide. The total area of the trial is 24.96 ha. Each treatment, of which there are four, will be replicated four times, in a random design (Figure 4). Treatment ('brew') will be applied to the full length of row. Sicot 70BRF will be planted.

The treatments include:

Treatment 1 = Control, no treatments applied.

Treatment 2 = 100 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 Units /ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring.

Treatment 3 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units/ ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring.

Treatment 4 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units ha)) - soil injection. No foliar application.

Preparation of the 'brew' began on the 25th October 2008. To prepare 'brew' 100 L of water was added to 1 L of Natural N and 1 L of C-Cat. The 'brew' was left to sit for 24 hours before application. Prior to field application, Easy N, a liquid N fertiliser, was mixed into the tank. Suggested rate was 6 L per 100 L, which will provide N as a food source for the bacteria.

The Control treatment was planted on Sunday Oct 26th. Treatment plots were planted Monday Oct 27th at the rate of 13 seeds per metre row. Recording plots were 10 m long in rows 4, 8, 12, 16 and 20. There was approximately 110 m between plots down the field (Figure 5).

Emergence and establishment counts were taken on the 10 November and 11 December 2008 respectively. Fov was observed to be present in at least 8% of plants at this stage. Cotton was harvested on the 5th May 2009, followed by disease assessment based on absence and presence of stem vascular discolouration.

Figure 4. Experimental plot design

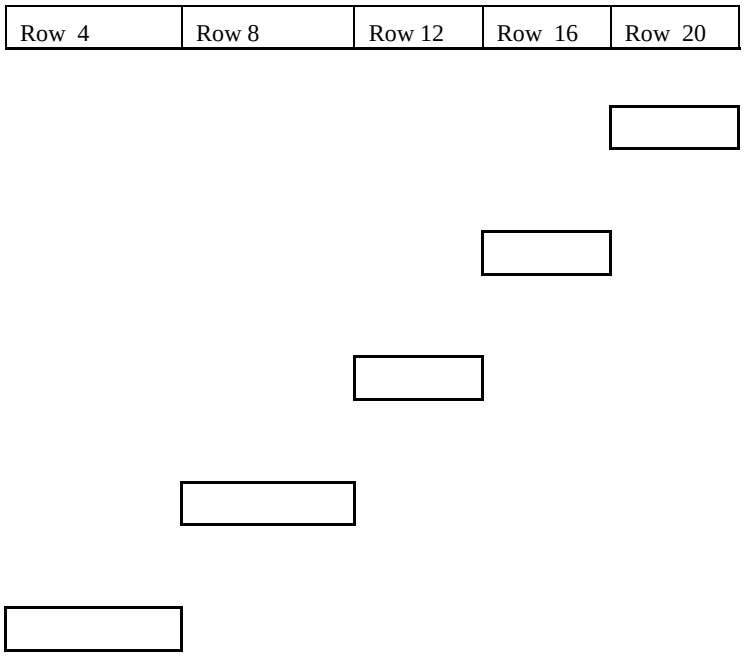


Figure 5. Plan showing recording plots selected for each treatment

Objective 4.

Develop and extend new information packages for disease management. Provide new information to industry through the extension network, cotton consultants and other industry forums when data becomes available. This objective has been achieved.

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.

Objective 5.

Training of staff in Amplified Fragment Length Polymorphism PCR, to be used as a tool to characterise *Fusarium oxysporum* f. sp. *vasinfectum* (Fov).

Culture Preparation
Fusarium oxysporum f. sp. *vasinfectum* cultures were plated from filter paper onto ½ PDA/S and incubated at 25C. Fungal growth was sub-cultured onto 2 plates each of ½ PDA/S and incubated at 25C until the plates were covered with fungal growth. In the laminar flow, mycelial growth (~45mg) was scraped using a sterile scalpel blade into a 2 ml safe lock tube.

DNA extraction
Genomic DNA was extracted using Wizard Genomic DNA Purification Kits according to the manufacturers' instructions. DNA was stored at 2-8C.

DNA concentration
Safe lock tubes (2 ml) were labelled and 72 µl of sterile water was added per tube plus 8 µl of DNA extract. The concentration of DNA was determined using a SmartSpec 3000 spectrophotometer and adjusted to 100 ng/µl.

Restriction digest
DNA (1 µl) was codigested with *Eco*R I and *Mse*I at 37C for 3 hours and overnight. Digest was run on a 3% agarose gel (gel red, 0.5xTBE buffer) and examined under UV.

Ligation
Oligo-adapters (*Eco*R I adapter and *Mse* I adapter) were ligated to DNA fragments at 37C for 90 minutes in restriction ligation mix (100 µM). Restriction was checked by visualisation of 5 µl of the restriction ligation on a 3% agarose gel.

Pre-selective and selective amplification
Pre-amplification used primers *Eco*R I +0 and *Mse* I+0, while the selective amplification contained selective primer *Mse* I+A and fluorescent selective primer *Eco*R I+AGT.

Beckman CEQ8800
Final PCR product was prepared using manufacturer's instructions and fragments analysed using a Beckman CEQ8800 instrument. The CEQ8800 dominant scoring algorithm automatically scores the presence or absence of AFLP-generated fragments in binary mode (1, 0) through an integrated binary process. The dominant scoring results are used for phylogenetic analysis.

Phylogenetic analysis
Similarity between isolates based on Dice coefficients were calculated using NTSYS-pc, version 2.1x (Exeter Software). The trees were generated using the sequential agglomerative hierarchal nested (SAHN) clustering program with the unweighted pair-group method (UPGMA) that used an averaging algorithm in NTSYS-pc.

Results

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

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.

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.

2007/08
Most cotton production areas experienced mild seasonal conditions, which were accompanied by significant periods of very wet and/or very dry weather. These conditions had a considerable effect on disease distribution, incidence and importance.

There were two new reports of Fusarium wilt from Theodore area. Fusarium wilt was most common in crops on the Darling Downs where the incidence was 11.4% and the disease was found in 9 out of 13 crops inspected – with 69% of plants infected in one field. Despite the avoidance of problem fields due to limited water, delayed planting and the use of more resistant varieties, this represents the highest mean incidence of Fusarium wilt in crops on the Darling Downs over the six years of surveys. Fusarium wilt was also found at low levels in two fields in the Theodore area and in one field in St George.

Several transects have been established to monitor the impact of seasonal conditions and farming practices on the development of Fusarium wilt. In St George, the use of irrigation water from the tail-drain of an infested field for subsequent irrigations of the same field has increased the incidence of disease from a few small patches to over 19% in three seasons.

No exotic diseases were recorded.

2008/09
Most cotton production areas experienced near average conditions with the exception of Emerald and the Burdekin. Cotton crops in the Burdekin received very high rainfall during the season and Emerald experienced only half of the average number of days with temperatures greater than 35C and higher than average rainfall.

There were no new reports of Fusarium wilt this season. Fusarium wilt was most common on the Darling Downs where the disease was found in 9 of the 11 crops inspected. However the average incidence of Fusarium wilt was reduced to only 1.4% of plants compared to 11.4% last season. This may be due to later planting on the Downs to avoid cooler conditions that can increase the incidence of Fusarium wilt. Other factors could include greater use of more resistant varieties and the more widespread use of BION seed treatment in Queensland cotton production.

Tobacco Streak Virus (TSV) was observed in 7 out of 14 crops inspected in November and in 6 of the 9 crops inspected in February in the Emerald area. TSV was also identified in a weed, Crownbeard, along the roadside. This is the first record of TSV in cotton outside of Emerald. There were no major disease problems and no new diseases were observed.

No exotic diseases were recorded.

2009/10

Early Season Disease Survey

Emerald and Theodore

Early season disease surveys were conducted on 3rd – 5th November 2009. Seedling disease caused by Pythium, Rhizoctonia and some wireworm was observed in nine out of 28 fields surveyed (32%).

Although not a new disease, cortical root disease caused by *Sclerotia rolfsii* is not a disease often observed in cotton during disease surveys. Southern blight is the term adopted for cortical root diseases caused by *S. rolfsii* in a wide range of crops in the United States. Cotton is not as susceptible to this pathogen as many other crops, but in the United States, cotton is often included in rotations with susceptible crops such as peanut. Eight fields out of 28 surveyed (29%) had plants that were severely wilted. When these plants were removed from the soil, dark brown lesions could be seen on the lower stem at and below soil level (Figure 6). A mat of white hyphae was observed on diseased tissue of these plants (Figure 7), often with sclerotia (Figure 8). These are typical symptoms of *S. rolfsii*.

Figure 6. Cotton seedling displaying symptoms of cortical root disease

Figure 7. White fungal hyphae adhering to diseased tissue

Figure 8. Sclerotia produced in culture

No Fusarium wilt was detected, even though fields with a history of Fusarium wilt were visited in Theodore. This is probably due to the warm, dry conditions, which are not conducive to this disease.

In general seedlings were performing well. There were no new diseases observed.

There was the occasional volunteer cotton plant in-crop, along roadsides and in channels. It is important to remove these unwanted plants as they can harbour pests and diseases; carrying them from season to season providing an inoculum source for re-infection of crops.

St George/Dirranbandi

Disease surveys of cotton in the St. George/Dirranbandi region were conducted mid November. Fusarium wilt was observed on one farm only. Conditions were very hot and dry, causing a small percentage of young seedlings to wilt and die due to lack of moisture in the soil rather than disease. No significant disease to report. No new diseases observed.

Darling Downs

Disease survey was conducted on 1st December 2009. No significant disease to report. No new diseases.

Burdekin

On January 19th 2010, two farms (5 fields) were visited, representing 95% of cotton grown in the Burdekin this season. Seedling establishment ranged from 79 to 100%. Uneven growth of seedlings was observed at both farms; however this was not due to disease. On one farm, uneven growth was observed in fields where cotton followed maize, which may be due to allelopathy.

Outbreaks of mealybugs have recently been reported from a number of cotton farms in central Queensland. While mealybugs are considered a minor pest of cotton, they have, on rare occasions, reached minor outbreak levels or 'hotspots' in commercial crops in central Queensland. Mealybugs are small, sucking insects related to aphids and form colonies on shoots, stems, and leaves developing into dense, waxy, white masses. Both adults and nymphs pierce and suck on plant tissue. They can affect any stage of crop development. Symptoms of mealybug infestations on cotton include; crinkled and twisted leaves, fewer flowers and fewer bolls, smaller bolls, and distorted and stunted plants. Boll opening may also be adversely affected, resulting in serious losses in yield. In 2009, during late season disease surveys, mealybugs were observed to be causing significant problems in the Burdekin. In January 2010, at the start of the cotton season numerous mealybugs were observed on volunteer cotton inter-row in close proximity to cotton seedlings (Figure 9). At present there are no registered insecticides for the control of mealybugs on cotton in Australia. Hence control of volunteer cotton and weed hosts is extremely important for the management of this pest.

Figure 9. Mealybug infested volunteer cotton plant adjacent to cotton seedlings

Late Season Disease Survey

Boll Rots

The incidence of boll rots in Queensland was higher this season compared to the last six seasons (Figure 10). At a district level, the incidence of boll rots was higher in all districts this season compared to the 2008/2009 season (Figure 11); particularly in Emerald and St George. Emerald had an incidence of 11.9% boll rots and St. George had 9.8%. Theodore had a 6.4% incidence of boll rot with the lowest incidence recorded on the Darling Downs at 1.3%.

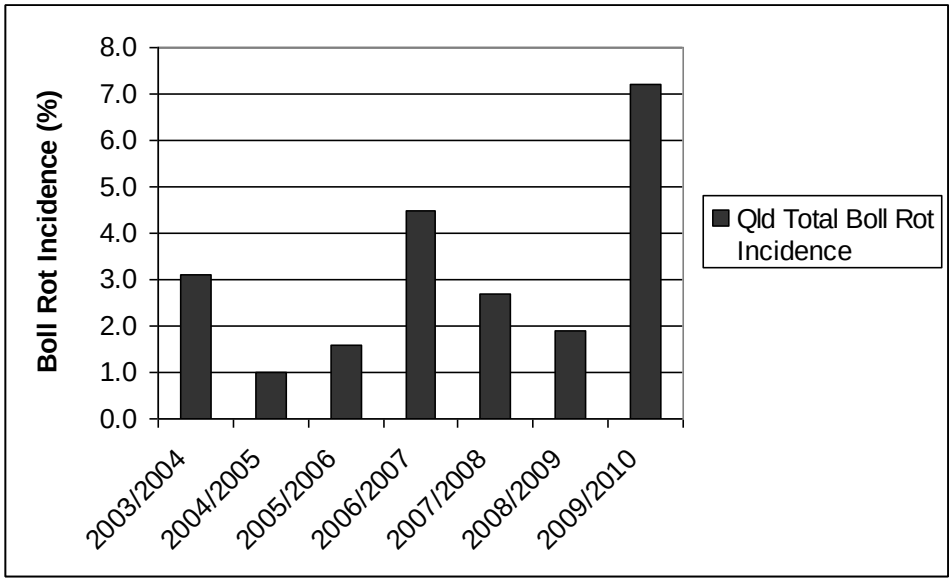


Figure 10. Comparison of the incidence of total boll rots in Qld over the last seven seasons

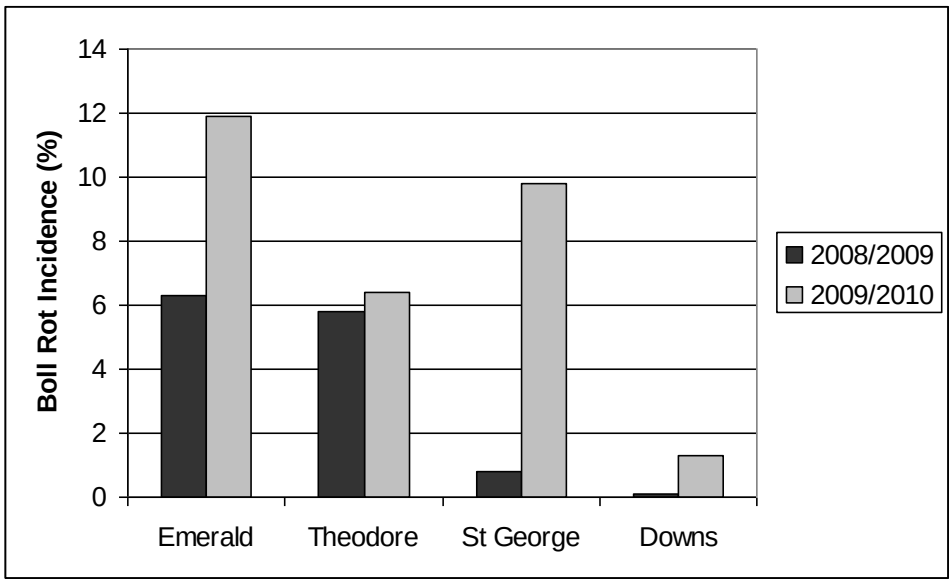


Figure 11. Comparison of boll rots in the 2008/09 and 2009/10 season

The increase in boll rot is likely due to the high summer rainfall in 2009/10 (Figure 12). In Emerald there was considerably greater rainfall in February 2010 when disease surveys were conducted compared to 2009 (Figure 13). In St George, the total summer rainfall for 2009/10 was greater than in 2008/09 (Figure 7.), particularly for December and January (Figure 14). Heavy rain in March 2010 saw much of south western and central Queensland undergo major flooding caused by rainfall generated by a monsoon trough. A number of towns along major rivers flooded, which included St George and Theodore. Over the period 1–3 March, rainfall totals of between 100-300 mm were observed in the area. This water ran into already saturated rivers and creeks in the area. For the month of March St George received 250 mm of rain (Figure 14), contributing significantly to the increase in boll rot incidence. Losses from cotton crops destroyed at Theodore and the area around St George and Dirranbandi are expected to be significant.

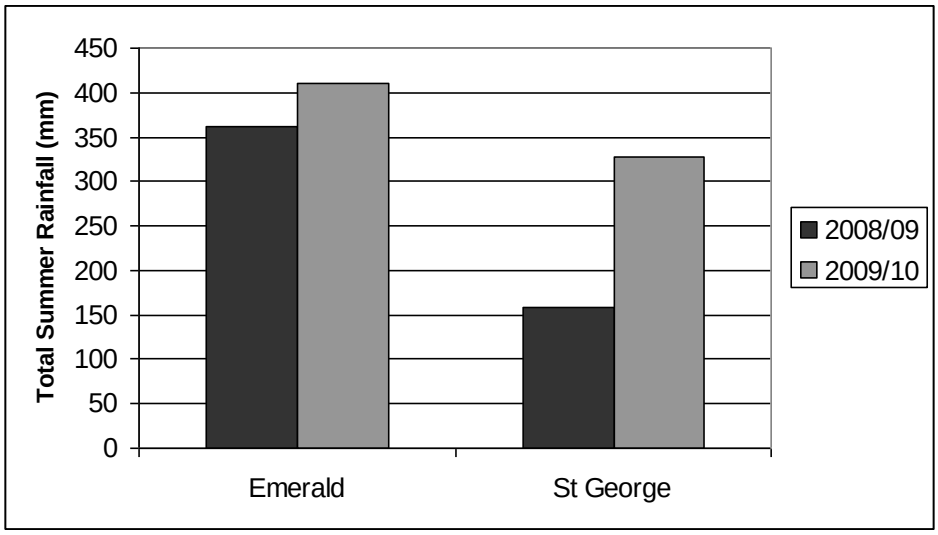


Figure 12. Total summer rainfall for Emerald and St George regions in 2008/09 and 2009/10

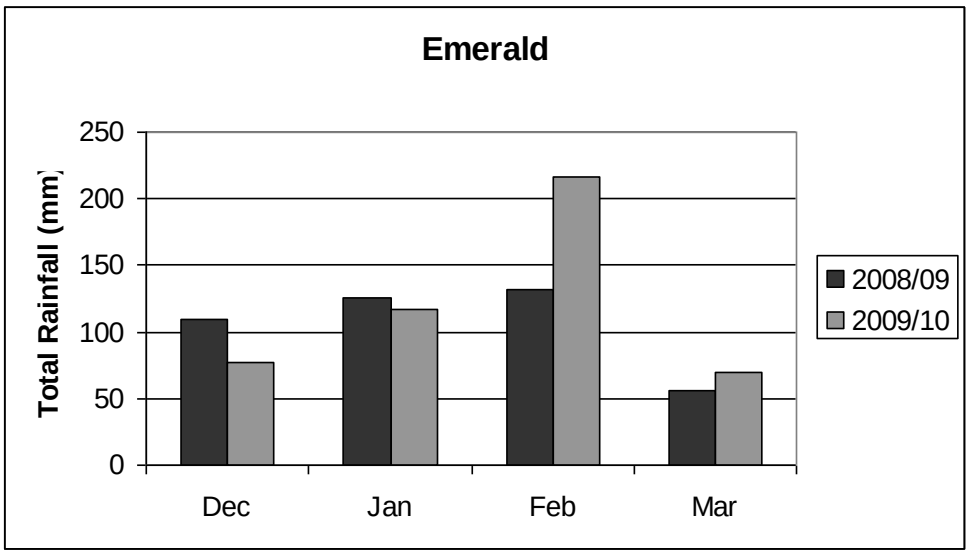


Figure 13. Total monthly rainfall from Dec to March in Emerald for 2008/09 and 2009/10

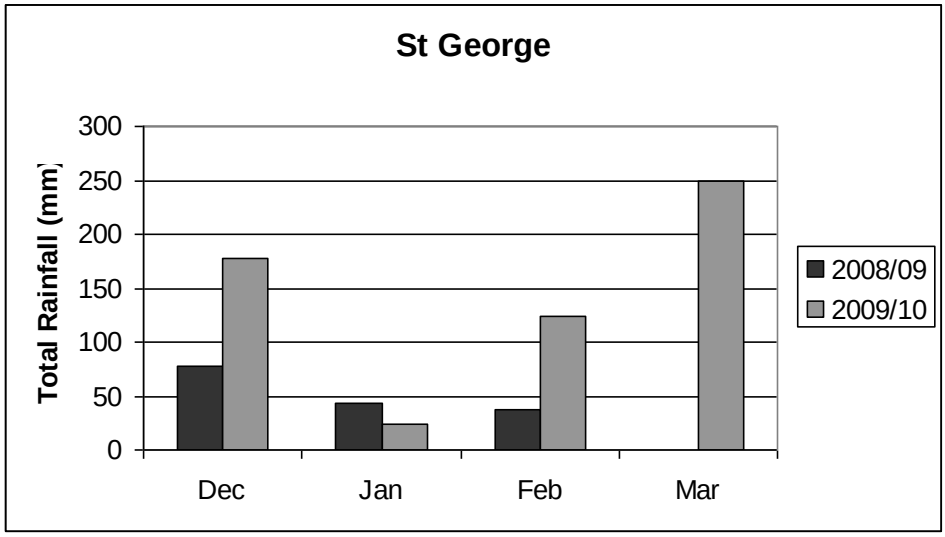


Figure 14. Total monthly rainfall from Dec to March in St George for 2008/09 and 2009/10

Fusarium wilt

The incidence of Fusarium wilt has significantly reduced over the last two seasons (Figures 15 and 16), when compared over eight seasons. Three factors that may have contributed to this trend are resistant varieties, delayed planting and Bion seed treatment.

The development and adoption of new varieties with increased resistance such as 71BR with an F-rank of 101(4), 70BRF with 116(17) and 71BRF with 118(8) will have contributed to the reduction in Fusarium wilt incidence.

On the Downs, many growers delay planting up to 2-4 weeks than previously practiced, as a management practice to reduce the effect of cold stress on seedling emergence and development. Later plantings assist rapid germination and good seedling growth and contribute to a reduction in the development of Fusarium wilt and seedling disease. On the Downs 6/13 fields were planted after 15/10/2009 and 6/13 fields were planted in November.

The use of Bion treated seed may also have contributed to the reduction in Fusarium wilt incidence. Over 1000 tonnes of planting seed carrying the Bion seed treatment was sold in Australia in 2009.

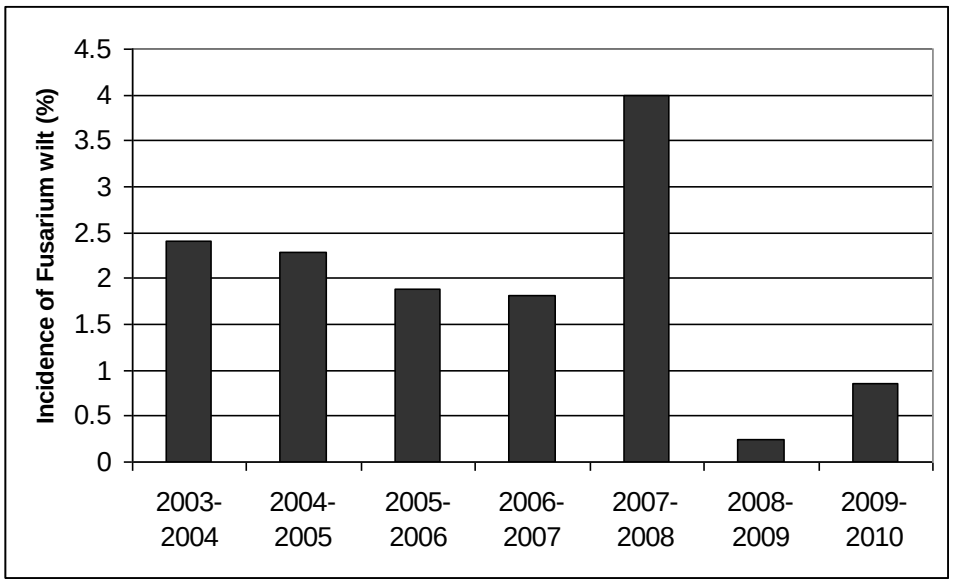


Figure 15. Comparison of the incidence of Fusarium wilt over eight seasons

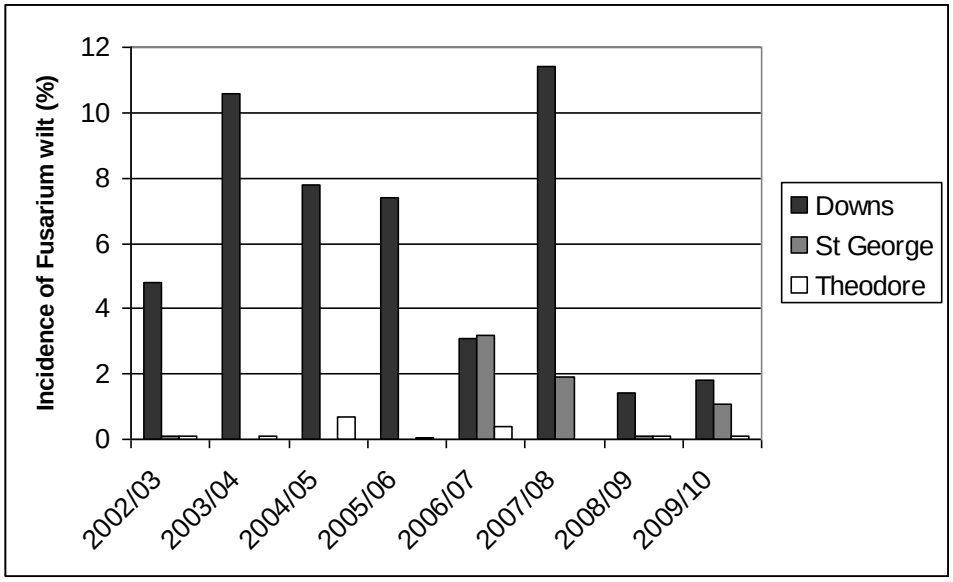


Figure 16. Comparison of the incidence of Fusarium wilt for each region over eight seasons

Surveillance for exotic pathogens

Texas root rot, cotton leaf curl virus, blue disease, defoliating Verticillium, hypervirulent bacterial blight and exotic Fusarium wilt are exotic pathogens listed by the Australian cotton industry as serious threats to the industry. These six exotics are listed on survey sheets for the annual surveys and are surveyed for twice yearly in addition to endemic pathogens. Absence data was collected for all six pathogens this season.

Volunteer cotton – carry over from previous season – Spring 2009

Information collected during Annual disease surveys by pathologists from DEEDI Qld & CSD and amalgamated by Stephen Allen (CSD) based on 28 farms in Queensland.

Includes:

1. Mature cotton plants surviving along roadsides, fence lines, along channels and in tail water return systems or drains
2. Mature cotton plants surviving from previous season or regrowth from stubs in rotation fields (eg cereal) and fallow fields
3. Mature cotton plants surviving from previous season or regrowth from stubs (Ratoon cotton?) in current cotton crops

	1.	**2.**	**3.**	**TOTAL**
	Channels, roads, fences	**In fallows or rotations**	**In the current crop**	
In Qld	11/28 (32%)	4/19 (21%)	15/28 (54%)	20/28 (71%)

Rare	2/28 (7%)	1/19 (5%)	2/28 (7%)
Common	7/28 (25%)	1/19 (5%)	10/28 (36%)
Numerous	2/28 (7%)	2/19 (10%)	3/28 (11%)
TOTAL	11/28 (32%)	4/19 (21%)	15/28 (54%)

Rare 1-10 volunteers/farm
Common 11-100 volunteers/farm
Numerous >100 volunteers/farm

In summary, 71% of all farms surveyed, had some form of volunteer cotton plant present. Mature cotton plants were observed to be surviving alongside roadsides, fencelines, along channels and in tail water return systems or drains in 32% of farms surveyed. A total of 21% of farms had mature cotton plants surviving from previous season or regrowth from stubs in rotation fields and fallow fields. In their current crop, 54% of farms had mature cotton plants surviving from the previous season in their current cotton crop. It is extremely important to remove these unwanted plants as they can harbour pests and diseases; carrying them from season to season providing an inoculum source for re-infection of crops. Volunteers will become a more significant issue with the recent introduction of the Solenopsis mealy bug.

Diagnostics

In the 07/08 season 24 diagnostic samples were received at the Indooroopilly laboratories. Of these, 10 samples were collected during the Darling Downs disease survey. Nine of these samples yielded *Fusarium oxysporum* (Fo), all of which were Fov and belong to VCG 01111. From one sample, Fo was not recovered. A further four samples were collected during the Central Queensland disease surveys, of which one sample yielded Fov (VCG 0111), one sample yielded Verticillium wilt and from two samples no pathogen could be recovered. The positive Fov sample was from a farm that had not had Fusarium wilt before. Samples received from Theodore, Goondiwindi and Dalby all belonged to VCG 01111. Verticillium wilt was recovered from two samples, one each from the Upper Namoi and Lower Namoi Valley, NSW. Samples collected from four F. rank trials all yielded Fov belonging to VCG 01111. Samples received from Chris Anderson that were collected from a seed production crop west of Moree that were destined for export, yielded Fov belonging to VCG 01111.

In the 08/09 season a total of 49 samples were received at Indooroopilly; sent from growers, consultants, disease surveys, field trials and researchers. Of the 49 samples, 20 were sent from growers and consultants, of which 25% were positive for Fov. A similar number of samples were received this season as last season. However the number of samples received has generally declined over the past seasons. Of the positive isolates 10% were from the MacIntyre and Gwydir Valleys, with 5% from the Namoi Valley. No positive samples were received from growers in the Macquarie Valley, St George, Darling Downs or Theodore. Eleven samples were received from the MacIntyre Valley region, of which two samples were positive for Fov (VCG 01111); two for Verticillium wilt, and from seven samples no pathogen was recovered. From the Gwydir Valley three samples were

received; two positive for Fov (VCG 01111) and one from which no pathogen was isolated. One sample was received from both the Macquarie Valley and Emerald. Symptoms of both samples were confirmed to be due to sudden wilt. One sample was received from the Namoi Valley and was positive for Fov (VCG 01111). Three samples were received from Theodore. All were negative for Fov; no pathogens were isolated. The remaining isolations were from samples collected during annual disease surveys and field trial assessments. A total of 25 samples were positive for Fov (VCG 01111) and one was positive for Verticillium wilt. From three samples no pathogen was recovered.

No new properties with Fusarium wilt were identified this season.

In the 09/10 season a total of 12 samples were received, 9 of which were collected during annual disease surveys. This is a significant reduction in samples for testing; however this may partly be because the spread of Fusarium has reduced significantly as indicated by very few new records of Fusarium during this project. Also there has been significantly decreased cropping area due to a lack of water and therefore Fusarium infested fields have not been farmed to cotton.

There were no samples received from NSW cotton fields, except from Stephen Allen's (CSD) field trials, hence diagnostic samples from growers have significantly reduced. The loss of staff involved with disease surveys may have contributed to this.

New disease detection

In 2010 a new disease was detected in the Burdekin cotton producing district. An unusually high level of boll rot had been observed in one field by the farm's consultant. The consultant notified Stephen Allen (CSD) prior to commencement of disease surveys to inform the team that there were some unusual disease levels for us to inspect. Samples of rotten and discoloured bolls were collected by Linda Smith for further investigation at the Indooroopilly laboratory. Tom Marney (DEEDI) isolated and identified *Nemtospora* (*Eremothecium*) *coryli* from a sample of cotton bolls. This fungus causes several serious diseases of cotton including seed rot, internal boll rot (stigmatomycosis) and tight lock. In the USA losses of 40-60% of fibre has been reported in cotton. The fungus is the only plant pathogenic yeast and is spread to bolls punctured by insects during feeding. Insect control is the best way to prevent infection, although improved cultivar resistance may be possible. *Nemtospora* (*Eremothecium*) *coryli* was first reported in Australia in 2004 from dry rot of citrus. In this paper it was proposed that the fungus had been present and undetected in Queensland for at least 90 years.

Biosecurity Queensland, CRDC and Plant Science (DEEDI) were notified of the isolation of this pathogen. We are waiting on advice from Biosecurity Queensland regarding action required.

Mungindi isolates – Pathogenicity Tests

Pathogenicity Test 1 -Isolate 24590

A pathogenicity test conducted by Wayne O'Neill in 2006 determined that isolate 24590 was pathogenic on cotton variety Siokra 1-4 (Figure 17).

Figure 17. External symptoms of Fusarium wilt visible 3 weeks after inoculating Siokra 1-4 with isolate 24590 from Wyadrigah

Pathogenicity test 2

7/12/09
Seventeen days after inoculation the only plants expressing symptoms of Fusarium wilt were those inoculated with the standard isolate 24500 VCG 01111. Symptoms included yellowing and necrosis of leaves plus wilting. Plants of all other treatments visually appeared healthy.

15/12/09
Twenty-five days after inoculation, plants inoculated with standard isolate 24500 were severely stunted with yellowing leaves. One plant treated with Wyadrigah isolate 25358 was stunted and wilting (Figure 18). The original Wyadrigah isolate 24590 showed severe disease symptoms three weeks after inoculation in the original pathogenicity test (Figure 17), however in this test there was no expression of disease symptoms (Figure 18).

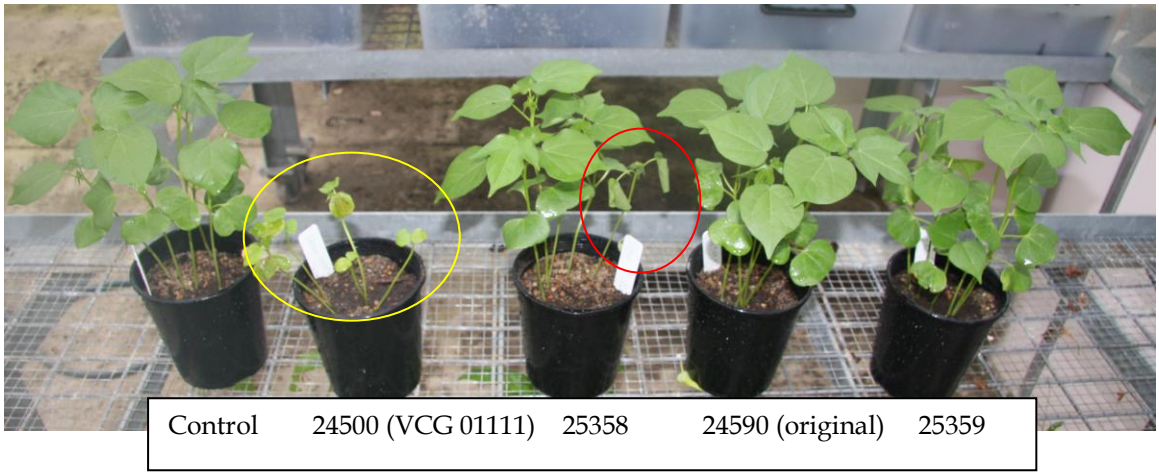


Figure 18. Disease development in cv. Siokra 1-4 25 days after inoculation with various isolates of *Fusarium oxysporum* f. sp. *vasinfectum*. Plants inoculated with isolate 24500 were severely stunted with yellowing leaves (circled yellow). One plant inoculated with 25358 was stunted and wilting (circled red).

At harvest
When examined internally, the stem and roots of the control treatment were 'healthy' with no vascular discolouration evident (Figure 19). Alternatively, four out of five plants inoculated with the standard Fov isolate 24500 had significantly less root mass compared to the control and other treatments (Figure 19). When examined internally, 4 out of 5 plants had vascular browning in the roots only.

Although in a previous experiment isolate 24590 caused severe wilting and vascular browning of cv. Siokra 1-4, in this experiment there was no external or internal evidence of disease.

The two Wyadrigah isolates recovered in 2009 caused distinctive vascular browning (Figure 19). For isolate 25358, four out of five plants had dark brown/black vascular discolouration. In two of these plants the vascular discolouration extended through the whole length of the plant (top node). For the remaining two plants, vascular discolouration was confined to the root. For plants inoculated with isolate 25359, three out of five plants were infected with Fov and vascular discolouration extended to the top node in all infected plants.

Re-isolation of *Fusarium oxysporum*
Fusarium oxysporum was isolated from discoloured vascular tissue from seedlings inoculated with Fov isolates 24500 (VCG 01111), 25358 and 25359.

Summary
This pathogenicity study has confirmed that Wyadrigah isolates 25358 and 25359 are pathogenic on cotton, however external symptoms of disease were mild compared to those caused by the standard isolate 24500. In a previous study, Wyadrigah isolate 24590 was shown to be highly pathogenic on cotton, however in this study no disease developed. This experiment will be repeated to confirm results.

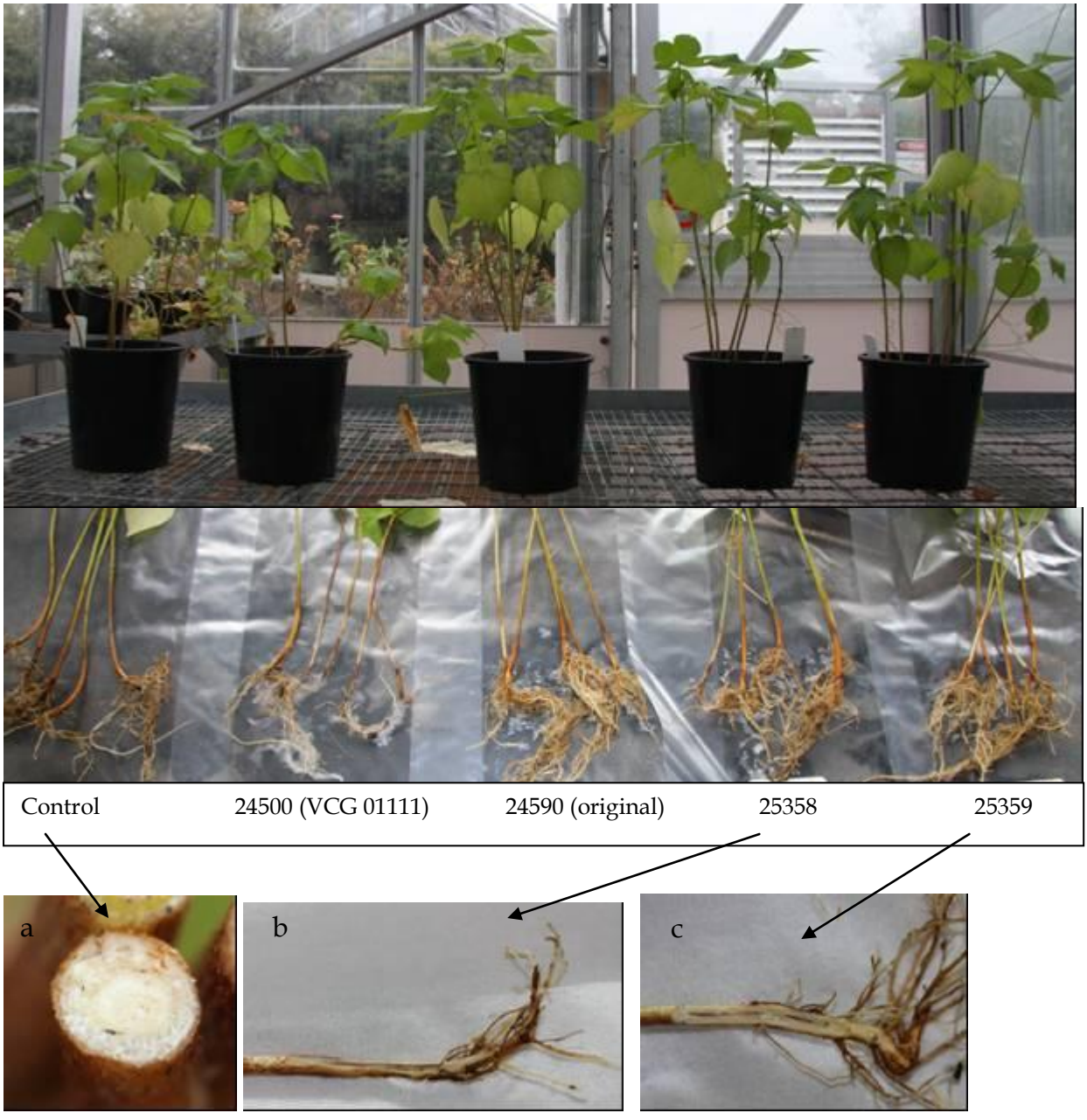


Figure 19. Plant and root growth 9 weeks after cotton cv. Siokra 1-4 was inoculated with various isolates of *Fusarium oxysporum* f. sp. *vasinfectum* (Fov). Bottom photographs show a) a cross-section of stem from the control treatment showing no vascular discolouration; b) and c) longitudinal cut along root and stem of plant inoculated with Wyadrigah isolates 25358 and 25359 respectively showing vascular discolouration.

Objective 2. Investigate the role of crop rotation and crop residue on the ecology of Fov and on subsequent disease development in cotton.

Field Rotation Trial

Disease counts and assessments of cotton treatments sown in the 2007/08 (Table 3) and 2008/09 (Table 4) seasons are shown below. Table 5 summarises disease assessments from all treatments oversown with cotton in 2009/10.

Table 3. Plant emergence, percentage plant survival to maturity, disease severity and yield of cotton treatments, 2007/08

Treatment	Emergence	% Plant survival to maturity	% 0s & 1s	Yield (bales/ha)
c-f-c	84 a	41 a	11 a	4.7 a
c-c-c	85 a	39 a	7 b	4.3 a

Numbers within columns followed by the same letter are not significantly different (p=0.05)

Table 4. Plant emergence, percentage plant survival to maturity, disease severity and yield of cotton treatments, 2008/09

Treatment	Emergence	% Plant survival to maturity	% 0s & 1s	Yield (bales/ha)
f-c-c (1)	102 a	80 a	39 a	7.4 a
f-c-c (2)	98 a	81 a	46 a	8.0 a
f-c-c (3)	102 a	79 a	44 a	8.0 a
c-c-c	97 a	60 b	22 b	6.9 a

Numbers within columns followed by the same letter are not significantly different (p=0.05)

Table 5. Final assessment of plant emergence, percentage plant survival to maturity, disease severity and yield of cotton following various rotations, 2009/10

Treatment	Emergence	% Survival	% 0s & 1s	Yield (bales/ha)
M(ret)-f-c	101 a	84 ab	57 a	9.2 ab
S(i)-f-c	100 a	82 abc	50 bcd	9.1 abc
c-f-c	100 a	80 cde	43 efg	8.8 abc
M(i)-f-c	99 a	85 ab	54 ab	8.6 bcd
f-c-c (2)	99 a	82 abc	52 abc	8.7 abc
f-c-c (1)	99 a	77 ef	38 g	8.4 bcd
S(ret)-f-c	98 a	85 a	47 cde	9.5 a
f-c-c (3)	97 a	81 bcd	48 bcde	8.8 abc
c-c-c	95 ab	78 def	39 fg	7.8 d
c-soy-c	88 b	75 f	44 def	8.3 cd

Numbers within columns followed by the same letter are not significantly different (p=0.05)

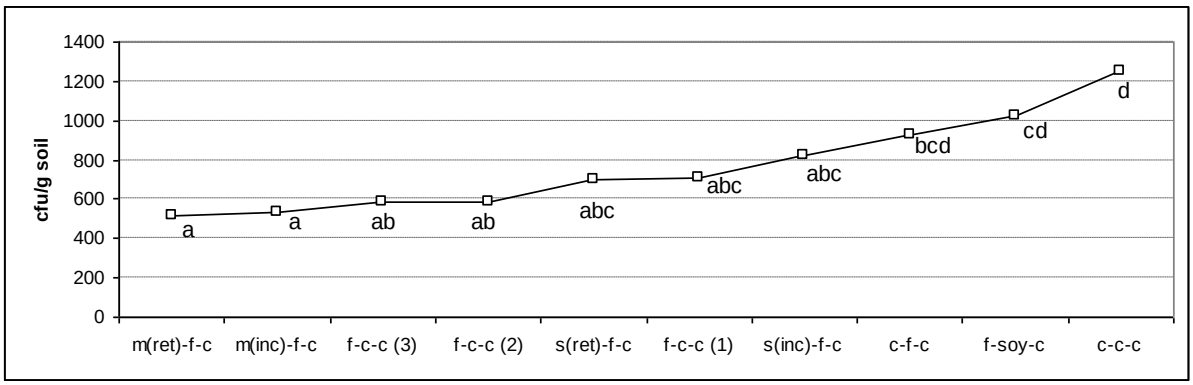
In the second year (2008/09, Table 4) of the trial when cotton was sown the treatment where cotton had been grown for 2 years had significantly more disease (less plant survival to maturity) and greater degree of stem discolouration (represented by lower % 0's and 1s) than each of the three treatments where the previous season was a bare fallow. Yield, although not statistically different, was lowest following 2 years of cotton compared to a fallow-cotton.

Third year (2009/10, all treatments oversown with cotton, Table 5)

- In terms of plant emergence the cotton-soybean-cotton rotation had a significantly reduced plant stand compared to all other treatments except three years of continuous cotton (which was not significantly different). The cotton-soybean-cotton rotation was slightly better than three years of continuous cotton in terms of yield, % plant survival to maturity and the number of 0's and 1's although the differences were not significant.
- Two of the fallow-cotton-cotton treatments differed from the third. Fallow-cotton-cotton(2) and fallow-cotton-cotton(3) had significantly greater plant survival to maturity and greater 0s and 1s (less disease) than fallow-cotton-cotton(1). The yields, however, were similar (8.4 – 8.8 bales/ha) and not significantly different.
- The continuous cotton treatment had the lowest yield of all treatments and was significantly lower than two of the fallow-cotton-cotton treatments (2) and (3) and the cotton-fallow-cotton treatment. In terms of disease severity (0s and 1s) two years of cotton (fallow-cotton-cotton(2) and (3)) had less disease than a cotton-fallow-cotton rotation (although was significantly better than (2) only) and both had significantly less disease than three years of continuous cotton.
- Treatments including maize or sorghum (where residues were either retained or incorporated) had significantly more plants surviving to maturity than three years of continuous cotton, cotton-soybean-cotton and fallow-cotton-cotton(1). In addition sorghum (retained)-fallow-cotton had significantly greater plant survival than cotton-fallow-cotton and fallow-cotton-cotton(3), and the two maize treatments had significantly more plants surviving than cotton-fallow-cotton.
- There was no significant difference between retaining maize residues on the surface or incorporating residues in any measured variable. Similarly there were no significant differences between where sorghum residues were retained or incorporated.

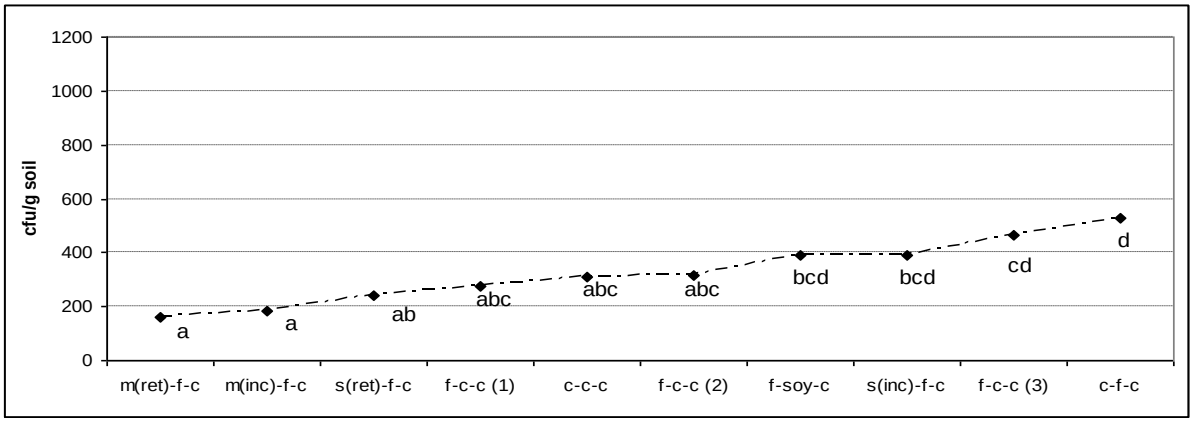
- Comparing the maize and sorghum treatments, percentage survival to maturity was similar between all four treatments regardless of how the residues were managed after harvest. Both maize treatments had the highest number of plants rating 0 and 1 (less disease) of all ten rotations examined. Maize (retained)-fallow-cotton had significantly more plants rating 0 and 1 than both sorghum treatments. Maize (incorporated)-fallow-cotton had significantly more 0s and 1s than sorghum (retained)-fallow-cotton but was not significantly different from sorghum (incorporated)-fallow-cotton. Sorghum (retained)-fallow-cotton had the highest yield of all treatments and was significantly greater than maize (incorporated)-fallow-cotton. The yield of maize (retained), sorghum (incorporated) and maize (incorporated) were not significantly different.

Results of Soil Population Counts:



Treatments followed by the same letter are not significantly different

Figure 20. Total soil population (cfu/g soil) of *Fov/Fo* in August 08



Treatments followed by the same letter are not significantly different

Figure 21. Total soil population (cfu/g soil) of *Fov/Fo* in August 09

Overall the total soil population of Fov/Fo was fairly low (less than 1250 cfu/g soil) at both sampling times but higher in 2008 than 2009 (Figure 20 and 21). In August 2008, the total Fusarium population in the continuous cotton treatment was significantly higher than all other treatments, except for the fallow-soybean-cotton and cotton-fallow-cotton treatment. A high population would be expected at this sampling time in treatments coming out of cotton (c-c-c or c-f-c) but not necessarily the fallow-soybean-cotton treatment which had been fallow prior to the sampling time. The adjustment in plot size at the start of the 2008 season meant some side soybean rows were coming out of cotton not fallow and this may have had an effect. In the August 2009 sample the cotton-fallow-cotton treatment was significantly greater than 2 years of cotton (ie. c-c-c treatment).

Where maize had been grown (2008) and then left fallow (2009) the Fusarium levels were the lowest. With soil-borne diseases pathogen population distribution in the soil can be quite patchy and variable. Only relative small sized soil samples were taken, and given the size of the plots larger soil samples and more replications would probably be required in the future to rule out any potential Fusarium hotspots and reduce variability.

In pathogenicity tests on a small representative sample of isolates in August 2009, greater than 88% of the total soil population was Fov in all rotation treatments.

Glasshouse Experiments

1. Artificial inoculation of rotation crops with a spore suspension of *Fov* using the root-dip technique

Experiment 1

Fov cannot be distinguished from Fo on culture plates without carrying out either pathogenicity tests or VCG analysis. Therefore in all five experiments all recovered Fo isolates obtained from the inoculated plants were tested for pathogenicity on cotton seedlings where they caused characteristic symptoms of Fusarium wilt (stem browning, stunting, wilting); Fo was re-isolated from the infected cotton plants confirming the presence of Fov.

In the first experiment Fov was isolated from the roots of cotton, soybean, sunflower, fababean, chickpea and canola, but was not recovered from roots of sorghum, fieldpea, maize or vetch. Fusarium was not recovered from any non-inoculated plants. There was no obvious symptom development (wilting, vascular stem discolouration) except in cotton.

Experiment 2

In the second experiment Fov was isolated from stem tissue of inoculated cotton, linseed, barley, wheat and pigeonpea plants and root tissue of all other crops except for oats, safflower and mungbean (Table 6). The number of plants Fov was recovered from was relatively small (less than 35%) compared to cotton (80%). Symptoms of wilting, stunting and stem browning only occurred on inoculated cotton plants.

Table 6. Experiment 2: Number of plants & height to which Fov was isolated from the stem of inoculated rotation crop species (3 weeks after inoculation)

Crop	Roots	0.5 cm stem	1 cm stem	Non-infected plants
Cotton	-	-	15	4
Sorghum	1	-	-	13
Maize	1	-	-	11
Wheat	-	2	2	8
Barley	1	1	-	10
Oats	-	-	-	12
Triticale	1	-	-	13
Safflower	-	-	-	14
Peanut	1	-	-	14
Linseed	-	1	-	6
Pigeonpea	-	-	1	13
Mungbean	-	-	-	13

Experiment 3

Fov was recovered from some root and/or stem tissue of all inoculated plants except for maize and panicum (Table 7).

Table 7. Experiment 3: Number of plants & height to which Fov was isolated from the stem of inoculated rotation crop species (3 ½ - 4 weeks after inoculation)

Crop	Roots	0.5 cm	1 cm	1.5 cm	2 cm	Non-infected plants
Cotton	-	-	-	-	14	1
Maize	-	-	-	-	-	15
Canary	3	-	1	-	-	11
Panicum	-	-	-	-	-	19
Jap. millet	1	1	-	-	-	18
Sunflower	-	2	2	1	-	9
Chickpea	1	-	1	-	-	13
Lupin	4	2	1	-	2	6
Fababean	1	-	-	-	1	12
Fieldpea	3	-	1	1	3	6
Vetch	-	-	3	-	2	7
Soybean	4	1	1	-	-	8
Lablab	1	-	1	-	8	5

Experiment 4

Fov was isolated from all plants except canary (Table 8). Infection was largely restricted to the root and crown region in some crops (eg sorghum and Japanese millet). In others (eg lablab, mungbean, soybean, vetch, pigeonpea, fieldpea) colonization was more extensive up the stem.

Table 8. Experiment 4: Number of plants & height to which Fov was isolated from the stem of inoculated rotation crop species (6 ½ weeks after inoculation)

Crop	Root	Crown	1 cm	2 cm	3 cm	4 cm	5 cm	6 cm	Non-infected plants
Cotton	x	-	-	-	-	-	-	11	3
Sorghum	-	7	-	-	-	-	-	-	7
Maize	-	2	2	-	-	-	-	-	5
Wheat	-	-	3	4	5	1	-	-	1
Barley	-	5	2	1	-	-	-	-	4
Oats	x	-	3	4	3	1	-	-	2
Triticale	x	-	-	-	1	-	-	-	11
Canary	-	-	-	-	-	-	-	-	14
Panicum	-	2	3	3	5	1	-	-	0
Japanese millet	-	7	-	-	2	-	-	-	3
Safflower	-	-	1	2	1	-	-	-	11
Canola	-	-	-	-	1	12	-	-	0
Sunflower	2	-	4	2	-	1	-	-	5
Chickpea	2	-	1	1	2	1	-	-	8
Peanut	-	1	2	3	3	1	-	-	4
Linseed	x	-	3	2	3	2	-	1	3
Lupin	1	1	2	5	1	1	-	-	3
Fababean	-	3	-	-	-	-	-	-	7
Fieldpea	-	-	-	-	1	10	2	-	0
Pigeonpea	-	-	-	1	1	11	6	-	0
Vetch	x	-	-	-	4	7	4	-	0
Soybean	-	-	-	4	2	5	2	-	1
Mungbean	x	-	-	4	2	3	4	2	0
Lablab	-	2	-	3	1	-	4	3	1

Table 9. Experiment 5: Number of plants & height to which Fov was isolated from the stem of inoculated rotation crop species (7 weeks after inoculation)

Crop	Root	1cm	2cm	3cm	4cm	5cm	6cm	7cm	8cm	9cm	10cm	Non-infected plants
Cotton	x	1	-	-	-	2	1	2	2	1	5	0
Sorghum	x	3 cr	-	-	1	-	-	-	-	-	-	11
Wheat	x	-	-	-	4	2	-	-	2	2	1	2
Barley	x	-	3	1	1	1	2	-	3	-	-	3
Oats	x	3	-	4	2	2	-	1	-	-	-	3
Triticale	x	-	2	3	2	2	-	-	-	-	-	5
Canary	x	1	-	-	2	3	-	-	-	-	-	8
Panicum*	x	1	12	2	x	x	x	x	x	x	x	0
Japanese millet	x	3	-	-	1	-	-	1	-	-	1	9
Safflower	x	2	1	2	3	4	1	1	-	-	-	0
Canola	x	-	-	5	5	1	1	-	-	-	-	1
Sunflower	3	9	-	-	x	x	x	x	x	x	x	3
Chickpea	x	-	-	2	2	3	1	-	3	1	-	2
Peanut	x	-	1	3	7	2	1	-	-	-	-	0
Linseed	x	-	-	4	3	1	1	-	-	-	-	2
Lupin	x	7	1	-	1	-	1	-	-	-	-	4
Fababean	x	1	-	3	1	2	1	2	1	-	-	1
Fieldpea	x	-	1	-	-	-	3	-	4	2	2	0
Pigeonpea	x	-	1	6	6	2	-	-	-	-	-	0
Soybean	x	-	3	6	2	-	-	-	-	-	-	3
Mungbean	x	-	4	6	1	-	1	-	-	-	-	3
Lablab	x	2	1	-	5	1	3	3	-	-	-	0

*Three weeks following inoculation 12 out of 15 panicum plants were severely stunted and dead; *Fov* was isolated from all 12 plants. The three remaining plants (3 cm of stem) were plated out at the completion of the experiment and *Fov* was recovered from all. There was some internal brown discolouration to 3 cm.

Overall summary: All 24 crop species artificially inoculated with the cotton wilt pathogen were susceptible to infection and the pathogen was isolated from stem tissue of all crops. The extent of colonization, however, did vary with the crop tested. With the exception of fieldpea (and panicum), most of the crops were generally not colonized to the same extent as cotton. *Fov* was isolated to 10 cm, the maximum stem length plated out in experiment five, in four crops. The pathogen was isolated from 93% of cotton plants in the 5-10 cm stem region; 92% of fieldpea plants in the 6-10 cm stem region; 54% of wheat plants in the 5-10 cm stem region and 13% of Japanese millet plants in the 7-10 cm stem section. The inoculation of panicum with Fov in the fifth experiment did essentially kill 80% of plants within 3 weeks of inoculation.

Maize, sorghum and sunflower were the least well colonized crops with infection largely restricted to the root/crown region or lower stem in a few plants (up to 4 cm in one sorghum plant, 1 cm in two maize plants and to a maximum of 4 cm in sunflower).

The spore concentration used in the later experiments was quite high and the inoculation technique of removing the plants would have provided an entry point for the fungus through wounded roots. The pots used were small and increasing stress would have been placed on the plants the longer they were left after inoculation before harvest, and this could have consequently allowed for more colonization to occur. The degree of colonization seen in these experiments may not reflect what actually occurs in the field under natural conditions. The susceptibility of the glasshouse plants may be a result of the plants being overwhelmed by inoculation with a high concentration of a particularly aggressive isolate of Fov. Therefore, further studies on natural infection are required. Growers, however, should be aware that the Fusarium wilt fungus is able to infect and persist on other crops, aiding in the survival of this pathogen. This is especially important to consider when planning crop rotation sequences in fields where there is a high level of Fusarium.

Symptom development in other crops

Determining symptomatic development in crops other than cotton was not clear cut when comparing inoculated and non-inoculated plants. There was evidence of internal stem brown discolouration in inoculated legumes such as lablab, fieldpea, fababean, mungbeans and chickpea roots/stems, however the non-inoculated plants also looked discoloured. Similarly inoculated canola plants sometimes were quite brown in the very centre of the root and stem, but there was also some discolouration in the non-inoculated plants. Therefore it was often inconclusive whether symptom development was a direct result of Fov infection.

The following symptoms were noted on inoculated plants in the last two experiments but there was uncertainty as to whether symptoms were caused by Fov infection in all cases. Stem browning was evident in cotton up to 10 cm.

Experiment 4

Lablab - stem browning was evident in eight plants 5-6 cm from the crown and the fungus was isolated up to 6 cm. Six lablab plant stems were clean; Fov was isolated from the crown/root region of two of these plants, up to 2 cm in three plants and not recovered from one plant.

Lupin - three plants were wilting slightly with reduced vegetative growth; Fov was isolated.

Panicum - one plant was stunted (12 cm); Fov was isolated 3 cm up the stem.

Soybean - slight brown streaking (?) in lower stem of two plants (Fov isolated to 3 cm)

Linseed - 1 stunted plant? (Fov isolated)

Canola – slight brown in central stem of a few plants (Fov isolated).

Experiment 5

Panicum – 12/15 plants died 3 weeks following inoculation as described above.

Lupin - 3 plants were wilted slightly with reduced vegetative growth; Fov was isolated.

Safflower - 1 wilting plant (isolated Fov to 4 cm)

Soybean - 3 plants wilting (isolated Fov to 2, 3 and 4 cm)

Lablab - 5 clean plants (isolated Fov to 1 cm, 1 cm, 2 cm, 4 cm, 6 cm). 10 plants with some degree of stem browning up to 8cm (Fov recovered up to 7 cm)

Mungbean – 1 plant with brown discolouration up to 7 cm (Fov isolated up to 6 cm)

Therefore because the pathogen was isolated from both 'clean' and 'symptomatic' plants (and because non-inoculated plants also show some stem discolouration) the symptoms, especially internal stem browning, may or may not have been a result of Fov infection.

2. Natural infection by Fov in crops other than cotton

The extent of colonisation may not occur naturally in the field as occurred in the artificial inoculation experiments, so some preliminary pot trials using naturally infected soil collected from 'Cowan" were set up to determine the extent of natural infection with all of these crops.

Natural infection was largely confined to the root and crown region in most plants in which Fov was isolated (Figure 22). Cotton plants were symptomatic and infected. Fov was isolated from the stem in a small number of mungbean, fieldpea and lablab plants but was confined to the lower 2 cm. Fov was not recovered from any of the following crops tested: pigeonpea, broccoli, Japanese millet, triticale, barley, safflower, panicum, soybean, peanut or maize.

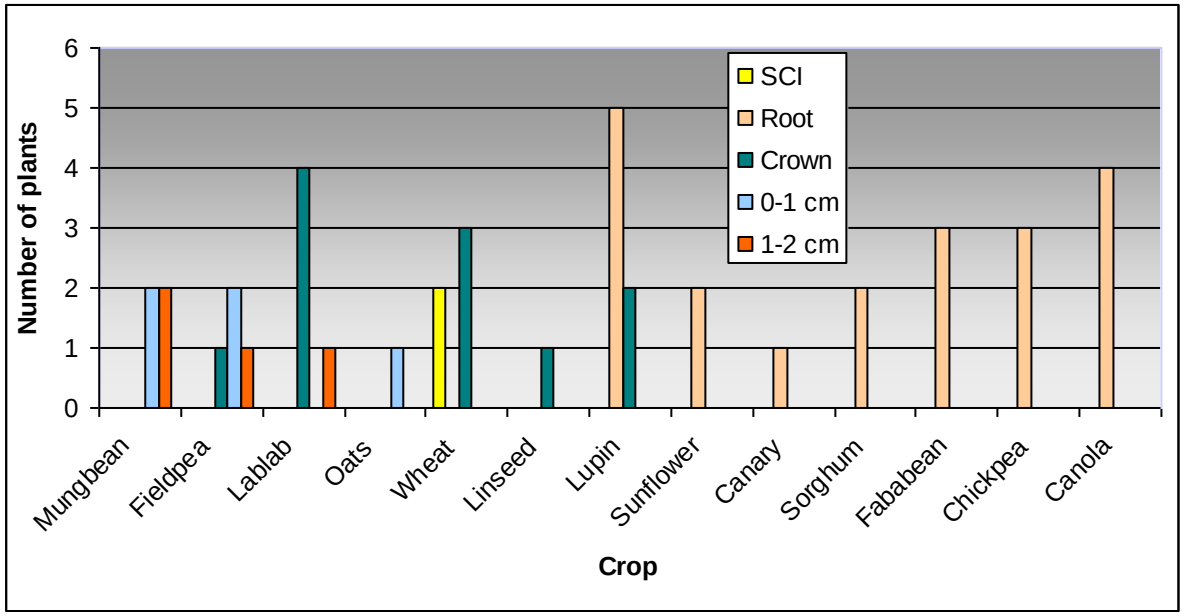


Figure 22. Number of plants infected by Fov growing in naturally infected soil in glasshouse pot trials, and tissue region *Fov* was recovered from

3. Green manure study

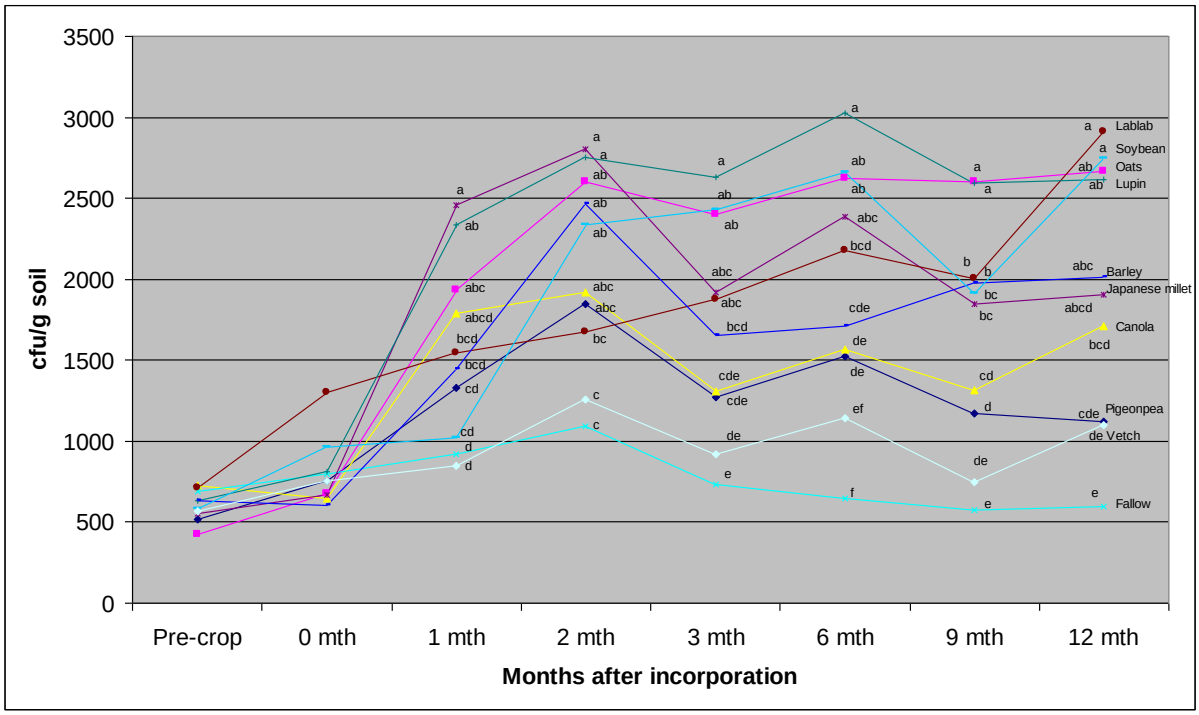


Figure 23. Total soil population of Fo and Fov over a 12 month period following green crop incorporation

Crop treatments within a month followed by the same letter are not significantly different (p=0.05)

Treatments were not significantly different before the crops were sown (pre-crop) or at the time of incorporation (0 months)

The total soil population of Fo/Fov before the crops were planted (pre-crop) and after the crops had been grown for 8 weeks prior to being incorporated (0 months) were similar between treatments with no significant differences (Figure 23). One month after the crops were incorporated, the soil population in the bare fallow, vetch, soybean and pigeonpea treatments were significantly lower than japanese millet and lupin. Where oats had been incorporated the soil population was significantly higher than a bare fallow and vetch treatment. Similar trends continued in the second month but with larger increases in the soil populations in treatments where barley and soybean had been incorporated. Although fluctuating, overall the soil population numbers then remained fairly consistent until the end of the experiment, with numbers decreasing slightly in the japanese millet, barley and pigeonpea treatments from month two.

After 12 months the Fov/Fo population in the bare fallow treatment was significantly lower than canola, japanese millet, barley, lupin, oats, soybean and lablab treatments, but not significantly different from pigeonpea or vetch. The fungal population was still relatively high in some crop treatments even after a 12 month fallow period. This study highlights that incorporation of green manure crops does increase the soil population of Fo/Fov by providing a residue substrate for the pathogen to survive on saprophytically and potentially multiply. Since the pathogen remains indefinitely in the soil, using rotations as a management tool may be limited but growers need to manage residues (and weeds) through fallow periods and use green manure/cover crops with caution where Fusarium is present.

Pathogenicity test results:

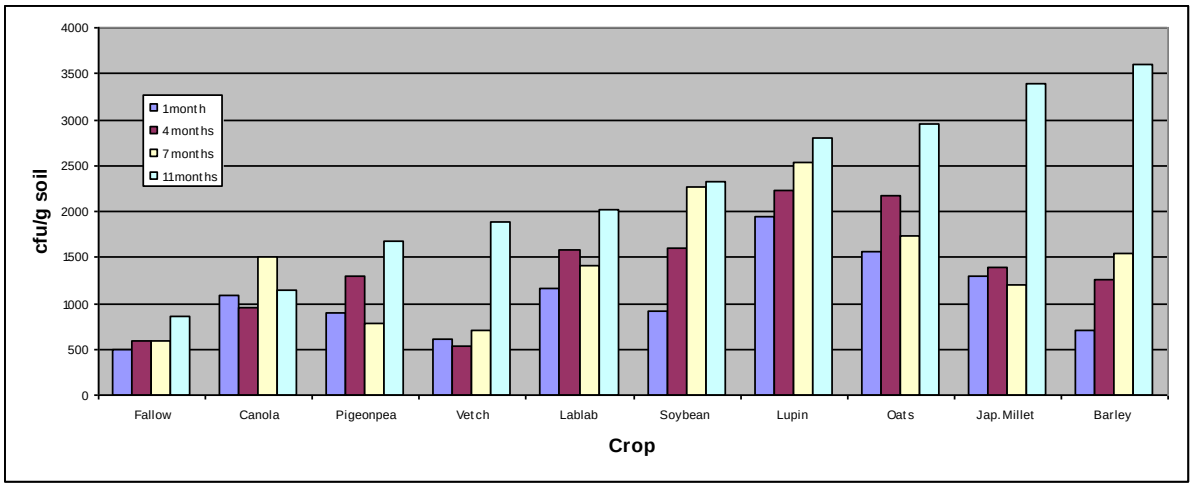


Figure 24. Total Fov population changes at 1, 4, 7 and 11 months following green crop incorporation (1 replication only)

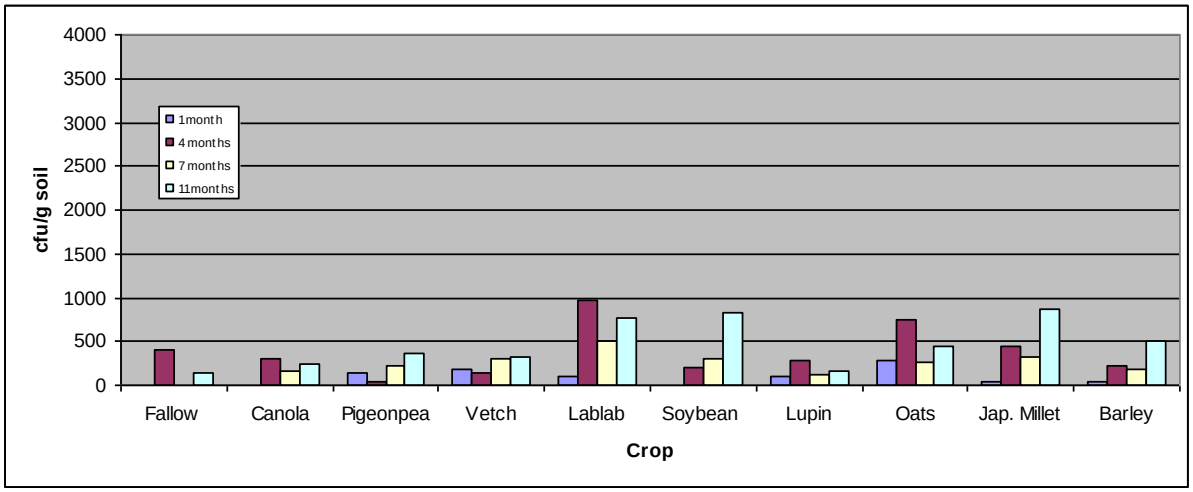


Figure 25. Total Fo population changes at 1, 4, 7 and 11 months following green crop incorporation (1 replication only)

Given that a subset of Fo isolates from one replication only of each treatment were examined at the four monthly times, the sample size was very small so data reflects general trends only. A larger sample of isolates undergoing pathogenicity tests would be needed for more conclusive comments.

The total soil population was comprised of greater than 60% Fov propagules in all treatments in all four months (and up to 100% at times).

The Fov population was generally greater at 11 months following crop incorporation than 1 month after.

Objective 3.

Investigate the role key nutrients play in host resistance, including the effect on mychorrizal colonisation. Nutrients of importance that require attention for nutrient balance and disease management are nitrogen, phosphorus and potassium.

Glasshouse trial 1

In sand culture, the cultivar with higher genetic resistance (Sicot 189) was less affected by mineral nutrition (Figure 26). For Siokra 1-4, as P increased disease severity increased, with significant differences being observed (Figure 26). Application of 60 kg/ha resulted in significantly higher mean VDI (MVDI) than when 0 or 5 kg/ha was applied. However, in Sicot 189 there was an initial increase in disease severity when P was applied, however differences were not significant but further increases had little effect (Figure 26).

Application of some nutrients, for example N, close to roots can cause root damage and this can result in an increase in disease severity. There was no root damage observed following P application at the rates tested (Figure 27).

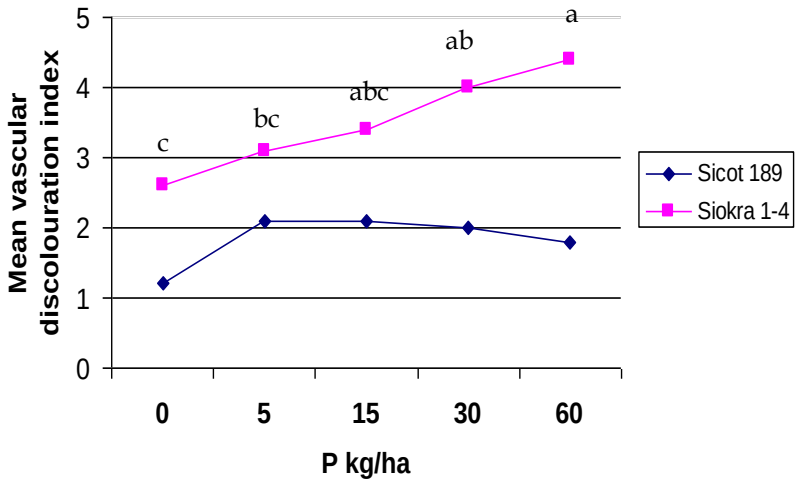


Figure 26. The effect of phosphorus fertilisation on severity of Fusarium wilt on cotton plants grown in sand culture

Figure 27. There was no evidence of root damage following application of P at 0, 5, 15, 30 and 60 kg/ha

Glasshouse trial 2

In Sicot 189, although adequate P was indicated by soil analysis (P-bic 29 mg/kg, critical value of soil P is 6 mg/kg), a low level of P (5 kg/ha) applied below the seed was beneficial significantly (P=0.02)

reducing mean vascular discolouration index (MVDI) compared to 0, 15 and 30 kg/ha (Figure 28). P is immobile in soil so it is possible that a low application assisted plant resistance. However as P was increased further disease severity increased, but the increase was not significant compared to the control treatment in which P was not applied.

There was no significant effect of P application on MVDI in Sicot F-1 (Figure 28).

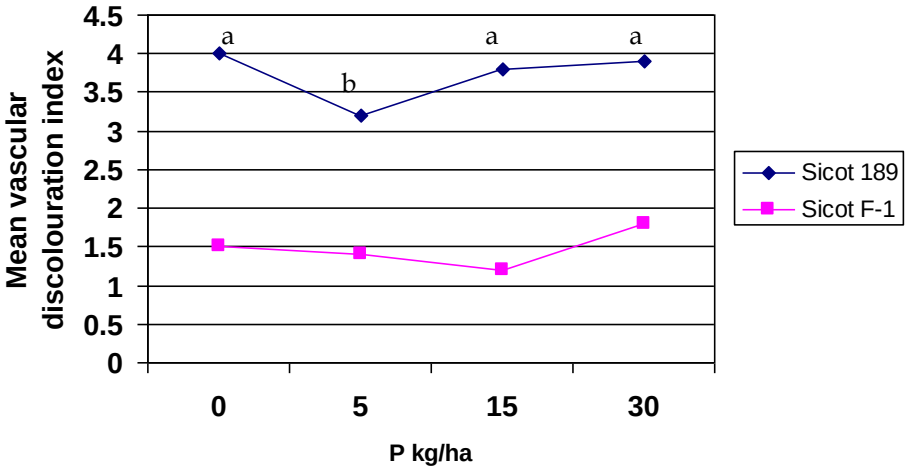


Figure 28. Effect of phosphorus application on mean vascular discolouration index of cultivars Sicot 189 and Sicot F-1
Rates followed by the same letter indicate MVDI that are not significantly different from one another.

Results of N application are interesting. Application of N at 120 and 250 kg/ha significantly reduced MVDI for both cultivars compared to 0 and 40 kg/ha (Figure 29). One of the most commonly assumed relationships of N to disease is that high N leads to more severe disease, however this was not observed in this trial indicating that N nutrition, which might be higher than local practices, may suppress disease. However, there are a lot of problems associated with over-fertilisation, as previously mentioned. N-NO3 measured in this soil/sand mix was 29.7 mg/kg, with the critical value of soil N being 20 – 30 mg/kg, hence adequate N was available without additional N application. The effect of N on plant growth was not measured in this trial, however field trials conducted at "Cowan" should highlight these problems if they arise.

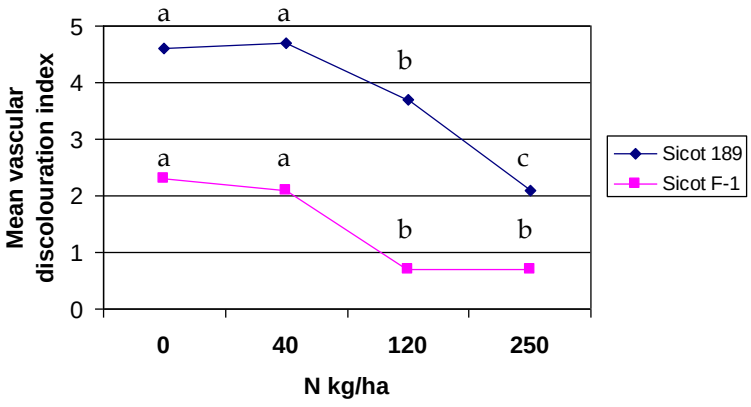


Figure 29. Effect of nitrogen application on mean vascular discolouration index of cultivars Sicot 189 and Sicot F-1
Rates followed by the same letter indicate MVDI that are not significantly different from one another.

In this trial K was abundant for plant growth (K-ammonium acetate 0.76 meq/100g , critical value of soil K is 0.2 – 0.4 meq/100g), and addition of K had no significant effect on MDVI for either cultivar (Figure 30), however there was a significant interaction (P=0.027) between P (5 kg/ha) and K (100 kg/ha) (Figure 30), resulting in a reduction in MVDI for Sicot 189 (Figure 31).

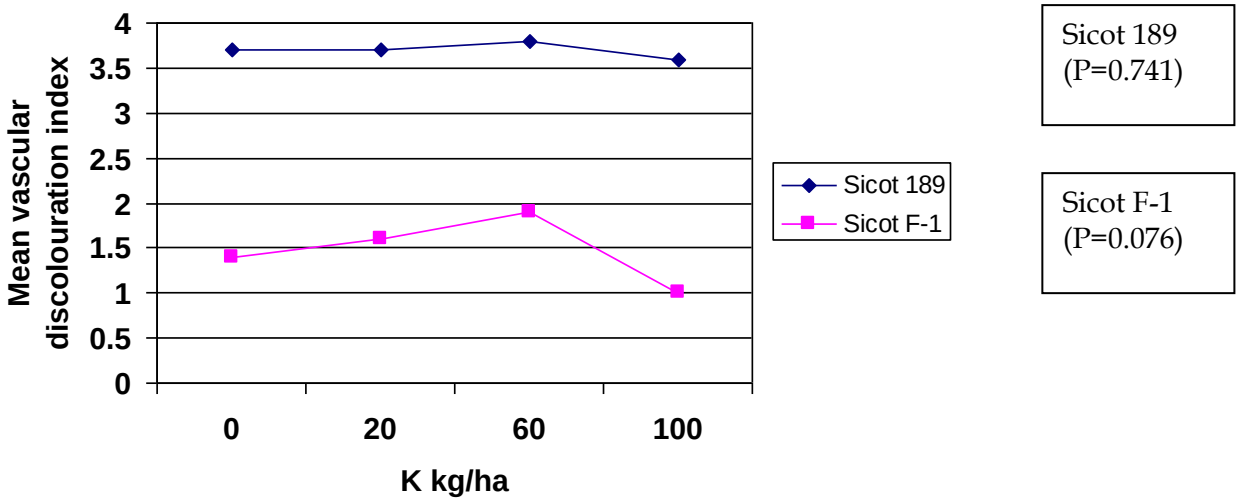


Figure 30. Effect of potassium application on mean vascular discolouration index of cultivars Sicot 189 and Sicot F-1

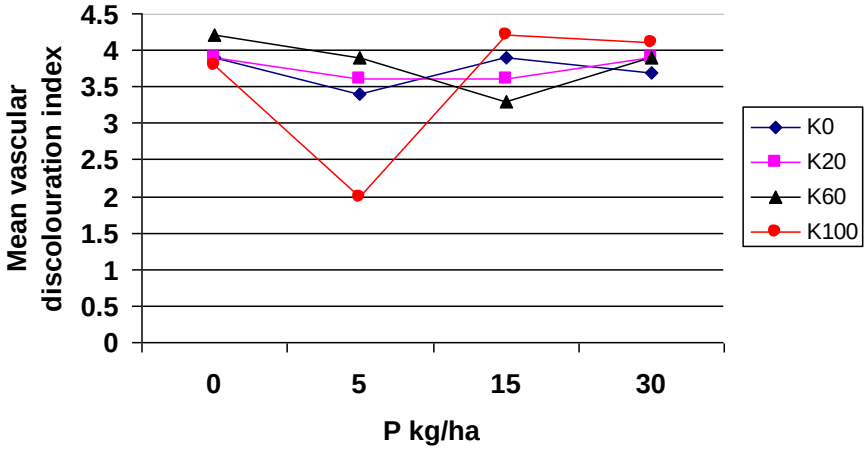
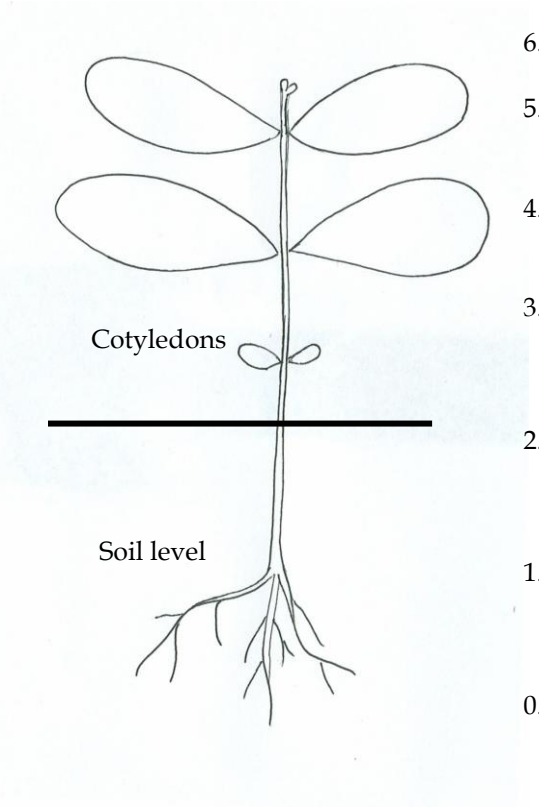


Figure 31. Interactive effect of phosphorus and potassium on mean vascular discolouration index for Sicot 189



		Sicot 189	Sicot F-1
6.	Dead plant	Rating 5-6 10 combinations Highest level of disease P1N2K4 MVDI 5.4 **P1N1K1** MVDI 5.0	Rating 5-6 2 combinations Highest level of disease P2N2K3 MVDI 6.0 P4N2K3 MVDI 5.7
5.	Complete vascular discolouration of stem	Rating 4-5 13 combinations	Rating 4-5 0 combinations
4.	Discolouration of stem above the cotyledons	Rating 3-4 13 combinations	Rating 3-4 5 combinations
3.	Discolouration of the stem up to the cotyledons	Rating 2-3 8 combinations	Rating 2-3 11 combinations
2.	Discolouration above soil level but below cotyledons	Rating 1-2 8 combinations Including **P4N4K4** MVDI 1.8	Rating 1-2 22 combinations
1.	Base of stem discoloured below soil level	Rating 0-1 1 combination Lowest level of disease **P2N4K4** MVDI 0.6	Rating 0-1 24 combinations **P4N4K4** MVDI 0.1 Lowest MVDI = 0 7 combinations P1N4K4 P2N3K1 P2N3K4 **P2N4K4**
0.	No vascular discolouration		

Figure 32. Summary of NPK on disease severity in Sicot 189 and Sicot F-1

In glasshouse trial 2, the effect of NPK on disease severity ranged from disease-free plants to dead plants for both cultivars. Disease severity was influenced by the balance of nutrients. For Sicot 189, the higher disease ratings tended to have lower levels of nutrients added, particularly low N, and lower disease ratings tended to have higher N. The lowest level of disease was observed when 5 kg/ha of P, 250 kg/ha of N and 100 kg/ha of K was applied. For Sicot F-1, disease-free plants mostly had been treated with the highest level of K at 100 kg/ha in combination with high N at either 120 or 250 kg/ha. P did not appear to effect disease severity.

In summary, under glasshouse conditions, disease severity was influenced by the balance of N, P and K; however cultivars differing in disease resistance were affected differently in some instances. For example, when P was applied without N and K, Siokra 1-4, which is highly susceptible to Fusarium wilt, had significantly greater levels of disease with increasing P. However, Sicot 189 which has mid-range resistance was not affected by increasing levels of P in the absence of N and K. Although P did not influence disease rating in Sicot 189 when grown in sand culture, in a field soil/sand mix, a low rate of P significantly reduced disease severity. This low rate may have balanced the level of N, P and K available, thereby resulting in a lower level of disease. Nitrogen significantly influenced the level of disease in both cultivars tested, regardless of resistance ranking. This may be due to an effect of N

(nitrite/ammonia) on pathogen populations. Further investigations are required to determine if N application reduce the population of Fov and whether ammonium and/or nitrite are responsible. The combination of K and N was important in the resistance of Sicot F-1. Trials conducted to investigate the rates of N, P and K that result in the lowest levels of disease highlight just how important nutrient balance is in relation to Fusarium wilt management. Plant health ranged from disease-free to dead, depending on the combination of N, P and K applied. Monitoring soil nutrient levels, nutrient loss through crop harvest and other means of nutrient loss is of utmost importance so that the correct levels of nutrition can be maintained that assist in the reduction of Fusarium wilt severity.

NPK Field Trial 1 2008/09

Nutrient levels pre-plant
Analysis of N, P and K indicates that availability of these nutrients exceeds the critical values required for adequate plant growth (Table 10). Any improvement in yield at harvest is not expected to be due to additional nutrition as there are already adequate supplies available for optimum growth and yield.

Table 10. Nutrient and pH analysis of soil collected from field site

Measurement	0-15 cm	15-60 cm	60-120 cm	Critical value (NUTRIpak)
pH	8.01	8.28	8.45	-
N mg/kg	22	40	30	20-30
P mg/kg	29	9	7	6
K mg/kg	331	199	276	100-150

Varietal effects on plant emergence and establishment, and seed cotton yield
Plant emergence is measured as the number of seedlings emerged per 10 m plot two to three weeks after planting. Plant establishment is measured as the number of plants established 6 weeks after planting per 10 m plot. There was no difference between varieties with respect to emergence (Table 11). There were significantly more plants established for Sicala 45 BRF (F-rank 126) than for Sicala 60 BRF (F-rank 102) (Table 11), highlighting the importance of planting varieties with a higher F-rank in Fusarium infested soils. Yield was significantly higher for Sicala 45 BRF than for Sicala 60 BRF (Table 11).

There were an additional 15, 000 plants/ha established for Sicala 45 BRF compared to Sicala 60 BRF which produced a difference of 2.1 bales/ha of lint produced when the higher F-ranked variety was sown (Table 12). This equates to approximately an additional $302 400 in cotton production for an averages sized farm of 300 ha (Table 12).

Table 11. The effect of variety/F-rank on number of plants emerged and established per 10m row and yield

Variety	Emergence	Establishment	Yield*
V1. Sicala 45 BRF (F-rank 126)	100	70 a	4.70 a
V2. Sicala 60 BRF (F-rank 102)	99	55 b	3.49 b
LSD (P=0.05)	ns	2.8	0.162

ns = not significant.
Data followed by different letters are significantly different from one another.
*Yield measured as seed cotton in kg per 10 m row.

Table 12. The effect of variety/F-rank on seed cotton yield, yield and difference in value of production

Variety	Seed cotton yield (Kg/10 m row)	Lint yield* (Bales/ha)	Lint yield difference (Bales/ha) and value ($)**
V1. Sicala 45 BRF (F-rank 126)	4.70 a	8.3	2.1 bales/ha difference 2.1 x $480 x 300ha = $302 400
V2. Sicala 60 BRF (F-rank 102)	3.5 b	6.2	
LSD (P=0.05)	0.162	-	

* Lint yield based on a 40% gin turnout
** based on 2008/09 sale of $480/bale

Nutritional effects on plant emergence and establishment, and seed cotton yield
N application had no effect on emergence (Table 13), however at 150 kg/ha, N significantly increased the number of plants established for both varieties and yield (Table 13). N application has been associated with reduced Fusarium wilt in cotton (Bo Wang 1994). This may be due to an effect of the form of N on the pathogen in the soil. Nitrate-N has been shown to reduce Fusarium wilt symptoms, whereas ammonium-N increases Fusarium wilt symptoms. The effect of each form may be associated with soil pH influences. Loffler *et. al*. (1986) determined that urea and ammonium chloride reduced the population of *Fusarium oxysporum* f. sp. *dianthi* (Fod), however it was not the ammonia, and nitrate had no effect on the Fusarium oxysporum population. They concluded that it was the nitrite rather than ammonia that was responsible for the decline effect of ammonia-generated compounds on populations of Fod. Ammonia is oxidised to nitrite by bacteria then subsequently to nitrate.

Table 13. The effect of N fertilisation on emergence and establishment of varieties Sicala 45 BRF and Sicala 60 BRF per 10m row and yield

Nutrient	V1. Sicala 45 BRF F-rank 126			V2. Sicala 60 BRF F-rank 102		
N kg/ha	Emergence	Establishment	Yield*	Emergence	Establishment	Yield*
0	100	67 a	4.54 a	99	51 a	3.30 a
150	100	**73 b**	**4.94 b**	98	**59 b**	**3.70 b**
LSD (P=0.05)	ns	3.4	0.227	ns	3.4	0.210

ns = not significant.
Data followed by different letters are significantly different from one another.
* Yield measured as seed cotton in kg per 10 m row.

Application of P at four rates (0, 20, 40 and 80 kg/ha) had no effect on emergence or establishment of Sicala 45 BRF (Table 14). P application also had no effect on emergence of Sicala 60 BRF (Table 14). However, at the highest rate of P (80 kg/ha) establishment of Sicala 60 BRF was significantly reduced compared to plants that had 0 and 40 kg/ha of P applied (Table 14). There was no effect of P application on yield for either variety (Table 14).

Table 14. The effect of P fertilisation on emergence and establishment of varieties Sicala 45 BRF and Sicala 60 BRF per 10m row and yield

Nutrient	V1. Sicala 45 BRF F-rank 126			V2. Sicala 60 BRF F-rank 102		
P kg/ha	**Emergence**	**Establishment**	**Yield***	**Emergence**	**Establishment**	**Yield***
0	98	69	4.79	98	58 b	3.40
20	102	73	4.64	97	54 ab	3.50
40	99	68	4.89	100	56 b	3.56
80	101	69	4.66	100	**51 a**	3.52
LSD (P=0.05)	ns	ns	ns	ns	4.8	ns

ns = not significant. Data followed by different letters are significantly different from one another. * Yield measured as seed cotton in kg per 10 m row.

Application of K at 100 kg/ha significantly increased establishment of Sicala 45 BRF, but had effect on emergence. There was no significant yield response to K application (Table 15).

There was no effect of K on emergence or establishment of Sicala 60 BRF; however yield was significantly increased following application of K (Table 15).

Table 15. The effect of K fertilisation on emergence and establishment of varieties Sicala 45 BRF and Sicala 60 BRF per 10m row and yield

Nutrient	V1. Sicala 45 BRF F-rank 126			V2. Sicala 60 BRF F-rank 102		
K kg/ha	**Emergence**	**Establishment**	**Yield***	**Emergence**	**Establishment**	**Yield***
0	99	68 a	4.65	98	54	3.36 a
100	101	**72 b**	4.84	99	56	**3.63 b**
LSD (P=0.05)	ns	3.4	ns (P=0.086)	ns	ns	0.210

ns = not significant.
Data followed by different letters are significantly different from one another.
* Yield measured as seed cotton in kg per 10 m row.

There was an interactive effect of N and K on establishment of Sicala 45 BRF. When neither N nor K fertiliser was applied, the number of plants established was significantly reduced compared to other NK treatment combinations (Table 16).

There was no interactive effect of N and K on emergence or establishment of Sicala 60 BRF (Table 16). None of the treatments significantly affected yield (Table 16).

Table 16. The interactive effect of N and K fertilisation on emergence and establishment per 10 m row and yield of varieties Sicala 45 BRF and Sicala 60 BRF

Nutrient		V1. Sicala 45 BRF F-rank 126						V2. Sicala 60 BRF F-rank 102					
		Emergence		**Establishment**		**Yield***		**Emergence**		**Establishment**		**Yield***	
K kg/ha	***N kg/ha***	***0***	***150***	***0***	***150***	**0**	**150**	***0***	***150***	***0***	***150***	**0**	**150**
0		97	103	**63 a**	73 b	4.41	4.88	98	100	51	57	3.24	3.48
100		101	99	70 b	74 b	4.68	5.01	99	98	51	61	3.35	3.91
LSD (P=0.05)		ns		4.8		ns		ns		ns		ns	

ns = not significant.
Data followed by different letters are significantly different from one another at P=0.05.
* Yield measured as seed cotton in kg per 10 m row.

There were no interactive effects of N and P on emergence for Sicala 45 BRF, however there was an effect of N and P on establishment of Sicala 45 BRF. When N was applied at 150 kg/ha and P at 20 kg/ha, plant establishment was significantly increased compared to all other NP treatments (Table 17). This increase in plant number did not transpire to a significant increase in yield compared to other treatments. However when P was applied at 80 kg/ha without the addition of N, yield was significantly reduced compared to all other treatments, with the exception of P applied at 20 kg/ha and no N (Table 17).

There was no interactive effect of N and P on emergence, establishment or yield of Sicala 60 BRF.

Table 17. The interactive effect of N and P fertilisation on emergence and establishment per 10 m row and yield of varieties Sicala 45 BRF and Sicala 60 BRF

Nutrient		V1. Sicala 45 BRF F-rank 126						V2. Sicala 60 BRF F-rank 102					
		Emergence		Establishment		Yield*		Emergence		Establishment		Yield*	
P	***N***	***0***	***150***	***0***	***150***	***0***	***150***	***0***	***150***	***0***	***105***	***0***	***150***
0 kg/ha		98	97	68	70 a	4.72 ab	4.86 a	98	99	54	62	3.12	3.69
20 kg/ha		101	103	65	**81b**	**4.32 bc**	4.97 a	96	97	49	59	3.41	3.59
40 kg/ha		103	96	66	71 a	4.89 a	4.88 a	100	99	54	59	3.50	3.62
80 kg/ha		98	103	67	71 a	**4.26 c**	5.06 a	101	99	47	56	3.15	3.89
LSD (P=0.05)		ns		6.8		0.453		ns		ns		ns	

ns = not significant.
Data followed by different letters are significantly different from one another.
* Yield measured as seed cotton in kg per 10 m row.

N fertilisation increased the profit margin of both varieties, however the increase in yield from application of K was not sufficient to cover the costs of K fertiliser purchased (Table 18). The yield increases and profit associated with increased N application was not sufficient to warrant the initial cost (Graham Clapham pers. comm.).

Table 18. The effect of N and K fertilisation on yield and profit

Nutrient	Yield Bales/ha		Estimated potential profit (based on 2008/2009 sale of $480/bale. Average size Darling Downs irrigated cotton production - 300 ha/farm)	
	Sicala 45 BRF	Sicala 60 BRF	Sicala 45 BRF	Sicala 60 BRF
0 Kg/ha N	8.0 a	5.8 a	Profit $128/ha	Profit $110/ha
150 Kg/ha N	**8.7 b**	**6.5 b**	**~$38 400 profit from N fertilisation**	**~ $33 000 profit from N fertilisation**
0 Kg/ha K	8.2 a	5.9 a		N application increased profit by $125/ha, whereas K decreased profit by $15/ha i.e. yield increase did not cover cost of K fertiliser
100 Kg/ha K	8.3 a	**6.4 b**		

Varietal effect on nutrient uptake

The higher F-rank variety, Sicala 45 BRF, had significantly higher levels of N, P and K in the 5th terminal leaf than Sicala 60 BRF (Table 19). The level of nitrogen and potassium in the 5th terminal leaves indicate sufficient availability and uptake of these nutrients. However, phosphorus levels were high in the leaves as the normal range is 0.28 – 0.5% Wt (Table 19).

Table 19. Effect of variety on N, P and K in the 5th terminal leaf

Variety	N % Wt	K % Wt	P % Wt
Sicala 45 BRF F-rank 126	**3.85 (0.2045 a)**	**1.97 (0.1066 a)**	**0.92 (0.0492 a)**
Sicala 60 BRF F-rank 102	3.79 (0.2014 b)	1.96 (0.1041 b)	0.90 (0.0481 b)
LSD (P=0.05)	(0.0014)	(0.0014)	(0.0005)

Data in parentheses are transformed (data/100, square root, arcsine).
Data followed by different letters are significantly different from one another.

Effect of nutrient application on uptake

When N was applied, there was a significant increase in N acquisition. There was also a significant increase in K uptake in association with N application. This may be due to N stimulating plant growth resulting in higher K uptake or it may be due to ammonia replacing some of the potassium on the clay particles as ammonia can help release potassium thereby making it more available. Increasing K with application of N is a common response in the field (Table 20).

When K was applied, K uptake was increased (Table 20). The levels of both K and N are good, that is, within the expected range for sufficient nutrition.

A lack of response to phosphorus application can be due to high sodium levels in the soil because in sodic soils a response to phosphorus is generally not observed. Sodic soils are those which have an exchangeable sodium percentage (ESP) of 15% or greater. Excess exchangeable sodium has an adverse effect on the physical and nutritional properties of the soil, with consequent reduction in crop growth. The ESP of this soil ranged from 5.7 to 7.8 % and sodium ranged from 575 to 847 mg/kg depending on soil depth of sample (Table 21) indicating problems with P uptake are not associated with sodium levels.

Table 20. The effect of N, P and K application on content of the 5th terminal leaf of varieties Sicala 45 BRF and Sicala 60 BRF

Treatment Applied	Sicala 45 BRF F-rank 126			Sicala 60 BRF F-rank 102		
	N	K	P	N	K	P
N0	0.2022 a	0.1046 a	0.0494	0.1979 a	0.1022 a	0.0481
N150	**0.2067 b**	**0.1085 b**	0.0491	**0.2049 b**	**0.1060 b**	0.0481
LSD (P=0.05)	0.0019	0.0021	ns	0.0019	0.0018	ns
P0	0.2042 a	0.1072	0.0492	0.2011	0.1029	0.0474
P20	0.2023 a	0.1054	0.0486	0.2023	0.1037	0.04832
P40	0.2041 a	0.1056	0.0493	0.2014	0.1053	0.04841
P80	0.2071 b	0.1081	0.0497	0.2009	0.1043	0.0484
LSD (P=0.05)	0.0027	ns	ns	ns	ns	ns
K0	0.2040	0.1049 a	0.0491	0.2009	0.1030 a	0.0478
K100	0.2049	**0.1083 b**	0.0494	0.2019	**0.1051 b**	0.0484
LSD (P=0.05)	ns	0.0021	ns	ns	0.0018	ns

ns = not significant.
Data followed by different letters are significantly different from one another.
Data presented is transformed data (data/100, square root, arcsine), original % Wt data not provided.

Table 21. Sodium (Na) and Exchangeable Sodium Percentage (ESP) of soil

Measurement	0-15 cm	15-60 cm	60-120 cm	Comment
Na mg/kg	575	847	820	<1000 good >2000 high >3000 problem
ESP %	5.7	7.8	6.8	≥ 15 % soil

Australian cotton-growing soils have a high clay content, high cation exchange capacity (CEC) and are alkaline (pH > 7.5). Under alkaline conditions, P availability is often low, despite the soil having high total P content and this may have contributed to the low P determined in the cotton plant.

Plant maturity and disease severity

A counted mature plant is one which produces four open bolls or more. The % of plants that reach maturity was measured as survival of plants through to maturity, expressed as a percentage of the emergence count. For both varieties, the application of N significantly increased the % of plants that reached maturity (Table 22). There was no effect of P on % maturity for Sicala 45 BRF, however for Sicala 60 BRF, the highest plant maturity was when no P was applied, with applications of 40 and 80 kg/ha significantly reducing % maturity (Table 23). There was no effect of K on % of plants mature (Table 24). Without N application, the application of P had no effect on % maturity. However when N was applied, P applied at 20 and 40 kg/ha significantly increased % maturity compared to plants treated with 80 kg/ha and all applications of P without N (Table 25).

Disease severity was assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count. There was no effect of N, P or K on disease severity measured as the % of plants that were rated 0 and 1 for vascular discolouration (Tables 21, 22 and 23 respectively). There was however an interactive effect of between N and K on disease severity, with the effect differing between varieties. For Sicala 45 BRF (F-rank 126) the application of K in the absence of N fertilisation significantly increased the % of plants rated 0 and 1. However when N was applied there was no effect of K on disease severity (Table 17). For Sicala 60 BRF (F-rank 102) the application of K without N had no effect on disease severity, however when both N and K were applied, the number of plants rated 0 and 1 was significantly increased (Table 27).

There are reports in the literature of increasing P levels both increasing (Dick and Tisdale, 1937; Young and Tharp, 1941) and decreasing severity of Fusarium wilt. Unfortunately little is understood about how P influences disease severity. In this trial, it was not possible to determine if P application had an effect on disease severity because there was no uptake of P following application. However these results suggest that P application did not influence the population of Fov in the soil as N application has been shown to do.

Table 22. The effect of N fertilisation on % of plants that reach maturity and disease severity of varieties Sicala 45 BRF and Sicala 60 BRF

Nutrient	V1. Sicala 45 BRF F-rank 126		V2. Sicala 60 BRF F-rank 102	
N kg/ha	**% maturity**	**% 0 and 1's**	**% maturity**	**% 0 and 1's**
0	65.7 (0.9457 a)	29.5 (0.5634)	54.8 (0.8452 a)	10.3 (0.3198)
150	**72.0 (1.0154 b)**	28.3 (0.5580)	**60.1 (0.8958 b)**	11.6 (0.3424)
LSD (P=0.05)	(0.0314)	(ns)	(0.0281)	(ns)

ns = not significant.
Data followed by different letters are significantly different from one another.
Data in parentheses is transformed data (data/100, square root, arcsine), original % maturity and % 0 and 1's data provided.
Disease severity is assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count.
A counted mature plant is one which produces four open bolls or more. The % of plants that reach maturity was measured as survival of plants through to maturity, expressed as a percentage of the emergence count.

Table 23. The effect of P fertilisation on % of plants that reach maturity and disease severity of varieties Sicala 45 BRF and Sicala 60 BRF

Nutrient	V1. Sicala 45 BRF F-rank 126		V2. Sicala 60 BRF F-rank 102	
P kg/ha	**% maturity**	**% 0 and 1's**	**% maturity**	**% 0 and 1's**
0	68.7 (0.9907)	28.4 (0.5635)	60.1 (0.9071 a)	11.4 (0.3429)
20	69.9 (0.9802)	30.8 (0.5741)	55.9 (0.8687 ab)	10.2 (0.3221)
40	68.5 (0.9912)	29.5 (0.5707)	**57.8 (0.8674 b)**	11.8 (0.3443)
80	68.3 (0.9601)	27.0 (0.5346)	**55.6 (0.8387 b)**	10.4 (0.3151)
LSD (P=0.05)	(ns)	(ns)	(0.0397)	(ns)

ns = not significant.
Data followed by different letters are significantly different from one another.
Data in parentheses is transformed data (data/100, square root, arcsine), original % maturity and % 0 and 1's data provided.
Disease severity is assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count.

Table 24. The effect of K fertilisation on % of plants that reach maturity and disease severity of varieties Sicala 45 BRF and Sicala 60 BRF

Nutrient	V1. Sicala 45 BRF F-rank 126		V2. Sicala 60 BRF F-rank 102	
K kg/ha	**% maturity**	**% 0 and 1's**	**% maturity**	**% 0 and 1's**
0	67.2 (0.9688)	28.0 (0.5531)	56.9 (0.8675)	10.7 (0.3257)
100	70.6 (0.9923)	29.9 (0.5683)	57.7 (0.8734)	11.2 (0.3365)
LSD (P=0.05)	(ns)	(ns)	(ns)	(ns)

ns = not significant.
Data followed by different letters are significantly different from one another.
Data in parentheses is transformed data (data/100, square root, arcsine), original % maturity and % 0 and 1's data provided.
Disease severity is assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count.

Table 25. The interactive effect of N and P fertilisation on % of plants that reach maturity for Sicala 45 BRF and Sicala 60 BRF

Nutrient		V1. Sicala 45 BRF F-rank 126		V2. Sicala 60 BRF F-rank 102	
		% maturity		% maturity	
P kg/ha	***N kg/ha***	***0***	***150***	***0***	***150***
0		(0.9671 bc)	(1.1043 ab)	(0.8697)	(0.9445)
20		(0.9223 c)	**(1.0381 a)**	(0.8534)	(0.8839)
40		(0.9351 c)	**(1.0474 a)**	(0.8561)	(0.8786)
80		(0.9582 bc)	(0.9620 bc)	(0.8014)	(0.8761)
LSD (P=0.05)		(0.06274)		(ns)	

ns = not significant.
Data followed by different letters are significantly different from one another.
Data in parentheses is transformed data (data/100, square root, arcsine), original % maturity data provided.

Table 26. The interactive effect of N and K fertilisation on disease severity for Sicala 45 BRF and Sicala 60 BRF

Nutrient		V1. Sicala 45 BRF F-rank 126		V2. Sicala 60 BRF F-rank 102	
		% 0 and 1's		% 0 and 1's	
K kg/ha	***N kg/ha***	***0***	***150***	***0***	***150***
0		**(0.5358 b)**	(0.5704 ab)	(0.3305 ab)	(0.3209 ab)
100		**(0.5911 a)**	(0.5456 ab)	**(0.3091 b)**	**(0.3639 a)**
LSD (P=0.05)		0.0480		0.0437	

Data followed by different letters are significantly different from one another.
Data in parentheses is transformed data (data/100, square root, arcsine), original % 0 and 1's data provided.
Disease severity is assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count.

Nutrition and cotton fibre quality

Nitrogen fertilisation significantly increased fibre strength of variety Sicala 45 BRF (Table 27); a fundamentally important characteristic of cotton in regard to spinning. There was no effect of P or K on aspects of cotton fibre quality (Table 27). For Sicala 60 BRF, the application of K significantly increased the degree of colour pigment (Table 28).

Table 27. The effect of N, P, and K fertilisation on aspects of cotton fibre quality for variety Sicala 45 BRF

Nutrient	Mic	Rd	SFI	Strength	UHML	Uniformity	+b
N0	4.3	69.7	7.5	30.3 a	1.2	83.8	6.3
N150	4.2	69.4	7.7	**31.0 b**	1.2	83.8	6.3
LSD (P=0.05)	ns	ns	ns	0.46	ns	ns	ns
P0	4.3	69.6	7.5	30.8	1.2	83.8	6.4
P20	4.2	69.9	7.9	30.6	1.2	84.0	6.3
P40	4.4	69.0	7.6	30.4	1.2	83.6	6.3
P80	4.3	69.8	7.4	30.8	1.2	83.8	6.2
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns
K0	4.3	69.4	7.5	30.6	1.2	83.9	6.2
K100	4.3	69.7	7.7	30.7	1.2	83.7	6.4
LSD(P=0.05)	ns	ns	ns	ns	ns	ns	ns

ns - not significant
Data followed by different letters are significantly different from one another.
Mic = Micronaire, Rd = Degree of reflectance, SFI = Short fibre index, UHML = Upper half mean length, +b =Yellowness, degree of colour pigment.

Table 28. The effect of N, P, and K fertilisation on aspects of cotton fibre quality for variety Sicala 60 BRF

Nutrient	Mic	Rd	SFI	Strength	UHML	Uniformity	+b
N0	4.1	68.4	7.1	32.1	1.21	84.6	6.2
N150	4.1	68.2	7.1	32.6	1.21	84.4	6.2
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns
P0	4.1	68.5	7.2	31.9	1.21	84.5	6.0
P20	4.1	68.2	7.2	32.5	1.21	84.5	6.3
P40	4.0	67.8	6.7	32.6	1.21	84.4	6.1
P80	4.1	68.7	7.3	32.3	1.21	84.7	6.2
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns
K0	4.1	68.0	6.9	32.2	1.21	84.6	6.06 a
K100	4.1	68.6	7.3	32.5	1.21	84.5	**6.27 b**
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	0.20

ns - not significant
Data followed by different letters are significantly different from one another.
Mic = Micronaire, Rd = Degree of reflectance, SFI = Short fibre index, UHML = Upper half mean length, +b =Yellowness, degree of colour pigment.

Summary of results

- The nutrient levels of N, P and K indicated that availability of these nutrients exceeded the critical values required for adequate plant growth. Any improvement in yield at harvest was not expected to be due to additional nutrition as there were already adequate supplies available for optimum growth and yield.

- Fertiliser application influenced the number of plants established
  - N at 150 kg/ha significantly increased the number of plants established for both varieties.
  - K at 100 kg/ha significantly increased establishment of Sicala 45 BRF.

- Varieties differ in nutrient uptake in the leaves.
  - Sicala 45 BRF (F-rank 126) had a significantly higher level of N, P and K in the 5th terminal leaf than Sicala 60 BRF (F-rank 102).

- Fertiliser application influenced nutrient uptake.
  - N fertilisation increased N uptake and also increased K uptake (This may be due to N stimulating plant growth resulting in higher K uptake, or it may be due to ammonia replacing some of the potassium on the clay particles, thereby making it more available. Increasing K with N application is a common response in the field).
  - K fertilisation increased K uptake.
  - No effect of P fertilisation on P uptake. This response is not due to soil being sodic. P availability can be low in alkaline soils despite soil having a high P content.

- The higher the F-rank, the higher the yield.
  - 1.21 kg/10 m row difference between Sicala 45 BRF F-rank 126 and Sicala 60 BRF F-rank 102.

- Fertiliser treatments influenced yield.
  - N fertilisation increased yield of both varieties by 0.4 kg/10 m row.
  - K fertilisation increased yield by 0.33 kg/10 m row for Sicala 60 BRF (F-rank 102).

- The higher the F-rank, the higher the % of plants rated 0 and 1 for vascular discolouration i.e. the higher the % of plants with less than 5% vascular discolouration.

- There was no effect of N or K fertilisation individually on disease severity at the end of the season.
  - Increased yields following N fertilisation may be a direct nutritional effect or an indirect effect from lower Fov populations at the start of the season resulting in higher plant establishment.
  - Increased yields from K fertilisation may be a direct nutritional effect or an effect of K on the various stages of pathogen establishment and development in the host, as K plays a critical role in the production and transport of fungus inhibiting phenolic compounds and flavanoids at sites of infection..

- There was an interactive effect of NK fertilisation on disease severity measured as % of plants rated 0 and 1 for vascular discolouration.
  - For Sicala 45 BRF, when K was applied without N, there were significantly more plants rated 0 and 1 for vascular discolouration than for plants not fertilised at all.
  - However for Sicala 60 BRF, significantly more plants were rated 0 or 1 when both N and k was applied compared to only K being applied.

- There were some nutritional effects on cotton fibre quality.
  - For Sicala 45 BRF, N application significantly increased fibre strength. For Sicala 60 BRF, K application significantly increased the degree of colour pigment (yellowness).

Conclusions

Results support previous results that higher F-ranked varieties have higher plant establishment, lower levels of disease and higher yields, when grown in Fusarium infested soil.

Two varieties differing in Fusarium resistance differed in N, P and K uptake. Sicala 45 BRF (F-rank 126) had a significantly higher level of N, P and K in the 5th terminal leaf than Sicala 60 BRF (F-rank 102). Does this higher uptake of nutrients contribute to greater disease resistance? The nutrition of a plant largely determines its resistance or susceptibility to disease because the metabolic expression of a plants' defence is regulated by mineral ions (Huber 1990). It would be prudent to evaluate a larger selection of varieties that differ in Fusarium resistance to determine if there is a relationship between higher nutrient uptake and disease resistance.

Varieties differing in Fusarium wilt resistance differed in their response to N and K fertilisation in regards to plant establishment, yield and disease severity. For Sicala 45 BRF (F-rank 126), N and K increased plant establishment, N increased yield, and disease severity was reduced when K was applied without N. For Sicala 60 BRF (F-rank 102) N increased plant establishment, N and K increased yield, and disease severity was reduced when both N and K were applied. Despite increases in yield, the profit margin was not sufficient to warrant the extra initial expenses.

Increased plant establishment may be due to an N effect on the Fov population; this requires further investigation. There may also have been a direct affect of N on plant growth which influenced host resistance.

Results strongly suggest that the application of N, above what is required for adequate plant growth, increased yield and consequently profit, which may be partly due to increased plant establishment and a reduction in disease severity.

K also influenced plant establishment, yield and disease severity; however the increase in yield was not sufficient to cover the costs of fertiliser application.

The influence of P on disease could not be determined from this trial as P application did not result in P uptake.

NPK Field Trial 2 2009/10

Nutrient levels pre-plant
Soil samples were collected from all of last seasons' control plots at three depths, 0-15 cm, 15-60 cm and 60-120 cm then analysed for macro- and micro- nutrients prior to trial commencement to determine nutrient availability (Table 29). Analysis of nitrogen (N), phosphorus (P) and potassium (K) indicates that availability of these nutrients exceeds the critical values required for adequate plant growth. Any improvement in yield at harvest is not expected to be due to additional nutrition as there were already adequate supplies available for optimum growth and yield.

Table 29. Nutrient and pH analysis of soil collected from field site

Measurement	0-15 cm*	15-60 cm*	60-120 cm*	Critical value mg/kg (NUTRIpak)
pH				-
Nitrate N mg/kg	26 (22)	34 (40)	75 (30)	20-30
Nitrate N kg/ha	104	134	301	-
Colwell P mg/kg	16 (29)	8 (9)	6 (7)	6
Colwell P kg/ha	63	31	26	-
K mg/kg	176 (331)	307 (199)	471 (276)	100-150
K kg/ha	703	1227	1883	-

*In parentheses, 2008/09 season's soil analysis results for comparison

To achieve optimum cotton yields an uptake of about 180 kg N /ha is needed. Most N taken up by the crop comes from the surface soil of a depth of 0 to 50 cm. At this site there was 234 kg/ha of nitrate N in the top 60cm which should be more than adequate N available for maximum yields without additional N. There was more than double the quantity of N available in the soil profile at a depth of 60 – 120cm in the 09/10 season compared to the previous season. This may be due to leaching of excess N down the soil profile.

For P, high yielding crops typically take up 25-30 kg /ha. The highest concentration of P occurs in the top 30 cm of soil. Phosphorus fertiliser should be applied when the Colwell P is less than 10 mg/kg. When it is less than 6 mg/kg higher rates of P may be warranted. With 16 and 8 mg/kg of P available in the 0-15 cm and 15-60cm respectively, there was adequate P available for maximum growth and yield at this site. P levels were similar last season to this season in the 15-60cm and 60-120cm profile. P was a little lower this season in the top 15 cm compared to last season.

Potassium is the second most abundant nutrient in cotton plants. Cotton can take up more than 200 kg/ha of K. There was more than adequate K in the soil profile for maximum plant growth and yield. There was more than double the level of K in the top 15 cm last season compared to this season. There are significantly greater levels of K in the 15-60cm and 60-120cm profile this season compared

to last season. High potassium fertilization can decrease the availability of magnesium to the plant and may result in magnesium deficiency of crops grown on soils that are already low in magnesium.

Varietal effects on plant emergence and establishment, maturity, disease severity and seed cotton yield

Plant emergence is measured as the number of seedlings emerged per 10 m plot two to three weeks after planting. Plant establishment is measured as the number of plants established 6 weeks after planting per 10 m plot. A mature plant is one which produces four open bolls or more. Maturity was measured as survival of plants through to maturity. There was a significant difference between varieties with respect to emergence and establishment (Table 30). The higher the F-rank, the greater the number of plants emerged and became established, highlighting the importance of planting varieties with higher F-rank in Fusarium infested soils. There was no significant difference between Siokra V18 BRF and Sicot 70 BRF with respect to the number of plants that reached maturity and the number of plants that had none or less than 5% vascular discolouration (rated 0 and 1) (Table 30). The lowest F-ranked variety Sicala 60 BRF had significantly fewer plants that reached maturity and a higher incidence of disease. Although a similar number of plants of varieties Siokra V18 BRF and Sicot 70 BRF reached maturity with similar levels of plants with less than 5% vascular browning, Sicot 70 BRF was significantly higher yielding than the higher F-ranked variety. Sicala 60 yielded significantly lower than both Siokra V18 BRF and Sicot 70 BRF (Table 30).

Table 30. The effect of variety/F-rank on plant emergence, establishment and maturity, disease severity and seed cotton yield

Variety	Emergence No. plants/10m	Establishment No. plants/10m	Maturity No. plants/10m	Total no. plants rated 0 and 1 for disease severity	Yield Seed cotton kg/10m
V1. Siokra V18 BRF (F-rank 125)	**135a**	**108a**	64a	30a	**2.9a**
V2. Sicot 70 BRF (F-rank 115)	**115b**	**100b**	68a	32a	**4.0b**
V3. Sicala 60 BRF (F-rank 105)	**85c**	**66c**	**49b**	**11b**	**2.4c**
LSD (P=0.05)	6.0	7.0	8.0	4.0	0.3

Data followed by different letters are significantly different from one another.

There was no effect of N application on plant emergence and establishment of the two higher F-ranked cultivars Siokra 18 BRF and Sicot 70 BRF; however N application did significantly reduce the number of plants emerged and established for Sicala 60 BRF; however this did not significantly affect yield. There was no effect of N application on yield for any of the varieties tested. These results differ to last season where N application significantly increased plant establishment and yield of both Sicala 45 BRF and Sicala 60 BRF, which was probably due to an increase in N uptake following N application and an effect on the pathogen population in the soil. However this season, N application did not significantly affect N uptake (Table 31).

Table 31. The effect of N fertilisation on emergence, establishment and yield of varieties Siokra V18 BRF, Sicot 70 BRF and Sicala 60 BRF per 10m row

Nutrient	V1. Siokra V18 BRF F-rank 125			V2. Sicot 70 BRF F-rank 115			V3. Sicala 60 BRF F-rank 105		
N kg/ha	Emergence Plants/10m	Establishment Plants/10m	Yield kg/10m	Emergence Plants/10m	Establishment Plants/10m	Yield kg/10m	Emergence Plants/10m	Establishment Plants/10m	Yield kg/10m
0	136a	108a	3.0a	115a	100a	3.84a	**90a**	**72a**	2.5a
150	133a	109a	2.8a	116a	100a	4.15a	**81b**	**60b**	2.3a
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	7.6	9.9	ns

ns = not significant.
Data followed by different letters are significantly different from one another.

There was also no effect of P fertilisation on plant emergence, establishment or yield. Application of P also did not result in an increase in P uptake (Table 32); which was the same result as last season (08/09).

Table 32. The effect of P fertilisation on emergence, establishment and yield of varieties Siokra V18 BRF, Sicot 70 BRF and Sicala 60 BRF per 10m row

Nutrient	V1. Siokra V18 BRF F-rank 125			V2. Sicot 70 BRF F-rank 115			V3. Sicala 60 BRF F-rank 105		
P kg/ha	Emergence Plants/10m	Establishment Plants/10m	Yield Kg/10m	Emergence Plants/10m	Establishment Plants/10m	Yield Kg/10m	Emergence Plants/10m	Establishment Plants/10m	Yield Kg/10m
0	139a	113a	3.0a	116a	100a	3.88a	86a	64a	2.5a
20	131a	104a	2.8a	114a	99a	4.11a	85a	68a	2.3a
LSD (P=0.05)	ns	ns (F=0.075)	ns	ns	ns	ns	ns	ns	ns

ns = not significant.
Data followed by different letters are significantly different from one another.

In the 08/09 season, K application significantly increased yield of Sicala 60 BRF. In the 09/10 season, K application had no effect on K uptake for any variety (Table 33).

Table 33. The effect of K fertilisation on emergence, establishment and yield of varieties Siokra V18 BRF, Sicot 70 BRF and Sicala 60 BRF per 10m row

Nutrient	V1. Siokra V18 BRF F-rank 125			V2. Sicot 70 BRF F-rank 115			V3. Sicala 60 BRF F-rank 105		
K kg/ha	Emergence Plants/10m	Establishment Plants/10m	Yield Kg/10m	Emergence Plants/10m	Establishment Plants/10m	Yield Kg/10m	Emergence Plants/10m	Establishment Plants/10m	Yield Kg/10m
0	134a	107a	3.0a	114a	98a	3.88a	85a	67a	2.4a
100	135a	109a	2.8a	117a	101a	4.11a	86a	65a	2.4a
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns

ns = not significant.
Data followed by different letters are significantly different from one another.

When N was applied to Sicala 60 BRF there was a significant reduction in the number of plants that reached maturity (Table 34). This is an indication of an oversupply of N, as this is known to delay maturity. However there was no effect of N application on maturity of Siokra V18 BRF or Sicot 70 BRF. An application of N also significantly reduced the % of plants rated 0 and 1 for disease, hence increasing disease severity in Sicot 70 BRF. There are reports that as the N content of many plants are increased beyond sufficient, or when N is out of balance with other nutrients, synthesis of defence related compounds decreases and this can lead to an increase in disease. It appears that different varieties can react differently to the same nutritional applications.

Table 34. The effect of N fertilisation on number plants that reached maturity and Fusarium wilt severity of varieties Siokra V18 BRF, Sicot 70 BRF and Sicala 60 BRF

Nutrient	V1. Siokra V18 BRF F-rank 125			V2. Sicot 70 BRF F-rank 115			V3. Sicala 60 BRF F-rank 105		
N kg/ha	**Maturity**	**No. plants rated 0 and 1 for disease**	**% 0 and 1's over emergence**	**Maturity**	**No. plants rated 0 and 1 for disease**	**% 0 and 1's over emergence**	**Maturity**	**No. plants rated 0 and 1 for disease**	**% 0 and 1's over emergence**
0	68	31	24 (0.52)	71.7	**36a**	**31.2 (0.59)a**	**55a**	12	13.3 (0.36)
150	61	29	23.3 (0.50)	64.8	**27b**	**27.1 (0.54)b**	**42b**	10	14.6 (0.39)
LSD (P=0.05)	ns	ns	ns	ns	7.2	0.046	9.0	ns	ns

Data followed by different letters are significantly different from one another.
Disease severity is assessed as number of plants rated 0 & 1 for disease which is measured as plants with 5% or less vascular discolouration (rating 0 and 1).
Disease severity is assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count.
A counted mature plant is one which produces four open bolls or more.

The application of P had no effect on plant maturity or disease rated as 0 &1 for any variety (Table 35).

Table 35. The effect of P fertilisation on number of plants that reached maturity and Fusarium wilt severity of varieties Siokra V18 BRF, Sicot 70 BRF and Sicala 60 BRF

Nutrient	V1. Siokra V18 BRF F-rank 125			V2. Sicot 70 BRF F-rank 115			V3. Sicala 60 BRF F-rank 105		
P kg/ha	**Maturity**	**No plants rated 0 and 1 for disease**	**% 0 and 1's over emergence**	**Maturity**	**No plants rated 0 and 1 for disease**	**% 0 and 1's over emergence**	**Maturity**	**No plants rated 0 and 1 for disease**	**% 0 and 1's over emergence**
0	66	32	25.2 (0.52)	70.2	32	28.8 (0.56)	47	11a	13.5 (0.36)
20	62	28	22.1 (0.48)	66.2	31	29.5 (0.57)	50	12a	14.5 (0.38)
LSD (P=0.05)	ns	ns	ns F=0.076	ns	ns	ns	ns	ns	ns

Data followed by different letters are significantly different from one another.
Disease severity is assessed as number of plants rated 0 & 1 for disease which is measured as plants with 5% or less vascular discolouration (rating 0 and 1).
Disease severity is assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count.
A counted mature plant is one which produces four open bolls or more.

The application of K had no effect on maturity or disease levels of Siokra V18 BRF, Sicot 70 BRF or Sicala 60 BRF (Table 36).

Table 36. The effect of K fertilisation on number of plants that reached maturity and Fusarium wilt severity of varieties Siokra V18 BRF, Sicot 70 BRF and Sicala 60 BRF

Nutrient	V1. Siokra V18 BRF F-rank 125			V2. Sicot 70 BRF F-rank 115			V3. Sicala 60 BRF F-rank 105		
K kg/ha	Maturity	No plants rated 0 and 1 for disease	% 0 and 1's over emergence	Maturity	No plants rated 0 and 1 for disease	% 0 and 1's over emergence	Maturity	No plants rated 0 and 1 for disease	% 0 and 1's over emergence
0	65	30	23.4 (0.50)	68.1	32	30.7 (0.57)	50	12	13.7 (0.36)
100	63	30	24.0 (0.51)	68.4	31	27.6 (0.55)	47	11	14.2 (0.38)
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns

Data followed by different letters are significantly different from one another.
Disease severity is assessed as number of plants rated 0 & 1 for disease which is measured as plants with 5% or less vascular discolouration (rating 0 and 1).
Disease severity is assessed as % 0 & 1's which is measured as plants with 5% or less vascular discolouration (rating 0 and 1), expressed as a percentage of the emergence count.
A counted mature plant is one which produces four open bolls or more.

Different varieties take up different levels of nutrients (Table 37). Sicala 60 BRF has a higher N content and Sicot 70 BRF has a lower K content. It is possible to compare nutrient uptake over two seasons for Sicala 60 BRF. In the 09/10 season N and K uptake were greater than in 08/09; however P uptake was greatly reduced compared to 08/09 season. The normal range for P in leaf analysis is 0.28 – 0.5%. Last season P levels were very high, this season they are just below the normal range (Table 37).

Table 37. Effect of variety on N, P and K in the 5th terminal leaf

Variety	N % Wt	K % Wt	P % Wt
Siokra V18 BRF F-rank 125	**4.049b**	**1.253a**	**0.292ab**
Sicot 70 BRF F-rank 115	**4.042b**	**1.085b**	**0.282b**
Sicala 60 BRF F-rank 105	**4.263a** ***3.79***	**1.278a** ***1.96***	**0.230a** ***0.9***
LSD (P=0.05)	0.108	0.070	0.011

Data followed by different letters are significantly different from one another.
Data in italics is data from 08/09 season

The application of N, P and K did not increase uptake of these elements, however, the application of K to Siokra V18 BRF significantly increased P uptake, and application of K to Sicala 60 BRF significantly increased N uptake (Table 38). Cotton will take up nutrients if they are present and there is a need for them. As there was more than sufficient nutrients available in the soil without fertiliser application, there was no increased uptake of N, P or K upon application of these nutrients. There is also an enormous variation in nutrient uptake between seasons (Ian Rochester pers. com.).

Table 38. The effect of N, P and K application on nutrient content of the 5th terminal leaf of varieties Siokra V18 BRF, Sicot 70 BRF and Sicala 60 BRF

Treatment Applied	Siokra V18 BRF F-rank 125			Sicot 70 BRF F-rank 115			Sicala 60 BRF 105		
	N Wt %	**P** Wt %	**K** Wt %	**N** Wt %	**P** Wt %	**K** Wt %	**N** Wt %	**P** Wt %	**K** Wt %
N0	4.031	0.288	1.215	3.993	0.281	1.064	4.228	0.289	1.258
N150	4.066	0.295	1.292	4.091	0.282	1.105	4.229	0.302	1.297
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns	ns
P0	4.052	0.292	1.277	4.074	0.282	1.112	**4.192a**	0.296a	1.235a
P20	4.045	0.292	1.229	4.01	0.281	1.057	**4.335b**	0.304a	1.320a
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	0.119	ns	ns
K0	4.031 a	**0.285a**	1.236a	4.026	0.281	1.102	4.278	0.303	1.269
K100	4.066 a	**0.298b**	1.270a	4.057	0.286	1.067	4.249	0.297	1.286
LSD (P=0.05)	ns	0.012	ns	ns	ns	ns	ns	ns	ns

ns = not significant.
Data followed by different letters are significantly different from one another.

Sodic soils are those which have an exchangeable sodium percentage (ESP) of 15% or greater. Excess exchangeable sodium has an adverse effect on the physical and nutritional properties of the soil, with consequent reduction in crop growth. The soil collected from this trial had an ESP less than 15% across the soil profile; hence there should not be any problems with P uptake associated with Na levels (Table 39). Sodium ranged from 228 to 1823 mg/kg depending on soil depth of sample (Table 11) indicating the Na levels are not too high as yet, but they have increased in the 5-120 cm profile compared to last season.

Table 39. Sodium (Na) and Exchangeable Sodium Percentage (ESP) of soil

Measurement	0-15 cm	15-60 cm	60-120 cm	Comment
Na mg/kg	228 (575)	1162 (847)	1823 (820)	<1000 good >2000 high >3000 problem
ESP %	5.0 (5.7)	6.9 (7.8)	7.5 (6.8)	≥ 15 % soil

*In parentheses, 2008/09 season's soil analysis results for comparison

Fibre length is defined as the upper-half mean length (UHML), which is the mean length of the longer half (50%) of the fibre by weight. Longer fibres allow finer and stronger yarn to be spun. The ideal range for UHML is in excess of 1.125 inches; for premium fibre 1.250. For Siokra V18 BRF, the application of P significantly increased the UHML (Table 40). Length uniformity is important as variations in length can lead to an increase in waste and deterioration in yarn quality. The ideal range for uniformity is greater than 80%. The application of P significantly increased fibre uniformity (Table 40).

Table 40. The effect of N, P, and K fertilisation on aspects of cotton fibre quality for variety Siokra V18 BRF (F-rank 125)

Nutrient	Mic	Rd	SFI	Strength	UHML	Uniformity	+b	% gin
N0	4.6	71.7	7.8	31.4	1.2	84.19	6.2	(0.74)
N150	4.7	72.2	7.8	31.5	1.2	84.25	6.3	(0.74)
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns
P0	4.6	71.5	7.9	31.1	**1.220b**	**83.94b**	6.2	(0.74)
P20	4.7	72.4	7.7	31.8	**1.232a**	**84.50a**	6.3	(0.74)
LSD (P=0.05)	ns	ns	ns	ns	0.012	0.4	ns	ns
K0	4.7	72.2	7.6	31.3	1.2	84.34	6.2	(0.74)
K100	4.6	71.7	7.9	31.6	1.2	84.10	6.3	(0.74)
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns

The application of N significantly decreased % gin turnout of Sicot 70 BRF, however application of P and K significantly increased % gin turnout (Table 41).

Sicot Table 41. The effect of N, P, and K fertilisation on aspects of cotton fibre quality for variety 70 BRF (F-rank 115)

Nutrient	Mic	Rd	SFI	Strength	UHML	Uniformity	+b	% Gin
N0	4.5	74.3	7.55	32.5	1.21	84.0	6.5	**(0.732)a**
N150	4.4	73.0	7.54	31.5	1.22	84.1	6.5	**(0.726)b**
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	(0.004)
P0	4.5	73.5	7.60	31.7	1.21	84.0	6.5	**(0.727)a**
P20	4.4	73.9	7.49	32.3	1.22	84.0	6.4	**(0.731)b**
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	(0.004)
K0	4.4	73.8	7.46	31.9	1.21	84.0	6.4	**(0.725)a**
K100	4.5	73.6	7.63	32.1	1.22	84.0	6.5	**(0.737)b**
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	(0.004)

The application of N significantly reduced the short fibre index (SFI) (Table 42). This is important as the presence of short fibre in cotton causes increases in processing waste and uneven and weaker yarns. There was no effect of N, P or K on other aspects of fibre quality for Sicala 60 BRF (Table 42).

Table 42. The effect of N, P, and K fertilisation on aspects of cotton fibre quality for variety Sicala 60 BRF (F-rank 105)

Nutrient	Mic	Rd	SFI	Strength	UHML	Uniformity	+b	% gin
N0	4.4	72.2	**7.71a**	33.2	1.22	85.0	6.6	(0.725)
N150	4.5	72.4	**7.10b**	32.7	1.22	84.9	6.7	(0.721)
LSD (P=0.05)	ns	ns	0.54	ns	ns	ns	ns	ns
P0	4.5	72.4	7.45	32.9	1.22	85.0	6.7	**(0.726)a**
P20	4.6	72.2	7.35	33.0	1.22	84.8	6.6	**(0.720)b**
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	0.004
K0	4.4	72.2	7.47	33.0	1.22	84.9	6.7	(0.723)
K100	4.5	72.5	7.34	33.0	1.22	84.9	6.7	(0.724)
LSD(P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns

Summary of results

- As with the 08/09 season, the nutrient levels of N, P and K indicated that availability of these nutrients exceeded the critical values required for adequate plant growth. Any improvement in yield at harvest was not expected to be due to additional nutrition as there were already adequate supplies available for optimum growth and yield.

- Fertiliser application did not influence nutrient uptake of the nutrient applied.
  - N, P or K application did not increase uptake of these nutrients.
  - K fertilisation increased P uptake of Siokra V18 BRF.
  - P fertilisation increased N uptake of Sicala 60 BRF.

- Fertiliser application influenced plant emergence and establishment of Sicala 60 BRF.
  - N fertilisation reduced plant emergence and establishment.

- Varieties differed in nutrient uptake in the 5th terminal leaf.
  - Sicala 60 BRF had the highest uptake of N, whilst Sicot 70 BRF had the lowest uptake of K.
  -
- The highest F-ranked cultivar did not yield highest.
  - Although Siokra V18 BRF had the highest F-rank (125) and had a significantly higher plant emergence and establishment than either Sicot 70 BRF (F-rank 115) or Sicala 60 BRF (F-rank 105), yield was significantly lower than Sicot 70 BRF, but higher than the lowest F-ranked cultivar tested.

- Fertiliser treatments did not affect yield.

- The lowest F-ranked cultivar, Sicala 60 BRF (F-rank 105) had the lowest number of plants rated 0 and 1 for vascular discolouration i.e. the highest incidence of disease. There was no difference in disease levels between Siokra V18 BRF and Sicot 70 BRF.

- There was no effect of K fertilisation individually on disease severity at the end of the season; however N significantly increased the number of Sicot 70 BRF plants with disease. An increase in the % of plants with disease did not reduce the yield of this cultivar.

- There were some nutritional effects on cotton fibre quality.
  - P increased fibre length and uniformity of Siokra V18 BRF.
  - P and K increased % gin turnout for Sicot 70 BRF, however N decreased it.
  - For Sicala 60 BRF, N reduced the short fibre index and P reduced the % gin turnout.

Conclusions

Results from trials in the 08/09 season strongly suggested that the application of N, above what was required for adequate plant growth, increases yield and consequently profit, which may be due to increased plant establishment and a reduction in disease severity. K also influenced plant establishment, yield and disease severity; however the increase in yield was not sufficient to cover the costs of fertiliser application. Results for the 09/10 were very different from 08/09. The application of N, P and K fertilisers had no effect on the uptake of these nutrients and no effect on yield. Excess nutrients are costly, can reduce yields and reduce fibre quality. Overall, results from glasshouse and field trial 1 strongly suggest that a balance of nutrients is the key to disease management and yield.

The trial site proposed for the next nutrient trials is going to be depleted of nutrients for one season through the growth of cotton across the site without any additional nutrients being applied, so that N,

P and K levels will be reduced sufficiently for application of fertiliser to be required in following trials.

Other trials

A field trial was conducted at 'Atleigh', Cecil Plains to investigate the potential of biological products Natural Nitrogen & C-Cat to reduce the severity of Fusarium wilt and to increase yield.

Application of 150 L/ ha of brew ((Natural N & C-Cat & Easy N (3 units/ ha)) soil injected, significantly increased the number of cotton plants established compared to the untreated plots. The application of C-Cat at squaring in Treatment 3 did not significantly affect plant establishment (Table 43 and Figure 33).

At harvest, there were small increases in yield following the application of Natural Nitrogen at 100 and 150 L/ha plus C-Cat compared to the untreated control. The increased number of plants established following the higher application of Natural Nitrogen did not translate into higher yields compared to plants that received 100 L/ha. Greater yield increases were observed however when 150 L/ha of Natural Nitrogen was applied without follow-up applications of C-Cat. None of these differences however were statistically significant (Table 43 and Figure 34). Despite the lack of significance it was interesting that the highest yield was observed in plants that did not receive C-Cat as a foliar spray.

The standard error for each treatment mean (bales/ha) was quite large; therefore differences between treatments would have to be large to be significantly different from one another (Figure 35). High variability between replicate blocks within a treatment is a common problem in field trials.

Table 43. The effect of Natural N & C-Cat on establishment of cotton plants and yield

Treatment[A]	Plant Establishment	Bales/acre	Bales/ha	Lint yield Kg	Lint yield Kg/ha	Seed cotton Kg
1	82.05 a	3.232 a	7.99 a	464 a	1812 a	1160 a
2	85.65 ab	3.320 a	8.21 a	477 a	1863 a	1192 a
3	**90.25 b**	3.330 a	8.22 a	478 a	1868 a	1195 a
4	**89.55 b**	3.552 a	8.78 a	510 a	1992 a	1275 a

Means with same subscript are not significantly different at the P = 0.05 level

[A]Treatment 1 = Control, no treatments applied; Treatment 2 = 100 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 Units /ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 3 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units/ ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 4 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units ha)) - soil injection. No foliar application.

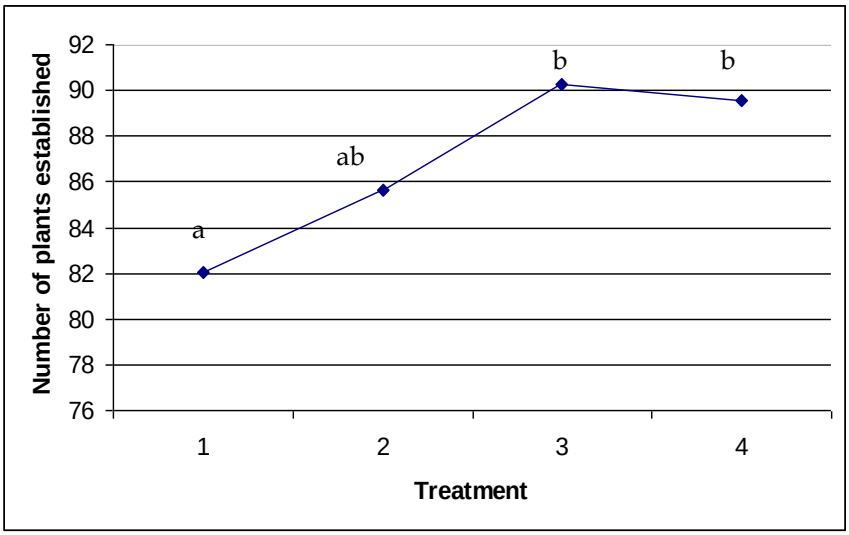


Figure 33. The effect of Natural N & C-Cat on establishment of Sicot 70 BRF

Means with same subscript are not significantly different at the P = 0.05 level

Treatment 1 = Control, no treatments applied; Treatment 2 = 100 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 Units /ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 3 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units/ ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 4 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units ha)) - soil injection. No foliar application.

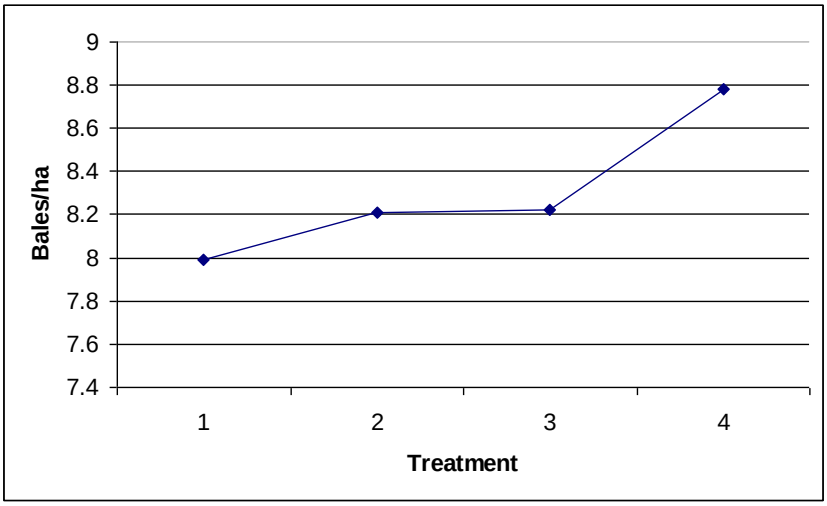


Figure 34. The effect of Natural N & C-Cat on yield of Sicot 70 BRF

Treatment 1 = Control, no treatments applied; Treatment 2 = 100 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 Units /ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 3 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units/ ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 4 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units ha)) - soil injection. No foliar application.

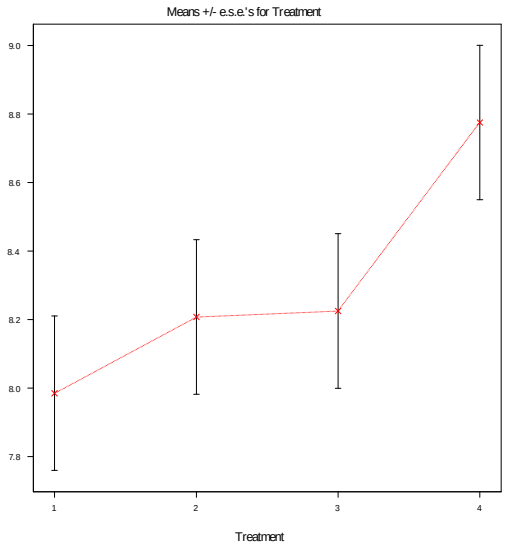


Figure 35. Standard error of the means for yield data bales/ha

A mature plant is one which produces four open bolls or more. Plant maturity is measured as the survival of plants through to maturity expressed as a percentage of the emergence count per metre row or expressed as a percentage of the number of seeds (13) planted per metre of row. There was no treatment effect on percentage of plants reaching maturity (Table 44).

Disease severity is determined by cutting stems after harvest and counting the stems that have either no vascular discolouration (rated 0), or less than 5% of the cut surface showing discolouration (rated 1). This data is expressed as % 0's and 1's, which is the number of plants with 5% or less vascular discolouration (rating 0 and 1) expressed as a percentage of the emergence count per metre of row, or expressed as a percentage over 13 seeds planted per metre row. In this trial there was no effect of treatments on the percentage of plants rated 0 and 1, that is, there was no effect on disease severity (Table 44).

Anecdotal evidence from Ian Smith, NSW who had Fusarium wilt on his property has used these products for many years, suggests that in the first year of treatment Fusarium wilt was still noticeable; however there was an obvious difference between rows. In the second year no infected plants were observed in the treated field. Because this is a biological product (contains bacteria) it may be necessary to apply the product for a second season to see benefit in our trials.

Table 44. The effect of Natural N & C-Cat on % plant maturity and % of plants rated zero and one for disease severity

Treatment[A]	[B] % maturity over 13 plants/m	Transformed data % maturity over 13 plants/m	[B] % maturity over emergence plants/m	Transformed data % maturity over emergence plants/m	[C] % 0's and 1's over 13 plants/m	Transformed data % 0's and 1's over 13 plants/m	[C] % 0's and 1's over emergence plants/m	% 0's and 1's over emergence plants/m
1	59.1	0.8767	73.3	1.028	43.4	0.7186	54.0	0.826
2	59.4	0.8797	73.9	1.035	41.6	0.7006	51.9	0.804
3	62.9	0.9162	74.1	1.038	44.6	0.7311	52.8	0.814
4	63.3	0.9206	74.1	1.039	43.6	0.7210	51.2	0.797
LSD (P=0.05)	-	NS	-	NS	-	NS	-	NS

[A] Treatment 1 = Control, no treatments applied; Treatment 2 = 100 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 Units /ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 3 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units/ ha)) - soil injection plus foliar C-Cat 1 Litre in 50 L Water/ ha at squaring; Treatment 4 = 150 Litres/ ha Brew (Natural N & C-Cat & Easy N (3 units ha)) - soil injection. No foliar application.

[B] % maturity = Survival of plants through to maturity, expressed as a percentage of the emergence count per metre of row or expressed as a percentage over 13 seeds planted per metre row.
A counted mature plant is one which produces 4 open bolls or more.

[C] % 0's and 1's = Plant with 5% or less vascular discolouration (rating 0 and 1) when stems are cut at the end of the season, expressed as a percentage of the emergence count per metre of row or expressed as a percentage over 13 seeds planted per metre row.

Objective 4. Develop and extend new information packages for disease management. Provide new information to industry through the extension network, cotton consultants and other industry forums when data becomes available.

Information generated from this project has been disseminated throughout the industry. Distribution has been through grower meetings and field-days and regional 'Cotton Tales' newsletters. Project staff are members of the Fusarium Management Committee (FUSCOM) and assisted with the development of the Integrated Disease Management Guidelines.

Information and updated disease management options generated by this project have been presented to industry via field days and farm walks at the 'Cowan' trial site at Norwin on the Darling Downs. Feedback from the Cotton Consultants Association members and cotton growers indicate a high value placed on these trials and their contribution to the management of Fusarium wilt of cotton in the Australian cotton industry.

Extension activities have ensured the widespread dissemination about the disease throughout the industry. The "COME CLEAN GO CLEAN" campaign has been waged in every cotton growing area in Australia to assist in slowing down the spread of pathogens and weeds; particularly following the detection of mealybugs at Emerald and the Burdekin.

Project staff have presented posters at international conferences, and papers at national conferences. Staff have presented at DEEDI/Australasian Plant Pathology seminar days to staff and students. Much of the information is published in conference proceedings and is freely available to the Cotton Industry.

Objective 5. Training of staff in Amplified Fragment Length Polymorphism PCR, to be used as a tool to characterise *Fusarium oxysporum* f. sp. *vasinfectum* (Fov).

AFLP methodologies to analyse the genetic diversity of Fov isolates was provided by Dr Bo Wang (CSIRO) and Cecilia O'Dwyer (DEEDI). To test the performance of these methodologies at the Indooroopilly Laboratories, 10 *Fov* isolates representing VCGs 01111 and 01112 plus the new

Mungindi strain were chosen for analysis. For the initial trial the selective primer combination used was EcoRI-AGT/MseI-A. Similarities between isolates based on Dice coefficients were calculated using NTSYS-pc. Trees were generated using the sequential agglomerative hierarchical nested (SAHN) clustering program with the unweighted pair-group method (UPMA) (Figure 36).

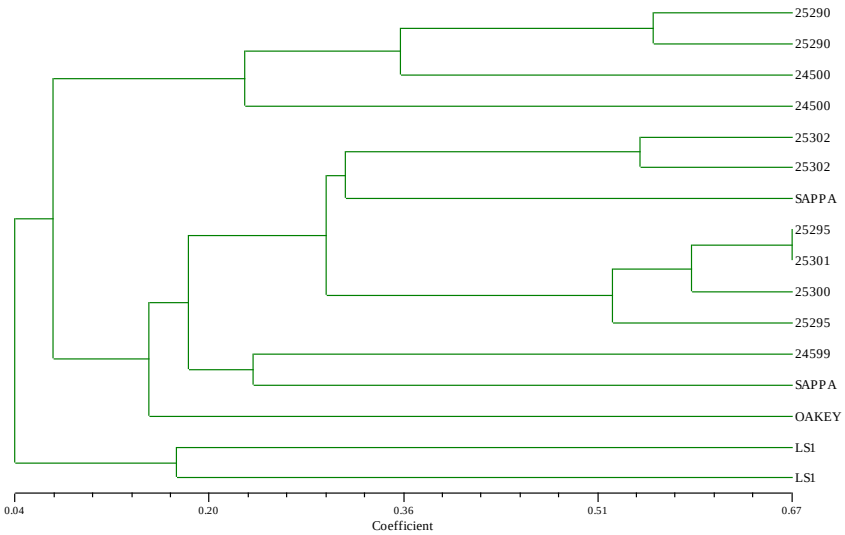


Figure 36. Dendrogram based on the Dice coefficient constructed by UPGMA cluster analysis of polymorphic bands from 10 haplotype representatives of *Fusarium oxysporum* f. sp. *vasinfectum* generated with one amplified fragment length polymorphism (AFLP) primer combination.

The results were encouraging. Isolates generally paired together as expected, with the exception of isolate Sappa, which had not been single spored. To increase confidence of detecting isolates within a VCG, additional primer combinations will be introduced into the analysis. A further 20 isolates are currently being single-spored and will be analysed using two additional primer combinations. This work will continue into the new CRDC project, but will not need funds from this project as training and consumables have already been purchased with funds from DAQ003.

Outcomes

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

The expected outputs have largely been achieved and have provided the cotton industry with some significant outcomes for the management of Fusarium wilt. Planned outputs/outcomes achieved include:

Outcome 1

Industry: *Quantify the relative performance of disease threats and ensure an adequate response by the cotton industry, communities and government agencies.*

Science: *An understanding of the factors that contribute to pathogen occurrence and development of epidemics.*

Output: *Knowledge of the occurrence of diseases in the field, including Fusarium wilt, Verticillium wilt, black root rot, seedling disease, boll rots, Alternaria leaf spot, cotton bunchy top, bacterial blight, nematodes and others.*

Knowledge of the occurrence of diseases in the field enables quantification of the relative performance of disease threats followed by an adequate response. The incidence of Fusarium wilt has significantly reduced over the last two seasons (08/09 and 09/10) when compared over eight seasons. Three factors that have contributed to this trend are resistant varieties, delayed planting and BION seed treatment. Despite this, Fusarium wilt still remains one of the most important diseases of cotton in Queensland. An integrated management strategy has continually been promoted to manage this disease.

The importance of irrigation water management was highlighted in this project. Use of irrigation water from the tail-drain of infested fields will increase the incidence of Fusarium wilt.

The incidence of boll rots has increased in Queensland, particularly in Emerald and St George. This is likely due to the high summer rainfalls.

There were a high percentage of farms surveyed that had some form of cotton volunteers. The observation of Solenopsis mealybug on volunteers growing along water channels and on volunteer cotton adjacent to cotton seedlings highlights the importance of managing volunteer cotton as they harbour pests and diseases; carrying them from season to season providing an inoculum source for re-infection of crops.

A new pathogen was identified during this project, *Nematospora coryli*, highlighting the importance of annual disease surveys. This fungus causes several serious diseases of cotton including seed rot, internal boll rot (stigmatomycosis) and tight lock. In the USA losses of 40-60% of fibre has been reported in cotton. The fungus is the only plant pathogenic yeast and is spread to bolls punctured by insects during feeding. Insect control is the best way to prevent infection, although improved cultivar resistance may be possible. Biosecurity Queensland is aware of this new disease and industry is currently waiting on their recommendation of action.

During the course of this project staff were also involved in the survey of two important plant pests. Solenopsis mealybug (*Phenacoccus solenopsis*), which has been identified affecting cotton in both the Emerald and Burdekin regions, and Tobacco Streak Virus (TSV), which has been affecting cotton in Emerald.

Texas root rot, cotton leaf curl virus, blue disease, defoliating Verticillium, hypervirulent bacterial blight and exotic Fusarium wilt are exotic pathogens listed by the Australian cotton industry as serious threats to the industry. Absence data was collected for these six exotics during annual disease surveys.

Outcome 2

Industry: *An understanding of pathogen diversity is very important when screening germplasm for resistance.*

Science: *Input to understanding those factors affecting the ecology of Fov and identification of new races as soon as they occur.*

Output: *Determine the occurrence of Fov in cotton growing areas and status of pathogen diversity, maintaining the distribution database.*

An understanding of pathogen diversity is very important when screening germplasm for resistance. A new strain of Fov was identified from the Macintyre valley in 2005. Molecular analysis of the isolate determined it was not exotic and had probably evolved locally from a spontaneous mutation. Sampling from the area has yielded further isolates of this type, indicating that this new isolate has established but does not appeared to have spread. A new VCG will not be designated to this new strain until a larger number of representative isolates are identified.

Training of staff in Amplified Fragment Length Polymorphism PCR has commenced. This technique will be used as a tool to characterise *Fusarium oxysporum* f. sp. *vasinfectum* (Fov).

Outcome 3

Industry: *Improved decision support information packages for growers to choose best options to manage Fusarium wilt.*

Science: *An understanding of the effect of different rotations and residue management on Fusarium wilt incidence in subsequent cotton crops.*

Output: *More research is required to obtain information on the effect of other rotations and treatment of crop residues on inoculum levels of Fov in the soil and subsequent disease development in cotton. Both field and glasshouse trials will be conducted.*

An understanding of the effect of different rotation and residue management on incidence of Fusarium wilt in subsequent cotton crops will provide growers and consultants with greater options and improved decision support packages to manage Fusarium wilt. Key findings include:

1) Continuous cotton treatment had the lowest yield of all treatments.

2) The highest yielding treatment was a maize (retained)-fallow-cotton rotation. This treatment also resulted in the least disease in the final cotton crop and high plant survival.

3). There was no significant difference between retaining maize or sorghum residues on the surface and incorporating residues in any measured variable. However, the current practice is to retain residues on the surface in support of minimum tillage.

4) Continuous cotton maintains a higher population of Fo/Fov in the soil. The lowest population of Fo/Fov was determined with a maize (retained or incorporated)-fallow-cotton rotation.

5) All crop species investigated in the glasshouse were susceptible to artificial inoculation of Fov; however the extent of colonisation varied between crops. Maize, sorghum and sunflower were the least colonized crops; supporting field studies in which maize/sorghum- fallow-cotton rotation yielded highest had significantly lower levels of disease than continuous cotton. In glasshouse trials with naturally infected soil, maize was not infected with Fov.

6) Previous studies have shown that when other crops are incorporated into the soil in the presence of Fusarium, the disease severity increases. These studies highlight that incorporation of green manure crops does increase the soil population of Fo/Fov by providing a residue substrate for the pathogen to survive saprophytically and potentially multiply.

6) Since the pathogen remains indefinitely in the soil, using rotations as a management tool may be limited; however a maize/sorghum-fallow-cotton rotation looks promising. Growers need to manage

residues (and weeds) through fallow periods and use green manure/cover crops with caution where Fusarium is present.

Outcome 4

Industry: *Improved knowledge and decision making tools with regard to fertiliser regime and management of Fusarium wilt.*

Science: *An understanding of the effect of nutrient fertilisation (NPK) on the incidence of disease.*

Output: *Both glasshouse and small-plot field trials will be conducted to gain information on the effect of fertilisation of key nutrients on disease severity of Fusarium wilt.*

Fertiliser recommendations are developed to optimise nutrient uptake and provide the crop with adequate nutrients for normal growth and yield. An understanding of how N, P and K influences Fusarium wilt severity will provide growers with improved decision making tools for the management of this disease.

In glasshouse trials, it was very clear that disease severity was influenced by the balance of nutrients; however cultivars differing in disease resistance were affected differently in some instances. Key findings include:

1) For Sicot 189 (F-rank 100), higher disease ratings were observed in plants fertilised with lower levels of nutrients, particularly low N, and lower disease ratings tended to have higher N. The lowest level of disease was observed when 5 kg/ha of P, 250 kg/ha of N and 100 kg/ha of K was applied.

2) For Sicot F-1 (F-rank 144), disease-free plants mostly had been treated with the highest level of K at 100 kg/ha in combination with N at either 120 or 250 kg/ha. P did not appear to effect disease severity.

3) P only increased disease severity in cultivar Siokra 1-4, which has a low level of Fusarium resistance.

In the field, fertiliser application only influenced nutrient uptake in trial 1. Key findings include:

1) Results support previous results that higher F-ranked varieties have higher plant establishment, lower levels of disease and higher yields, when grown in Fusarium infested soil.

2) Varieties differing in Fusarium resistance differed in N, P and K uptake. For example, Sicala 45 BRF (F-rank 126) had a significantly higher level of N, P and K in the 5th terminal leaf than Sicala 60 BRF (F-rank 102). Does this contribute to greater disease resistance?

3) Varieties differing in Fusarium wilt resistance differed in their response to N and K fertilisation in regards to plant establishment, yield and disease severity. For Sicala 45 BRF, N and K increased plant establishment, N increased yield, and disease severity was reduced when K was applied without N. For Sicala 60 BRF, N increased plant establishment, N and K increased yield, and disease severity was reduced when both N and K were applied.

4) Increased plant establishment may be due to an N effect on the Fov population; this requires further investigation. There may also have been a direct affect of N on plant growth which influenced host resistance.

5) Results strongly suggest that the application of N, above what is required for adequate plant growth, increased yield, which may be partly due to increased plant establishment and a reduction in disease severity.

6) K influenced plant establishment, yield and disease severity.

7) There was an interactive effect of NK fertilisation on disease severity. For Sicala 45 BRF, when K was applied without N, there were significantly more plants with disease than for plants not fertilised at all, hence balance of N and K may be important. However for Sicala 60 BRF, significantly more plants were disease free when both N and k was applied compared to only K being applied.

8) The influence of P on disease could not be determined as P application did not result in P uptake.

In trial 2, there was no significant uptake of N, P or K upon application of these nutrients; as more than adequate amounts of these nutrients were available in the soil profile hence the effect of these nutrients could not be determined. Excess nutrients, particularly N, are prone to leaching and run-off and can become a water-quality concern for agriculture. For cotton growers this is a fibre quality, financial and environmental issue.

These early trials in the investigation of N, P and K on disease management highlight the importance of nutrient balance in the soil. This is achieved through knowledge of what is present in the soil and what is being removed, and developing an appropriate nutrient replacement program.

An additional trial was conducted to evaluate the potential of Natural Nitrogen & C-cat to reduce severity of Fusarium wilt. There was no evidence in this study that the biological products Natural Nitrogen & C-Cat reduced the severity of Fusarium wilt or effected yield.

6. Please describe any:-
   - technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);

   There were no patents or licences applied for.
   - other information developed from research (eg discoveries in methodology, equipment design, etc.); and

   Agricultural practices that may reduce the incidence of Fusarium wilt have been identified.

   Following training in the United States of America to learn how to diagnose *Verticillium dahliae* using molecular techniques, there is now the opportunity to conduct gel based PCR using specific primers to identify VCGs 01111 and 01112. A gel based PCR assay was developed for VCGs 01111 and 0112 in projects funded by the CRCTPP, ACCRC and CRDC (DAQ107C). There is the opportunity to use this diagnostic tool in project DAQ1103 Fusarium wilt management to identify VCGs 01111 and 01112, which would be significantly faster than the current methods (VCG analysis).

   - required changes to the Intellectual Property register.

   No

Conclusion

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

Strategies to manage Fusarium wilt are being developed as a result of this research. Specific highlights include:

- Monitoring of disease levels and distribution enables appropriate action to be taken to reduce impact.
- The assessment of pathogen diversity ensures that the whole spectrum of diversity within the pathogen's population is known so that cultivars are evaluated for resistance to these.
- Continuous cotton builds up the Fov population in the soil resulting in higher disease and lower yields. However, some agricultural practices have been identified that may reduce the incidence of the disease.
  - Maize/sorghum (retained residues) – fallow – cotton rotation was highest yielding and had significantly lower levels of disease compared to continuous cotton.
  - Maize was not infected with Fov when grown in naturally infested field soil, and maize, sorghum and sunflower were least colonised by Fov in artificial inoculation trials. These crops will not rapidly increase the Fov population in the soil.
- In DAQ130C, the investigation of residue management suggested that when maize residues were retained on the soil surface there was greater plant survival to maturity and a lower disease incidence compared to residues that were burnt or incorporated. In this study, there was no difference between retaining maize or sorghum residues on the surface or incorporating residues on emergence, plant survival disease severity or yield of cotton.
- Some agricultural practices were identified that may increase disease severity.
  - Incorporation of green manure crops increased the soil population of Fo/Fov by providing a residue substrate for the pathogen to survive saprophytically and potentially multiply. Hence growers need to use green manure crops with caution.
- Growers need to manage residues and weeds through fallow periods to assist in keeping the population of Fov as low as possible.
- These early trials highlight the importance of nutrient balance for disease management; however cultivars differing in disease resistance were affected differently in some instances.
  - Increasing P without application of N or K significantly increased disease severity in a cultivar with low disease resistance (Siokra 1-4), however in cultivars with higher levels of resistance (Sicot 189 and Sicot F-1), increasing P did not significantly increase disease severity.
- In glasshouse trials in general, higher disease ratings were observed in plants with lower levels of nutrients applied, particularly low N (0 and 40 kg N/ha), and lower disease ratings tended to have higher N (120 and 250 kg N/ha) and K (100 kg K/ha) applied.

- In the field, N and K application increased plant establishment and yield, with an interactive effect of these nutrients on disease severity. Importantly, the interactive effect differed between cultivars.

- Better agricultural practices, together with more tolerant cultivars, will provide the best strategy to manage this disease.

Extension Opportunities

8. Detail a plan for the activities or other steps that may be taken:
   (a) to further develop or to exploit the project technology.
   (b) for the future presentation and dissemination of the project outcomes.
   (c) for future research.

A new project DAQ1103 will exploit and extend some of the technologies developed in this project. The aims of this new project are: to monitor cotton diseases in Queensland as well as monitoring the diversity and distribution of strains of *Fov* in cotton-growing areas in Australia; investigate rotation options and the effect of crop residue, organic matter and green manuring of crops in relation to pathogen survival and subsequent disease development; the influence of crop nutrition on disease development, the effect of fungicide seed treatments on Fusarium wilt severity; the effect of BION seed treatment and silicon on Fusarium wilt severity and develop and extend new information packages for disease management. This project aims to reduce the impact of Fusarium wilt on cotton production ensuring a more sustainable, profitable and competitive industry in Australia.

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

A presentation delivering research results was given to the Regional Cotton Extension Team Workshop in Toowoomba in May 2008.

Field trial results have been included in a Darling Downs Field Trial Booklet.

A poster presented at the Australian Cotton Conference highlighting the importance of contacting a plant pathologist to investigate unusual or unfamiliar symptoms of disease within a crop was displayed at UNE in 2008.

Field walk 2009 – trial results were presented and discussed with farmers on-site at "Cowan" near Cecil Plains.

Two facts sheets on the exotic pathogens *Verticillium dahliae* – defoliating strain and *Fusarium oxysporum* f.sp. *vasinfectum*- exotic strains, were developed with PHA for inclusion in the National Cotton Biosecurity Plan.

Conference papers

Smith, L.J and Carrick, D.F (2007). The effect of silicon and phosphorus amendment on severity of Fusarium wilt of cotton. 16th Biennial Australasian Plant Pathology Society Conference, Adelaide, South Australia.

Smith, L.J., Swan, L.J. and Lehane, J. (2008) Potential of silicon amendment to reduce severity of Fusarium wilt. Proceedings of the 14th Australian Cotton Conference, Gold Coast Convention and Exhibition Centre, Broadbeach, Queensland, 12 - 14 August 2008.

Smith, L.J., Gambley, C. and Scheikowski, L.J (2008) Can you identify these exotic diseases? 14th Australian Cotton Conference, Gold Coast Convention and Exhibition Centre, Broadbeach, Queensland, 12 - 14 August 2008.

Smith, L.J and Lehane, J.K. (2009). NPK fertilisation influences severity of Fusarium wilt of cotton. 5th Australasian Soilborne Diseases Symposium. Thredbo Alpine Hotel, Thredbo, NSW 5-7 February.

Smith, L.J. and Lehane, J.K. (2009). Fertilisation with N, P and K above critical values required for adequate plant growth influences plant establishment of cotton varieties in Fusarium infested soil. 17th Biennial Australasian Plant Pathology Society Conference, Newcastle, NSW.

Scheikowski, L.J, Smith, L.J. and Lehane, J. (2010) Artificial inoculation of crop species in Australia with *Fusarium oxysporum* f.sp. *vasinfectum*. Beltwide Cotton Conference, New Orleans, Louisiana, 4-7 January 2010.

Smith, L.J., Lehane, J. and Scheikowski, L.J. (2010) NPK fertilisation influences severity of Fusarium wilt of cotton. Beltwide Cotton Conference, New Orleans, Louisiana, 4-7 January 2010.

Smith, L.J., Lehane, J. and Scheikowski, L.J. (2010) The potential of silicon soil amendment to reduce severity of Fusarium wilt of cotton. Beltwide Cotton Conference, New Orleans, Louisiana, 4-7 January 2010.

Allen, S., Anderson, C., Lonergan, P. Scheikowski, L. and Smith, L. (2010) 20 to 1 - Issues in cotton pathology. Proceedings of 15th Australian Cotton Conference, Gold Coast Convention and Exhibition Centre, Broadbeach, Queensland, 10 - 12 August 2010.

Smith, L., Scheikowski, L. and Lehane, (2010) Fusarium wilt IDM. 15th Australian Cotton Conference, Gold Coast Convention and Exhibition Centre, Broadbeach, Queensland, 10 - 12 August 2010.

Disease Survey Reports

Allen, S.J., Anderson, C.M.T., Lonergan, P.A., McNamara, G., Swan, L.J. and Smith, L.J. (2008) Cotton Pathology 2007-2008. 2008 Variety Trial Results, CSD: 74-77.

Allen, S., McNamara, G., Anderson, C., Lonergan, P., Swan, L. and Smith. L. (2008) Cotton Pathology Survey 2007/08. 2008-2009 Cotton Pest Management Guide, NSW Department of Primary Industries: 123-126.

Allen, S.J., Anderson, C.M.T., Lonergan, P.A., Scheikowski, L.J. and Smith, L.J. (2009) Cotton Pathology Survey 2008/09. Cotton Pest Management Guide 2009-10, NSW Department of Industry and Investment: 124-127.

Allen, S.J., Anderson, C.M.T., Lehane, J., Lonergan, P.A., Scheikowski, L.J. and Smith, L.J. (2010) Cotton Pathology Survey 2009-2010. Cotton Pest Management Guide 2010-11, Greenmount Press: 122-124.

Annual disease survey results are also published on the CSD website and in annual CSD Variety Trial Results.

Early Disease Survey summary 2009 for Emerald/Theodore was published in the Central Queensland Cotton Tales.

Disease survey summary 2009/10 was published in the CRDC magazine Spotlight.

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

No.

Part 4 – Final Report Executive Summary

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

Fusarium wilt continues to be an important constraint to sustainable cotton production. This project, 'Cotton Fusarium Wilt Management', had a number of objectives to obtain data to improve the management of this disease. The three year project resulted in several important outcomes with direct consequence for the industry.

Outcome 1. *Collection of disease incidence data annually has enabled the quantification of the relative performance of disease threats and the effectiveness of response, and the detection of new pathogens and problems.*

Planting resistant varieties, delaying planting to avoid cold shock and sowing seed treated with BION have contributed to the reduction in the incidence of Fusarium wilt over the last two seasons compared over eight.

Irrigation water management from Fusarium infested fields is important as use of irrigation water from the tail-drain will increase the incidence of Fusarium wilt.

It is important to manage volunteer cotton as they harbour pests and diseases; carrying them from season to season providing an inoculum source for re-infection of crops.

The identification of a new cotton pathogen in Australia during disease surveys highlights the importance of these surveys. *Nematospora coryli* is a fungus that causes several serious diseases of cotton including seed rot, internal boll rot (stigmatomycosis) and tight lock. The fungus is the only plant pathogenic yeast and is spread to bolls punctured by insects during feeding. Insect control is the best way to prevent infection, although improved cultivar resistance may be possible.

Outcome 2. *The diversity of Fov in cotton growing regions is changing.*

Further isolates of the Mungindi strain detected in 2005 were again detected in 2009 from the same field. Pathogenicity tests determined that these isolates were pathogenic on a susceptible cotton host. AFLP analysis determined that the isolates did not belong to VCG 01111 or 01112, but were similar

to the original Mungindi strain. An understanding of pathogen diversity is very important when screening germplasm for resistance.

To enhance the diagnostic capabilities of DEEDI staff, training in Amplified Fragment Length Polymorphism PCR has commenced. This technique will be used as a tool to characterise *Fusarium oxysporum* f. sp. *vasinfectum* (Fov).

Outcome 3. *Since the pathogen remains indefinitely in the soil, using rotations as a management tool may be limited; however a maize/sorghum-fallow-cotton rotation looks promising. Growers need to manage residues (and weeds) through fallow periods and use green manure/cover crops with caution where Fusarium is present.*

An understanding of the effect of different rotation and residue management on incidence of Fusarium wilt in subsequent cotton crops will provide growers and consultants with greater options and improved decision support packages to manage Fusarium wilt.

Outcome 4. *Fusarium wilt severity is influenced by the balance of nutrients; however cultivars differing in disease resistance are affected differently in some instances.*

Fertiliser recommendations are developed to optimise nutrient uptake and provide the crop with adequate nutrients for normal growth and yield. An understanding of how N, P and K influences Fusarium wilt severity will provide growers with improved decision making tools for the management of this disease.

These early trials in the investigation of N, P and K on disease management highlight the importance of nutrient balance in the soil. This is achieved through knowledge of what is present in the soil and what is being removed, and developing an appropriate nutrient replacement program.

In general, higher disease ratings were observed in plants with lower levels of nutrients applied, particularly low N (0 and 40 kg N/ha), and lower disease ratings tended to have higher N (120, 150 and 250 kg N/ha) and K (100 kg K/ha) applied.