Australian Government
Cotton Research and Development Corporation

Cotton Catchment Communities CRC

FINAL REPORT

Severity factors in Fusarium wilt of cotton

CRDC Project No DAN176C

July 2003 to June 2006

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Part 1 - Summary Details

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Executive summary

“Severity Factors in Fusarium Wilt of Cotton” aimed to investigate the factors that affect the severity of Fusarium wilt, and by doing so enable the development of more effective integrated disease management (IDM) strategies. The three year project resulted in several important outcomes with direct consequences for the industry.

Outcome 1. *Cool, wet early season conditions are the single biggest factor influencing the severity of Fusarium wilt.*

Disease observed in the crop late in the season is the result of infection early in the season, say within the first 8 weeks after sowing. Therefore, when early season conditions are conducive to infection, disease severity will be increased. High rainfall and cool temperatures provide the most conducive conditions for infection of plants by the pathogen. When spring rainfall is high (say >200mm before December 31), disease severity will be high. A prolonged period of water-logging later in the season may also induce the appearance of external symptoms (eg. wilting, yellowing, plant death).

Therefore, the best way to reduce the impact of cool, wet early season conditions is to avoid them by planting later (say mid-October or later). This strategy enabled us to reduce disease severity in two out of three years. Benefits from delayed sowing are reduced when disease-conducive conditions are prolonged and cannot be avoided. We recommend delayed sowing as a “best bet” strategy for minimising the impact of Fusarium wilt.

Outcome 2. *Fusarium is carried in large numbers on floating trash during irrigations.*

We measured over 160 million colony forming units of *Fusarium oxysporum* per kg of floating trash during irrigation. Floating trash is the primary means of transport of the Fusarium wilt fungus around the farm during irrigations. Therefore it is important to 1) stop trash from leaving the field by using a trash-retaining drop box, and/or remove trash from channels using trash racks. The process of passaging irrigation water back through a storage dam or settling pond also aids in removing most of the pathogen from the water.

Outcome 3. *Remove stubble, or retain slashed stubble on the surface for a month or longer before incorporation.*

Fusarium wilt will be less severe where stubble from previous crops has been raked and burnt or slashed and retained on the surface for a month or longer prior to incorporation. These practices are highly recommended for growers with Fusarium wilt.

Other Outcomes

- Glyphosate (Roundup™ etc) and Roundup Ready™ technologies do NOT increase the severity of Fusarium wilt.
- Stress from heavy boll loads does NOT increase the severity of Fusarium wilt.
- There is NO interaction between black root rot and Fusarium wilt.
- Nematodes are NOT a problem in Australian cotton.

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Full Report

1. BACKGROUND

NSW is currently experiencing an epidemic of Fusarium wilt (Figure 1). At the time this project was proposed, no effective measures for disease control were available to the industry. The recent release of cotton cultivars with increased levels of resistance to the disease has provided growers with one effective option for disease management. However, none of these cultivars are immune to Fusarium wilt, and disease severity varies considerably between seasons. Therefore an integrated approach to disease management remains important.

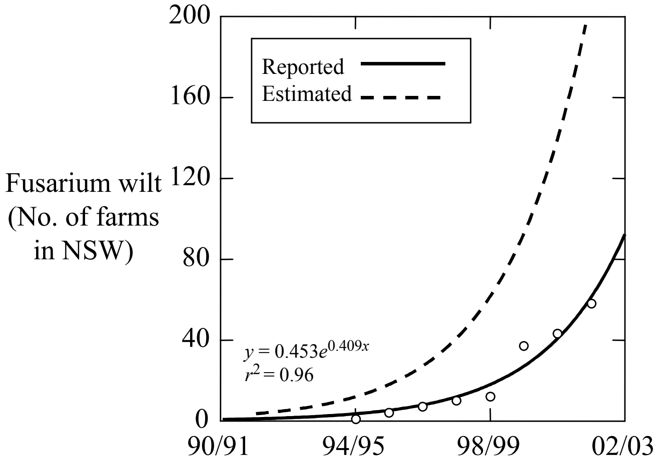

Figure 1. Increasing incidence of reported cases of Fusarium wilt in NSW. The estimated incidence assumes a three year lag between the time a farm is first infested and development of sufficient disease to be noticed. i.e. the same curve moved back three years.

This project aimed to investigate the factors that affect the severity of Fusarium wilt, and by doing so enable the development of more effective integrated disease management (IDM) strategies.

2. OBJECTIVES AND EXTENT TO WHICH THEY HAVE BEEN ACHEIVED

Objective 1. *Identify and evaluate factors contributing to the spread and increase of severity of Fusarium wilt, including: soil type; climate; irrigation; the location of inoculum in cotton residues; movement of inoculum in irrigation water; timing of infection and spread throughout the plant in the field; relative importance of host resistance in roots and stems; interaction with other pathogens.*

We identified two important factors that contribute to the spread and increase of severity of Fusarium wilt: 1) cool, wet early season environmental conditions; and 2) the movement of inoculum in mud that adheres to floating trash during irrigations. In addition, we were able to discount the importance of potential factors including heavy boll loads, the use of glyphosate, and interaction with the black root rot pathogen. We also demonstrated the potential for systemic acquired resistance to reduce the severity of Fusarium wilt in heavily infested fields.

We were not able to fully investigate the importance of plant parts in the carry-over of the pathogen between seasons, or the impact of soil type on disease severity because of problems associated with access to laboratory and glasshouse facilities, arising from farm hygiene policies at several sites (detailed in Section Results).

An investigation of the potential for an association between nematodes and vascular wilts of cotton, specifically Verticillium wilt, indicated no relationship between disease incidence and severity and nematode numbers (results detailed in final report CRC 1.01.02).

It was not possible to evaluate inoculum density of *Fov*, as originally proposed because the transport of contaminated materials to ACRI, and the culturing of the pathogen at ACRI in the laboratory were not permitted for the first two years of the project. Moreover, glasshouse space at ACRI cannot be used for experiments involving the Fusarium wilt pathogen. Several attempts were made to access alternative glasshouse space for pot trials. After much negotiation, a small glasshouse was constructed at the DIPNR depot in Narrabri West. Upon completion of this new glasshouse in 2005, we were informed that the depot had been transferred to State Water and that we could no longer use the facility for experiments involving pathogens, so we were forced to look elsewhere. Finally, in 2006 we were granted access to glasshouse space at the IA Watson Grains Research Institute in Narrabri where experiments are currently being conducted.

Objective 2. *Evaluate the use and/or modification of cultural practices for management of Fusarium wilt.*

Disease severity was decreased substantially by delaying sowing, in two out of three seasons. This practice has potential to control Fusarium wilt in most years by decreasing the period of exposure to early-season climatic conditions that favour disease development. Delayed sowing may be less effective in seasons that have extended periods of cool wet conditions through November.

Disease severity was reduced by raking and burning cotton trash and by retaining mulched trash on the surface for a month before incorporation into the soil.

Several novel products were evaluated and found to be ineffective in controlling Fusarium wilt. These included silicon based products applied as soil amendments, in-furrow sprays, seed coatings and "over the top" sprays, and a plant growth regulator containing gibberellic acid, applied as an in-furrow spray.

In a winter-crop rotation experiment, wheat, faba bean, chick pea, safflower and canola did not impact upon the severity of Fusarium wilt in the following cotton crop. In other field experiments, there was no difference in the severity of Fusarium wilt in cotton that was sown on soil moisture or irrigated after sowing.

Objective 3. *Design control strategies that will enhance the existing integrated disease management guidelines for Fusarium wilt of cotton, including measures appropriate for cooler production regions.*

Changes (highlighted in **bold**) to the IDM guidelines are recommended based on the results of this project:

PLANNING

- If your farm is free from this disease, try to keep it that way! – See 'Farm Hygiene'; 'Come clean-Go clean'
- Use the most resistant cotton varieties available, especially if Fov occurs in your district
- Ensure that seed is treated (eg. Quintozene and Apron)
- **Design or modify irrigation reticulation systems to return water from infested fields directly to the storage used for their supply or install settling ponds before on-flow to other fields.**

PLANTING

- Plant **as late as possible, within the planting window, to minimise exposure to cool wet** conditions **that favour disease**.

IN CROP

- Control weeds during and between crops
- Avoid mechanical inter-row cultivations if possible during the crop (eg. use shielded sprayer to control weeds)
- Manage the crop to avoid stresses such as waterlogging, over-fertilisation, root damage
- Maintain farm hygiene and awareness of incoming traffic through the season
- Conduct regular inspections to allow early detection of any suspicious looking plants. If any are found, send immediately to QDPI for analysis. Educate farm workers what to look for and encourage reporting
- If Fov is confirmed, rogue and burn for small patches
- Solarisation may also be an appropriate treatment for small affected patches detected early in the season.
- Isolate affected areas from irrigation flows and traffic to avoid spreading the fungus **or m**inimise tail-water **exiting** from those areas.
- **Minimise the exit of floating residues, of all crops, in tail water in affected fields eg. use trash-racks or baffles in drop boxes** (see example in Figure 2).

LATE SEASON

- Ensure that harvesting machinery is clean
- If Fov has been confirmed on your farm notify all relevant parties so that measures can be taken to avoid

spreading the fungus to other fields on your property and to other regions

AFTER HARVEST

- After harvest, retain crop residues on the surface for as long as possible before incorporation

ROTATIONS

• Selection and management of rotation crops is important as the pathogen is able to survive in association with

the residues of non host crops. **Bare fallows will always result in lower disease severity in the following crop**.

• Summer flooding, where possible, has been shown to be effective but does not eradicate the pathogen.

A design concept for a trash retaining drop box (Figure 2) was also devised.

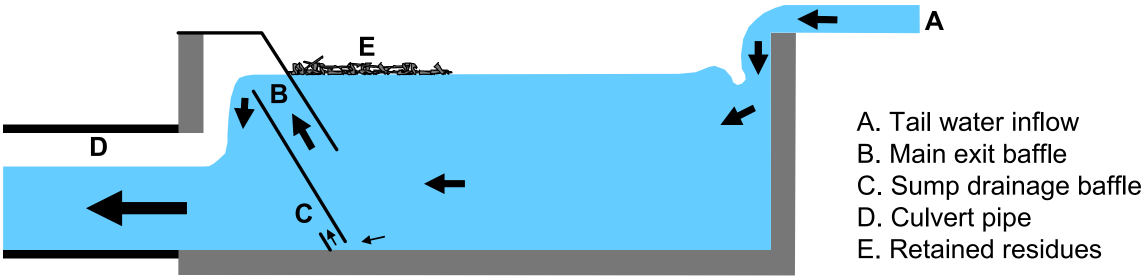

Figure 2. Design concept for crop-residue-trapping culvert box for irrigated fields. Arrows represent volume and direction of flow. The box is constructed to a length that will ensure that the main exit baffle has a cross-sectional area sufficiently larger than the area of the culvert pipe to prevent flows through the baffles being strong enough to suck floating residues through the system. The drainage baffle at the base allows the box to empty for later removal of residues and soil. The box is sloped at one end and wide enough to allow residue removal with a front end loader.

Objective 4. *Facilitate delivery and deployment of management strategies for Fusarium wilt of cotton through: close collaboration with the cotton extension network; presentation of results at meetings and industry forums; publication of extension material and media releases; implementation of demonstration trials in the field to aid in the adoption of management practices.*

Findings of the project were extended through grower meetings, articles and papers as below:

Grower meetings and presentations

2004 Farm walk at Moree organised by McGregor Gourlay
2004 talk to the Macquarie Valley Cotton Growers Association at Trangie
2004 Cotton Conference Hands on Research session for Fusarium wilt
2006 Cotton Consultants Association tour of ACRI
2006 Cotton Conference Hands on Research session for Fusarium wilt
2006 CSD Web on Wednesday interview with Mr David Kelly about trash management options for growers with Fusarium wilt
2006 Final Report Presentation to the ACGRA at Narrabri

Articles and posters

2006 Cotton Grower article "Fusarium Floats"
2004/5/6 Gwydir Valley field trials results book compiled by Julie O'Halloran

Papers and Posters

2004 Cotton Conference poster "Factors affecting the severity of Fusarium wilt: environmental factors of the disease"
2005 Australasian Plant Pathology Society meeting in Geelong poster "First record of the root lesion nematode *Helicotylenchus dihystera* in cotton"

2006 Cotton Conference posters "Delayed sowing to minimise the impact of Fusarium wilt", "Cotton trash management to minimise the impact of Fusarium wilt", and "Nematodes in cotton"

2006 Paper: OGG Knox, CMT Anderson, SJ Allen, DB Nehl. *Helicotylenchus dihystera* in Australian cotton roots. *Australasian Plant Pathology*. **35(2)** pp287-288

3. Methodology and Justification

Site selection and disease assessment

Field experiments were used to compare disease incidence with farm management practices and environmental factors. We mapped the distribution of disease at potential trial sites by cutting the stems of plants prior to picking and recording the presence or absence of vascular discolouration along transect lines at various distances from the tail drain. This enabled us to create colour maps detailing disease incidence from which we could determine the most effective areas to place experiments (Figure 3).

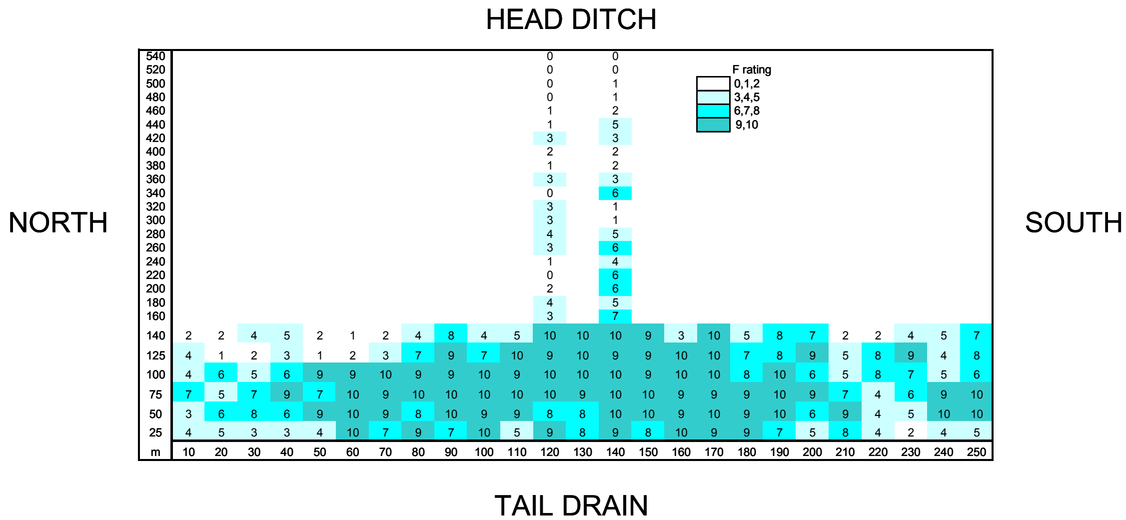

Figure 3. Map of a potential trial site showing the incidence of Fusarium wilt assessed after picking: darker colours represent higher incidence.

Farming practices were evaluated in commercial fields, where available, in fully-controlled experiments. Seedling mortality was determined by expressing plant stand, 3-6 weeks after sowing, as a percentage of the seed sown. Disease incidence was assessed as the percentage of plants with vascular discolouration in the stem at ground level. Disease severity was scored on a scale of 0-4, where: 0 = no vascular discoloration; 1 = less than 5% discoloration; 2 = between 5% and 20% discoloration; 3 = between 20% and 40% discoloration; 4 = greater than 40% discoloration). Disease incidence and severity was assessed in pre-determined plots of 10m or 15m in length in which plant stand is assessed at the beginning of the season. Data from Fusarium wilt experiments was expressed as three parameters, as follows.

- *Seedling survival* = the percentage of the original plant stand, in October, that was still alive at the end of the season.
- *Adult survival* = the percentage of plants at the end of the season that had little or no infection (0's and 1's in the 0-4 stem rating scale)
- *Total survival* = the percentage of the original plant stand, in October, that survived to the end of the season with little or no disease (0 and 1 on the 0-4 stem rating scale)

Unless stated otherwise, only *Total survival* data is presented in this report.

Disease Progress Mapping

Disease progress was mapped across the season in selected experiments by randomly selecting up to 200 plants on any given date, removing the plants from the ground and 1) measuring shoot height and root length, 2) recording the presence or absence of chlorotic, necrotic and/or wilting leaves and branches, then 3) shaving back the bark and measuring vascular discolouration up the shoot and down the root.

Hand Planting

Several experiments required multiple planting dates throughout the season. The easiest way to achieve this was to plant small plots by hand using a custom made 1m long dibber (Figure 4). This enable seeds to be sown at a uniform depth of 40mm at a rate of 14 seeds/m. After sowing, plots were irrigated with a watering can at a rate of 2L per metre, and covered back over with dry soil to prevent evaporation.

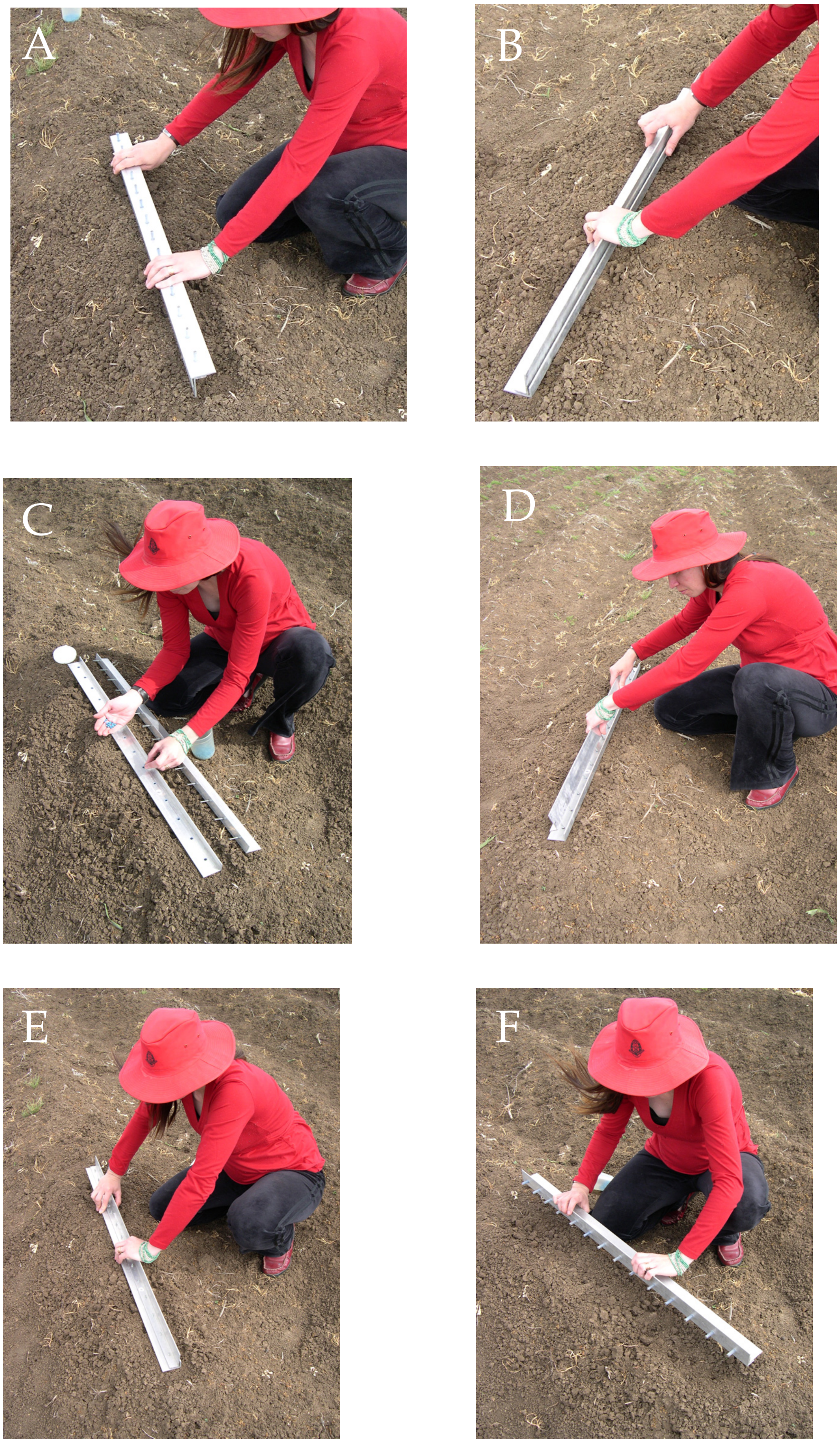

Figure 4. Planting with a dibber: A) dry soil is scraped away until moisture is reached; B) the dibber is pushed into the ground: C) the top section is removed and seeds placed into the holes; D) the top section is replaced and E) downward pressure is applied to plant the seeds; and F) the dibber removed from the soil.

Outdoor pot experiments

The results of pot experiments are often criticised because they are gathered by growing plants in an artificial environment. One way to overcome this is to place pots outside in the field where they can be exposed to identical environmental conditions experienced by the crop. We buried pots up to the rim to ensure that soil temperatures in the pots were identical to those in the field. Pots were always buried immediately adjacent to a cotton field to ensure wetting up during irrigations, but never within the field due to the high risk of contamination by the Fusarium wilt pathogen.

Isolation of Fungi

When isolating fungi from soil, we first diluted soil samples by adding 10g of dried soil to 90ml of sterile 2% water agar, producing a 1:10 dilution. This suspension was agitated for three minutes. 10ml of the agitated suspension was then added into another bottle containing 90ml of sterile 2% water agar, producing a 1:100 dilution, and agitated again for three minutes. This process was repeated once more to generate a 1:1000 dilution, from which 1ml aliquots were added to 9cm Petri dishes into which selective media were poured. The most commonly used selective media was Komada's Medium (Fusarium Laboratory Manual) which is selective for *F. oxysporum*.

When isolating from plant parts, parts were first surface sterilised in a solution of 70% ethanol and sodium hypochlorite (4% Cl). Surface sterilised parts were then rinsed in sterile distilled water and incubated on potato dextrose agar (PDA) for 48 hours after which emergent fungi were subcultured onto PDA that had been amended with the antibiotic streptomycin. After further incubation on the streptomycin PDA, isolates were transferred to PDA for storage at 4°C or carnation leaf agar (CLA) for morphological identification.

Design and analysis

Field and outdoor pot experiments to be analysed using ANOVA used either completely randomised block, completely randomised, Latin square, or split plot designs. Data was screened for normality and heterogeneity of variance before analysis. Analysis of variance with spatial analysis (ASREML) was applied to field experiments with planned comparisons of treatments. Linear and non linear regression models were fitted to time-scale experiments and for comparison between symptoms and other parameters.

4. Results and Discussion

4.1 Identify and evaluate factors contributing to the spread and increase of severity of Fusarium wilt.

4.1.1 Progression of disease within the crop

To determine the factors that affect the severity of Fusarium wilt the development of disease within plants was mapped during two seasons.

2003/04 SEASON

In 2003/4 at Moree and Boggabilla, we measured the movement of vascular discolouration up and down the stem, and the appearance of external symptoms including wilting, chlorosis and necrosis, throughout the season (Figures 5 and 6).

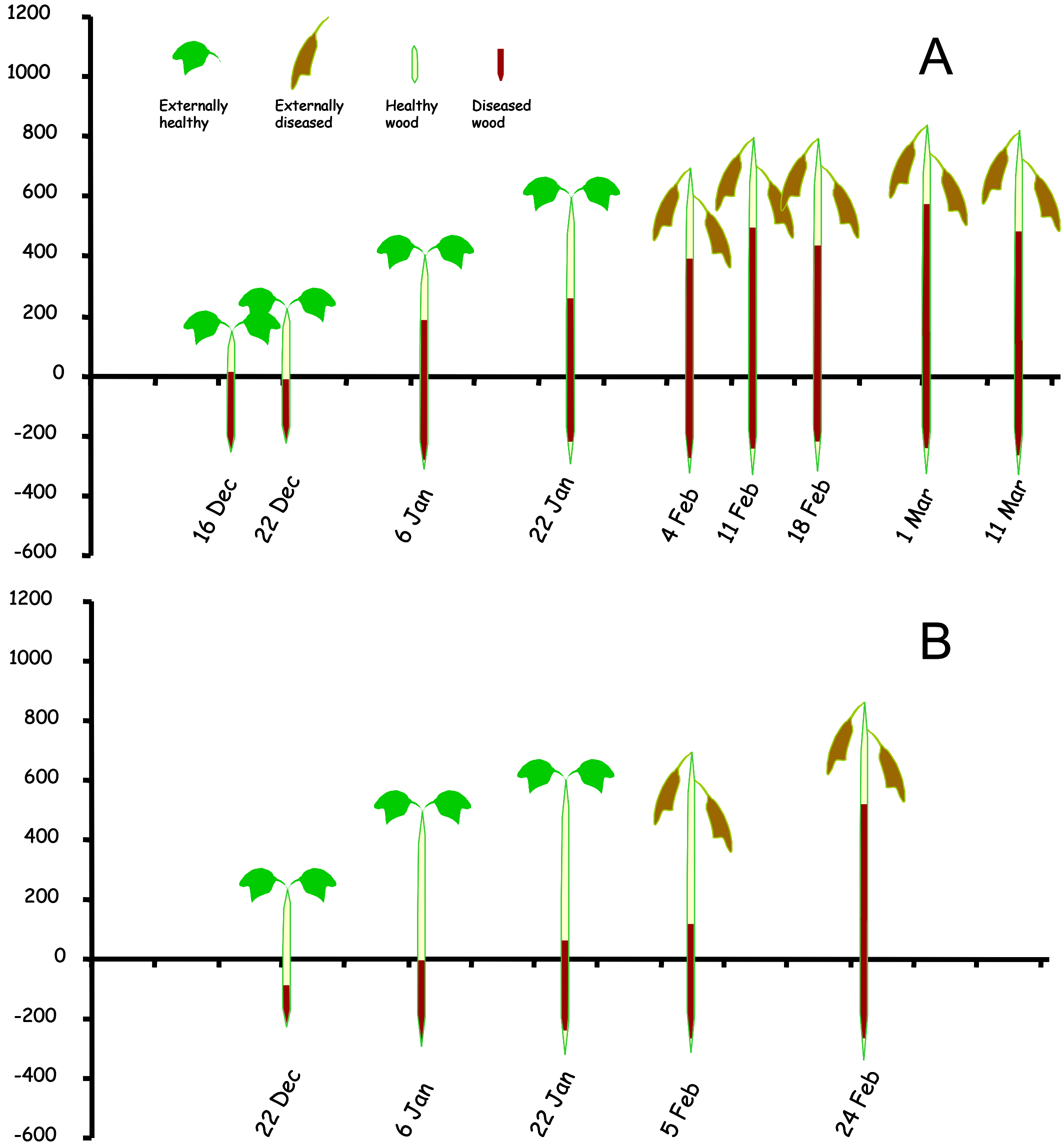

Figure 5. The movement of vascular discolouration and the appearance of external symptoms in cotton crops at Boggabilla (A) and Moree (B) in the 2003/4 cotton season. *Y* axis indicates plant height and root depth in millimetres.

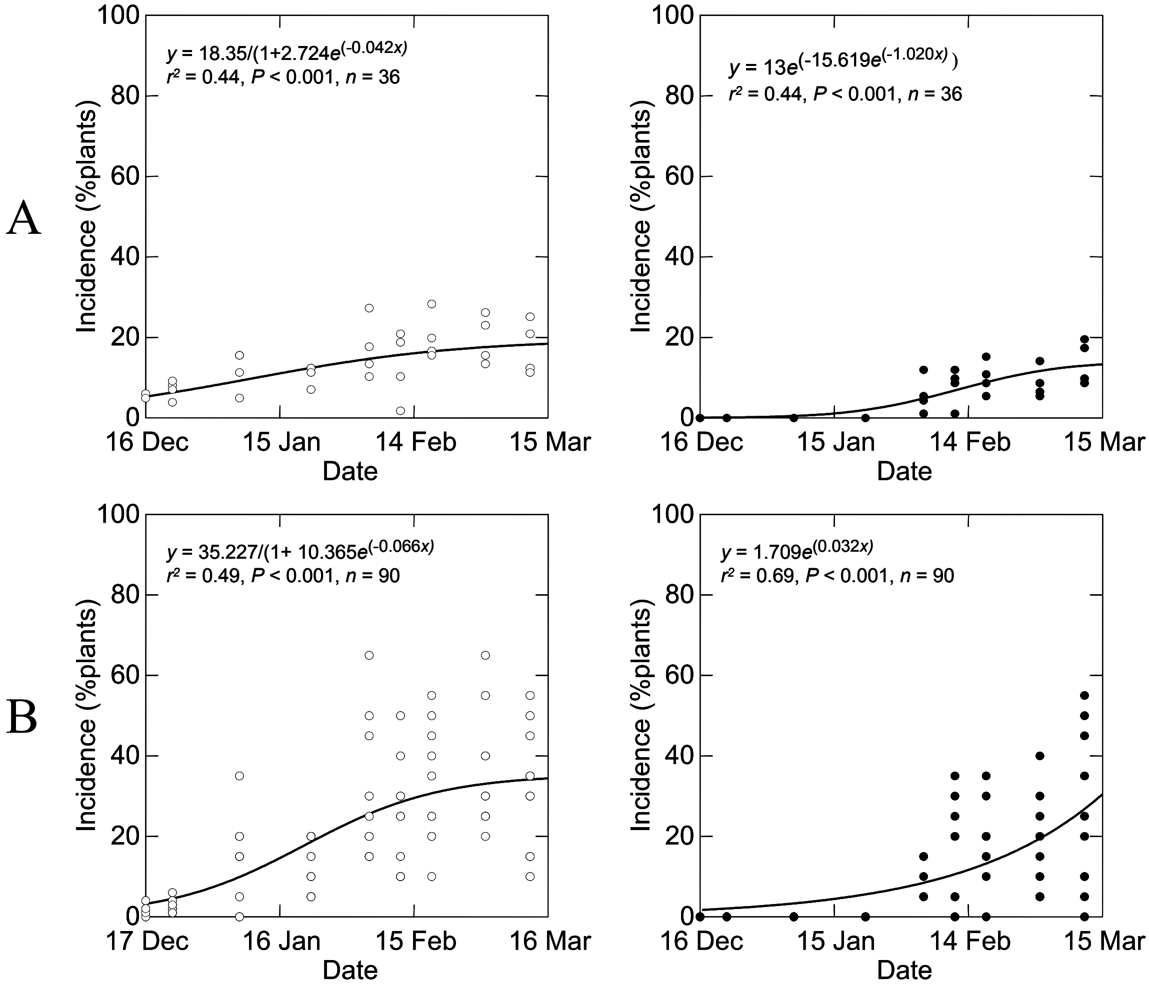

Figure 6. Incidence of vascular discolouration and external symptoms of Fusarium wilt of cotton at Moree (A) and Boggabilla (B) in the 2003-04 season.

Disease incidence was lower at the Moree site that at the Boggabilla site (Figure 6). Even though 5 to 10% of plants had vascular discolouration in December (Figure 6), the discolouration did not extend above the soil surface until January (Figure 5). Furthermore, at both sites, external symptoms did not appear until early February (Figures 5 and 6). While the appearance of external symptoms appeared to follow a period of wet weather (from 12-16 January;168 mm at Moree and 125.6 mm at Boggabilla – data courtesy SILO) this wet period was not correlated with the rise of vascular discolouration up the cotton stems (Figure 5). These observations are consistent with the hypothesis that the majority of infection occurs early in the season and progresses upwards in the plant. In plants that were ostensibly disease free above ground in December, infection was well under way deeper in the root system.

At the Boggabilla site, the spatial distribution of roots and vascular discolouration was mapped in the roots systems of 12 plants on 4 February 2004. There were no significant relationships between the percentage length of lateral roots infected and either i) depth in the soil profile, ii) angle of lateral root relative to the direction of cultivation and iii) the downward angle of the lateral root relative to the tap root (data not presented). In the majority of lateral roots the vascular discolouration was in contact with the tap root (Figure 7), suggesting that infection may have moved into the lateral roots by secondary spread. However, a few roots had vascular discolouration that was not in contact with the tap root (Figure 7), indicating that primary infections had occurred in these roots independently of the main infection in the tap root. These observations are also consistent with the hypothesis that infection occurs early in the season, with few primary infections occurring later in the season (see also discussion of 2004/05 season study, below)

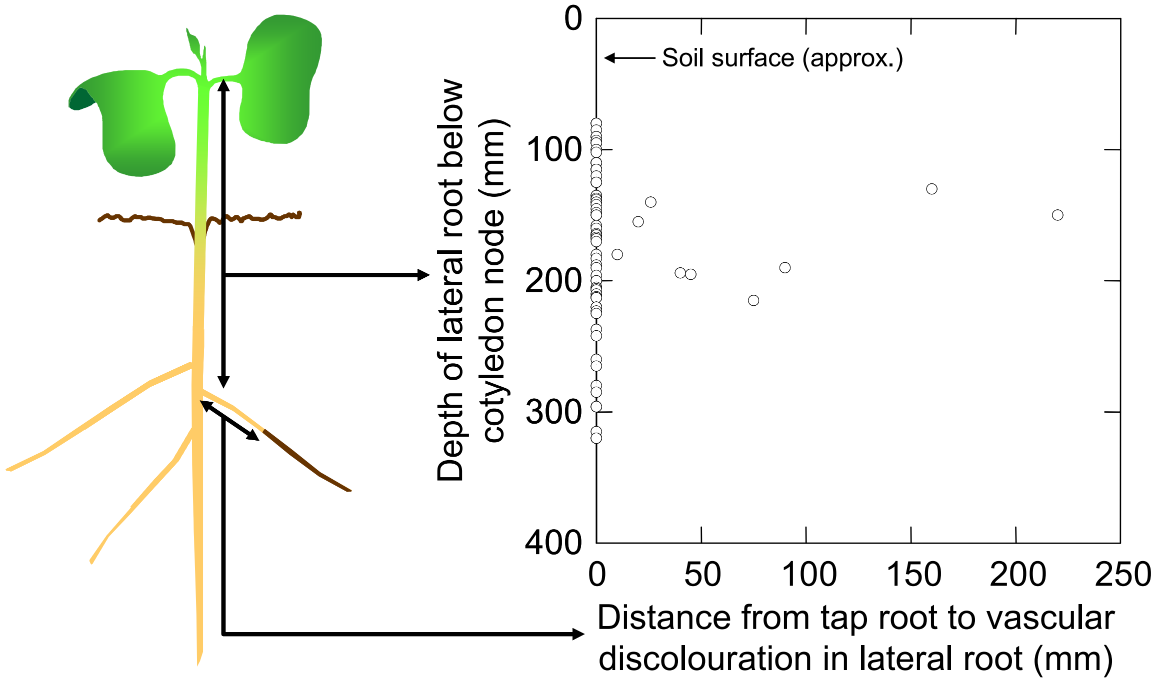

Figure 7. Relationship between lateral root depth and location of vascular discolouration within lateral roots at Boggabilla in the 2003-04 season.

2004/05 SEASON

In the 2004/05 season we measured the presence and absence of 1) internal vascular discolouration when stems were cut at ground level and 2) external symptoms including wilting, chlorosis and necrosis, throughout the season at Moree, and Boggabilla (Figures 8 and 9). The field used at Moree in 2004/05 was adjacent to that used in the previous year, whereas the field at Boggabilla in 2004/05 was on an adjacent farm.

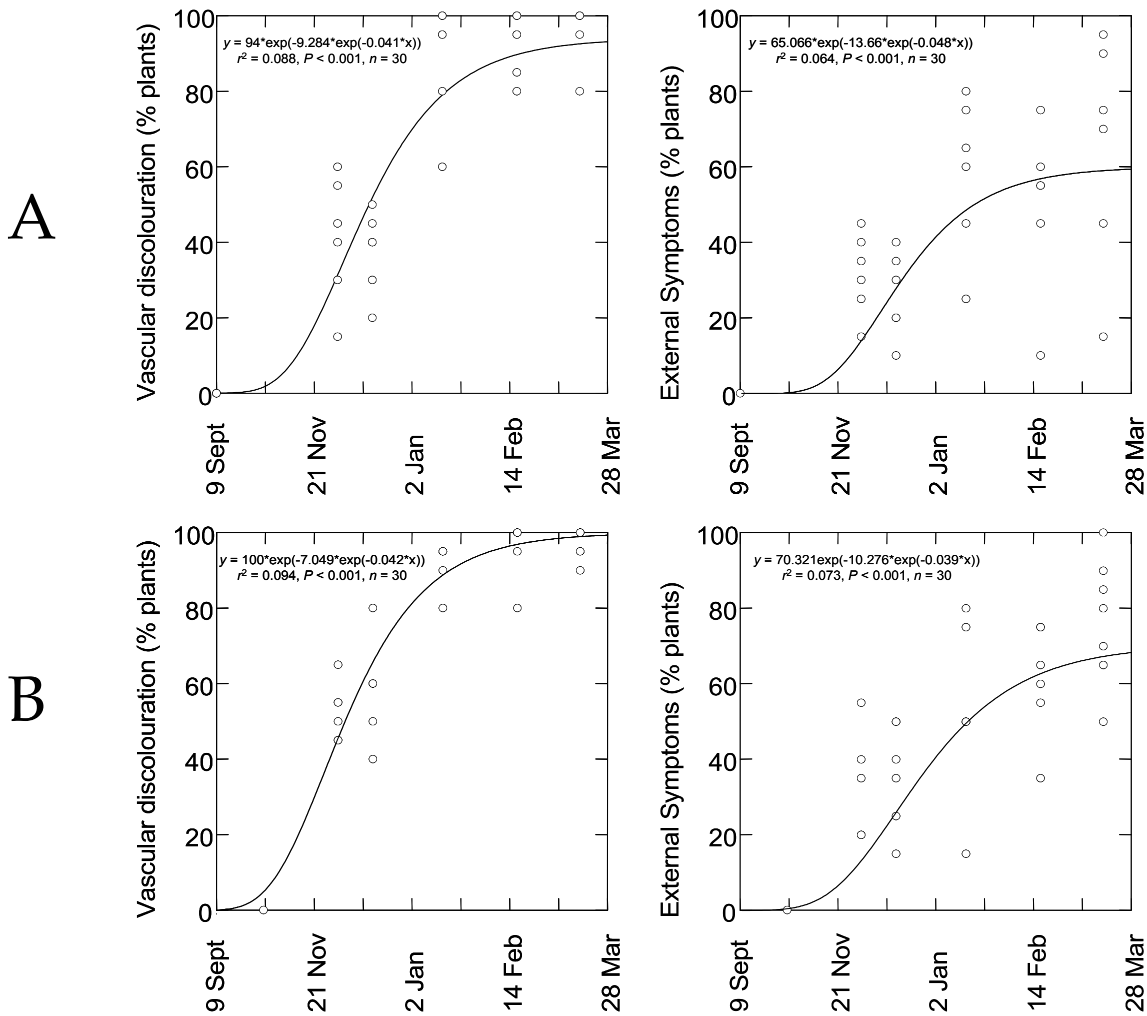

Figure 8. The incidence of plants displaying vascular discolouration and external symptoms throughout the season at Boggabilla. Data collected from a sowing date experiment where cotton was sown (A) 9/10/2004 and (B) 29/10/2004.

At both sites, the rate of increase in internal symptoms was much greater in the period preceding 31 December compared to the period following 31 December (Figures 8 and 9). Moreover, whilst the appearance of internal and external symptoms did not differ greatly between sites, external symptoms were visible much earlier in the 2004/5 season compared to the 2003/4 season and this was associated with a more rapid movement of vascular discolouration up the stem (Figures 8 and 9). This suggests that early season conditions in the 2004/5 season were much more conducive to disease development than conditions in the 2003/4 season. Therefore, we decided to compare the ability of *Fov* to infect cotton and cause disease across the season by staggering plantings of cotton in 10m plots from September through to February in fields at Moree and Boomi (Figure 10).

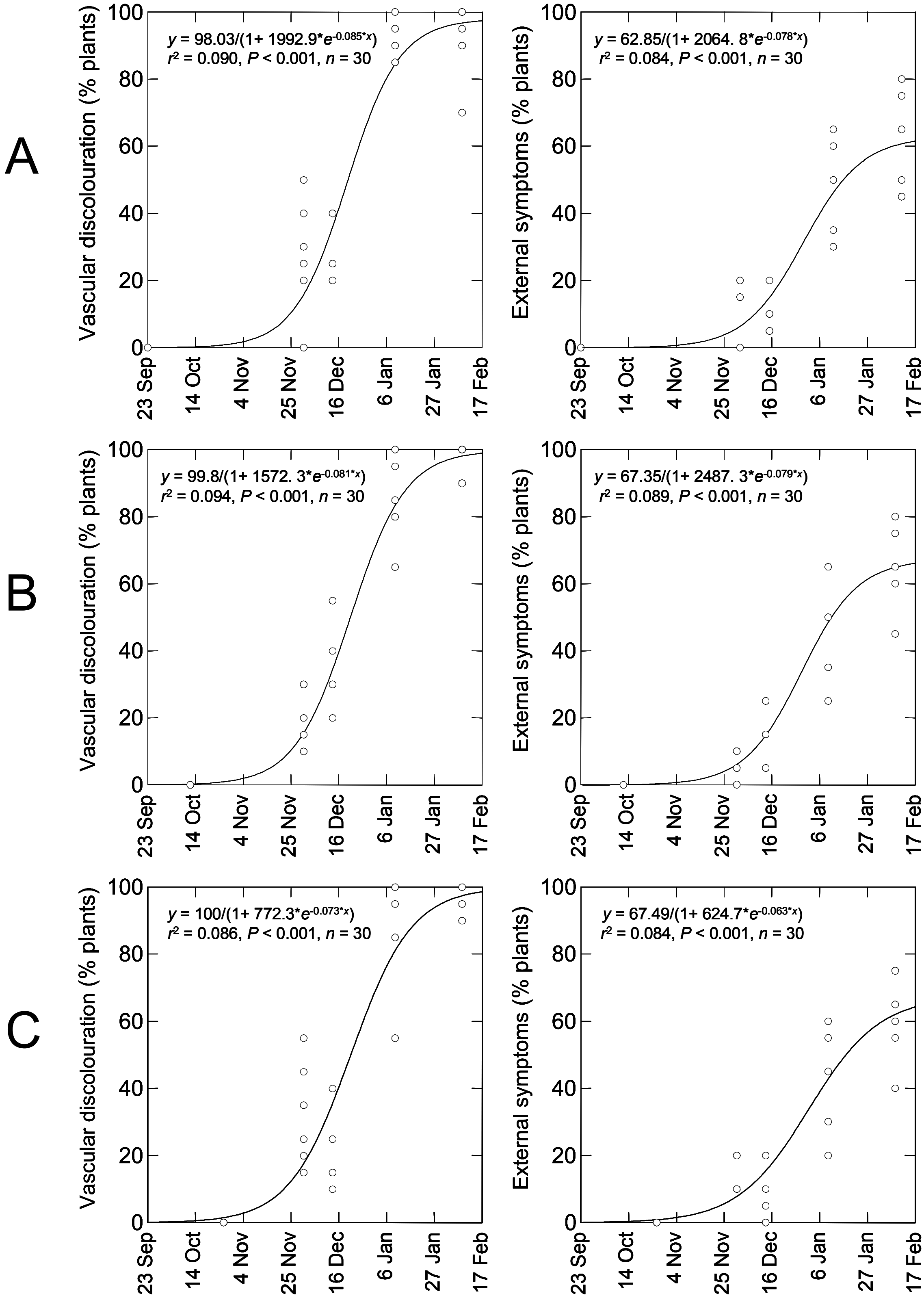

Figure 9. The incidence of plants displaying vascular discolouration and external symptoms throughout the season at Moree. Data collected from a sowing date experiment where cotton was sown (A) 23/9//2004, (B) 12/10/2004 and (C) 27/10/2004.

At both sites, total survival tended to be higher on later sowing dates. As cotton variety and pathogen levels in the soil differed between sites, a direct comparison is not valid. However, the data from Moree illustrate most clearly that the period before December was associated with the lowest levels of survival, again indicating that early season conditions were most conducive to disease (Figure 10).

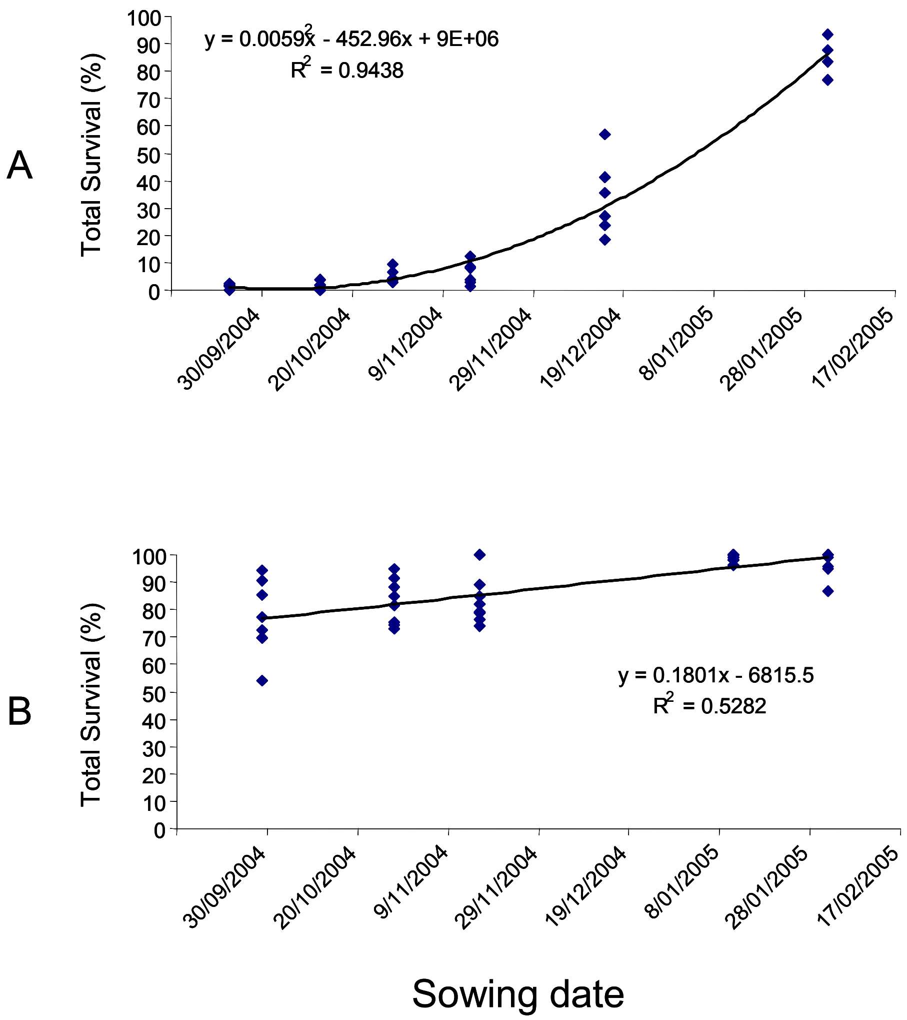

Figure 10. Total survival of cotton sown at various dates throughout the 2004/5 season at (A) Moree and (B) Boomi. Note that the varieties sown were Sicot 289BR and Sicot F1 at Moree and Boomi respectively.

2005/06 SEASON

In the light of the observation in 2004/05 that most infection occurred before December, the study was repeated in 2005/06 at threee sites, with emphasis on the period before December 31. Cotton (var. Sicot 71BR) was sown in 10m plots on eight different days across October, November and December 2005 at Moree (Figure 11).

We were also interested in comparing the influence of early season conditions on varieties with different levels of resistance to Fusarium wilt. Therefore we staggered plantings of a less-susceptible variety, Sicot F1 (F. rank 209), and a moderately susceptible variety, Sicot 71 (F. rank 108) over October, November and December 2005, in a field at Boomi, NSW (Figure 12). The study was also conducted at Boggabilla, using a less-susceptible variety, Sicot 43BR (F. rank 118), and a moderately susceptible variety, Sicot 71BR (F. rank 110), (Figure 13.

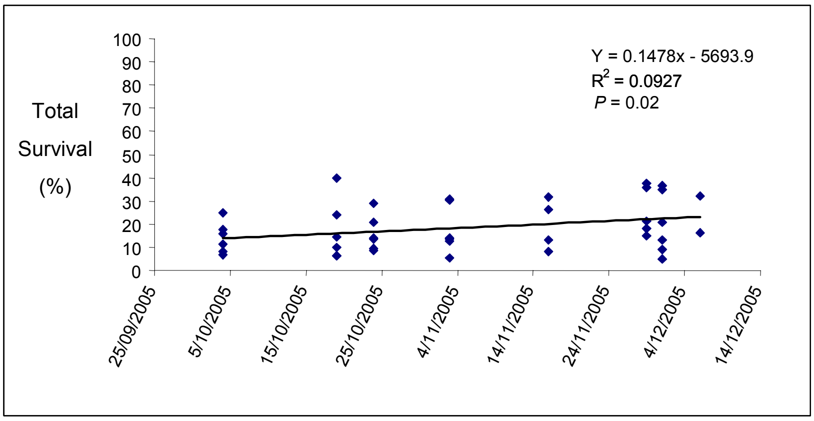

Figure 11. Total survival in cotton var. Sicot 71BR planted at Moree in October, November and December 2005.

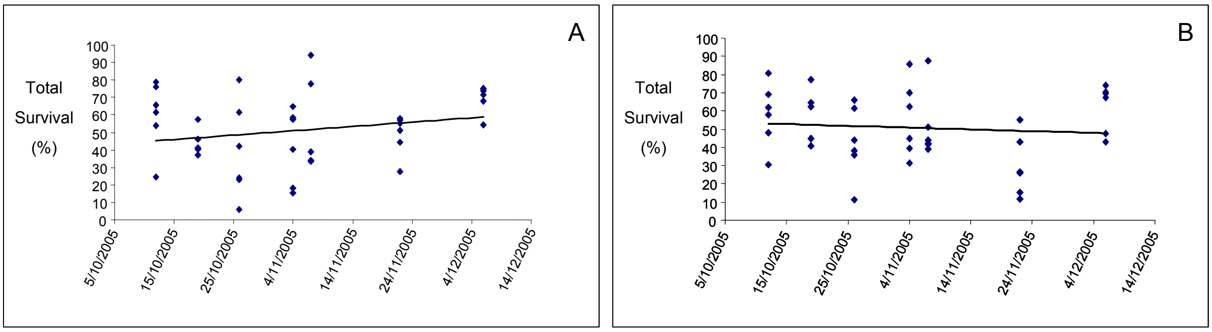

Figure 12. Total survival in cotton varieties Sicot 71BR (A) and Sicot 43BR (B) planted at Boggabilla in Ocotber, November and December 2005. Note regressions not significant.

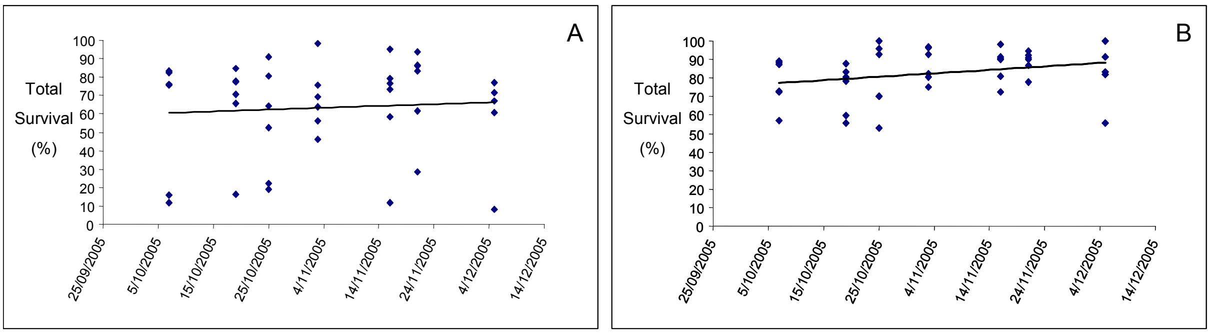

Figure 13. Total survival in cotton varieties Sicot 71 (A) and Sicot F1 (B) planted at Boomi in Ocotber, November and December 2005. Note regressions not significant.

We were unable to detect any correlation between sowing date and total survival at Boggabilla and Boomi and were therefore unable to draw any conclusions about the impact of early season conditions on different varieties (Figures 12 and 13). However, a weak positive correlation between sowing date and total survival was detected at Moree where disease severity was much higher: i.e. the later plantings tended to have higher total survival (Figure 11). Our inability to detect a trend and Boggabilla and Boomi, and the detection of only a weak trend at Moree was probably due to a sparse and variable distribution of the pathogen in the soil and/or variation in soil moisture between sowing dates.

In conclusion, the incidence of Fusarium wilt increases most quickly during the spring and then tends to level out over the remainder of the season. Early season conditions appear to be most conducive to disease development.

4.1.2 Early season conditions affecting disease development

Cotton var. Sicot 189 was grown repeatedly over five seasons in a field near Pampas on the Darling Downs. Total survival was recorded at the end of each season and then correlated with a range of environmental variables. Cumulative rainfall in October and November explained 96.7% of the variation in total survival of Sicot 189 in any given year (Figure 14). The linear decrease in total survival shown in Figure 14 was not related to the build up of the pathogen over successive seasons of cotton (Figure 15). Intense rainfall events during other parts of the season may also increase the severity of Fusarium wilt as appeared to be the case at Moree and Boggabilla in January 2004 (Figure 5).

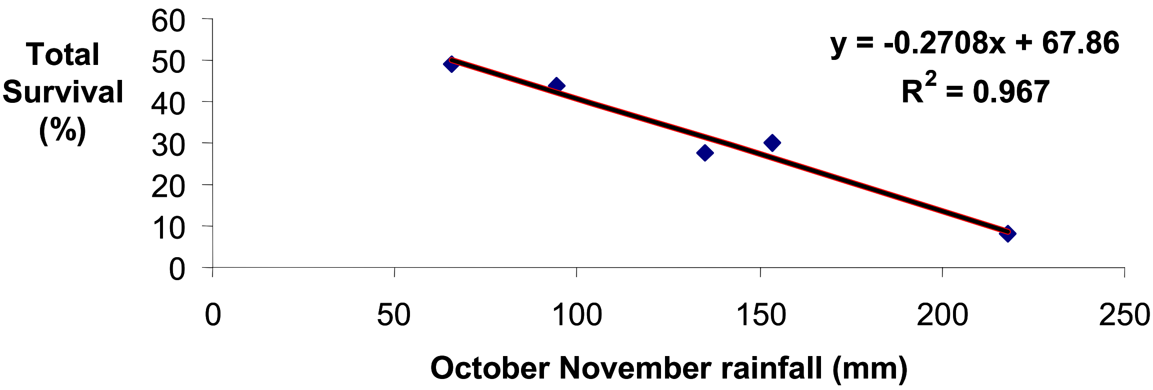

Figure 14. Negative correlation between total survival of cotton, cv. Sicot 189, and cumulative rainfall in October and November in a field infested with *Fusarium oxysporum* f.sp. *vasinfectum* at Pampas, QLD.

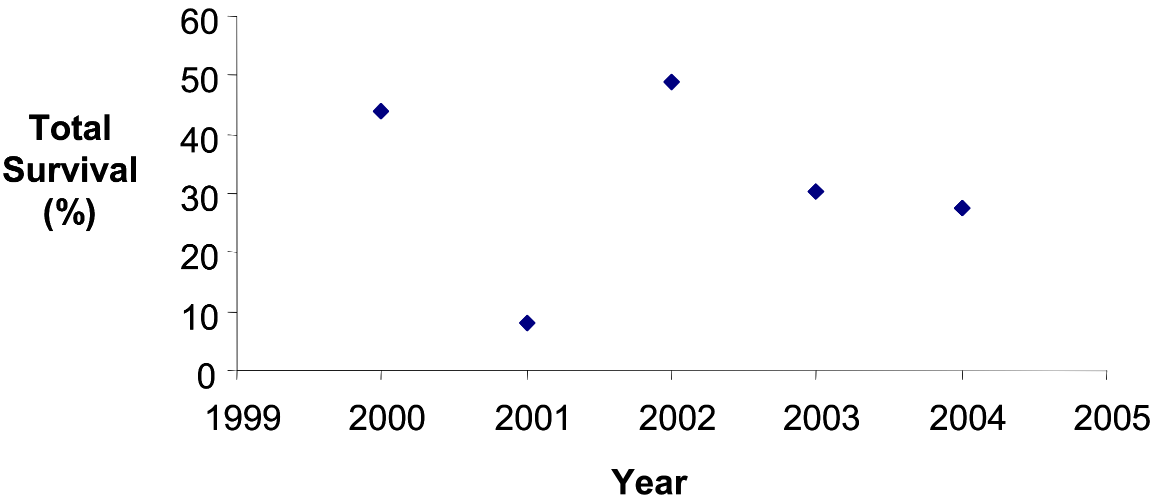

Figure 15. Lack of correlation between total survival of cotton, cv. Sicot 189, and growing season in a field infested with *Fusarium oxysporum* f.sp. *vasinfectum* at Pampas, QLD.

It is likely that rainfall in October and November increases the severity of Fusarium wilt because seedling roots are actively growing through zones in the soil where levels of *Fov* are high, and soil temperatures are conducive to fungal proliferation.

4.1.3 Contribution of irrigation to disease development and dispersal of *Fusarium oxysporum* f.sp. *vasinfectum*

It has long been thought that *Fov* is carried around the farm during irrigations and that this may contribute to the spread of the pathogen between fields. Therefore we measured the population density of *F. oxysporum* (number of colony forming units or CFU's) present in water and on trash at various points (Figure 16) in the irrigation reticulation system on a farm at Boggabilla. It is important to note that only some strains of *F. oxysporum* are *Fov*, so overall numbers of *F. oxysporum* are only indicative of the numbers of *Fov* in any sample.

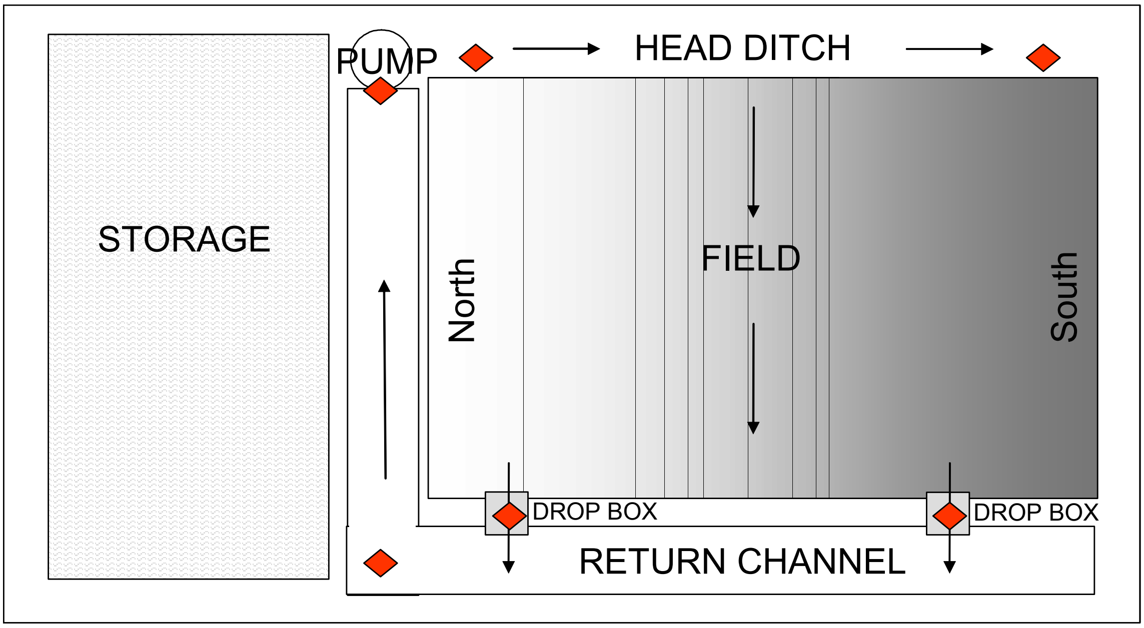

Figure 16. Sampling points ◆ in a water reticulation system during irrigation of a field heavily infested with the Fusarium wilt fungus. The direction of water flow is indicated by arrows. The incidence of Fusarium wilt in the field is indicated by shading, the darker area to the south having a higher incidence of disease compared to the lighter area to the north.

Levels of *F. oxysporum* in the water and on mud adhering to floating trash were lowest at the head ditch, then increased as water passed through the field, being highest in the south drop box, corresponding with high levels of disease at that end of the field (Figures 17 and 18). Levels then dropped off as water and trash moved back towards the lift pump (Figures 17 and 18). The substantial difference between levels of *F. oxysporum* at the lift pump and head ditch suggests that the passage of water through the storage dam removes most of the fungal material, probably through a process of settling out. Whilst approx. 25,000 CFU's of *F. oxysporum* were detected per litre of irrigation water, more than 160 million CFU's were detected in the mud adhering to 1kg of floating trash. This demonstrates the potential for floating cotton trash to carry the Fusarium wilt pathogen in high numbers from field to field with the flow of irrigation water.

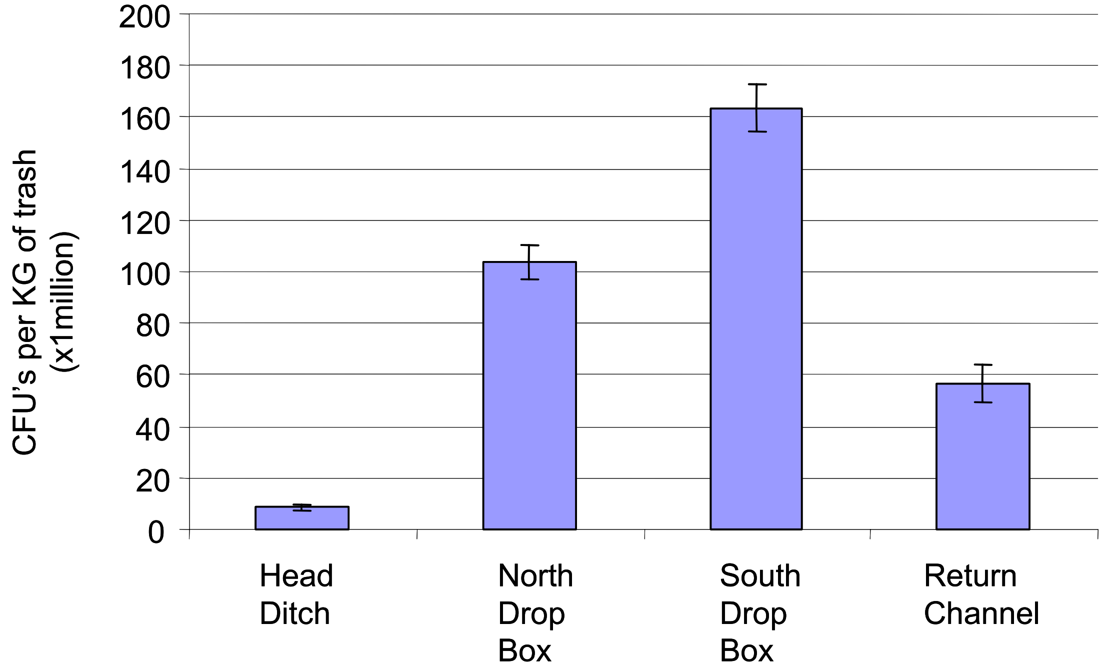

Figure 17. Colony forming units (CFU's) of *Fusarium oxysporum* per kilogram of floating trash.

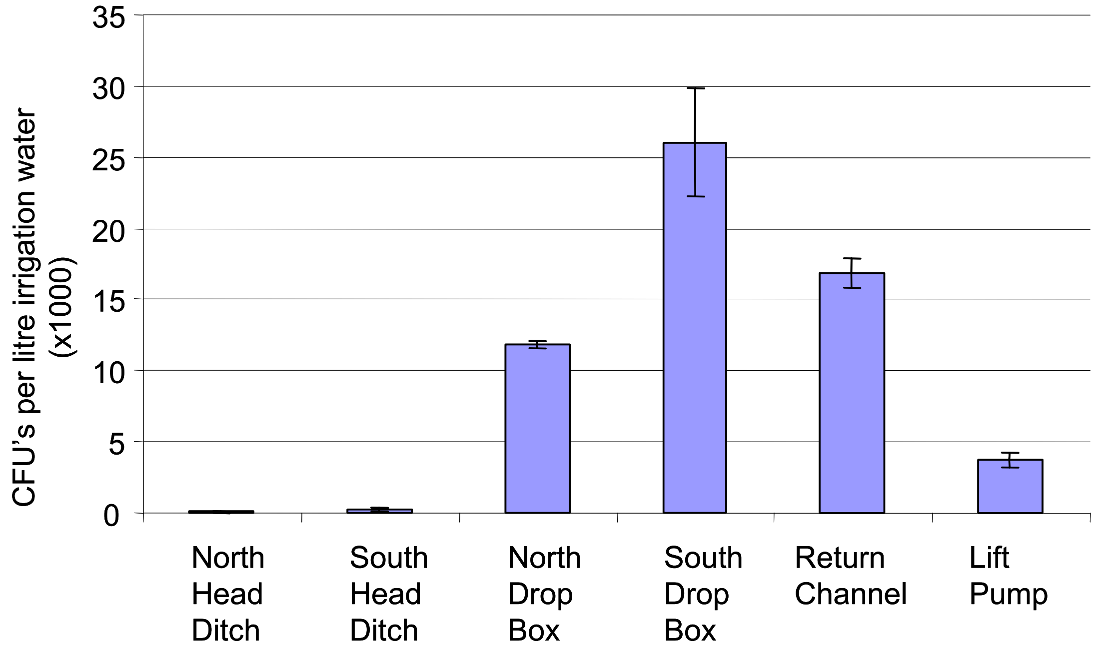

Figure 18. Colony forming units (CFU's) of *Fusarium oxysporum* per litre of irrigation water.

In conclusion, the Fusarium wilt pathogen is transported in both water and on floating trash during irrigations with the greatest number of propagules being carried on rafts of floating trash. Therefore it is important to reduce the amount of trash flowing off fields and through the water reticulation system during irrigations.

4.1.4 Contribution of late-season stresses to disease development

It has been commonly suggested that Fusarium wilt is a disease of late-season reproductive stress caused by heavy boll loads. We conducted two experiments in the 2003/4 season in which we reduced the reproductive stress of plants by removing bolls. In the first experiment, we physically removed the bolls from plants by hand on 11/02/2004. This resulted in a significant reduction in yield measured as lint weight per meter of cotton but did not affect plant survival (Figure 19).

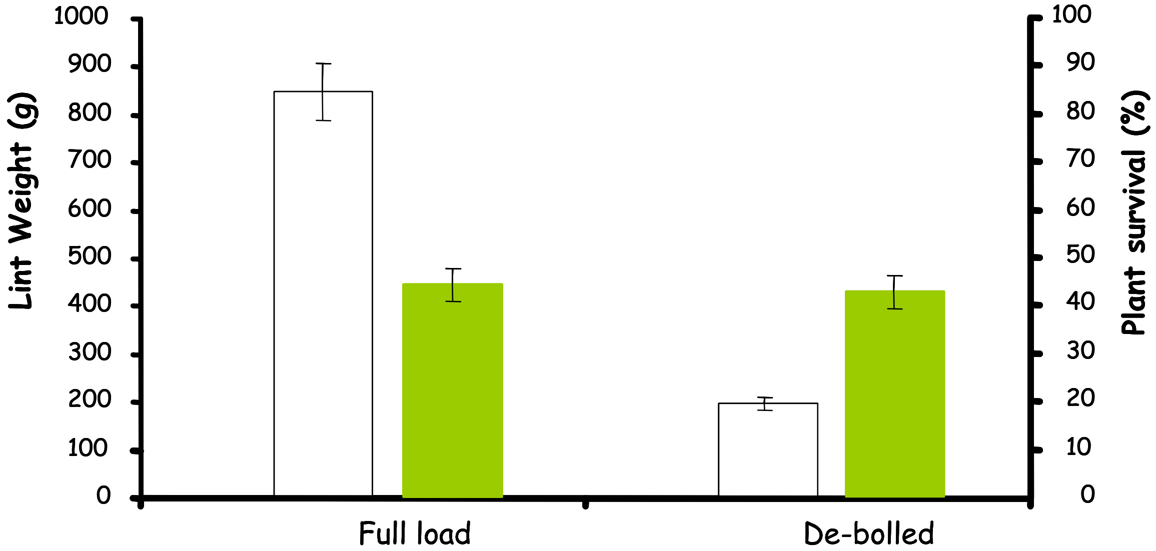

Figure 19. Lint weight (□) and total survival (■) in cotton with a full boll load, and cotton from which bolls had been removed, in a field infested with *Fusarium oxysporum* f.sp. *vasinfectum* at Boggabilla, NSW.

In the second experiment, we sprayed glyphosate over the top of a Roundup Ready crop in order to sterilise anthers and thereby prevent fruit from setting. Plants were unsprayed, sprayed once, or sprayed twice after the four true-leaf stage. Once again, we observed a reduction in yield that was attributable to reduced numbers of fruit on the sprayed plants, but did not observe any difference in plant survival (Figure 20).

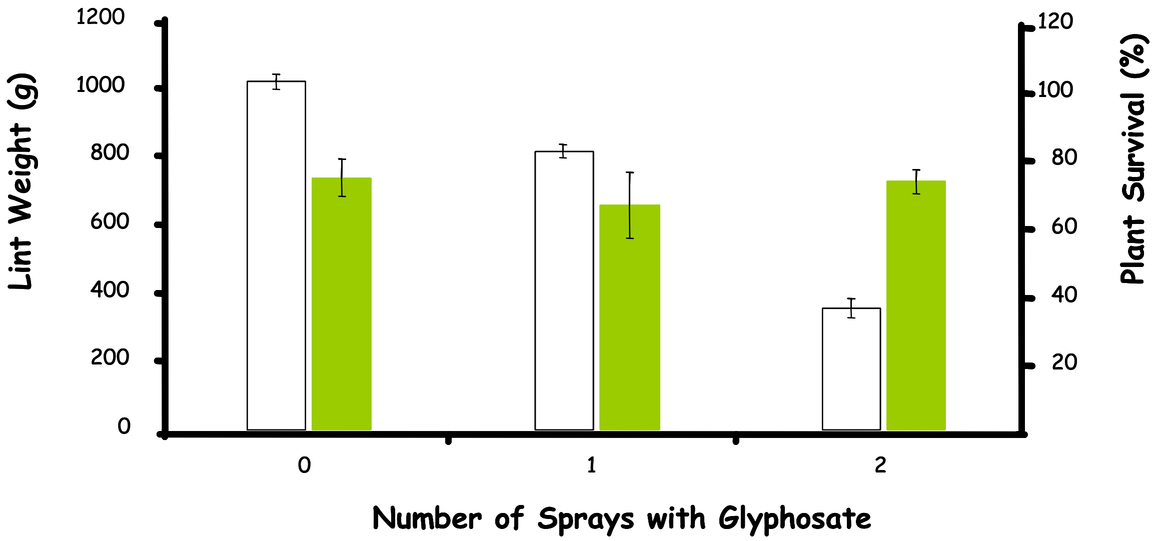

Figure 20. Lint weight (□) and total survival (■) in cotton that received zero, one or two consecutive sprays of glyphosate over the top after the four true leaf stage, in a field infested with *Fusarium oxysporum* f.sp. *vasinfectum* at Moree, NSW.

In both experiments, disease severity was not affected by reproductive stress. Moreover, we did not observe any correlation between the off-label use of glyphosate and disease severity.

It has often been claimed that the use of glyphosate increases the severity of various diseases caused by species of *Fusarium*. Our experiment provides further evidence that this is not the case. If the use of glyphosate increases the severity of Fusarium wilt, then we might expect the F. rank of Roundup Ready cotton varieties to be lower than non-Roundup Ready varieties, as the F. rank is a measure of total survival. However, this is not the case (Figure 21), providing further evidence that glyphosate does not increase the severity of Fusarium wilt.

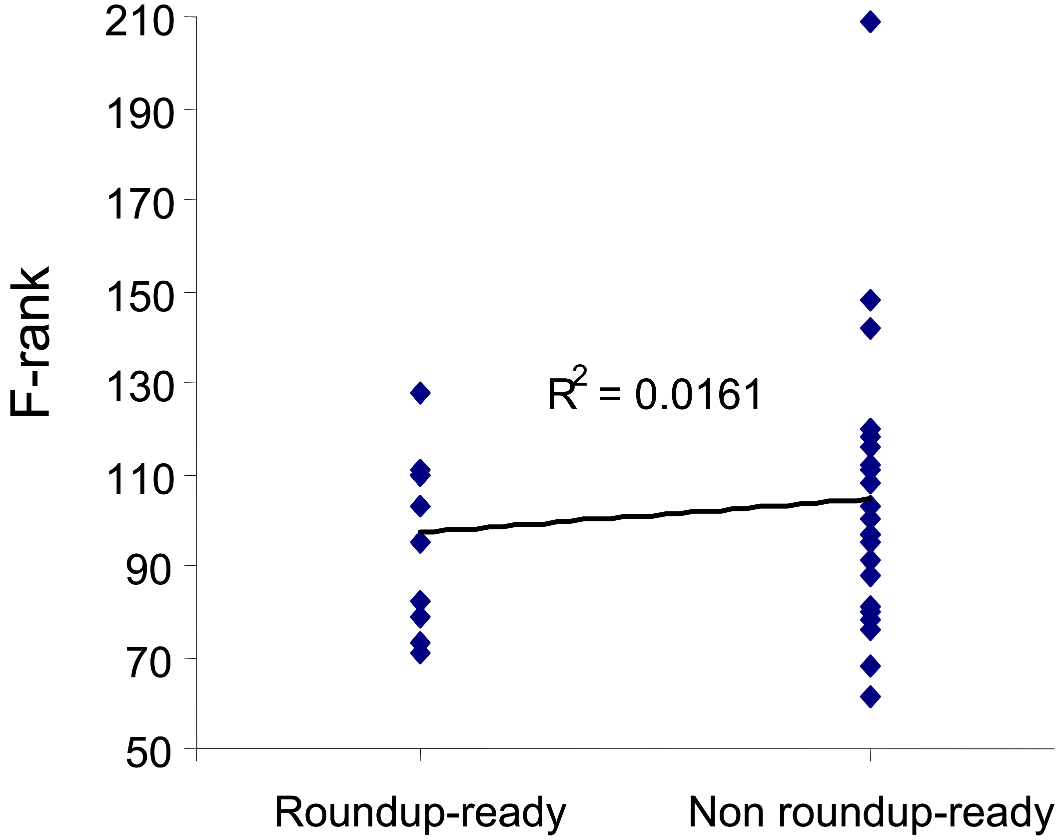

Figure 21. There is no relationship between relative resistance to Fusarium wilt of cotton and the presence or absence of the Roundup Ready gene in cotton varieties.

In conclusion, there is no relationship between late season reproductive stress caused by a heavy boll load and the severity of Fusarium wilt. Moreover, there is no evidence that the severity of Fusarium wilt is increased by the use of glyphosate.

4.1.5 Interaction between black root rot and Fusarium wilt

Disease complexes occur when two or more pathogens interact to increase the severity of disease experienced by a plant. *Fov* and the black root rot fungus are both widespread soil-borne pathogens of cotton in Australia, and commonly occur in the same fields. Therefore, we investigated the potential for high levels of the black root rot fungus to increase the severity of Fusarium wilt. Soil with a naturally high level of *Fov* was collected and put into eight inch pots that were then buried in the field. Inoculum of the black root rot fungus was then added to half the pots, mixed into the top 10cm of soil, and cotton sown. The experiment was run at Moree, Boomi and Boggabilla in the 2005/6 season.

There was no difference in the severity of Fusarium wilt between plants inoculated with the black root rot pathogen and un-inoculated plants (Figure 22).

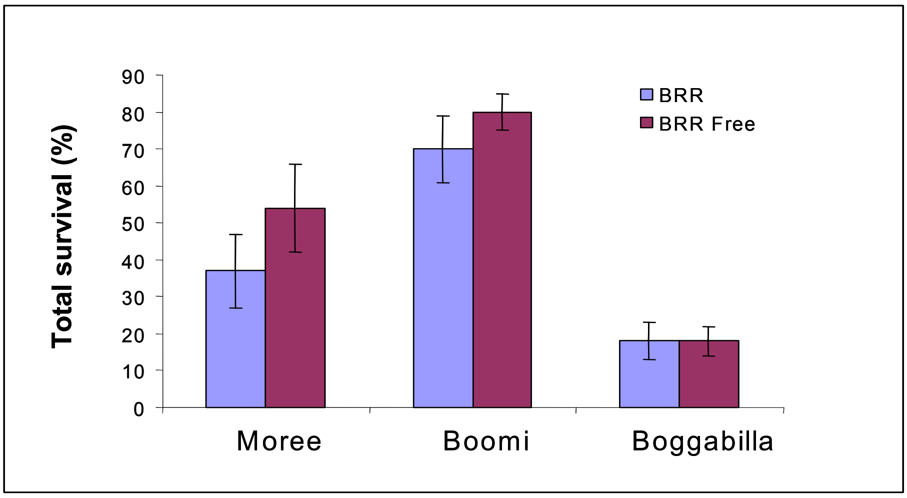

Figure 22. Average (standard error) total survival of cotton (var. Sicot 71BR) sown into soil with and without the black root rot pathogen.

The severity of black root rot was significantly higher among inoculated plants at Boomi (P<0.001) and Boggabilla, but not at Moree, indicating that a natural infestation of the black root rot pathogen was present in the soil at Moree (Figure 23).

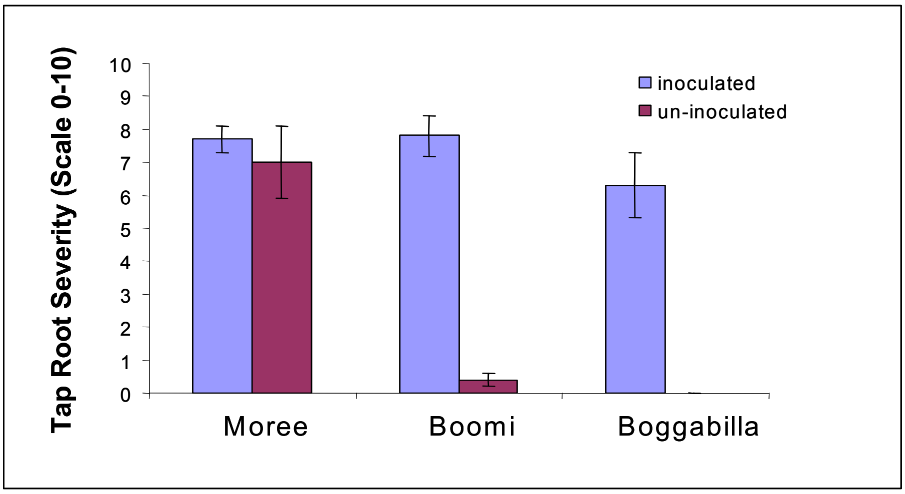

Table 23. The severity of black root rot of cotton plants either inoculated with the black root rot pathogen or un-inoculated expressed on a scale of 0-10, where 0 represents 0-10% of the tap root blackened, up to 10 representing 100% of the tap root blackened.

These experiments provide evidence that the presence of the black root rot pathogen in soil that is naturally infested by *Fov* does not increase the severity of Fusarium wilt.

4.1.6 Contribution of different plant parts to carry-over of the pathogen

Disease carry-over refers to the reoccurrence of a disease in a crop after a break-period wherein the crop is not grown. In the case of Fusarium wilt, reoccurrence can be attributable to survival of the pathogen in 1) the soil, 2) plant parts and/or 3) both. We examined the potential contribution of different plant parts to the carry-over of Fusarium wilt in two experiments conducted at Boggabilla.

In the first experiment, a cotton crop that had been picked was divided into large plots measuring 8x30m and either: 1) mulched and root cut to retain the whole plant, 2) pulled and raked to remove most plant parts, 3) root cut and raked to leave only tap-roots, and 4) pulled to remove roots and stems but retain the leaves and trash on the ground. A wheat crop was sown into the field immediately following the cotton crop, and then the field was fallowed for 12 months until October 2005 when it was sown back into cotton. Beds were also knocked down and re-listed into 1m beds during the break.

No difference was found in total survival between treatments indicating that plant-parts probably do not influence the carry-over of Fusarium wilt in this field (Figure 24).

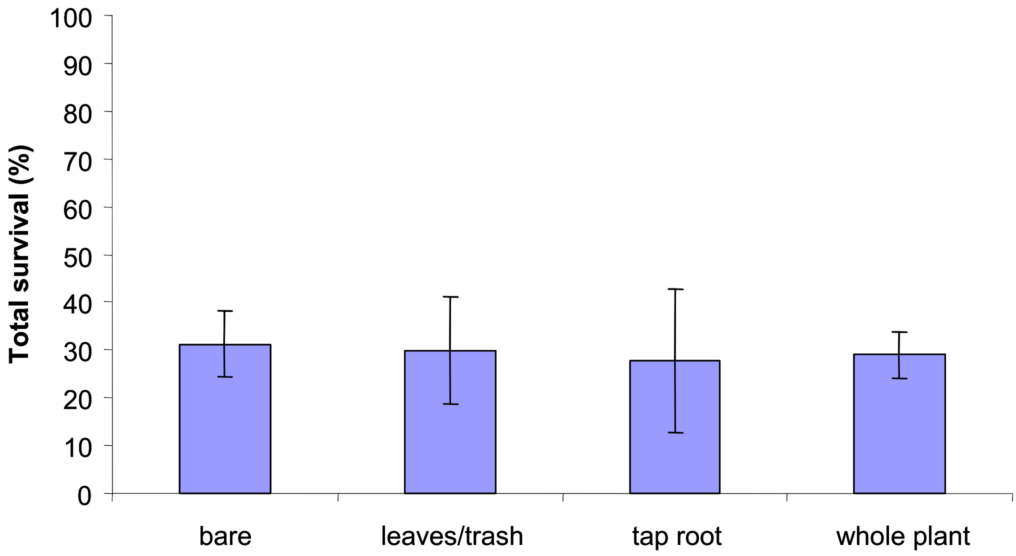

Figure 24. Total survival (standard error) of cotton sown into plots from which most plant material had been removed (bare), only leaves and trash were present (leaves/trash), only tap roots were present (tap root) and plots wherein cotton stubble had been mulched so as to retain the whole plant (whole plant).

However, the re-listing of 40inch beds into 1m beds would have moved the planting line and therefore the trash treatments enough to cause uncertainty about the true position of the original plots. Moreover, the winter wheat crop would have added large amounts of organic matter to the soil, potentially masking the impact of cotton stubble from the previous cotton crop.

It is possible that plant parts may play a more important role in fields with lower populations of *Fov* by contributing to the build up of the pathogen. This field had a high level of *Fov* in the soil, which may be too high to be influenced by the contribution of inoculum from infected plant parts. Therefore we cannot draw any solid conclusions about the role of plant parts and the carry-over of Fusarium wilt from this experiment.

In the second experiment, infected tap roots, stems and leaf/trash were collected at the end of the 2003/4 season and weighed into 1g portions. The portions were then added to soil that was assumed to be free of *Fov*, either buried or placed on the surface, in 20cm pots. Pots were placed outside in a cotton field for 18 months before being buried in another field that was thought to be free of *Fov* and planted with cotton (var. Sicot 71BR) in the 2005/6 season. Control pots contained no trash portions.

The type of plant part and whether or not it was buried or weathered on the soil surface for 18 months did not affect the total survival of cotton sown in the 2005/6 season (Figure 25).

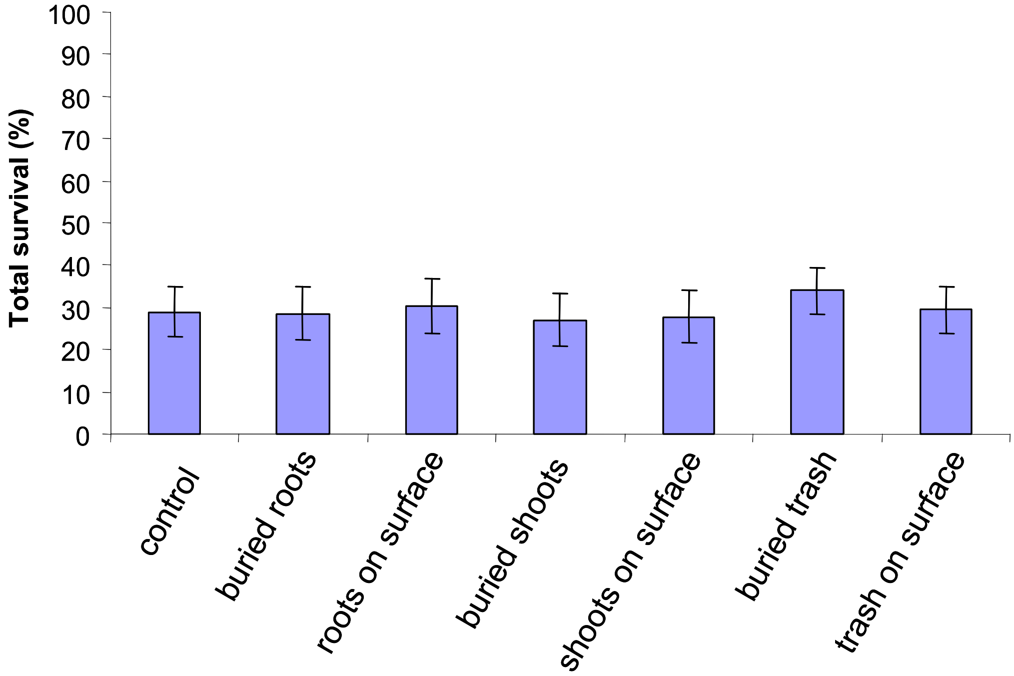

Figure 25. Total survival (standard error) of cotton in *Fov* free soil containing different *Fov*-infested trash portions, which had either been weathered on the soil surface or buried for 18 months prior to planting.

The control pots appeared to be contaminated by *Fov* indicating that the field into which the pots were buried for the duration of the 2005/6 season was probably infested with *Fov*. Moreover, many plants were killed by herbicide, introducing another confounding factor into the experiment. Despite the high level of replication in this experiment (30 pots per treatment), we cannot draw any conclusions as to the contribution of each trash portion to disease carry-over.

4.1.7 Systemic acquired resistance

Host plant resistance is an important factor that affects the severity of diseases. One method of increasing host plant resistance is to induce an immune response in the plant that up-regulates the expression of defence compounds active against pathogens. This can be achieved by applying compounds that induce this response, such as acibenzolar-S-methyl (Bion™). We applied Bion™ to the seedcoat of cotton before planting at Moree and Boggabilla in the 2005/6 season.

The level of resistance systemically acquired by cotton following the application of Bion™ to the seed coat increased the total survival of cotton at Boggabilla (P=0.043), but not at Moree (P=0.312) (Figure 26).

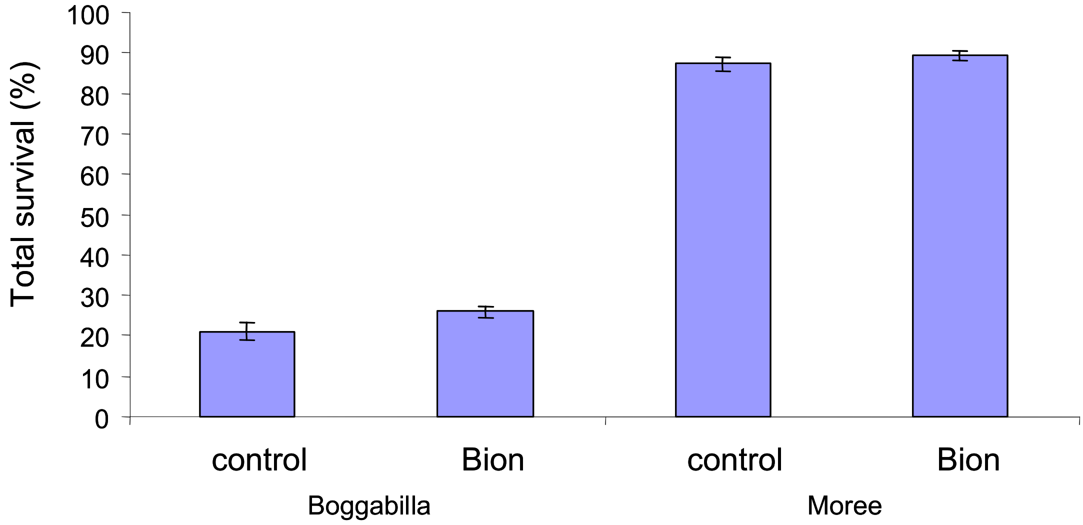

Figure 26. Total survival of cotton either treated with Bion™ or untreated at Boggabilla and Moree.

It seems counterintuitive that the level of resistance induced by Bion™ would protect plants against Fusarium wilt at Boggabilla but not at Moree, given that Boggabilla was a site with much higher levels of disease. One explanation may be that Bion™ prepares a plant for pathogen attack, but does not actually switch on the production of defence compounds. If this were the case, we might expect Bion™ treated plants to respond to pathogens more vigorously than non-treated plants, but not until actual contact with the pathogen is made. Therefore, at sites with only low levels of the pathogen, the effects of Bion™ may not be expressed to the same extent as at sites with high pathogen levels. Alternatively, the low level of disease at Moree probably reflected an uneven distribution of the pathogen in the soil, masking any effect that Bion® may have had on the small number of individually infected plants with plots.

These experiments raise questions about the efficacy of Bion™ in soils with low levels of *Fov*, and this will be investigated as part of DAN190. The induction of SAR can reduce the severity of Fusarium wilt, but more research is needed to understand factors that affect the efficacy of this process.

4.1.8 Potential for interactions between vascular wilts and nematodes in Australia

Nematodes are soil-dwelling animals that feed on a range of substrates including plants. Many nematodes cause diseases of plants (eg. root knot of Tomato) and many increase the severity of diseases caused by other pathogens. One form of Fusarium wilt of cotton in the USA is greatly enhanced by the presence of the root knot nematode *Meloidogyne incognita*. Another nematode, *Rotylenchus reniformis* enhances the severity of black root rot in Arkansas. We sampled fields with a history of severe Verticillium wilt in the Namoi valley. If we found a relationship between Verticillium wilt and nematodes then it would be imperative to look more closely at Fusarium wilt. However, there was no correlation of nematode species or numbers with the severity of Verticillium wilt, indicating that at present, there are no known incidences of a disease complex forming between nematodes and wilting diseases of cotton in Australia. We did however discover high numbers of the nematode *Helicotylenchus dihystera* in roots of cotton on a farm near Breeza (Figure 27). These nematodes are opportunistic grazers that had probably built up in numbers on a past wheat crop. They were not causing disease in the cotton crop.

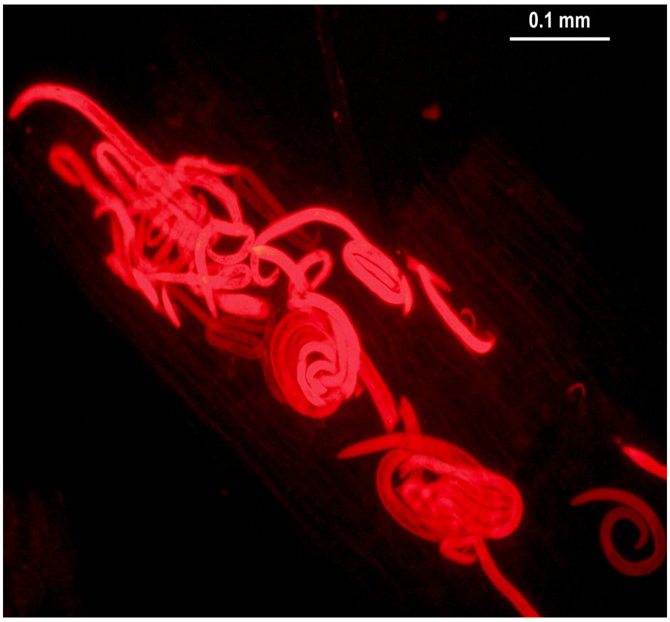

Figure 27. An aggregation of *Helicotylenchus dihystera* inside a cotton root.

Results are expanded upon in the following publication which can be found in Appendix 1:

OGG Knox, CMT Anderson, SJ Allen, DB Nehl **(2006)**. *Helicotylenchus dihystera* in Australian cotton roots. *Australasian Plant Pathology*. **35(2)** pp287-288.

4.2 Evaluate the use and/or modification of cultural practices for management of Fusarium wilt.

4.2.1 Modify soil temperature with plastic mulch for control Fusarium wilt

Cotton was planted in single-row replicated plots and beds covered with degradable plastic (Figure 28). Plastic was to be removed after two weeks. Soil temperature was monitored during the period while plastic was present. Plant stand was to be assessed in pegged areas of each plot as soon as possible after emergence and disease severity to be assessed in the same areas by cutting stems after harvest (before slashing/mulching). However, all seedlings died soon after emergence, probably due to the high humidity and high temperature under the plastic.

Figure 28. Plastic mulch covering the top of a bed.

We cannot recommend the use of plastic mulches in cotton production until a viable system is developed that ensures the survival of seedlings.

4.2.2 Modify soil temperature for disease control by changing bed shape

Cotton was planted in single-row replicated plots and bed shape modified manually, such that the seed remained at the same depth but the bed had a wider surface being perpendicular to the rays of the sun. Soil temperature was monitored during the early part of the season. Plant stand was assessed in pegged areas of each plot as soon as possible after emergence and disease severity assessed in the same areas by cutting stems after harvest (before slashing/mulching).

The average daily temperature from sowing to December 31 in normal and sloping beds was 24C and 23C respectively and this did not influence total survival of cotton ($p=0.372$). Therefore we cannot recommend the modification of bed shape as a tool for reducing the severity of Fusarium wilt.

4.2.3 Delayed sowing for control of Fusarium wilt

Since cool, wet early-season conditions are important in the development of Fusarium wilt (Section 4.1), then a logical control measure would be to minimise the period of exposure of cotton to these conditions by delaying sowing. We completed five delayed sowing experiments over the duration of the project with variable results.

In 2003/4 at Moree we sowed cotton on 10 October and then delayed sowing until 10 November in the first experiment, and 30 October in the second experiment. In both cases, we achieved an increase in total survival by delaying sowing (Table 1). Moreover, there was no difference in module weight between cotton sown on 10 October and 10 November (Table 1). In 2005/6 we sowed cotton on 4 October and then delayed sowing until 24 October and thereby substantially increased total survival (Table 1). However, in 2004/5 we were unable to increase total survival by delaying sowing (Table 1). A similar result was observed in 2004/5 at Boggabilla where we initially sowed on 9 October and then delayed the second planting until 29 October with no effect on total survival (p=0.18).

Table 1. Sowing dates, total survival, and yield in cotton for experiments conducted at Moree.

Experiment	Sowing date	Total survival (%)	Probability	Yield (ba/Ha)[A]
1	10/10/2003	65	<0.001	8.0
1	10/11/2003	83		8.0
2	10/10/2003	38	<0.001	Not recorded
2	30/10/2003	54		
3	23/09/2004	3	NS	7.7
3	12/10/2004	2		6.9
3	27/10/2004	7		6.4
4	4/10/2005	16	0.045	Not recorded
4	24/10/2005	24		

[A]Not replicated.

Therefore, delaying sowing was an effective means of reducing the severity of Fusarium wilt in the 2003/4 and 2005/6 seasons, but not in the 2004/5 season. Why?

Given that the biggest factor influencing the severity of Fusarium wilt is early season rainfall, we correlated the increase in total survival as a result of delayed sowing (the benefit from delayed sowing) in the experiments at Moree with early season rainfall and temperature. The benefit from delayed sowing was increased by decreases in 1) cumulative rainfall and 2) the number of days with a minimum temperature below 20°C, counted over the period from initial sowing in each experiment to 31 December (Figure 29). This observation explains the ineffectiveness of delayed sowing in the 2004/5 season where early season rainfall was high and minimum temperatures were cool.

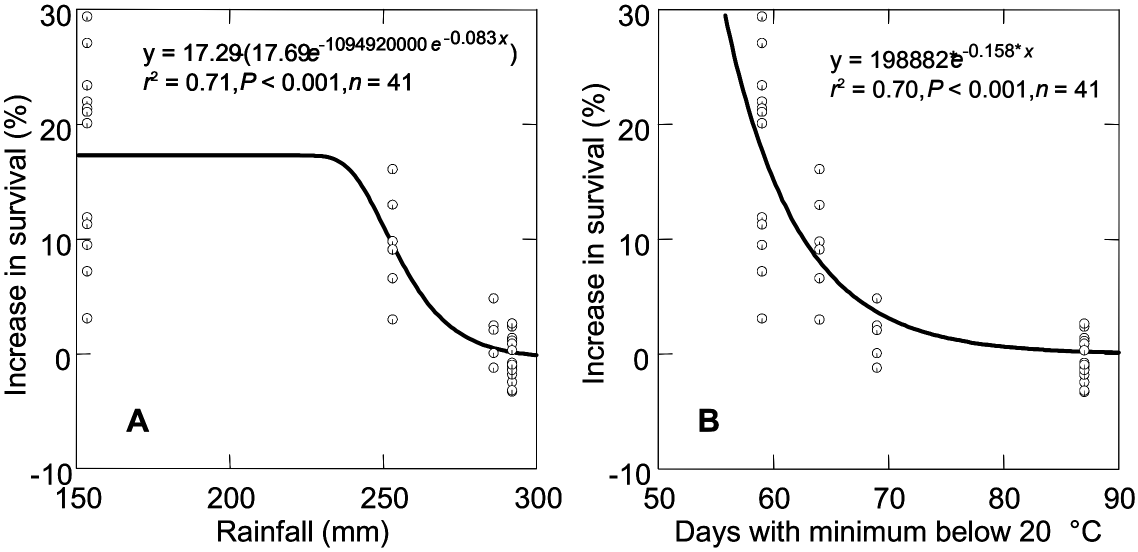

Figure 29. The benefit from delayed sowing diminishes with increases in 1) rainfall and 2) the number days with a minimum below 20C over the period from the initial sowing date to 31 December.

A benefit from delayed sowing will only be achieved if the adverse conditions that are experienced by cotton sown at the usual time can be avoided, and these conditions will only be avoided if they cease at the time of or soon after the delayed sowing date. Delaying sowing in a year where a prolonged period of cool wet conditions is expected can lead to yield loss. Therefore it is important to consider forecasts of temperature and rainfall for October and November so as to assess the risk of delaying sowing.

4.2.4 Burning cotton stubble for control Fusarium wilt

The Fusarium wilt fungus grows well on organic matter in the soil. Therefore, removing organic matter from the field by burning stubble should be an effective method for reducing the build up of the pathogen between cotton crops.

Cotton stubble was either root-cut, raked and burnt or root-cut and mulched in alternating strips the length of the field on a farm near Boggabilla in the 2004. Cotton was re-sown into the field in the 2005/6 season and assessed for total survival.

Total survival was higher in the plots where cotton stubble had been burnt compared to the mulched plots (Figure 30).

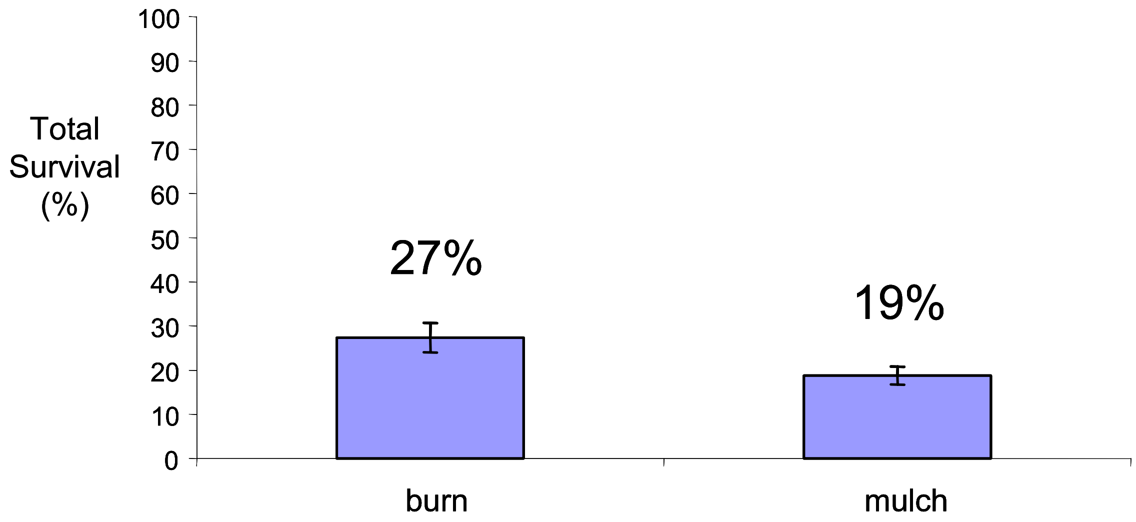

Figure 30. Mean total survival (standard error) of cotton was significantly higher where cotton stubble had been burnt as opposed to mulched at the completion of the previous crop (p=0.05).

This provides further evidence that raking and burning stubble can be an effective tool for reducing the severity of Fusarium wilt in future cotton crops. However, burning stubble removes much of the organic carbon that is returned to the soil following mulching. As organic carbon is important for maintaining soil structure, an alternative means of managing stubble in Fusarium fields is needed.

4.2.5 Cotton stubble incorporation practices for control Fusarium wilt

It has been suggested that slashing stubble and exposing it to the external environment for a prolonged period before incorporation could reduce the severity of Fusarium wilt compared to the incorporation of fresh infested material. This may be because 1) the incorporation of fresh infested material results in a rapid increase in pathogen numbers in the soil, or 2) because the exposure of infested plant parts to the external environment results in a decrease in inoculum of *Fov* and organic carbon present in those plant parts before incorporation into the soil, or 3) a combination of both.

We tested this hypothesis on a farm near Moree in 2005. Cotton was mulched and incorporated immediately, or retained on the surface for a month before incorporation.

The total survival of cotton was higher where stubble had retained on the surface for a month before being incorporated (Figure 31). We recommend this practice as a control strategy for Fusarium wilt, especially when growers do not want to burn stubble.

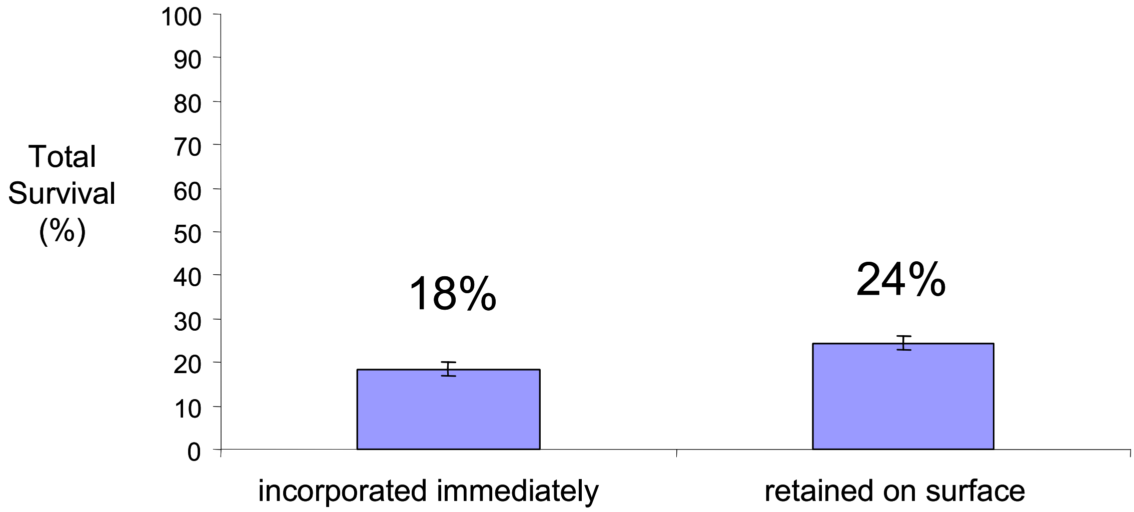

Figure 31. Mean total survival (standard error) of cotton was significantly higher where cotton stubble had been retained on the surface for 1 month as opposed to incorporated immediately at the completion of the previous crop (p=0.05).

4.2.6 Winter-crop rotation for control Fusarium wilt

The impact of winter crop rotations on the severity of Fusarium wilt is relatively unknown. We grew wheat, canola, faba bean, chick pea and safflower in 8x30m plots as part of a rotation experiment near Boggabilla in June 2004. Each crop was replicated six times in a Latin Square design. Control plots were fallowed.

The experiment was slashed in November 2004, before beds were re-listed from 40 inch beds into 1m beds, and cotton sown in October 2005. Total survival was assessed in each plot at the completion of the 2005/6 season.

Highest total survival occurred in plots where Faba bean had been grown as rotation and this was not different from bare fallow but was higher than canola, chick pea, safflower and wheat (Figure 32). Total survival in safflower plots was lower than Faba bean and bare fallow, but not lower than canola, chick pea and wheat (Figure 32).

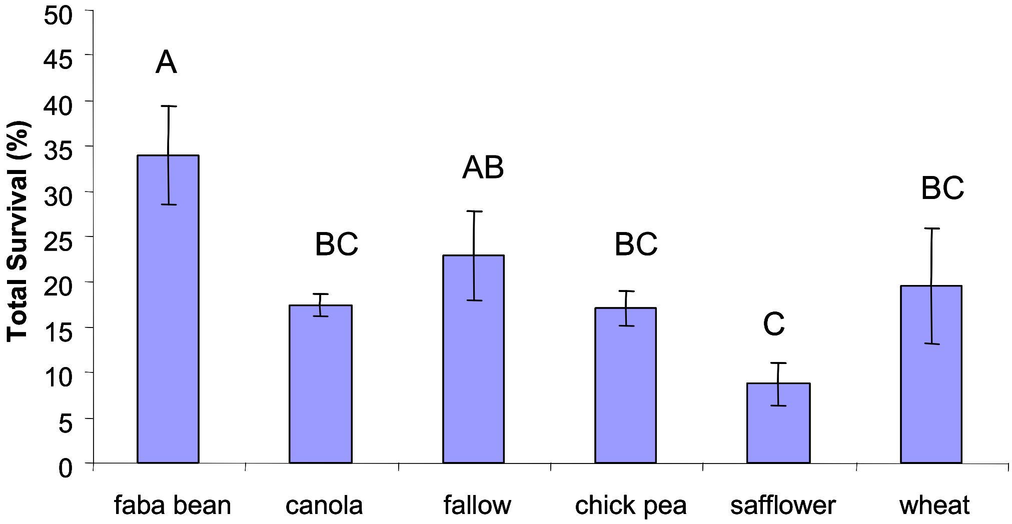

Figure 32. Total survival in cotton following rotation with faba bean, canola, chick pea, safflower, wheat and bare fallow (outliers removed). Significant differences (Fishers LSD, p=0.023) indicated by letters A,B,C.

This experiment indicates that faba bean may provide a viable alternative to bare fallow as a rotation with cotton in fields affected by Fusarium wilt. This is contrary to the results of other research, which have indicated that legumes increase the severity of Fusarium wilt. However, there was a high level of variation within the trial site, probably caused by the re-listing of beds following the winter rotation. Therefore, it would be necessary to repeat this experiment on a larger scale, ideally comparing faba bean to wheat and bare fallow, before drawing conclusions. It is anticipated that this question may be addressed as part of CRDC project DAN190C, *Survival and reproduction of the Fusarium wilt fungus*.

4.2.7 Irrigation practices at sowing for control Fusarium wilt

Anecdotal evidence suggests that the severity of Fusarium wilt is lessened by sowing on moisture when compared to watering up. This is thought to be due to the stimulation of soil microbial activity and associated competition/suppression of the Fusarium wilt fungus by microbes prior to sowing into moisture. Furthermore, cotton that is sown into moisture experiences a comparatively shorter period of cool soil temperatures compared to a crop that is watered up.

It is very difficult to test this hypothesis experimentally, especially on private farms, as cotton must be sown on the same date in the same field, but partly watered up and partly pre-irrigated, preferably in randomised plots. This was achieved by pre-irrigating a field, apart from selected runs of 16 rows, near Moree two weeks prior to sowing, then sowing the entire field, and watering up the selected runs of 16 rows.

There was no difference in the total survival of cotton that was sown on moisture compared to cotton that was watered up (Figure 33). It is possible that the heightened severity of Fusarium wilt during the 2004/5 season at Moree may have masked any impact of irrigation. Moreover, due to the difficulties involved in trying to run a complicated experiment on a commercial farm, the intended comparison of water-up versus pre-irrigate was potentially confounded by the wetting up of soil across the runs of 16 rows selected for watering-up during pre-irrigation. Seedling emergence at three weeks after sowing was significantly lower in the plots that had been pre-irrigated compared to the plots that were watered-up indicating that moisture in pre-irrigated plots was variable and that seedling disease may have been exacerbated by pre-irrigation (Figure 34).

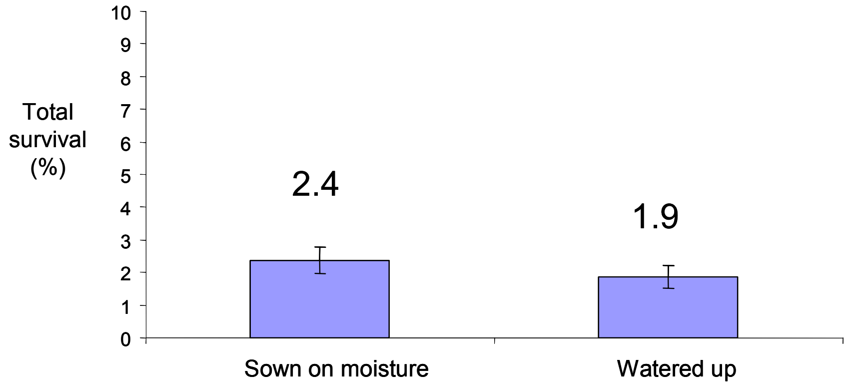

Figure 33. Mean total survival (standard error) of cotton that was either sown on moisture or watered up (p=0.355).

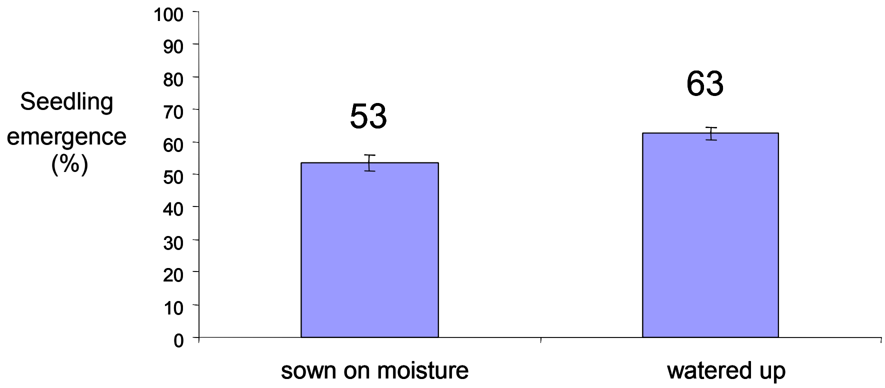

Figure 34. Seedling emergence was significantly higher in plots that had been watered up compared to plots that had been pre-irrigated (p=0.008).

4.2.8 Novel chemical products for control Fusarium wilt

SILICON

Silicon is an element that is involved in plant structural defences and that may induce systemic acquired resistance against pathogens (Fauteux *et al*., 2005). Several novel products are available that purport to reduce the severity of plant diseases. We tested three of these products, including Silvine™, Kasolv™ and Kasil™ as potential inducers of resistance against Fusarium wilt of cotton.

Silvine™ is a powder or granule that is made from crushed rock containing 4% soluble silicon present as monosilicic acid, and 5% soluble magnesium sulphate. Silvine™ powder was applied at sowing as an in-furrow spray and one week prior to sowing as granules banded below the planting line at a rate of 150kg/Ha in the following combinations: in-furrow Silvine™, in-furrow Silvine™ + granulated Silvine™, granulated Silvine™ + in-furrow water, and in-furrow water. Silvine™ applied as an in-furrow spray, as granules, and as a combination of both at 150kg/Ha did not influence the severity of Fusarium wilt (Figure 35).

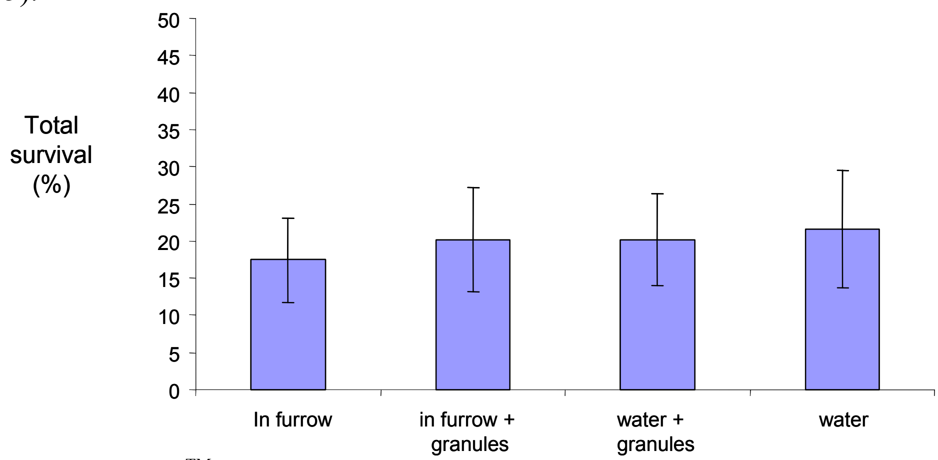

Figure 35. Silvine™ applied as an in-furrow spray and granules at the rate of 150kg/Ha of Silvine powder did not affect the severity of Fusarium wilt (p=0.97).

We also tested the potential for Silvine™ applied to the seed coating to induce resistance against Fusarium wilt. Cotton seed was treated with 1) powdered Silvine™, 2) the soluble fraction of powdered Silvine™ when mixed with water, and 3) a control of $MgSO_4$. An $MgSO_4$ control was used along with an untreated control to account for the high levels of $MgSO_4$ naturally present in Silvine™. Powdered Silvine™ was applied at a rate of 1.5g/kg seed. The soluble fraction of Silvine™ after dissolving 10g in 100ml of water was applied at a rate of 6ml/kg seed. A stock solution of $MgSO_4$ containing 10.238g of $MgSO_4$ per 100ml water was added to seed at a rate of 6ml/kg. The treated seed was sown in a box trial near Moree but there were no significant differences among the treatments (Figure 36).

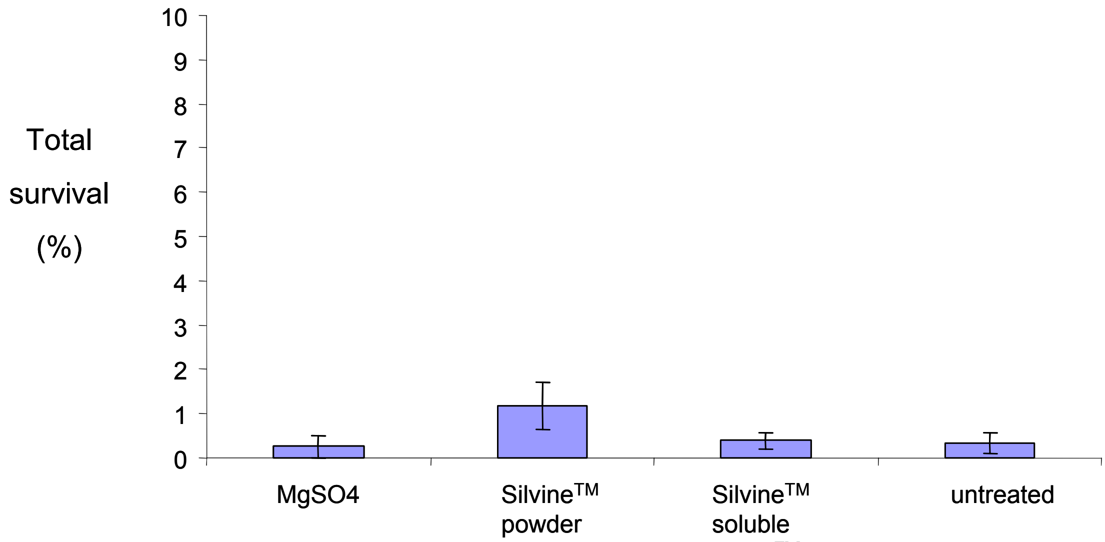

Figure 36. Mean (standard error) total survival of cotton treated with Silvine™ (p=0.20).

Silvine™ applied at the specified rates as a seed treatment did not reduce the severity of Fusarium wilt (Figure 35).

Another more commonly available form of soluble silicon is potassium silicate, commercially available as Kasolv™ (52% available $Si0_2$) powder and Kasil™ (26.5% available $Si0_2$) liquid. We tested both of these products, banding Kasolv™ 10cm below the planting line one week before sowing, and applying Kasil™ as a foliar spray approximately 2, 4 and 6 weeks after sowing in the following combinations: Kasolv™ banded, Kasolv™ banded + Kasil™ foliar spray, Kasil™ foliar spray, and no treatment. Kasolv™ was applied at 75kg/Ha and Kasil™ at 2.5L/Ha. There was no difference in the total survival of cotton that was treated with Kasolv™ and Kasil™ at the specified rates and untreated cotton (Figure 37).

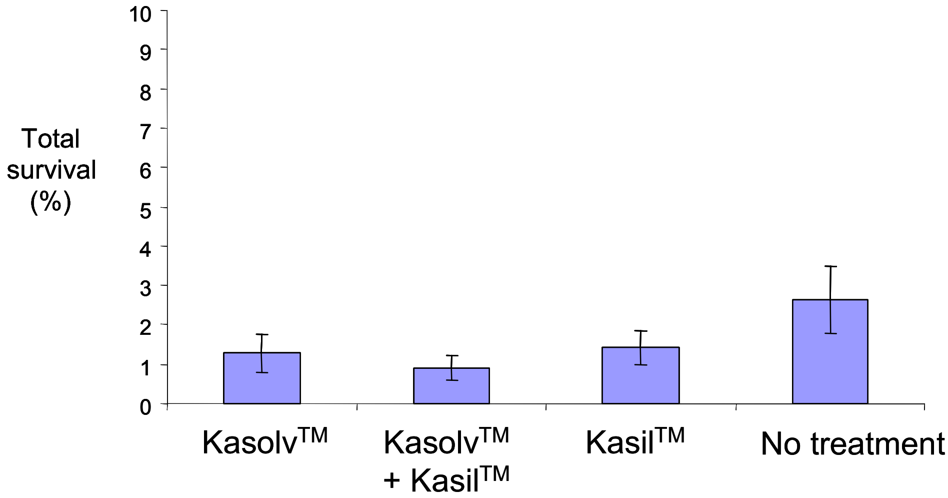

Figure 37. Mean (standard error) total survival of cotton treated with Kasolv™ and Kasil™ (p=0.08).

Our studies indicate that silicon applied as potassium silicate and as silicilic acid at the rates used in these experiments does not influence the severity of Fusarium wilt. Other studies have shown that various factors including phosphorous availability and pH can influence the uptake and availability of silicon and these factors may have confounded our experiments. There is a vast body of literature that suggests that silicon can be successfully used to control plant diseases by inducing plant defences and so further research in this area is warranted.

Reference: F Fauteux , W Remus-Borel, JG Menzies, RR Belanger (**2005**). Silicon and plant disease resistance against pathogenic fungi. *FEMS Microbiology Letters*. **249**, pp1-6.

PHYTOHORMONES

Gibberellic acid is a plant growth regulating hormone responsible for stem elongation and germination. Indole-butyric acid is also known as auxin and is a hormone responsible for many aspects of plant growth including cell elongation, apical dominance and ethylene synthesis. Cytokinins are plant hormones that regulate cell division, differentiation and other growth related processes.

We were supplied with a novel product (Early Harvest™) containing all three of the aforementioned plant hormones at unspecified rates. Early Harvest™ was applied as an in-furrow spray at sowing and controlled using water. Stand was assessed at approximately 3 and 4 weeks after sowing and total survival assessed at the end of the season.

Early Harvest™ was associated with higher germination rates at 3 weeks after sowing ($p=0.045$) but not at 4 weeks after sowing ($p=0.453$) indicating that treated seed may have germinated faster than un-treated seed (Figure 38). Early Harvest™ did not reduce the severity of Fusarium wilt ($p=0.455$).

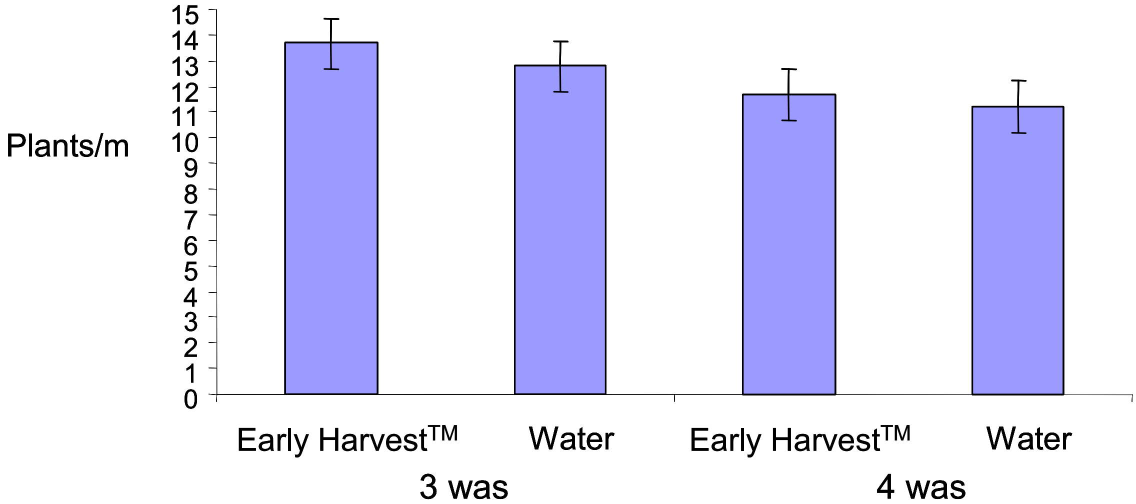

Figure 38. Mean (standard error) plants per metre at 3 weeks and 4 weeks after sowing following treatment of seed with Early Harvest™ or water.

5. OUTCOMES

5.1 Contribution of the projects outputs to the planned outcomes identified in the project application

ANTICIPATED OUTCOME 1

Industry: *Ensure adequate response to the disease in cooler regions.*

Science: *Quantify increase in disease severity and distribution.*

We were able to confirm the link between cool wet early season conditions and increased severity of Fusarium wilt. As southern cotton growing regions including the Macquarie, Lachlan and Murrumbidgee valleys experience cooler starts to the season, we expect that disease severity will be higher in these regions compared to areas further north.

Prolonged drought conditions and a consequent lack of experimental sites prevented us from completing any experiments in the southern regions. However, the results and recommendations of this research are applicable to the cooler regions. An adequate response to Fusarium wilt in the cooler regions of the industry should consist of:

- Delaying sowing to avoid the cool wet early season conditions that favour disease.
- Minimising the movement of trash around the farm during irrigations.
- Burning stubble, or retaining mulched stubble on the surface for a prolonged period before turning in, following the cotton crop.
- Maintaining good farm hygiene practices, especially in the areas where the Fusarium wilt pathogen is not yet present.

ANTICIPATED OUTCOME 2

Industry: *Integrated crop management to minimise/avoid the impact of severity factors according to infection progress within the crop.*

Science: *Determine if late-season symptoms result from latent infections that occur early-season. Measure impact of stresses (eg. irrigation, fruit set, weather events) on disease. Greater understanding of resistance.*

Delaying sowing provides an effective integrated crop management strategy to avoid the cool wet conditions that favour disease and thereby reduce the severity of Fusarium wilt. Late season symptoms result from progressive infections that are initiated early in the season. The severity of Fusarium wilt is not exacerbated by heavy boll loads. High rainfall events may be associated with the appearance external symptoms later in the season, especially in years where early season conditions are not conducive to the expression of external symptoms.

ANTICIPATED OUTCOMES 3 AND 4

Industry: *Minimise effects of irrigation on disease; Methods to minimise movement of inoculum within and between fields.*

Science: *Understanding of interaction between irrigation and climate etc; Greater understanding of mechanisms and rate of pathogen dispersal in water.*

Our studies did not link the method of initial irrigation, i.e. pre-irrigate versus water-up, with differences in disease severity. However, other studies have found that disease severity is lessened by pre-irrigating or sowing on moisture when compared to watering-up. It was not practical to complete experiments examining the impact of the number of irrigations on disease severity because experiments could only be completed on commercial farms. However, we demonstrated the potential for mass transport of the pathogen around the farm during irrigations and identified that the control of floating trash is the most effective means of minimising this process.

ANTICIPATED OUTCOME 5

Industry: *Improved integrated disease management that accounts for the impact of other pathogens on Fusarium wilt.*

Science: *Greater understanding of pathogen interactions.*

We demonstrated that there is no interaction between black root rot and Fusarium wilt. Therefore, there is no need to take such interactions into account when considering new IDM strategies. However, we know that many IDM strategies for black root rot including crop rotations and bio-fumigation can increase the severity of Fusarium wilt, so caution is required when both pathogens are present in the same field. Delaying sowing provides a solution for minimising the impact of both diseases.

ANTICIPATED OUTCOME 6

Industry: *Prediction of likelihood of disease in different soils.*

Science: *Indicate factors involved in disease suppression.*

We measured disease in several transects that crossed different soil types and did not find any correlation between soil colour and disease severity. However, we were unable to have soil samples chemically and biologically characterised, so cannot draw any correlation between abiotic soil factors and disease severity. We did not study the potential for biological suppression because we were unable to isolate from soil that was known to be contaminated with the Fusarium wilt fungus for a large part of the project.

ANTICIPATED OUTCOME 7

Industry: *Recommend sowing dates to minimise disease; optimum bed configuration/shape; cover crop options.*

Science: *Greater understanding of the effect of the soil environment on disease severity.*

We recommend that growers plant as late as is practicably as possible within the planting window. The modification of beds and the laying down of plastic mulch does not impact on disease severity. We did not study cover crops.

ANTICIPATED OUTCOME 8

Industry: *Practices to minimise the survival and dispersal of the fungus between crops.*

Science: *Greater understanding of survival strategy of the fungus and potential for dispersal.*

The most effective means of minimising the survival and dispersal of the fungus between crops is to burn stubble or retain stubble on the surface for a prolonged period (≈1month) before incorporating into the soil. We were unable to successfully quantify disease carry-over in plant parts.

NON-ANTICIPATED OUTCOMES:

- Confidence that the use of glyphosate both on and off-label does not increase the severity of Fusarium wilt.

- Confidence that heavy boll loads do not increase the severity of Fusarium wilt.
- An understanding of the role of early season environmental conditions, particularly spring rainfall, in influencing the severity of Fusarium wilt.
- An understanding of the impact of rainfall and cool temperatures on the effectiveness of delayed sowing as a tool for minimising the impact of Fusarium wilt.
- Further support that Bion® is an effective tool for controlling Fusarium wilt.
- Further confirmation that there is no relationship between nematodes and wilting diseases of cotton in Australia.
- Early indications that with further research faba bean may prove to be an acceptable rotation in fields with Fusarium wilt.
- Testing of several novel products, none of which provided any control of Fusarium wilt.

5.2 Intellectual property

5.2.1 Technical advances

A design concept for a trash retaining drop box (Figure 2) was developed after consideration of the role of floating crop residues in movement of the pathogen. After consultation with the CRDC, it was decided that this concept should be made available to the cotton industry, rather than proceeding along a course patenting or design registration.

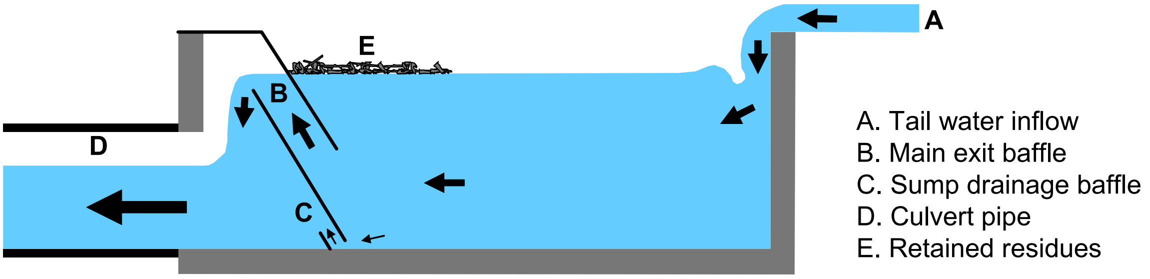

Figure 39. Design concept for crop-residue-trapping culvert box for irrigated fields. Arrows represent volume and direction of flow. The box is constructed to a length that will ensure that the main exit baffle has a cross-sectional area sufficiently larger than the area of the culvert pipe to prevent flows through the baffles being strong enough to suck floating residues through the system. The drainage baffle at the base allows the box to empty for later removal of residues and soil. The box is sloped at one end and wide enough to allow residue removal with a front end loader.

5.2.2 Other information

Hand planting using one-metre dibbers was proven to be a useful technique for small plot experiments in the field where and even distribution of seed is required. This method has already been utilised by researchers at the University of Melbourne in the 2006/07 season (see Section 3).

We also developed an effective method to conduct pot experiments in the field for inoculation studies, also described in Section 3..

5.2.3 Changes to the Intellectual Property Register

None anticipated.

6. Conclusion

We anticipate that this research will result in a greater awareness among growers of the impact of cool, wet early season conditions on the severity of Fusarium wilt. Practically, we expect to see sowing delayed until mid-late October; the burning of or retention of stubble on the surface for prolonged periods prior to turning in; improved control of floating trash during irrigations; and more widespread use of Bion® as a disease management tool.

Take home messages include:

1. The biggest single factor to increase the severity of Fusarium wilt is early season rainfall. When cumulative rainfall in October and November is high, disease severity will be high and *vis-versa*.
2. Delay sowing to avoid the conditions that favour disease. Sowing after mid-October will usually result in the avoidance of cool, wet conditions that favour disease. However, delaying sowing may not be effective in years when disease-conducive conditions cannot be avoided due to a prolonged cool, wet start.
3. Late season disease symptoms are the results of a progressive infection that begins early in Spring. Moreover, most plants that are infected before December 31, probably in the first 8 weeks after sowing.
4. The appearance of external symptoms lags behind the incidence of internal infection. The true incidence of Fusarium wilt can be masked by late onset of external symptoms.
5. High rainfall later in the season may induce the appearance of external symptoms, especially in years when early season conditions are not conducive to the appearance of external symptoms.
6. The use of glyphosate does not increase the severity of Fusarium wilt.
7. Heavy boll loads do not increase the severity of Fusarium wilt.
8. The Fusarium wilt fungus is carried in large numbers on floating rafts of trash that move around the farm during irrigations. The best means of minimising the spread of the fungus around the farm is to remove floating trash from the irrigation system and/or stop the trash from leaving the field using a trash-retaining drop box.
9. The passage of irrigation water through a storage dam removes most of the Fusarium wilt pathogen from the water. Therefore, when water is to be cycled from field to field, it should first be passaged though a storage dam or settling pond.
10. There is no interaction between black root rot and Fusarium wilt.
11. Farmers should burn stubble or mulch stubble and leave it on the surface for up to a month before incorporating. This will reduce the severity of Fusarium wilt in the following crop.

7. EXTENSION OPPORTUNITIES

7.1 Plan for activities and other steps

7.1.1 Further develop or exploit the project technology

The design concept for a trash retaining drop box will require engineering development and testing in the field.

7.1.2 Future presentation and dissemination of outcomes

We anticipate the continued extension of the project outcomes through field days and extension articles. It is also anticipated that revised disease management guidelines, incorporating results from CRDC projects DAN153C, DAN154C, DAN176C and DAN177C, will be prepared and endorsed at the next meeting of the Fusarium wilt coordination committee. Changes (highlighted in **bold**) to the IDM guidelines are recommended based on the results of this project:

PLANNING
- If your farm is free from this disease, try to keep it that way! – See 'Farm Hygiene'; 'Come clean-Go clean'
- Use the most resistant cotton varieties available, especially if Fov occurs in your district
- Ensure that seed is treated (eg. Quintozene and Apron)
- **Design or modify irrigation reticulation systems to return water from infested fields directly to the storage used for their supply or install settling ponds before on-flow to other fields.**

PLANTING
- Plant **as late as possible, within the planting window, to minimise exposure to cool wet** conditions **that favour disease**.

IN CROP
- Control weeds during and between crops
- Avoid mechanical inter-row cultivations if possible during the crop (eg. use shielded sprayer to control weeds)
- Manage the crop to avoid stresses such as waterlogging, over-fertilisation, root damage
- Maintain farm hygiene and awareness of incoming traffic through the season
- Conduct regular inspections to allow early detection of any suspicious looking plants. If any are found, send immediately to QDPI for analysis. Educate farm workers what to look for and encourage reporting
- If Fov is confirmed, rogue and burn for small patches
- Solarisation may also be an appropriate treatment for small affected patches detected early in the season.
- Isolate affected areas from irrigation flows and traffic to avoid spreading the fungus **or m**inimise tail-water **exiting** from those areas.
- **Minimise the exit of floating residues, of all crops, in tail water in affected fields eg. use trash-racks or baffles in drop boxes** (see example in Figure 2).

LATE SEASON
- Ensure that harvesting machinery is clean
- If Fov has been confirmed on your farm notify all relevant parties so that measures can be taken to avoid spreading the fungus to other fields on your property and to other regions

AFTER HARVEST
- After harvest, retain crop residues on the surface for as long as possible before incorporation

ROTATIONS
- Selection and management of rotation crops is important as the pathogen is able to survive in association with the residues of non host crops. **Bare fallows will always result in lower disease severity in the following crop**.
- Summer flooding, where possible, has been shown to be effective but does not eradicate the pathogen.

7.1.3 Future research

Acibenzolar-S-methyl requires further evaluation to its potential for activation of resistance in cotton against Fusarium wilt at low levels of the pathogen. A long-term experiment with large plots (data not presented) was commenced in this project and it is anticipate that this experiment will be continued as part of the proposed CRDC project *Diseases of cotton IX* commencing in July 2007.

The impact of crop rotations on the severity of Fusarium wilt requires further evaluation before firm recommendations can be made. Future research should evaluate the potential for Faba bean as a rotation crop. Further research is required to quantify the carry-over of disease in plant parts, including the rhizsosphere of different cotton varieties and rotation crops, and it is anticipated that this question will be partly addressed in CRDC project DAN190C *Survival and reproduction of the Fusarium wilt fungus*. The impact of soil-type and the potential for biological suppression remains unknown.

7.1.4 Publication list

Anderson C, Nehl D (2006) Research finds *Fusarium* floats. *Australian Cottongrower* **27**, 10-12.

Anderson CMT, Nehl DB, Allen S (2004) Factors affecting the severity of Fusarium wilt: environmental aspects of the disease. In 'Proceedings of the 12th Australian Cotton Conference'. Broadbeach, Australia. (Australian Cotton Growers Research Association)

Anderson CM, Knox OGG, Nehl DB, Allen SJ (2005) First record of the root lesion nematode *Helicotylenchus dihystera* in cotton in Australia. In '15th Biennial Australasian Plant Pathology Society Conference Handbook'. Geelong p. 108. (Australasian Plant Pathology Society) (see Appendix I)

Anderson CM, Nehl DB, Allen SJ (2005) Delayed sowing as a tool for minimising the impact of Fusarium wilt on cotton. In '15th Biennial Australasian Plant Pathology Society Conference Handbook'. Geelong p. 351. (Australasian Plant Pathology Society)

Knox OGG, Anderson CMT, Allen SJ, Nehl DB (2005) *Helicotylenchus dihystera* in Australian cotton roots. *Australasian Plant Pathology* **35**, 287-288.

Nehl DB, Mondal AH, Anderson CM (2005) Control of black root rot in cotton by delayed sowing. In '15th Biennial Australasian Plant Pathology Society Conference Handbook'. Geelong p. 206. (Australasian Plant Pathology Society)

Anderson CMT, Nehl DB (2006) Delayed sowing as a best-bet approach to minimise the impacts of Fusarium wilt. In 'Proceedings of the 13th Australian Cotton Conference'. Broadbeach, Australia. (Australian Cotton Growers Research Association)

Posters at the 2006 Cotton Conference: “Delayed sowing to minimise the impact of Fusarium wilt”, “Cotton trash management to minimise the impact of Fusarium wilt”, and “Nematodes in cotton” Trial results were submitted to the “2004/5/6 Gwydir Valley field trials results” book compiled by Julie O’Halloran. We intend to publish a paper in 2007 reporting the relationship between Spring rainfall and Fusarium wilt in Australia.

7.1.5 On-line resources

It is anticipated that the background paper on delayed sowing from the 13^{th} Australian Cotton Conference will be posted on the Cotton CRC website. Journal articles are available on-line at:

http://www.publish.csiro.au/paper/AP06010.htm

http://www.publish.csiro.au/paper/DN06018.htm

APPENDIX

Publications in scientific journals.

CSIRO PUBLISHING
www.publish.csiro.au/journals/app *Australasian Plant Pathology*, 2006, **35**, 287–288

DISEASE NOTES OR NEW RECORDS

Helicotylenchus dihystera in Australian cotton roots

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Abstract. We report for the first time in Australia, observation of the plant-parasitic nematode *Helicotylenchus dihystera* within roots of cotton (*Gossipium hirsutum*). The incidence of this and other nematodes in cotton roots did not correlate with the incidence of Verticillium wilt that was present within the same crops. The lack of reports of plant-parasitic nematodes in cotton in Australia has previously been attributed to the high clay content of the soils used to grow cotton. Our observations indicate that these heavy soils do not preclude the survival of plant-parasitic nematodes on cotton and reinforce the importance of quarantine and farm hygiene practices.

Synergistic interactions between wilting diseases of cotton and root lesion nematodes have been reported in cotton in the southern United States of America and India (Gazaway and Mclean 2003; Prasad and Padaganur 1980). In Australia, Verticillium and Fusarium wilt are important diseases of cotton, but there is very little information on the abundance, incidence or ecology of nematodes in the Australian cotton production system. To investigate whether there is potential for plant-parasitic nematodes to interact with Verticillium wilt, we sampled roots from four cotton crops in the Namoi Valley that had presented with severe Verticillium wilt symptoms during the summer of 2004–05.

Two of the sites were in the lower Namoi valley, Narrabri Shire, NSW (30°26.134′S 149°58.446′E; 30°06.640′S 149°33.666′E, GPS Datum AGD 66) and the other two were in the Upper Namoi Valley, Gunnedah Shire (30°46.438′S 150°07.128′E; 31°11.725′S 150°25.909′E). Using previous disease survey data, collected by NSW Department of Primary Industries, we identified patches in the crop at each site that were either severely affected by Verticillium wilt or free of disease. We also used a stem cut method to confirm the presence or absence of Verticillium wilt within these patches. At each sampled point, stems of 10 plants were cut below the first node and disease severity rated on a scale of 0–4; zero being disease free, 1, 2, 3 and 4 being <5%, 5 to <20%, 20 to <40% and 40% or more, respectively, of the stem cross sectional area displaying vascular discoloration. Three soil cores (15 cm diameter × 20 cm in depth) were then taken from between the 10 cut cotton plants and returned to the laboratory. Live roots were extracted from about 1 kg of soil in the ACRI laboratory using the following technique. Soil was soaked in a Calgon solution (0.2% w/v, a.i. sodium hexametaphosphate) for 2 h, then sieved and washed under tap water to facilitate the recovery of roots trapped on the sieves. Approximately 10 g of fresh roots from each sample were sent to Biological Crop Protection (Moggill, Queensland) for nematode extraction, quantification and trophic group identification. The remainder of the recovered roots were cleared, stained with acid fuchsin (Byrd *et al*. 1983), and observed under a dissecting microscope at 120× magnification for the presence of nematodes. Stained nematodes within the roots were counted.

Disease severity in cotton stems correlated well with the choice of sampling positions as either with, or without, Verticillium wilt ($r = 0.90$, $n = 36$). There was no correlation between the total number of nematodes in cotton roots and either disease incidence or mean severity (coefficients of 0.03 and 0.15, respectively). In addition, identification of nematodes recovered from the roots of plants between the lower and upper Namoi Valley were distinctly different, however, levels of Verticillium disease severity were the same regardless of the nematode species recovered.

Nematode populations recovered from cotton roots at both farms in the upper Namoi sites contained an average of 87% plant-parasitic nematodes. The predominant plant-parasitic nematode from these roots was the spiral nematode

 10.1071/AP06010 0815-3191/06/020287

Helicotylenchus dihystera (identified by Dr J. Nobbs, South Australian Research and Development Institute). *H. dihystera* has been recorded on cotton in the USA, but is not considered to be a major pathogen (Johnson *et al*. 2000). The presence of this nematode in Australian cotton has not been previously reported and its potential to either cause disease or interact with pathogens of cotton under Australian conditions is unknown. In contrast, nematodes recovered at both farms in the lower Namoi from cotton roots were predominantly bacteriophagous (*Rhabditidea*) and mycophagous (*Aphelenchidea*). Only 1% of the total number of nematodes extracted from these roots were plant parasitic, belonging to the genus *Pratylenchus*. The high proportion of free living nematodes extracted from plant roots was considered unusual, but nematode entry may have been assisted by either late season root senescence or damage caused by the shrinking and swelling of the heavy clay soils. Observations from all four sites were confirmed by partial dissection of the stained root samples, allowing nematode identification into functional groups based on their anterior morphology. While no correlation was seen between nematode levels and disease severity, observations were made towards the end of the growing season. To determine if interactions between nematodes and Verticillium may be occurring, further investigation is required both at additional sampling times and to assess whether populations of plant parasitic or free living nematodes can affect disease severity.

The occurrence of high numbers of *H. dihystera* within the roots of cotton from the upper Namoi sites did not appear to be associated with Verticillium wilt. However, the presence of these nematodes should not be ignored. The reniform nematode, *Rotylenchulus reniformis*, was first discovered in cotton growing regions of Alabama in 1958 but did not become a recognised pest until 1986. As of 2002, *R. reniformis* was above economic threshold levels in 47% of Alabama cotton fields (Gazaway and Mclean 2003). In the light of the USA experience, ongoing monitoring and investigation of nematodes in Australian cotton would be prudent. The most likely means of dispersal of nematodes between farms is in soil on machinery, tools and vehicles. In recent years, the Australian cotton industry has promoted the use of farm hygiene to restrict the spread of fungal pathogens and these practices will also assist with nematode control if these pathogens are found to be of economic concern. Our observations reinforce the need for such practices to continue.

Acknowledgements

This work was conducted under the support of CSIRO Land and Water, CSIRO Plant Industries and the NSW Department of Primary Industries through projects funded by the Cotton Research and Development Corporation.

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Prasad KSK, Padaganur GM (1980) Observations on the association of *Rotylenchulus reniformis* with Verticillium wilt of cotton. *Indian Journal of Nematology* **10**, 91–92.

Accepted 26 November 2005

CSIRO PUBLISHING

www.publish.csiro.au/journals/apdn *Australasian Plant Disease Notes*, 2006, **1**, 47–48

Observation of *Tylenchorhynchus ewingi* in association with cotton soils in Australia

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Abstract. We report for the first time the recovery of the plant parasitic nematode *Tylenchorhynchus ewingi* Hooper 1959 from field soils used to grow irrigated cotton (*Gossipium hirsutum*) in New South Wales, Australia. The observation was made while investigating the possible existence of an association between nematodes and Verticillium wilt of cotton. No interaction was evident between nematode population sizes, species composition or the number of nematodes in cotton roots and the severity of Verticillium wilt. The samples collected over the course of the investigation contained few plant parasitic nematodes. Although the plant parasitic nematode population was small it was comprised almost entirely of the stunt nematode, *Tylenchorhynchus ewingi*. This is the first observation of this nematode in an Australian cotton production system.

Verticillium wilt, caused by *Verticillium dahliae*, is an economically important fungal disease of cotton in Australia, particularly in the Namoi valley, New South Wales (NSW). Interactions between vascular wilts of cotton and root lesion nematodes have long been established in the United States of America (USA) and India (Prasad and Padaganur 1980; Gazaway and Mclean 2003), but there is little to no knowledge of nematodes in Australian cotton production systems. We undertook a pilot study in the Namoi Valley during the 2004–05 cotton season to establish if there is an association between severity of Verticillium wilt of cotton and occurrence of plant parasitic nematodes (Knox *et al*. 2006). That study resulted in the first observation of the spiral nematode, *Helicotylenchus dihystera* (Cobb, 1891) Sher, 1961 in cotton roots in Australia, but nematode frequency was not correlated with the incidence of Verticillium wilt. However, sampling was conducted late in the growing season and any interaction between the nematodes and *V. dahliae* during earlier stages of disease development would not have been observed. To establish if earlier season interactions were occurring between nematodes and *Verticillium*, observations were taken three times over the course of the following cotton season (2005–06) and reported herewith.

The two field locations investigated in the 2004–05 season were sampled again in the 2005–06 season; one in the lower Namoi valley (30°06.640S; 149°33.666E, GPS Datum AGD 66) and the other in the upper Namoi Valley (E; 31°11.725S; 150°25.909E). Six sample sites in each field were visited at 2 days before planting, and at 64 and 146 days after planting. GPS was used to return to the same sample sites as those assessed in the previous season. Incidence of Verticillium wilt within the cotton crop was assessed at 64 and 146 days after planting. This was done by cutting the stems of 10 plants over 1 m at each sample site and assessing the cut-stem tissue for discolouration and necrosis on a scale of 0–4 as previously described (Knox *et al*. 2006). Soil samples were collected at each sample point by taking three cores (15 cm diameter × 20 cm deep) between the assessed plant stand on the planting hill. The three cores were pooled and returned to the laboratory for assessment. Live cotton roots were extracted from 1 kg of each soil sample, cleared and stained with acid fuchsin. Nematode occurrence and frequency in the stained roots was assessed under the dissecting microscope (× 120 magnification). Soil (150 g) from each sample was sent to Biological Crop Protection (Moggill, Queensland) where nematodes were extracted in Whitehead trays for trophic group identification.

Helicotylenchus dihystera was not observed in any of the Whitehead tray assayed soil samples, even though it had been isolated from the roots of cotton at the upper Namoi site in the previous season (Knox *et al*. 2006). Plant parasitic nematodes accounted for only 3% of the total nematode population recovered from soil and was mostly comprised of a stunt nematode, later identified as *Tylenchorhynchus ewingi*

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(Dr J. Nobbs, South Australian Research and Development Institute). Of the 24 soil samples assessed, 21 were found to contain stunt nematodes, with its abundance accounting for 98% of the total plant parasitic nematodes recovered. When the samples were divided into the upper and lower regions of the Namoi further differences were observed. In the lower Namoi, 94% of soil samples contained *T. ewingi* (mean of 18 recovered per 150 g of soil), whilst for the upper Namoi the value was 75% (mean of three *T. ewingi* per 150 g).

Verticillium wilt was not observed at the lower Namoi field location in the 2005–06 crop, nor in the previous crop. At the upper Namoi location Verticillium wilt was not observed until the third sampling at 146 days post sowing and occurred at five of the six sampling sites within the field with a mean disease severity of 2.5 (disease severity scale of 0–4). This compared with a mean severity rating of 1.3 for the previous season with disease occurrence at four of six sample sites.

The frequency of nematodes within the stained cotton roots was lower than in the 2004–05 season samples. Nematodes were only observed in root material from the third and final sampling. An average of 1.4 g of fresh root material was recovered from each of the 1 kg samples with approximately one nematode observed in every 8 g of root material. Partial dissection of the stained root samples revealed that, based on anterior morphology, 45% of these endophytic nematodes were the spiral nematode *H. dihystera*, with the remainder belonging to bacteriophagous and mycophagous functional groups. Further identification was not possible due to the tissue fixation and preparation methodology utilised in this study. Nematodes recovered from both soil and root samples, at either field location, did not correlate with the incidence or severity of Verticillium wilt, suggesting that an interaction between Verticillium wilt and nematodes within cotton farming systems in the Namoi valley is unlikely.

The decrease in nematode numbers observed in cotton roots between the two seasons is consistent with the successive use of the biocide Aldicarb (Temik, Bayer Crop Science) at commercial rates of 7 kg/ha (Spurr and Sousa 1974). The wheat rotations, which preceded the 2004–05 cotton crops, did not have Aldicarb applied and (are likely to have) supported greater nematode populations. In cotton, Aldicarb application provides systemic control of the early season sucking pests, true wireworm (*Agrypnus variabilis*) and false wireworm (*Pterohelaeus darlingensis*). The impact of these sucking pests within the cotton industry has increased with the introduction of Bt insecticidal cotton varieties, probably resulting from decreased competition with other pests. This shift in the farming system has meant that application of Aldicarb at sowing is now commonplace and is likely to be responsible for the overall decline in nematode populations and their isolation only in the latter part of the growing season (i.e. 146 day sampling).

Our observations of *T. ewingi* represent the first record of this nematode in association with cotton farming systems in Australia. Previously *T. ewingi* has only been observed in association with wheat in Australia (McLeod *et al*. 1994). The system we investigated had been under monocultures of cotton for up to 28 months since the haying-off of the previous wheat crop, suggesting that *T. ewingi* is surviving on cotton despite not being observed within the recovered root material. In the USA, stunt nematodes have been found in association with cotton (Gazaway and Mclean 2003), but are considered to be either weak or non-pathogenic. They have, however, been implicated in cereal crop losses, particularly in the low rainfall regions of Oregon. The results reported in this study neither indicated the existence of an interaction between nematodes and Verticillium wilt nor the occurrence of large populations of potentially cotton pathogenic nematodes. While Australian cropping systems and soils might differ from those in other countries, observations of previously unrecognised plant parasitic nematodes serve as a reminder that we still know very little about our soil microfauna and continue to highlight the importance of farm hygiene practices to restrict the spread of potential pathogens.

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