INTERIM REPORT

2010 to 2012

Diseases of Cotton X

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Contents

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Signature of Research Provider Representative:

Intellectual Property developed within the projects.

No new intellectual property was developed within this project.

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The principle researcher also extends gratitude to Cotton Catchment Communities CRC, Cotton Research and Development Corporation and Cotton Australia, who provided substantial investment in the professional development of the principle researcher, and in the project.

Background

Australian cotton may be infected by a range of diseases that continue to threaten sustainability. Cotton production is also threatened by possible incursions of exotic diseases and potentially new diseases evolving from native population strains. The Diseases of Cotton projects have been conducted since 1984 playing an important role in indicating long term trends in disease incidence and severity. The result of this project adds to the existing long term data set and is improving the knowledge of diseases of cotton.

Three of the most economically damaging and recalcitrant diseases affecting cotton in New South Wales are Fusarium Wilt, Verticillium Wilt and black root rot. Soil-borne diseases are difficult to treat with traditional methods and for practical purposes are impossible to eradicate. However by gaining a better understanding of the pathogens ecology or life cycle, management practices can be modified in an economically and environmentally sustainable way to reduce disease impact.

Previous work has indicated that the soil type cotton is grown in has an impact on the disease expression of these three fungi. Soil borne fungal plant pathogens can overwinter as either saprophytes or is dormant structures such as chlamydospores and sclerotia. Having these mechanisms means the fungi are difficult to control and virtually impossible to eradicate. All three fungi are thought to be able to survive for long periods, for example *Verticillium dahliae* has been reported to survive for at least 10 years as micro-sclerotia. Survival however is mitigated by number of environmental factors such as soil type. There is little information available on the survival of these fungi in different soil types grown under cotton. The proposed research would look at the survival of these pathogens in different soil types. This fundamental information on the ecology of the pathogens is vital for making informed management decisions on crop rotation.

The increase in the price of fertilisers as well as a growing understanding for the need of environmentally sustainable agriculture has meant a growing interest in the use of nitrogen fixing crop species for crop rotation with cotton. It is one of the aims of this proposed research to investigate the potential of alternative crop plant species.

Crop rotation has been shown to affect the chemistry, physics and biology of the soil. Indeed crop rotation is an accepted way of potentially improving soil health and disease suppression. Work is currently under way at Queensland DPI looking at the effect of crop rotation on Fusarium wilt in cotton. However, there is little information on the interaction between crops and Verticillium wilt and black root rot.

The research in this project was designed to continue to monitor disease incidence and severity across production areas, further development of disease management strategies and transfer this information to growers for improved control of cotton diseases.

Objectives and extent to which they have been achieved

Objective 1 - Continue surveillance of endemic and exotic plant pathogen, monitoring incidence and severity of diseases of cotton and recording the absence of exotic diseases.

Disease surveys were conducted in November/December and March/April each year of the project including all production areas of NSW. Survey data was added to the existing database. Analysis included regional means and trends in distribution and or severity of seedling disease, black root rot, Alternaria leaf spot, Fusarium wilt, Verticillium wilt, boll rots and cotton bunchy top. The trends in the long term data highlight the changing status of diseases over time providing insights into possible factors affecting distribution and severity. Results reported in Chapter 1.

Objective 2 – Continue to evaluate existing and novel fungicide treatments for seedling disease and black root rot.

Seed treatments were evaluated each year of the project including evaluating potential of novel fungicides for effectiveness on reducing seedling mortality. The effect of seed treatments and planting dates was evaluated each year. Results of these seed treatment trials are reported in Chapter 2 and are available for growers in order to help with management decisions.

Objective 3 – Continue to evaluate IDM strategies for the control of black root rot including crop rotation and soil amendments.

A long term biofumigation trial and a long term rotation trial were carried out at ACRI, Narrabri. These trials are designed to be ongoing. Results to date for the long term biofumigation trial are reported in Chapter 3 and the long term rotation trial results to date are reported in Chapter 4.

Objective 4 – Continue to investigate the effect of long bare fallows on mycorrhizal colonisation of cotton.

The long term experiment on the impact of continuous bare fallows on the survival of mycorrhizal fungi was continued. Results are reported in Chapter 5.

Objective 5 – Provide information on the survival of pathogen inoculum in soil.

Several experiments investigated the survival of inoculum in soil, particularly *Thielaviopsis basicola*, the results of which are reported in Chapter 6. Research in this area should continue.

Objective 6 – Investigate *Verticillium dahliae* interaction with black root rot.

Fields were identified with significant levels of both Verticillium wilt and black root rot through annual surveys. Soil from these fields will be able to be used in future research investigating the possible interaction between these two diseases. Several experiments have been proposed and reported in Chapter 7. Methods are continuing to be evaluated for upcoming glasshouse and field experiments.

Objective 7 – Investigate bacterial seed rots identifying the pathogen(s) and potential means of infection.

Due to time constraints and staff changes it became apparent that this objective was not going to be met in time. It was decided that working on this in collaboration with Dr Moazzem Khan would provide the best outcome. Bolls were collected during the surveys and sent to Dr Khan where potential means of infection would be investigated. A full description of this is reported in Chapter 8.

Objective 8 – Re-establish Australia's capacity to screen for exotic races of bacterial blight.

The 10 cotton differential lines needed to screen races of bacterial blight were imported from United States of America in March 2012 and reported in Chapter 9. This has re-established Australia's capacity to screen for exotic races of this disease.

Chapter 1 - Disease Surveys

The objective was to continue surveillance of endemic and exotic plant pathogen, monitoring incidence and severity of diseases of cotton and recording the absence of exotic diseases.

Introduction:

The 2011/2012 cotton season marked the 29th consecutive year that bi-annual (early and late season) disease surveys have been conducted in the cotton production areas (Bourke/Walgett, Macintyre, Gwydir, Namoi, Macquarie, Lachlan and Murrumbidgee) of NSW. Disease surveys were conducted in November/December and later in the season in March/April each year of the project to assess the incidence and severity of diseases at different stages of crop development. Data collected included seedling mortality, incidence and severity of Black root rot, incidence of Verticillium wilt, Fusarium wilt, Alternaria leaf spot and boll rots along with hormone damage.

Seedling mortality is a collective term referring to seedling death as a result of a suite of circumstances. Losses can fluctuate each year and are largely influenced by biotic and abiotic factors. Inoculum levels in soil, insect pressure, soil temperature, moisture, seed viability and cultural practices can all have significant effects on seedling mortality. Seedling mortality includes the impact of seedling disease caused by *Rhizoctonia solani* and *Pythium* spp. Minimising losses due to seedling disease is important if the increased costs of replanting are to be avoided.

Black root is caused by the soil-borne fungus *Thielaviopsis basicola* and may result in plants with delayed development (up to four weeks) and stunting with yield reductions of up to 40% (Allen, Nehl, & Moore, no date). Infected plants are characterized by blackening of the roots due to destruction of the root cortex (Figure 1.1). Some roots may die, however black root rot does not kill cotton seedlings by itself. Black root rot in severe cases enhances seedling death caused by *Rhizoctonia* or *Pythium*. The pathogen was first detected in 1989 in the Gwydir as an internal ring rot and the first detected as a seedling pathogen in the Namoi Valley in 1990. This disease has since been recorded in all cotton production regions of NSW. This disease is favoured by

cool weather conditions; however the crop may compensate and yield well if the conditions warm up later in the season.

Figure 1.1 Black root rot in cotton seedlings

Verticillium wilt is caused by the fungal pathogen *Verticillium dahliae* and is widespread and common in Australian cotton growing regions. Symptoms include diffuse or angular leaf mottle where leaves yellow at the margins and between the veins (Figure 1.2) and vascular discolouration or browning throughout the stem (Allen, et al., no date). Dead tissue develops at the leaf edges and may replace the mottled areas. The impact of Verticillium wilt depends on the time of infection which is also dependent on the occurrence of cool/wet conditions. Under ideal weather conditions yield reductions of up to 30% are possible (Allen, et al., no date).

Figure 1.2 Verticillium wilt in cotton

The widespread adoption of resistant varieties during the 1990's effectively reduced the incidence if the disease. However the 1999/00 season was ideal for the disease with up to 50% of plants being affected in some fields. Repeated use of resistant varieties in California has shown selection for more virulent strains of the pathogen leading to a breakdown in cultivar resistance

(Nehl, 2004). It is important to continue to monitor the incidence of Verticillium wilt with repeated use of resistant varieties.

Fusarium wilt of cotton was first described in the USA by Atkinson (1892) and it now occurs in all the main cotton growing regions of the world (Hillcocks, 1992c). Fusarium wilt in cotton is caused by the soilborne fungus *Fusarium oxysporum* f.sp. *vasinfectum* (hereafter *Fov*). This pathogen enters the cotton plant via the roots, colonizing the vascular tissue in the stem spreading throughout. Australian strains of *Fov* are different to the isolates found overseas. DNA fingerprinting techniques and Vegetative Compatibility Group (VCG) analyses has identified three strains unique to Australia: VCG 01111 (known as the Darling Downs strain) is widespread in NSW and QLD, VCG 01112 (known as the Boggabilla strain) has only been found on a few farms near Boggabilla and one field at Moree and a new strain detected in the Macintyre Valley (referred to as the Mungindi strain).

Symptoms may appear at any stage of crop development and are dependent on temperature, host susceptibility and inoculum levels in the soil. Symptoms include stunting, wilting and death. Infected plants are characterized by a brown discolouration in the stem (Figure 1.3) and branches (Allen, et al., no date). Typically symptoms become apparent between one and two months after planting. When Fusarium wilt spreads unchecked throughout fields it can render whole farms unsuitable for cotton production. It remains essential that the spread of this disease be minimised and progress be monitored in newly infested production areas.

Figure 1.3 Fusarium wilt

There are over 100 micro-organisms that have been isolated from rotted bolls; most causing boll rot after the boll has been damaged with some secondary invaders of already infected tissue (Hillcocks, 1992a). The most common boll rot under Australian conditions is Phytophthora boll rot (*Phytophthora nicotianae* var *parasitica*), capable of causing significant yield losses. Infection occurs when soil containing zoospores is splashed onto lower bolls or when bolls are temporarily submerged under water. Generally lower bolls infected with *Phytophthora* spp. quickly turn brown, becoming blackened before opening prematurely (Figure 1.4). Other boll rots develop when bolls are exposed to damp conditions in a dense canopy. Boll rot observations need to be continually monitored.

Figure 1.4 Boll rot

Cotton bunchy top (CBT) was first observed in Australian cotton crops in the 1998/1999 growing season. The disease is transmitted by the cotton aphid (*Aphis gossypii*, Glover) with symptoms including small leaves with pale angular mottle visible on upper and lower leaf surfaces, short internodes (Figure 1.5) and small bolls (Reddall, et al., 2007). Cotton bunchy top was widespread and severe in 1998/99 season but has only occurred at low levels since then.

Figure 1.5 Cotton Bunchy Top

Hormone damage is most prevalent during the early stages of development and symptoms vary with the type and rate of chemical used. Symptoms may include yellowing between the veins on cotyledons and lower leaves, stunting, defoliation and distortion of leaves, poor root development and root pruning (Figure 1.6).

Figure 1.6 Hormone Damage

Alternaria leaf spot is the most common foliar disease of cotton (Hillcocks, 1992b). The earliest symptoms are apparent on cotyledons of young seedlings. Periods of high humidity encourages sporulation, with infection spreading from cotyledons to lower leaves. The pathogen survives between crops on undecomposed infected trash and within infected seeds. Primary infection of the lower canopy can result from conidia being splashed up from infected trash residues or blown into the crop. Symptoms include brown, grey or tan lesions 3-10mm in diameter (Figure 1.7). Plants with a high fruit load are more susceptible than plants with a low fruit load.

Figure 1.7 Alternaria leaf spot

The presence/absence of exotic cotton diseases was recorded during the early and late season surveys. Cotton leaf curl disease (CLCuD) is caused by a number of different virus species (Begomovirus) and a DNA beta satellite (DNA- ß). The disease has been documented in Pakistan, India, Egypt, Nigeria, Tanzania and Sudan; however both the virus and DNA-ß vary by region (Gambley, 2011).

Cotton blue disease (CBD) has been reported in Africa, Asia and the Americas and is suspected to be caused by aphid-transmitted viruses. CBD has symptoms similar to cotton bunchy top disease, anthocyanosis and cotton leaf roll all spread by the cotton aphid *Aphis gossypii* (Gambley, Wilson, & Allen, 2011). Fusarium wilt is caused by the soil-borne fungal pathogen *Fusarium oxysporum*. f.sp. *vasinfectum* (Fov) and has been recorded in every cotton growing region of the world with eight known races (genotypes).

Texas root rot is caused by the soil-borne fungus *Phymatotrichopsis omnivore* causing sudden wilt and death of infected plants, affecting over 2000 species of plants including cotton. Symptoms of this disease may be confused with sudden wilt, Fusarium wilt and lightning strikes (Anderson, 2011b). Verticillium wilt is caused by a widespread soil-borne fungus *Verticillium dahliae*. Strains of *V. dahliae* have been classified into two pathotypes: non-defoliating and defoliating strains. Non-defoliating strains are mildly virulent and cause wilt and partial or no defoliation. Defoliating strains are highly virulent and can cause complete defoliation of infected plants. There are no defoliating strains found in Australia (L. A. Smith, 2011).

Hyper-virulent bacterial blight caused by the bacterium *Xanthomonas axonopodis* pv. *malvacearum* has not been detected in commercial cotton crops in Namoi Valley since 1999/2000 season. Most varieties grown in Australia have good resistance to local races, with older US and some Pima varieties being susceptible. Two races originating in Africa are described as Hypervirulent and are not found in Australia (Anderson, 2011a).

Method and Materials:

Early Season Disease Surveys:

Fields from each cotton region (Bourke/Walgett, Macintyre, Gwydir, Namoi, Macquarie, Lachlan and Murrumbidgee) were sampled in November/December 2010/2011 and 2011/2012 seasons. A total of 200 plants from each field were sampled using a step-point method across two transects. In summary, 100 plants were sampled per transect line from each field. The first sampling was taken 50 m into the field at the tail drain end where a GPS coordinate was recorded. The second sampling was done by walking across 10 m and up the row 20 m. This pattern was repeated until 10 sampling sites were covered (Figure 1.8).

At each sampling point, the number of plants present along 1 m of the row was recorded to calculate seedling mortality rates. Seedling mortality was calculated as the percentage of established plants relative to the number of seeds planted.

Figure 1.8 Disease survey transect used in disease surveys. 100 plants are surveyed along each transect with two transects per field. (Image C. Anderson, 2010).

At each of the 10 sampling points, 10 plants were carefully removed from soil using an asparagus knife to loosen the soil. Each plant was inspected for Black root rot by carefully digging up the plant and examining the tap roots for symptoms. Black root rot severity was rated on a scale of 0 to 10 where 0 = total absence of disease symptoms and 10 = tap root completely black. Plants were also examined for the presence of Rhizoctonia, Pythium and exotic diseases.

Late Season Disease Surveys:

Fields from each cotton region (Bourke/Walgett, Macintyre, Gwydir, Namoi, Macquarie, Lachlan and Murrumbidgee) were sampled in March/April 2011 and in March/April 2012. Transect sampling was similar to that described for early season surveys, however stems were cut near the base of 10 plants and assessed for symptoms of Verticillium wilt, Fusarium wilt, boll rot, cotton bunchy top, herbicide/fertilizer damage and presence/absence of exotic disease. Confirmation of pathogens was made using laboratory isolation techniques on selective media.

Results & Discussion:

1.1 Seedling mortality:

Seedling mortality continues to fluctuate each year (Figure 1.1.1) as result of the pathogens present in the soil, insects present, soil temperature, moisture, crusting, planting times and bed preparation to name a few factors. In 2010/11 season a total of 89 fields were assessed on plants with an average growth stage of two and six true leaves per plant during the early season survey. The early season survey was completed later than usual this season due to the cool, wet start which delayed sowing and later forced replanting on many farms. After factoring in replanting, seedling mortality averaged 32% across NSW, which was similar to the 2009/2010 season of 33%. Seedling mortality ranged from 27 to 37% across the production areas surveyed with Bourke/Walgett 33%, Gwydir 28%, Lachlan 27%, Macintyre 30%, Macquarie 35%, Murrumbidgee 37% and Namoi 33%. The Murrumbidgee recorded the highest level of seedling mortality at 37%, up 1% on last season. Mortality was also high (37%) in the Macquarie, also up 1% on 2009/2010 season.

In 2011/12 season a total of 91 fields were assessed on plants with an average growth stage of two and six true leaves per plant during the early season survey. The early season survey was completed before Christmas this season. Germination and establishment problems led to large areas needing to be replanted on many farms. After factoring in replanting, seedling mortality averaged 32% (Figure 1.1.2) across NSW.

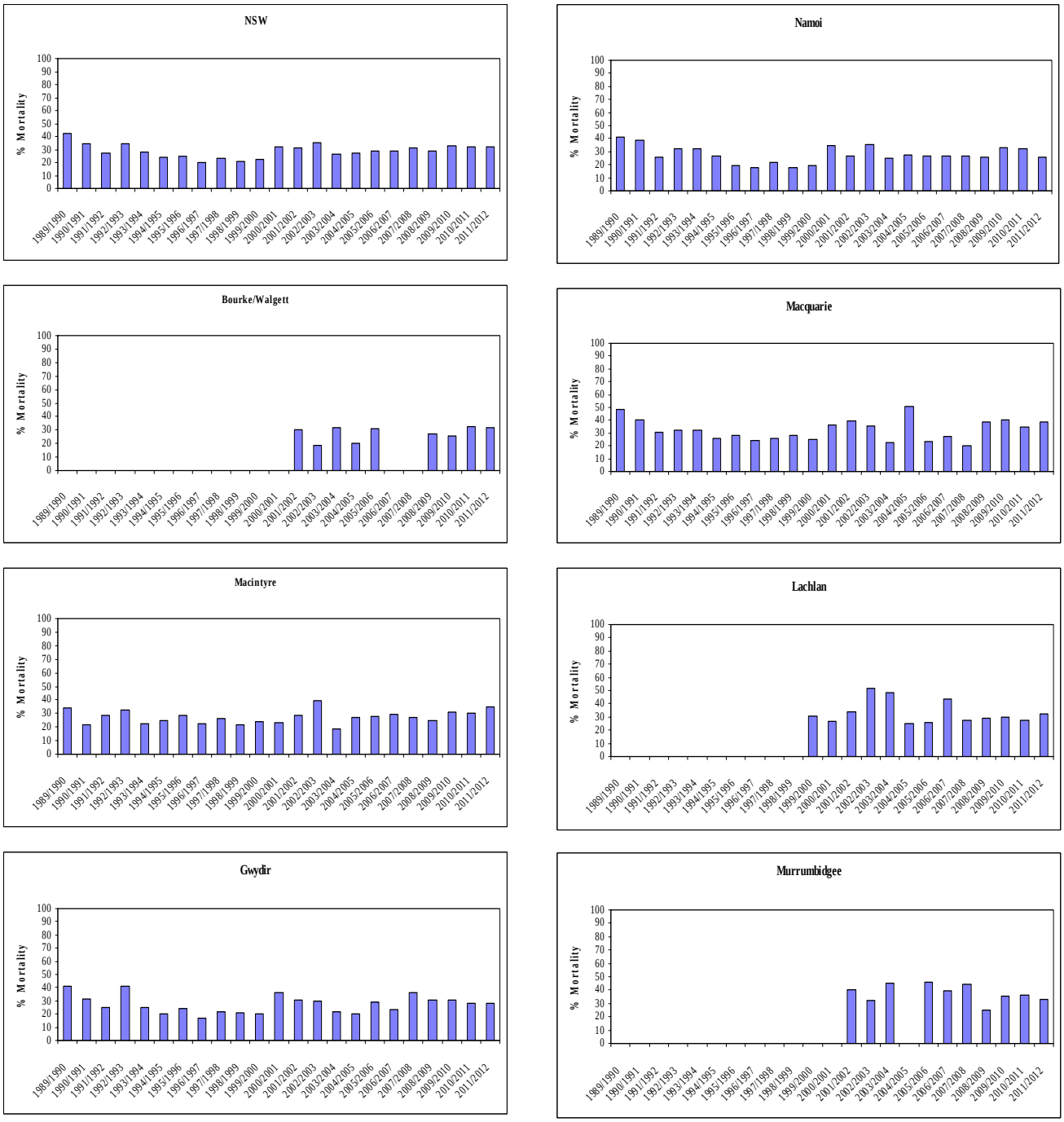


Figure 1.1.1 Long term trends in average seedling mortality (%) across each cotton growing region of NSW.

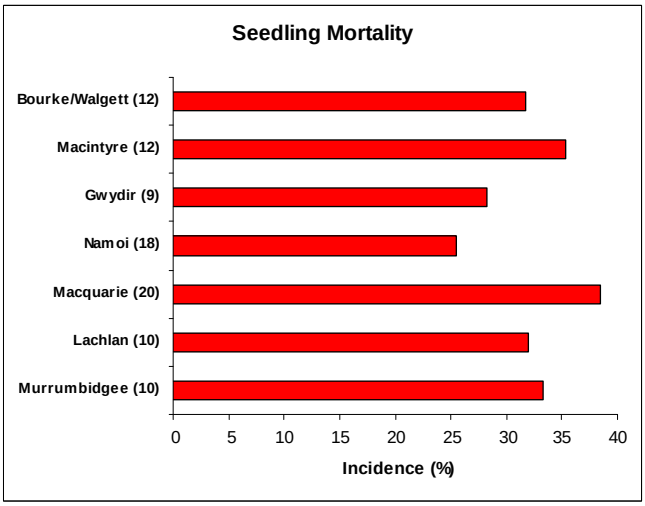


Figure 1.1.2 Average seedling mortality and number of fields (in parentheses) sampled within NSW 2011/2012 season.

Seedling mortality ranged from 25.5 to 38.4% across the production areas surveyed with Bourke/Walgett 31.7%, Gwydir 28.2%, Lachlan 32.0%, Macintyre 35.3%, Macquarie 38.4%, Murrumbidgee 33.3% and Namoi 25.5%. The Macquarie recorded the highest level of seedling mortality at 38.4%. Mortality was also high (35.3%) in the Macintyre.

1.2 Black root rot:

In 2010/11 season a total of 13,800 plants were assessed for black root rot during the early season survey. The incidence (% plants) of black root rot was most prevalent in the Namoi (80%) and Macintyre Valleys (47%). Incidence in NSW increased from 24% in 2009/2010 to 41% in 2010/2011 season. Highest incidence was in Namoi with 80% plants affected. Disease severity was also highest in the Namoi region with a severity rating of 2.7, Macintyre 1.1, and Gwydir, Macquarie, Lachlan and Murrumbidgee mean severity all less than 1.

The percentage of crops and plants with symptoms of black root rot has steadily increased over the years. Black root rot was detected in 41% of plants, 83% of fields and on 93% of the farms surveyed in 2010/2011 across NSW. The average severity of black root rot observed on tap roots for NSW was 1.2. The lower levels seen in the previous seasons may be attributed to the drier weather and years of drought prior to the wet start for this 2010/2011 season.

In 2011/12 season a total of 18,200 plants were assessed for b lack root rot during the early season survey. The incidence of b lack root rot (Figure 1.2.1) was most prevalent in the Namoi (76.2%) and Macintyre valleys (75.3%). Incidence in NSW increased from 24% in 2009/2010 to 41% in 2010/2011 season and 40% in 2011/2012 season. Highest incidence was again in Namoi with 76% plants affected. Severity was highest in the Macintyre region with a severity rating of 3.05, Namoi 2.98, Gwydir 2.38 and Macquarie, Lachlan and Murrumbidgee mean severity all less than 0.55 (Table 1.2.1).

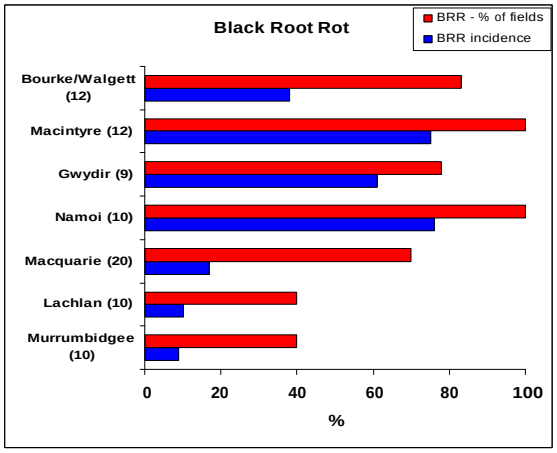


Figure 1.2.1 Incidence and percentage of fields infected with Black root rot in NSW (2011/2012) cotton season.

Table 1.2.1 Severity (scale 0-10) of Black root rot in the 2011/2012 season in NSW.

Region	Fields assessed	Black root rot severity
Bourke/Walgett	12	1.02
Macintyre	12	3.05
Gwydir	9	2.38
Namoi	10	2.98
Macquarie	20	0.51
Lachlan	10	0.35
Murrumbidgee	10	0.23

The percentage of crops and plants with symptoms of black root rot increased this season when compared to the long term data (Figure 1.2.2 and 1.2.3). Black root rot was detected in 76% of fields surveyed in 2011/2012 across NSW. The average severity of black root rot for NSW was 1.4. The lower levels seen in the previous seasons may be attributed to the drier weather and years of drought prior to the wet start of 2010/2011.

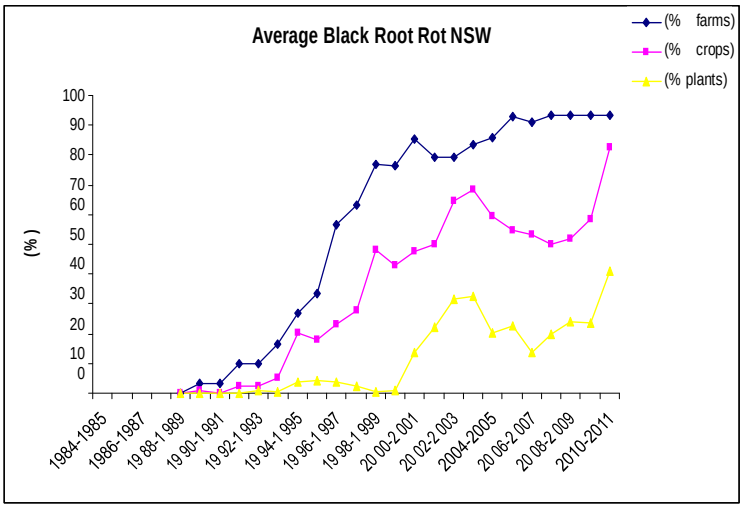


Figure 1.2.2 Long term trends for black root rot across NSW.

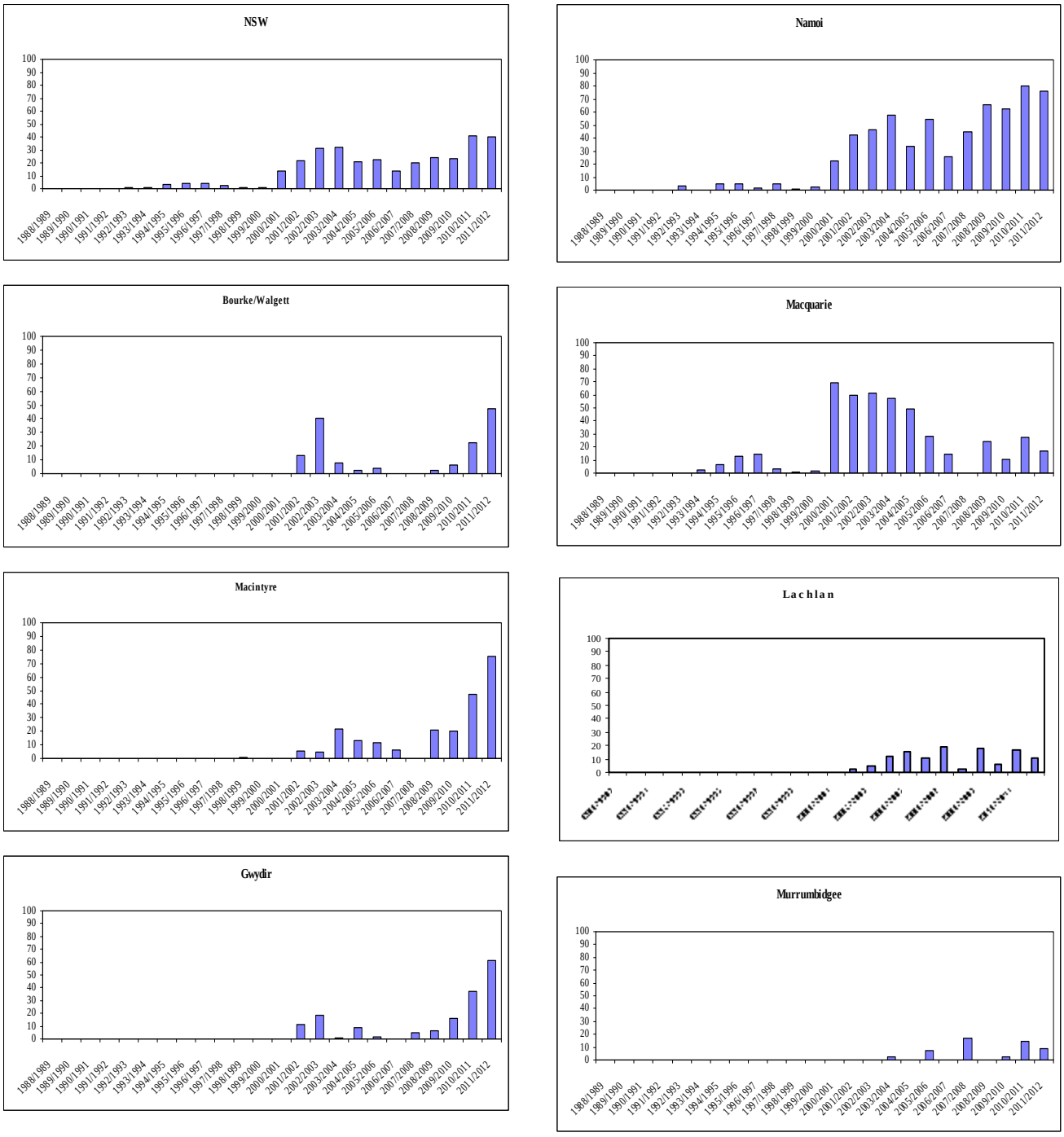


Figure 1.2.3 Long term trends in average seedling mortality (%) across each cotton growing region of NSW.

1.3 Verticillium wilt:

In 2010/11 season a total of 89 fields were assessed in NSW for Verticillium wilt. Verticillium wilt was present in 86% of fields in the Namoi (13% plants infected), 67% fields in the Gwydir (0.9% plants infected), 50% fields in the Macintyre (3.2% plants infected), 43% fields in Bourke/Walgett (2.0% plants infected) and 25% fields in Macquarie (0.2% plants infected). Verticillium wilt was not observed in the Lachlan or Murrumbidgee regions during 2010/11 sampling (Figure 1.3.1). Verticillium wilt (% fields) across NSW averaged 47.2% while incidence averaged 4.1% plants infected. These are the second highest levels in over 15 years, with the highest in 2007/08 season (Table 1.3.1).

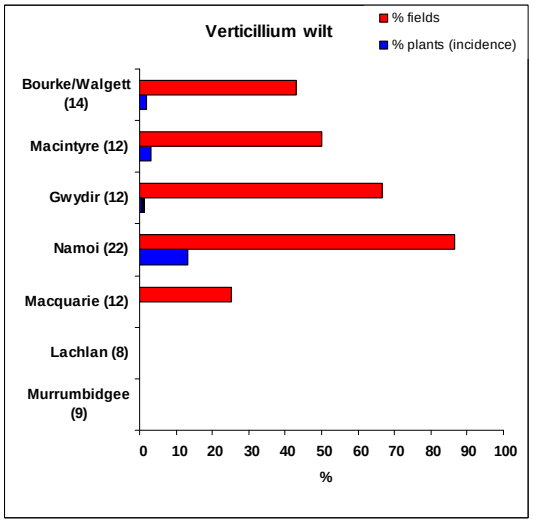


Figure 1.3.1 Incidence and percentage of fields infected with Verticillium wilt in NSW.

Table 1.3.1 Incidence of Verticillium wilt in fields and plants since 2007/08 cotton season.

	Verticillium wilt (% fields)			
	2007/08	2008/09	2009/10	2010/11
Bourke/Walgett	no data	25	36.4	43
Macintyre	0	50	41.7	50
Gwydir	57.1	28.6	54.5	67
Namoi	100	93.8	80	86
Macquarie	0	27.3	25	25
Lachlan	0	0	16.7	0
Murrumbidgee	20	0	0	0
NSW	51.4	40	36	47.2
	Verticillium wilt (% plants)			
	2007/08	2008/09	2009/10	2010/11
Bourke/Walgett	no data	0.7	0.3	2
Macintyre	0	2.6	1.5	3.2
Gwydir	2.2	0.9	2	0.9
Namoi	28.9	14	12.7	13.1
Macquarie	0	0.8	0.2	0.2
Lachlan	0	0	0.3	0
Murrumbidgee	0.1	0	0	0
NSW	11.2	3.8	2.4	4.1

At the time of writing this report the 2012 late season surveys had not been fully completed, therefore the data has not been presented for Verticillium wilt levels for 2011/12 season. Preliminary results show a few fields suffered from severe Verticillium wilt where in some cases up to 60 % of fields were affected. A detailed report on this will be available later in 2012 as the data is further analysed.

1.4 Fusarium wilt:

The incidence of Fusarium wilt was highest in the Gwydir (10%) up 1% from 2009/10, followed by the Macintyre (9%) up 3% and Bourke/Walgett (0.2%) where there was none reported in 2009/10. Fusarium wilt was detected for the first time this season in the Lachlan, with one field infected out of eight (12.5%), with 2/1600 plants infected (0.1%). There was no record of Fusarium wilt in Murrumbidgee, Macquarie or Namoi regions. Fusarium was detected in 24.7% of fields and 2.6% plants across NSW (Figure 1.4.1, Table 1.4.1). The total number of reported cases of this disease has now reached 85 in NSW. The percentage of fields infected with Fusarium wilt is the highest recorded in over 15 years. At the time of writing this report the 2012 late season surveys had not been completed, therefore the data has not been presented for Fusarium wilt levels for 2011/12 season.

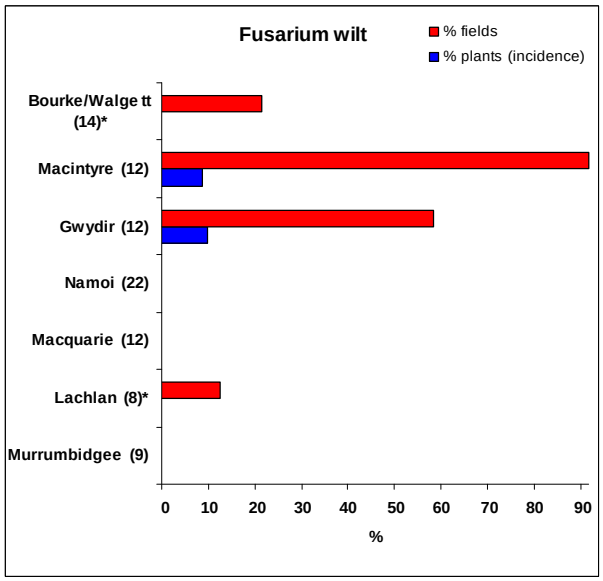


Figure 1.4.1 Incidence and percentage of field infected with Fusarium wilt in the cotton growing regions of NSW (*Incidence 0.2% Bourke/Walgett and 0.1% Lachlan).

Table 1.4.1 Incidence of Fusarium wilt in fields and plants since 2007/08 cotton season.

	Fusarium wilt (% fields)			
	2007/08	2008/09	2009/10	2010/11
Bourke/Walgett	no data	0	0	21.4
Macintyre	50	60	58.3	91.7
Gwydir	57.1	57.1	45.5	58.3
Namoi	0	0	0	0
Macquarie	0	0	0	0
Lachlan	0	0	0	12.5
Murrumbidgee	0	0	0	0
NSW	17.1	19.2	14.6	24.7
	Fusarium wilt (% plants)			
	2007/08	2008/09	2009/10	2010/11
Bourke/Walgett	no data	0	0	0.2
Macintyre	29.3	5.7	6.3	9.0
Gwydir	3.4	9.4	9.3	10.0
Namoi	0	0	0	0
Macquarie	0	0	0	0
Lachlan	0	0	0	0.1
Murrumbidgee	0	0	0	0
NSW	4	2.6	2.2	2.6

1.5 Boll rots and Alternaria Leaf Spots:

Percentage of fields infected with boll rots in 2010/11 season averaged 84% in NSW while the average incidence (% of plants) affected in NSW was 0.8%. In order of highest to lowest: boll rots in the Macintyre were down from 17% last year to 1.3%, Gwydir down from 13 to 1.1%, Bourke/Walgett down from 8 to 1.0%, Lachlan down from 6 to 0.7%, Macquarie down from 8.2 to 0.5%, Murrumbidgee down from 8 to 0.4% and Namoi down from 7 to 0.4% (Figure 1.5.1). Alternaria leaf spot was present in very low levels in all crops (less than 0.01%).

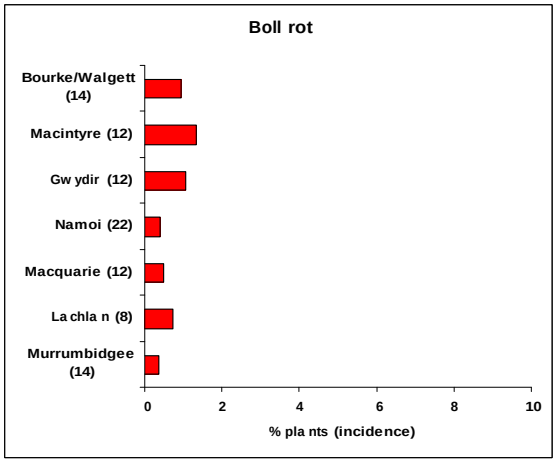


Figure 1.5.1 Incidence of boll rots in NSW cotton growing regions 2010/2011 season.

Alternaria: *Alternaria macrospora* was observed at very low levels throughout NSW, with average trace levels of less than 0.1% in every field surveyed (Figure 1.5.2).

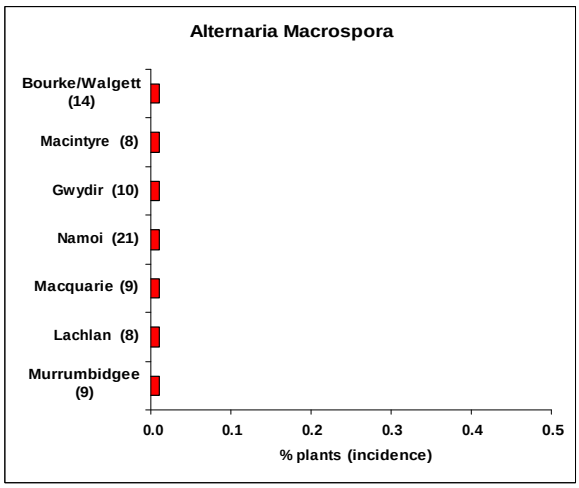


Figure 1.5.2 Trace amounts (less than 0.1%) of Alternaria recorded in NSW.

1.6 Cotton bunchy top and hormone damage:

Cotton bunchy top was observed in 43.0% crops inspected in NSW in 2010/11 season with the average incidence being 2.1%. The mean incidence for Bourke/Walgett region was high (6.9%), with two fields recording 48.5% and 43%. The Gwydir and Namoi regions were much lower (both 2%), Macquarie (1%) and Lachlan, Macintyre and Murrumbidgee less than 1%. While the incidence was low, the percentage of fields infected was high (Figure 1.6.1). The percentage of fields infected was highest in Bourke/Walgett (57%), Macintyre (50%), Namoi (46%), Lachlan (38%), Macquarie (25%), Murrumbidgee (22%) and lowest in the Gwydir (17%).

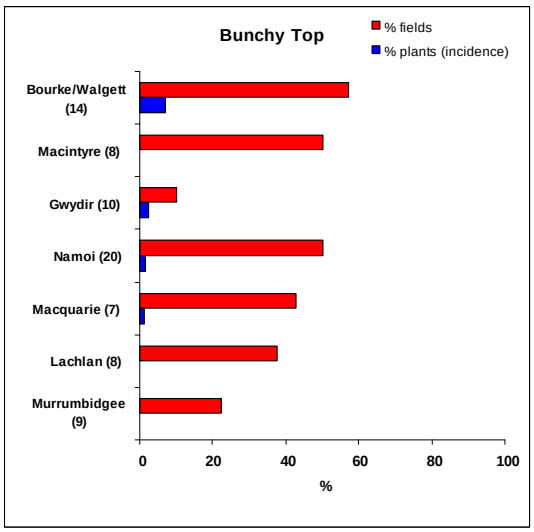


Figure 1.6.1 Incidence and percentage of fields infected with cotton bunchy top in NSW.

Damage from hormones throughout NSW 2011/12 season was very low, less than 2% (Figure 1.6.2).

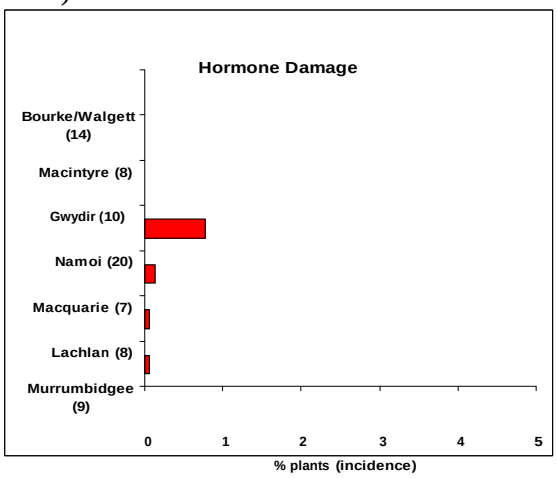


Figure 1.6.2 Percentage of plants affected by herbicide and or fertilizer in NSW.

1.7 Exotic diseases:

No exotic diseases have been observed during the course of this project. Dr Kirkby undertook a 3 week trip to Texas, USA during July/August 2011 funded by the Department of Agriculture, Fisheries and Forestry (DAFF). The purpose of the trip was to undergo training in diagnosing these two diseases and gaining knowledge to prepare National Diagnostic Protocols for these two diseases. The overseas travel commenced on 16th July 2011 and finished on 8th August 2011. Between these dates Dr Kirkby visited:

- 16th July – 22nd July 2011 - Narrabri to Sydney, then to Dallas Fort Worth, then College Station. Picked up by Clay Lewis (Assistant Researcher) from A&M University.
- Dr Steve Hague (Plant Breeder) - shown around the university, discussed the upcoming activities and tour of the farm. Steve organised meetings with several key scientists, both at Texas A&M University and with the USDA.
- Professor James Starr (Plant Pathologist) - discussed protocols for bacterial blight, then I met with Dr Richard Percy (Research Leader), Dr John Yu (Geneticist), Dr Lori Hinze (Research Geneticist), Dr All Bell (Pathologist).
- USDA germplasm curator Dr James Frelichowski, Dr David Stelly (Geneticist) and Associate Professor Keerti Rathore (Institute for Plant Genomics & Biotechnology). Toured the facility, growth rooms, glasshouses and field trials as well as met with graduate students.
- Dr Gino Medrano (USDA Research Plant Pathologist), Dr Jinggao Liu (Research Chemist – Cotton Pathology), Dr Robert Stipanovic (Research Leader), Associate Professor Gaylon Morgan (State Extension Cotton Specialist). Had discussions and initiated conversation regarding collaborative work. USDA are very keen to do this.
- 23rd July – 29th July 2011 – College Station and a 1030 mile road trip with Dr. Thomas Isakeit visiting farms with disease.
- 30th July – 8th August 2011 – Lubbock to work with Dr Terry Wheeler on Bacterial Blight then back to Narrabri

Travelling to the natural range where these diseases occur in the USA allowed Dr Kirkby to gain first hand experience in diagnosing the diseases, experience that could not be gained in Australia. The benefit to the cotton industry is having a local diagnostician with specialist experience with these organisms, hence improving the biosecurity of the industry. Development of national

standard diagnostic protocols for Texas root rot and hypervirulent bacterial blight has increased Australia's capacity to respond quickly should an exotic disease incursion occur. Acquisition of the full set of bacterial blight differential cultivars now enables designation of race to bacterial isolates in the event of an incursion.

The development of and leadership in assembling and delivering the National Diagnostic Protocols for these two pathogens ensures that the methods are validated and maintained by NSW DPI. These protocols will be implemented should an incursion occur in Australia. The biosecurity profile of Australian Cotton Research Institute (ACRI) and NSW DPI will be enhanced with the cotton industry and with the Commonwealth Department Agriculture, Fisheries and Forestry (DAFF). This will be of benefit in attracting external funding in the future.

1.8 Other disease issues:

There were occasional cases of Sclerotinia reported later in March and April 2012. Currently there is no chemical registered for the control of this disease in cotton. The conditions where the outbreaks occurred included rank growth, high humidity and generally after rainfall. There was also a trend with previous crop history of susceptible hosts being planted.

Conclusions:

No exotic diseases were detected during the disease surveys of 2010/2011 or 2011/2012. Mean seedling disease for fields inspected in NSW was 31.9% in 2010/2011 season and 32.3% in 2011/2012 season. Rain delayed harvests in winter meant a quick turnaround after harvest into planting cotton, coupled with a cold spell mid October lead to crop establishment problems in 2010/2011 season. The highest incidence of seedling mortality was in the Macquarie (38.4%) and the lowest in the Namoi (25.5%) in 2011/2012 season.

The seasonal conditions spring and summer of the 2010/2011 cotton season were very favourable for black root rot. In 2010/2011, black root rot was observed in 93% of the farms surveyed and in 83% of the fields in NSW with a mean severity of 2.69 (26.9% of each tap root blackened). The disease was most common in the Namoi Valley where it was observed in 100% of fields surveyed in 2010/2011 and 2011/2012. The incidence of fields in the Namoi, Gwydir and Macintyre valleys was substantially higher than previously recorded.

Verticillium wilt was observed in 47.2% of the fields with an average incidence of 4.1% plants infected in 2010/2011 season. This figure is up from 3.7% recorded in the 2009/2010. Incidence of Fusarium wilt increased in 2010/2011 season with the wet spring and cool, wet summer favourable for the disease. Obvious patches in fields were seen where infected seedlings had died early in the season. There were two new reports of Fusarium wilt in the Gwydir valley of NSW and the first report of Fusarium wilt on a farm in the Lachlan Valley and Emerald area in 2010/2011. A second report of Fusarium was also recorded in the Lachlan Valley in 2011/2012.

In NSW, bunchy top was observed in 43.0% crops inspected with the average incidence in these crops being 2.1%. The incidence of bunchy top was apparent in some fields in the Bourke/Walgett region where average incidence was 6.9%. The Bourke/Walgett region recorded the highest incidence with two crops recording 48.5% and 43%. Namoi valley and Gwydir recorded 28.5% and 25.5% respectively. Importantly, it was noted that where large numbers of volunteer cotton plants were found with bunchy top near fields, high incidence of bunchy top was also recorded. Hormone damage was minimal in 2010/2011 season being less than 2%. There were no exotic diseases and/or pathogens observed during the surveys of 2010/2011 or 2011/2012 seasons.

For a disease to occur there must be a susceptible host, a virulent pathogen and favourable environmental conditions, a concept referred to as the "Disease Triangle". The weather conditions play a huge role in what diseases we see present and how severe they are expressed. Cool wet starts followed by summer rainfall and fluctuating humidity provide favourable conditions particularly for fungal diseases. Growers are referred to the Cotton Pest Management Guide for Integrated Disease Management Strategies.

Chapter 2 - Seed Treatments

Objective 2 – Continue to evaluate existing and novel fungicide treatments for seedling disease and black root rot.

Introduction:

Seedling diseases threaten the productivity and sustainability of cotton production in Australia. Seedling disease occurs when cotton is invaded by a number of soil-borne fungi including *Pythium*, *Rhizoctonia*, Black root rot, and *Fusarium* causing seed rot and/or pre and post-emergent damping off (collapse and death of seedling). Cotton plants in the early stages of growth are more susceptible to seedling disease as cells in the hypocotyl and tap root continue to thicken. As seedlings mature (after two-leaf stage) plants become more resistant, particularly to *Rhizoctonia* and *Pythium*, except under wet and very cool conditions (Nehl, Allen, Mondal, & Lonergan, 2004a).

Each year NSW DPI evaluates the effectiveness of seed treatment fungicides and combinations against seedling disease. The standard fungicide for cotton for many years was Terraclor® (Quintozene- PCNB) (active against *Rhizoctonia*) and Metalaxyl-M (Apron®) (active against *Pythium* spp.). Following the release of the new seed treatment from Syngenta® in 2005/2006 registered to protect seeds, roots and emerging seedlings, Dynasty® replaced the historical industry standard. PCNB was included in the 2010/2011 and 2011/2012 seed treatment trials as a measure of *Rhizoctonia* pressure.

Method and Materials:

The 2010/11 and 2011/12 trials each had a total of 20 treatments (19 seed treatments and a control of black seed) to evaluated two cultivars (Sicot 71BRF and conventional Sicot 71). In 2010/11 field sites were located at ACRI Narrabri, Hillston, Warren and Mungindi. At the ACRI Narrabri site Sicot 71BRF was sown at 100 seeds per 13m plots over two planting dates i.e. early (17/09/10) and normal (13/10/10). At Hillston and Warren seeds were sown (25 to 26/9/10 and 11/10/10 respectively) at 120 seeds per 14m plots. At Mungindi Sicot 71 was sown

(30/09/10) at 120 seeds per 14m plots. In 2011/12 field sites were located at ACRI Narrabri, Breeza, Hillston, Warren, Mungindi and QLD. At the ACRI Narrabri site Sicot 71BRF was sown into moisture at 100 seeds per 12m plots over 2 planting dates i.e. early (07/09/11) and normal (12/10/11). Planting of Sicot 71 BRF was done at Breeza (13/10/11) into moisture, Hillston (4/10/11) into dry and Warren (12/10/11) into moisture at 120 seeds per 14m plots. At Mungindi Sicot 71 was sown 24/09/11 into moisture at 120 seeds per 14m plots. Planting date for the QLD Downs site was 25/10/11.

With the assistance of CSIRO Plant Industry, seeds were planted into late incorporated Namoi Wooly Pod Vetch (ACRI Narrabri), into moisture using a cone seeder at all trial sites. Details of seed treatments and product formulation for 2010/11 season are detailed in Table 2.1 and Table 2.2 and for 2011/12 season in Table 2.3 and Table 2.4. Intra-field variation in pathogen populations was accounted for by using completely randomised block designs at each site. The number of replicates for each site was: ACRI Narrabri (8), Hillston (9), Warren (10) and Mungindi (14). In 2011/12 season the number of replicates for each site was: ACRI Narrabri (8), Breeza (4), Hillston (6), Warren (6) and Mungindi (8). Surviving plants were counted at 3 and 6 weeks after sowing at ACRI Narrabri site and after 6 weeks at the remaining sites. Data was analysed using GenStat, 11th Edition, REML spatial modelling.

Table 2.1 Seed Treatments used in seasonal trials

Treatment
1. Untreated
2. Quintozene(PCNB) (Terraclor®)
3. Fludioxonil + Metalaxyl + Azoxystrobin (Dynasty® Cotton Seed Treatment)
4. Dynasty® CST + SYN524 (Dynasty® Plus)
5. Fludioxonil (Maxim® 100FS) – component of Dynasty® CST
6. Metalaxyl-M (Apron® XL 350ES) – component of Dynasty® CST
7. Azoxystrobin (Dynasty® 100FS) – component of Dynasty® CST
8. SYN524 – experimental fungicide Syngenta®
9. Fludioxonil + Metalaxyl-M
10. Fludioxonil + Azoxystrobin
11. Fludioxonil + SYN524
12. Metalaxyl-M + Azoxystrobin
13. Metalaxyl-M + SYN524
14. Azoxystrobin + SYN524
15. Untreated + Thiamethoxam (Cruiser® 600FS)
16. Trifloxystrobin (DC-094) (Rate 1) – experimental fungicide Bayer CropScience
17. Trifloxystrobin (DC-094) (Rate 2)
18. Trifloxystrobin (DC-094) (Rate 3)
19. 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Bion® Plant Activator)
20. Fludioxonil + Metalaxyl + Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Dynasty® CST + Bion® Plant Activator)

Table 2.2 Product formulation, active ingredients and recommended rates

Source	Formulation (proprietary names)	Active Ingredient	Recommended Rate (product)
Syngenta®	Dynasty® Cotton Seed Treatment	Metalaxyl-M 37 g/L Fludioxonil 12 g/L Azoxystrobin 75 g/L	2.00 mL/kg seed
Syngenta®	Apron® XL 350ES	Metalaxyl-M 350 g/L	0.43 mL/kg seed
Syngenta®	Dynasty® 100FS	Azoxystrobin 100 g/L	1.50 mL/kg seed
Syngenta®	Maxim® 100FS	Fludioxonil 100 g/L	0.25 mL/kg seed
Syngenta®	Syn524464 100 FS B-var	Syn-508210 + Syn-508211 100 g/L	0.5g/kg seed
Chemtura Agrosolutions™	Terraclor®	Quintozene(PCNB) 500 g/L	2.15 mL/kg seed
Syngenta®	Cruiser® 600FS	Thiamethoxam 600 g/L	4.60 mL/kg seed
Syngenta®	Bion® Plant Activator	1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	0.012g/kg seed
Bayer CropScience	DC-094	Trifloxystrobin 13.3%w/w	0.325 mL/kg seed 0.650 mL/kg seed 0.975 mL/kg seed

Table 2.3 Seed Treatments used in 2011-2012 season trial

	Treatment
1.	Untreated
2.	Quintozene(PCNB) (Terraclor®)
3.	Fludioxonil + Metalaxyl + Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Dynasty® Complete)
4.	1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester (Bion® Plant Activator) – component of Dynasty® Complete
5.	Fludioxonil (Maxim® 100FS) – component of Dynasty® Complete
6.	Metalaxyl-M (Apron® XL 350ES) – component of Dynasty® Complete
7.	Azoxystrobin (Dynasty® 100FS) – component of Dynasty® Complete
8.	Fludioxonil + Metalaxyl-M
9.	Fludioxonil + Azoxystrobin
10.	Fludioxonil + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
11.	Metalaxyl-M + Azoxystrobin
12.	Metalaxyl-M + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
13.	Azoxystrobin + 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester
14.	Untreated + Thiamethoxam (Cruiser® 600FS)
15.	Trifloxystrobin (DC-094) (Rate 1) – experimental fungicide Bayer CropScience
16.	Trifloxystrobin (DC-094) (Rate 1) + Metalaxyl-M
17.	Trifloxystrobin (DC-094) (Rate 2)
18.	Trifloxystrobin (DC-094) (Rate 2) + Metalaxyl-M
19.	Trifloxystrobin (DC-094) (Rate 3)
20.	Trifloxystrobin (DC-094) (Rate 3) + Metalaxyl-M

Table 2.4 Product formulation, active ingredients and recommended rates

Source	Formulation (proprietary names)	Active Ingredient	Recommended Rate (product)
Syngenta®	Dynasty® Complete	Metalaxyl-M 37 g/L Fludioxonil 12 g/L Azoxystrobin 75 g/L 1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	2.00 mL/kg seed 0.012g/kg seed
Syngenta®	Apron® XL 350ES	Metalaxyl-M 350 g/L	0.43 mL/kg seed
Syngenta®	Dynasty® 100FS	Azoxystrobin 100 g/L	1.50 mL/kg seed
Syngenta®	Maxim®100FS	Fludioxonil 100 g/L	0.25 mL/kg seed
Chemtura Agrosolutions™	Terraclor®	Quintozene(PCNB) 500 g/L	2.15 mL/kg seed
Syngenta®	Cruiser® 600FS	Thiamethoxam 600 g/L	4.60 mL/kg seed
Syngenta®	Bion® Plant Activator	1,2,-benzodiadiazole-7-thiocarboxylic acid-S-methyl-ester 500 g/L	0.012g/kg seed
Bayer CropScience	DC-094	Trifloxystrobin 13.3%w/w	0.325 mL/kg seed 0.650 mL/kg seed 0.975 mL/kg seed

Results:

2010/2011 annual seed treatment fungicide trials:

Plant stands were not significantly different regardless of seed treatment at the three week count, however after six weeks in the early planted trial mean number of plants were significantly higher (P=0.048) in plots treated with Dynasty® Plus, Dynasty® CST and Apron® + Azoxystrobin (76, 76 and 75 plants respectively) in comparison to the untreated control with 68 plants (Figure 2.1.1). Seedling mortality after six weeks averaged 29% across the trial.

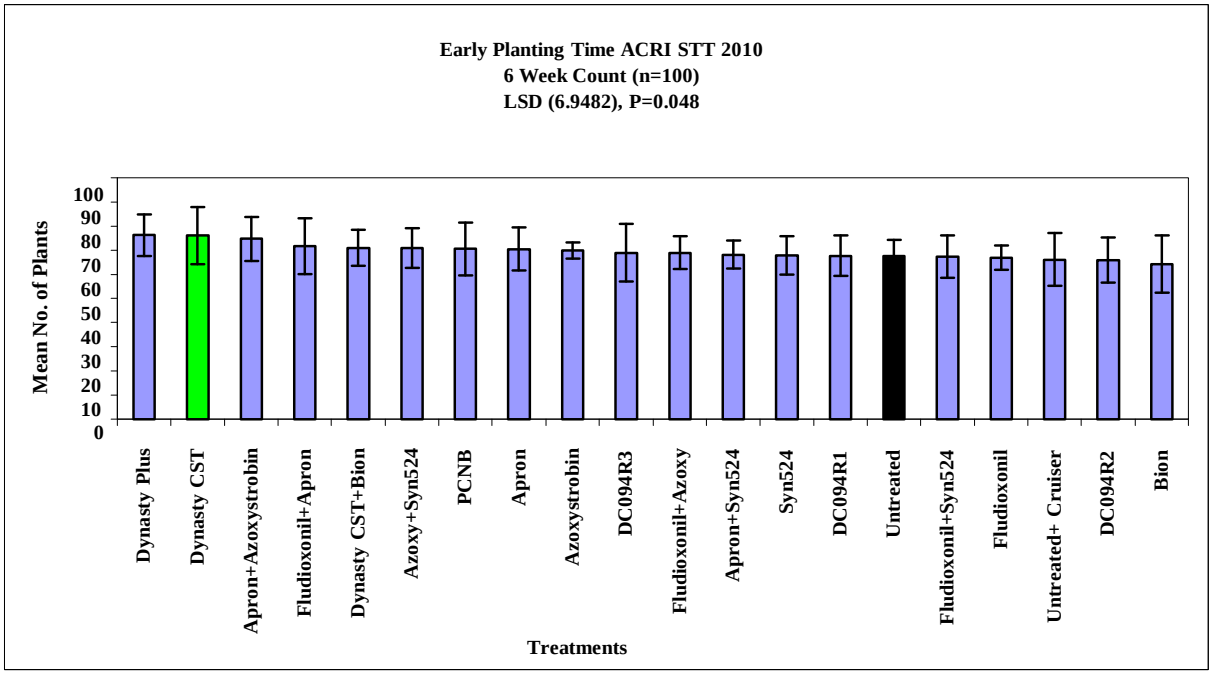


Figure 2.1.1 Mean stand counts six weeks after early sowing (17/09/10) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments (P=0.048). Industry standard seed treatment highlighted in green.

In the trial planted later (Normal) on the 13/10/10, plots treated with Dynasty® CST, Azoxystrobin, Apron® + Azoxystrobin, Dynasty® Plus, Fludioxonil + Apron® and Azoxy + Syn524 had significantly (P<0.001) higher mean number of plants (83,81,81,81,80,80 plants respectively) after six weeks in comparison with the untreated control with 73 plants (Figure 2.1.2). Seedling mortality after six weeks averaged 25% across the trial.

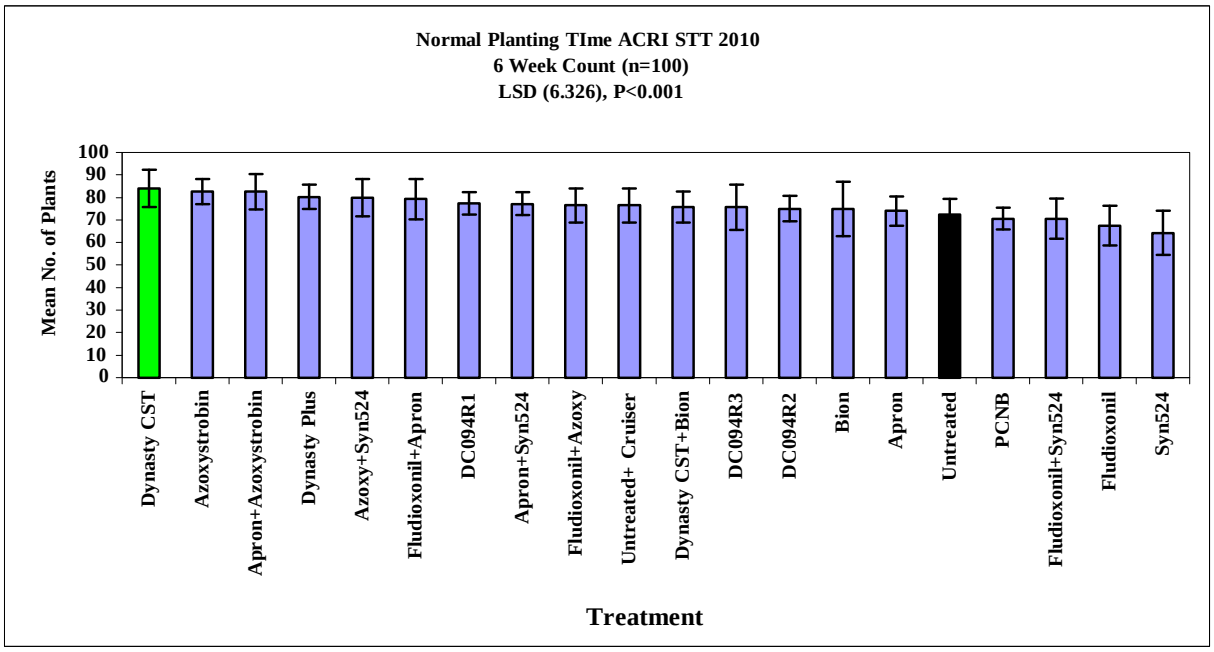


Figure 2.1.2 Mean stand counts six weeks after normal sowing date (13/10/10) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments (P<0.001).

At the Mungindi site, seed treatments had a significant (P<0.001) effect on reducing seedling mortality in comparison to untreated plots (Figure 2.1.3). PCNB and Syn524 had no effect; however plots treated with the remainder of seed treatments had significantly higher stand counts compared to untreated control which had the lowest mean number of plants of 53. The lack of affect of PCNB (known to be effective against *Rhizoctonia*) indicated that *Pythium* spp. may have been the dominant pathogen early this season. This was consistent with observations of Pythium damage on plants. Seedling mortality averaged 37% across the trial which was 11% higher than last season.

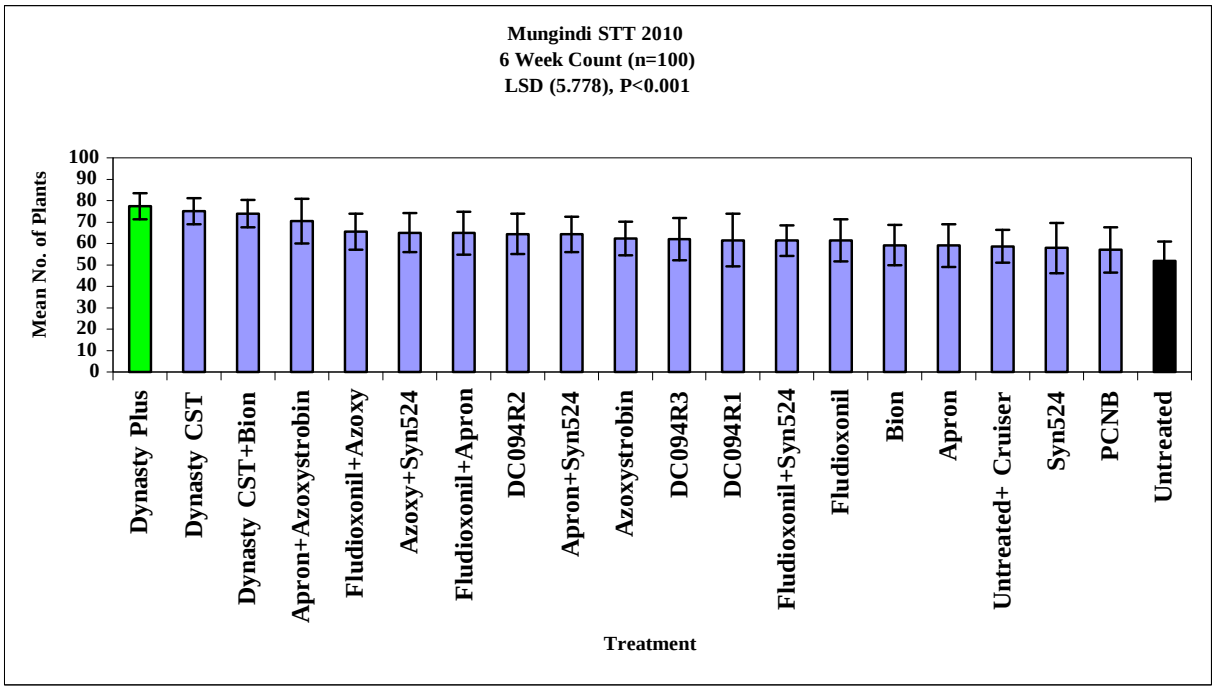


Figure 2.1.3 Mean stand counts six weeks after sowing (30/09/10) following seed treatment with various fungicides at Mungindi site. Significant differences between seed treatments (P<0.001).

At the Hillston site, stand counts were much lower with untreated plots recording a mean number of plants of 46. Of the 20 treatment, 12 had higher mean number of plants than that of the untreated control (Figure 2.1.4). At Hillston, seedling mortality averaged 47%.

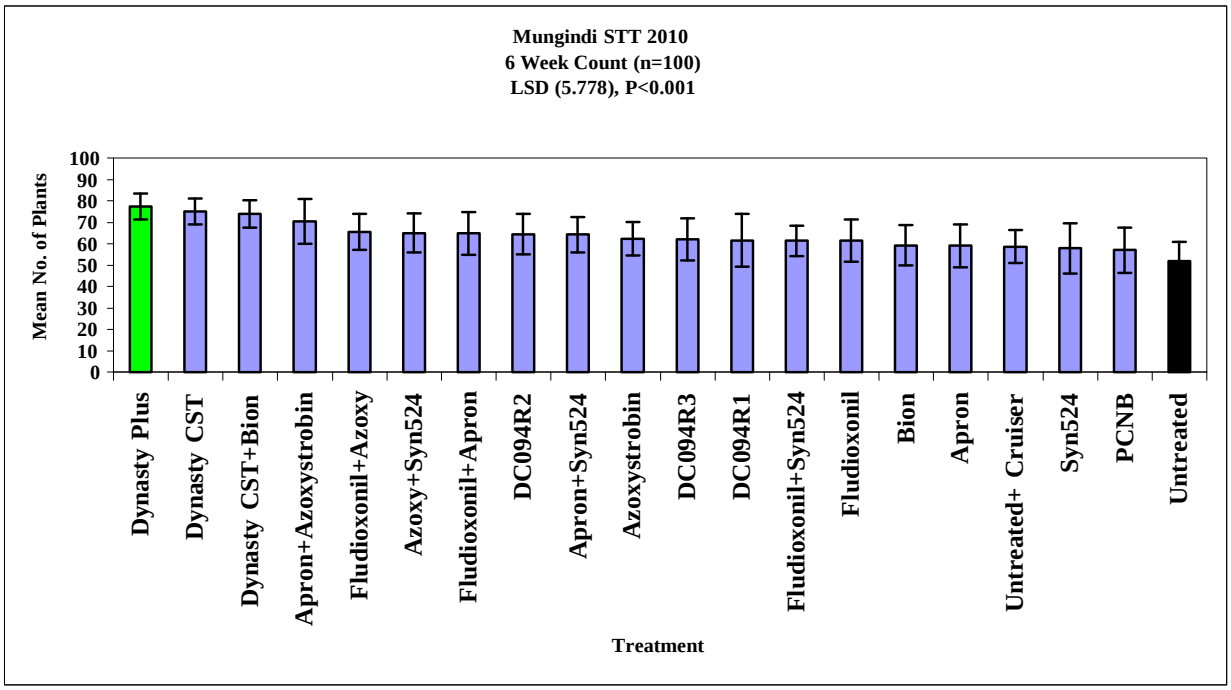


Figure 2.1.4 Mean stand counts six weeks after sowing (25/09/10 & 26/09/10) following seed treatment with various fungicides at Hillston site. Significant differences between seed treatments (P=0.002).

2011/2012 annual seed treatment fungicide trials:

At ACRI Narrabri, the trails were sown on the 7th September 2011 and again on the 12th October 2011. Plant stands treated with Bion alone were significantly lower than all other treatments at the three week count. There was no difference between the standard seed treatment Dynasty® Complete and the remaining treatments (Figure 2.2.1). The mean number of plants in the plots treated with Dynasty® Complete, untreated and Bion® was 38, 35 and 26 plants respectively.

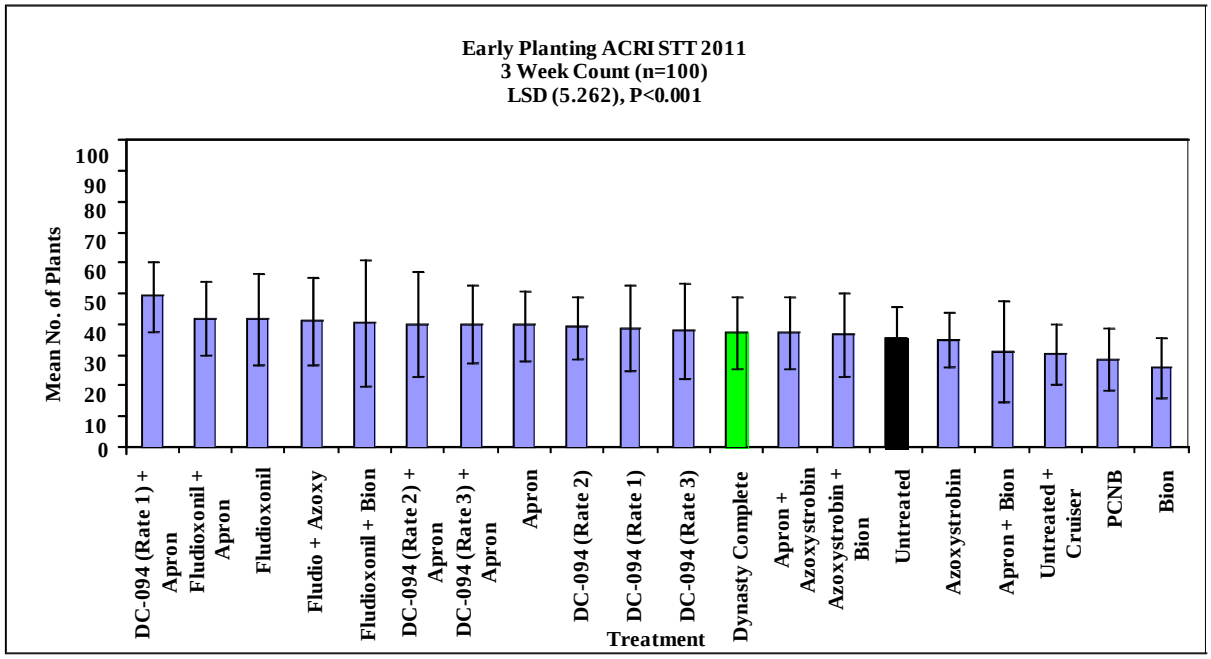


Figure 2.2.1 Mean stand counts three weeks after early sowing (07/09/11) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments (P<0.001).

The mean number of plants after six weeks growth in the early planted trial remained low with the mean for the trial being 32.4 plants. Soil and air temperatures were low providing favourable conditions for pathogens such as *Rhizoctonia*. The number of plants was significantly lower (P=0.004) in plots treated with Bion®, PCNB, DC-094 (Rate 3), Apron® + Bion®, Azoxystrobin + Bion® and Untreated (Figure 2.2.2). Seedling mortality after six weeks averaged 68% across the trial.

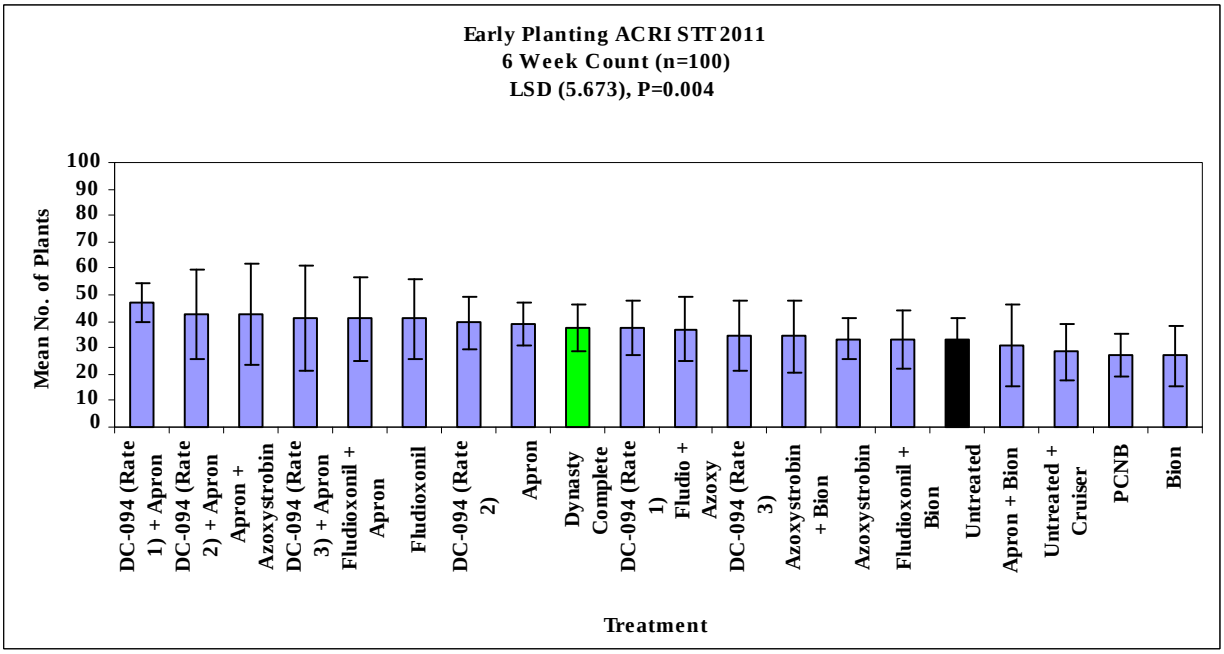


Figure 2.2.2 Mean stand counts six weeks after early sowing following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments (P<0.004).

In the trial planted later, considered normal time for planting (12/10/11), after three weeks there was no significant difference (P=0.083) between treatments (Figure 2.2.3). Seedling mortality after six weeks averaged 27% across the trial. The six week count recorded no significant difference (P=0.125) between treatments with a mean mortality of 28% (Figure 2.2.4).

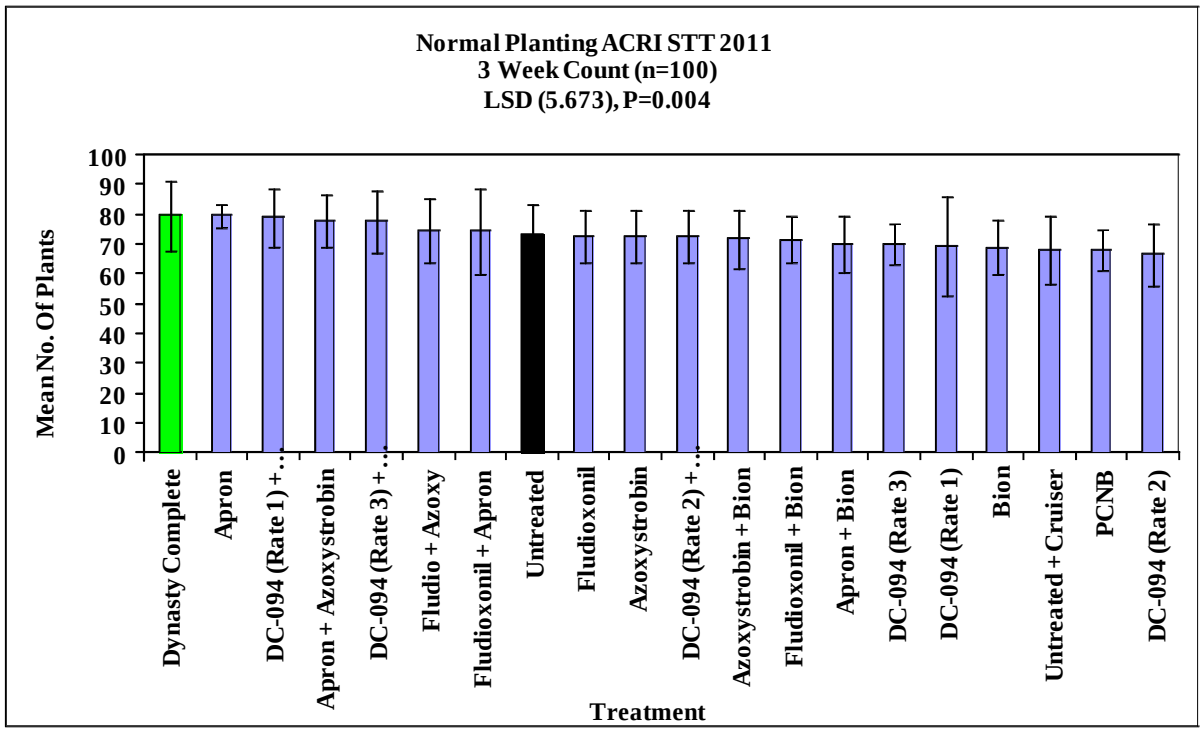


Figure 2.2.3 Mean stand counts three weeks after normal sowing date (12/10/11) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments (P=0.083).

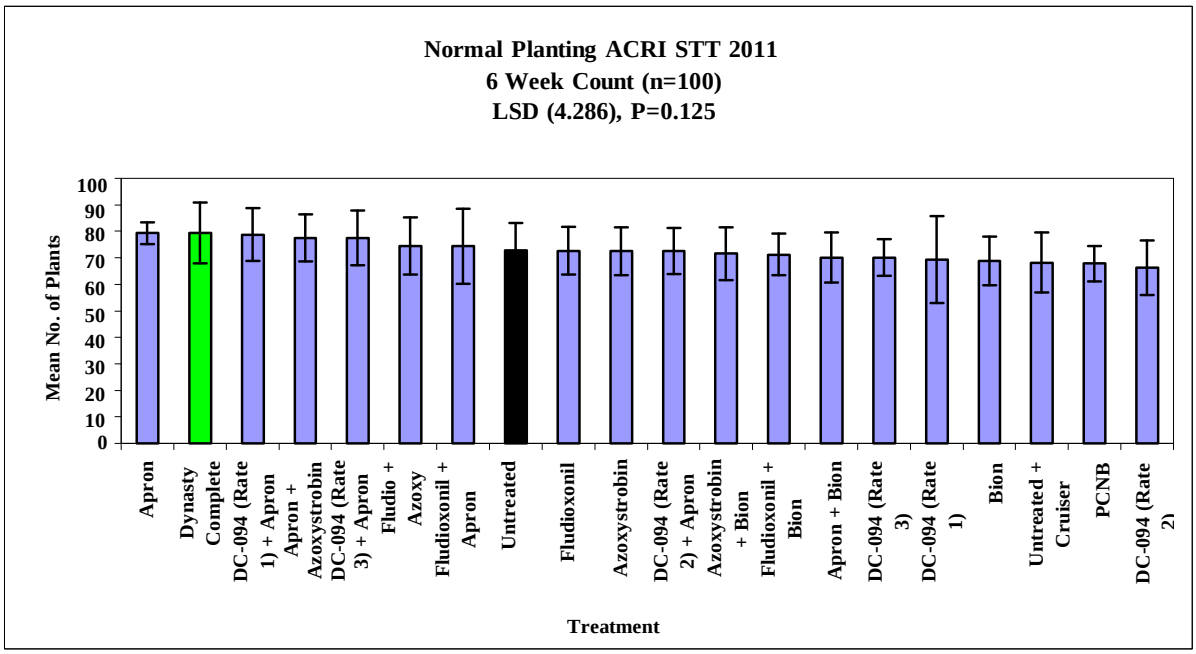


Figure 2.2.4 Mean stand counts six weeks after normal sowing date (12/10/11) following seed treatment with various fungicides at ACRI Narrabri site. Significant differences between seed treatments (P=0.125).

At the Mungindi site, seedling mortality was extremely high approximately 70%) regardless of treatment (Figure 2.2.5). The lack of affect of PCNB (known to be effective against *Rhizoctonia* indicated that *Pythium* spp. may have been the dominant pathogen early this season. This was consistent with observations of *Pythium* damage on plants. Mean % of plants across the trial was low at 24%.

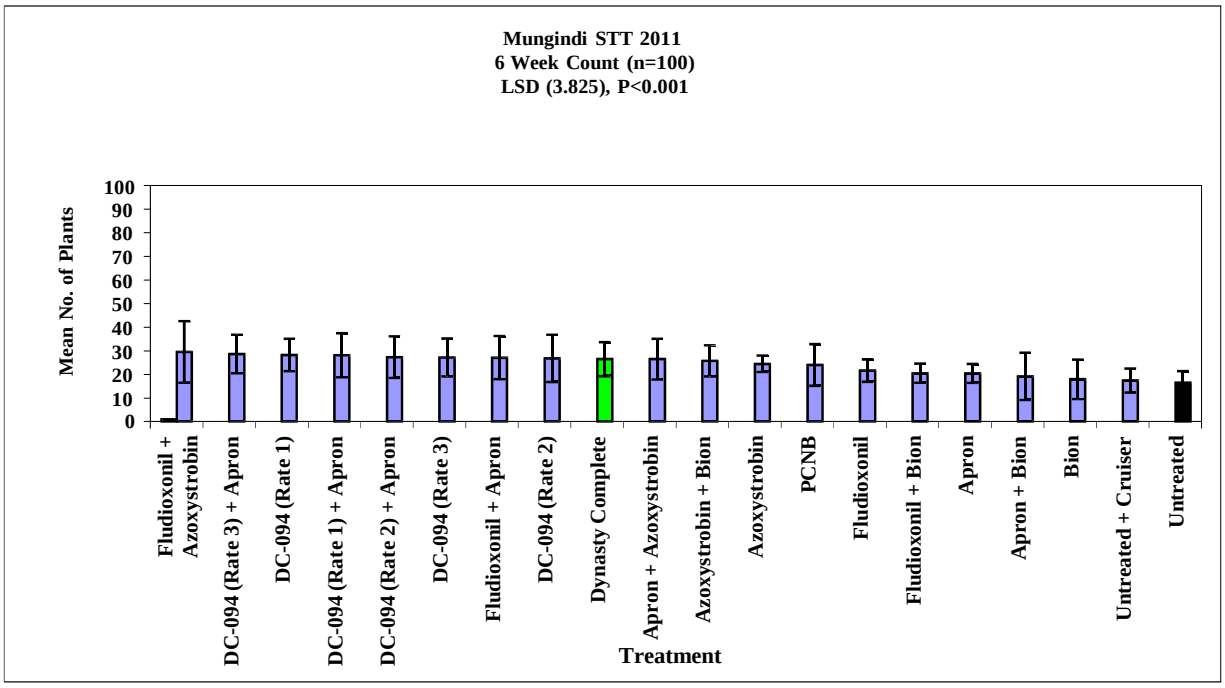


Figure 2.2.5 Mean stand counts six weeks after sowing (24/09/11) following seed treatment with various fungicides at Mungindi site. Significant differences between seed treatments (P<0.001).

At the QLD Downs site, seedling mortality was moderate (Figure 2.2.6). The lack of affect of PCNB at this site also indicated that *Pythium* spp. may have been the dominant pathogen early this season. Mean % of plants across the trial was 69%.

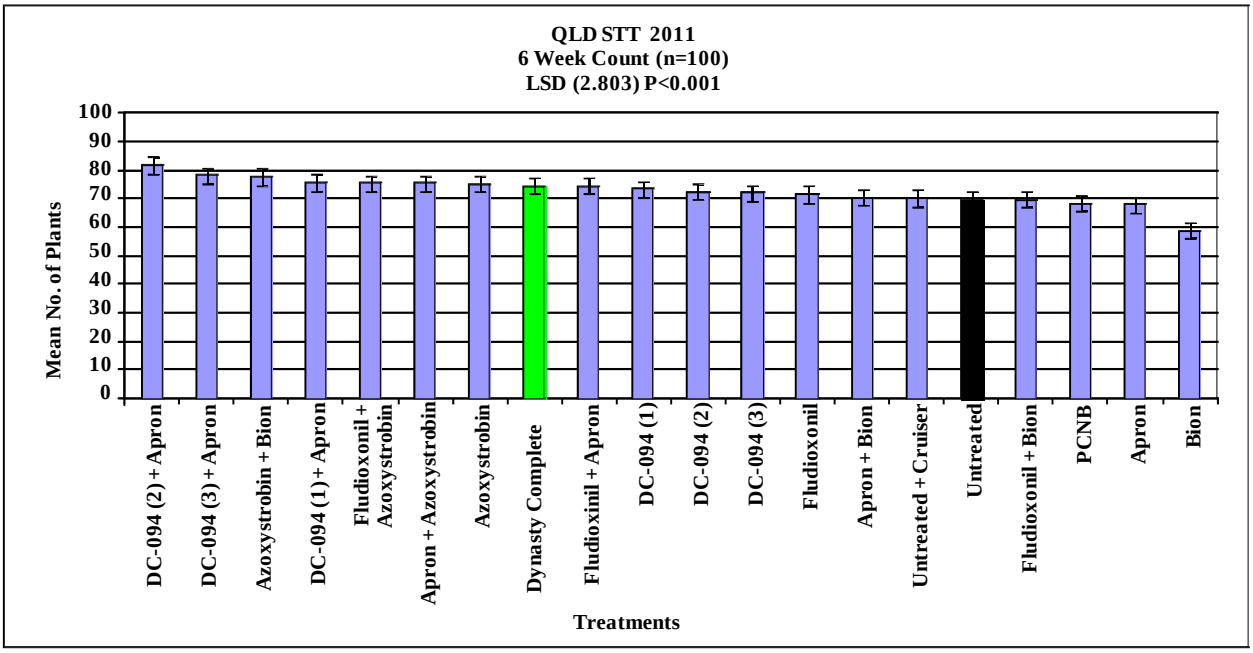


Figure 2.2.6 Mean stand counts six weeks after sowing (25/10/11) following seed treatment with various fungicides at QLD site. Significant differences between seed treatments (P<0.001).

Due to the wet weather conditions in the early season, the trial sites at Breeza, Hillston and Warren were not evaluated. These sites will (if possible) be assessed in the late season.

Conclusions:

Seed treatments did reduce seedling mortality, however the results were varied across the different geographic locations in which the trials were conducted. Dynasty® CST and Dynasty® Plus consistently reduced seedling mortality in the early planting and normal planting trials at ACRI Narrabri and the Mungindi trial site. Seed treatment efficacy can vary depending on the time of planting, geographical area and local conditions in which seed are planted.

Sowing should be delayed as much as possible in order to minimize the effects of seedling diseases such as black root rot, *Pythium* spp. and *Rhizoctonia solani*. Growers should continue to treat seed with fungicides and insecticides in order to minimize the risk of stand loss. Dynasty® CST was the most consistent seed treatment 2010/2011 season and Dynasty Complete in 2011/12 season. These findings are consistent with 2009/2010 seed treatment results. Pressure from pathogens varied between trial sites and is strongly influenced by temperature and soil moisture. Lesions on the hypocotyl were observed both below the soil level and protruding above the soil surface on many plants, consistent with the wet start in September and continued heavy falls of rain throughout November and early December 2010.

Use of seed treatments should be integrated with other management practices outlined in the Integrated Disease Management Guidelines produced by the Cotton CRC and Cotton R&D Corporation.

Chapter 3 – Long term biofumigation

Objective 3 – Continue to evaluate IDM strategies for the control of Black root rot including crop rotation and soil amendments.

Introduction:

Black root rot is caused by the pathogenic fungus *T. basicola* infecting a large number of host plants including field pea, chickpea, lupins, mung bean and tobacco (Simmonds, 1966). The first report of infection in cotton (*Gossypium barbadense* L.) was in Sacaton, AZ in 1922 (King & Presley, 1942). The earliest detection of this soil-borne fungus in Australian cotton (*Gossypium hirsutum*) seedlings was in 1989 (Allen, 1990). The pathogen invades the roots of young seedlings (up to 14 days after planting) (King & Presley, 1942), causing a dark-brown to black discoloration of the roots and occasionally the hypocotyl resulting in reduced root and shoot growth. In severe cases, this can lead to delayed maturity and reduced crop yields (Allen, 1990).

Black root rot is most severe in the early cotton season, when soil temperatures are below 24^{o}C and soil water content is high (Rothrock, 1992). Whilst some roots will die, *T. basicola* does not directly kill seedlings. Instead the pathogen will enhance seedling death caused by *Pythium* or *Rhizoctonia*. As temperatures increase, seedlings resume normal growth and the diseased tissue sloughs off *T. basicola* is characterised by thick-walled chlamydospores in chains and thin-walled endospores. This pathogen can only reproduce on living host plants and do not grow on crop residues. Cropping sequence is directly related to density of pathogen in the soil given that copious quantities of spores are produced on cotton roots and in the adjacent soil during each growing season.

Method and Materials:

In a completely randomized plot design, 4 treatments (fallow, canola, chickpea and vetch) were replicated 6 times. The trial covered 96 rows, consisting of 24 plots of 8 rows. Two transects were taken from each plot, one at the head ditch end and the other at the tail ditch end. The trial was divide into tiers with plots 1 to 12 in tier one and 13 to 24 in tier two (Figure 3.1.1). In summer 2008/2009 sunflower was sown across the entire trial to establish an approximately

uniform starting point of Black root rot inoculum. In February 2009, the sunflowers were turned in and left to fallow. On the 4th May 2009, a total of 192 soil cores were taken using a 5 cm diameter down pipe. For each sampling position 4 x 4 cm cores were taken from rows 3, 4, 5 and 6 in the 0-15 cm region of each bed top. The 4 cores were merged into one sample. Soil cores from the 4 rows were blended at both head and tail ditch ends giving 48 samples in which the Black root rot inoculum levels were quantified.

On the 5th May 2009, Ag-Muster canola, Tyrone chickpeas, Namoi vetch were planted across the trial including plots of bare fallow in a randomized design. Thereafter, 48 soil samples from the 24 plots were analysed each season. Samples from plots 1 to 12 (tier one) were taken 15 m from the head ditch and the second transect taken 15 meters toward the head ditch from field mid point maker. Samples from plots 13 to 24 (tier two) were taken 15 m toward the tail drain from field mid point maker and the second transect taken 15 m from the tail drain.

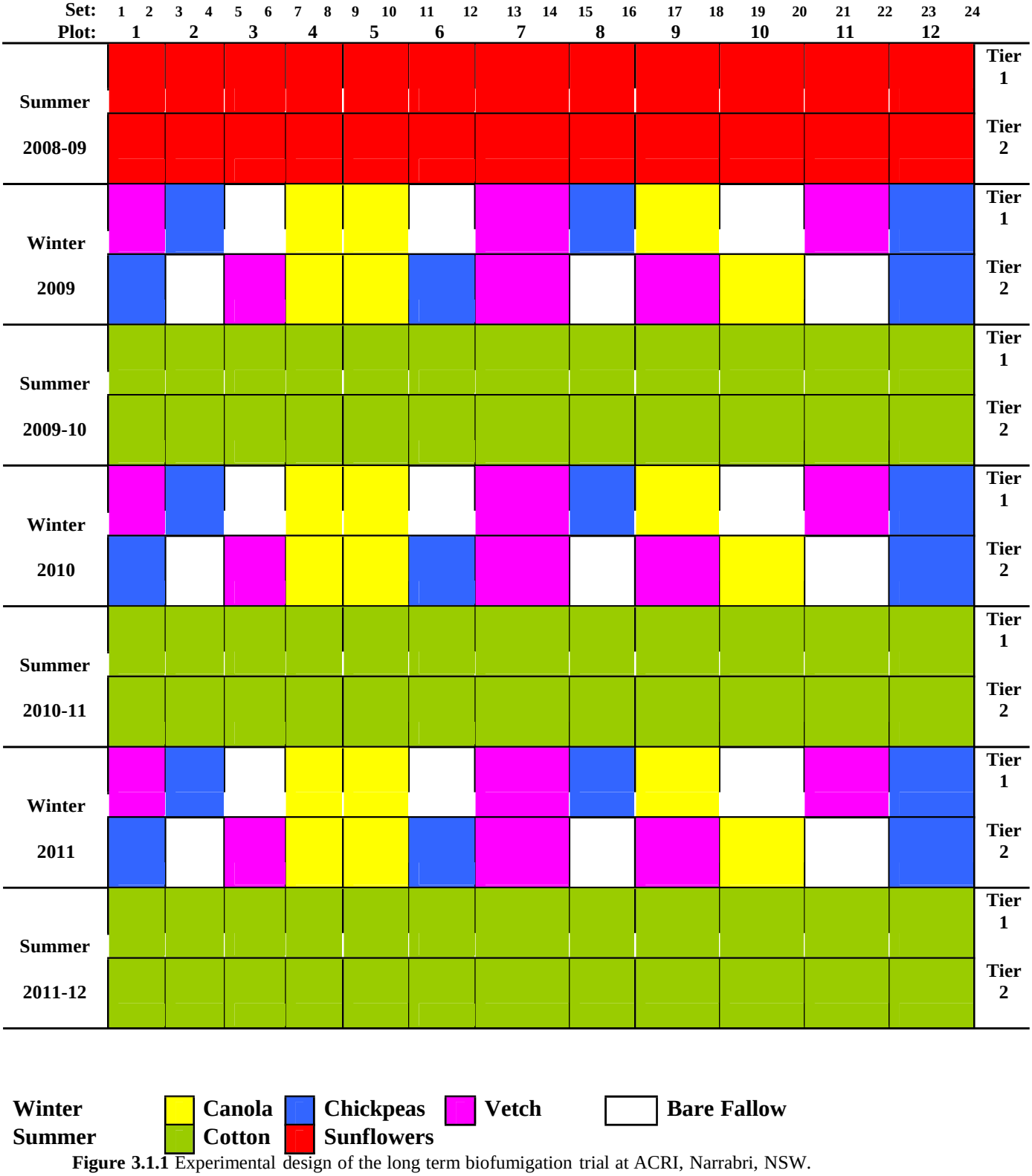


Figure 3.1.1 Experimental design of the long term biofumigation trial at ACRI, Narrabri, NSW.

Subsamples of the soil from the cores were split and used in the selective medium TbCEN to quantify *T. basicola* chlamydospore numbers in the soil using the method described by Specht and Griffin (1985) and Chittaranjan and Punja (1993). Reagents and concentrations used are listed in Table 3.1.2 In short, 10 g of sub-sampled soil was suspended in 0.1% distilled water agar (DWA), providing a 1 in 10 soil dilution. Using a mechanical shaker, the samples were shaken vigorously for approximately 10 minutes.

One mL of soil suspension was pipetted into each Petri plate (90 mm), after which 25 mL of molten medium was dispensed into each Petri dish using sterile tubing attached to a peristaltic pump, using a swirling motion to mix soil and medium whilst dispensing. Five plates were used for each soil collection and 5 containing no soil as controls. Plates were incubated at 23^{o}C under continuous darkness and inspected after 7 to 10 days for the presence of colonies growing on the medium. Counts were adjusted to colony forming units (CFU) per gram of soil by multiplying by the dilution factor of 10.

Inconsistent enumeration of Black root rot inoculum levels in soil using the TbCEN assays and discussions with international pathologists, it was decided that relative comparisons of infectivity of soils would be estimated using the carrot disc method described by Honess (1994) for soil samples taken after 2011. Although the carrot disc method does not accurately quantify inoculum levels in soil it does provide conservative estimates for comparison. In short carrots, approximately 20-30 mm in diameter were prepared first by transversely slicing into discs of equal width (5-6 mm thick), then surface sterilizing and rinsing in sterile water. Discs were then stored in sterile water for up to 30 minutes before use. Five wells were made in the cortical tissue of each carrot disc using a 6mm cork borer. Care was taken not to bore all the way through the disc. There were 5 carrot discs per plate with 4-6 replicates. From the shaken 1 in 10 soil dilutions per treatment, 0.1ml of soil was placed in each well of the carrot discs. Pathogen populations can be expressed on a weight or volume basis known as colony forming units (CFU) per gram of soil or CFU/cm^3 of soil. Estimated population per cm^3 of *T. basicola* is calculated as the average number colonies per plate multiplied by the dilution factor.

Table 3.1.2 Reagents used in TB-CEN semi-selective medium for determining soil populations of *T. basicola* in soil collected from ACRI Field 4.

Reagent	Concentration
Agar (commercial grade)	1.50%
Calcium carbonate ($CACO_3$)	1.0 g/L
Streptomycin sulphate	500 mg/L
Penicillin G potassium salt	100,000 U/L
Chlortetracycline hydrochloride	50 mg/L
Nystatin	125,000 U/L
Etridiazol (added as Terrazole 35 WP)	400 mg/L
Carrot extract (100%)	100 mL/L

The remainder of the soil was used in glasshouse and growth room pot experiments to establish the effect of treatments on severity of Black root rot on cotton. For each pot experiment, a completely randomized design was conducted in a controlled environment to examine the effects of each treatment on Black root rot. Grapevine tubes were lined with patty cake liners and filled with soil collected from each treatment. Into these, 12 Sicot 43 BRF seeds were placed on level soil surface and covered with vermiculite and sand mix (1:1 ratio). Tubes were watered from below using small individual take away containers under each tube. To ensure the seed being used was viable, germination tests were carried out by placing 300 Sicot 43 BRF seeds on wet paper towel (100 on each paper towel) and rolled into a tube, placed into a plastic container then into an incubator set at 22^{o}C, no light. After 10 days, seed viability (90.3%) was calculated as the average percentage of seeds that had germinated.

Severity of disease was established twenty one days after emergence using the method described by (Nehl, et al., 2004a), with the roots of up to 10 seedlings per treatment being rated for disease severity on a 0 to10 scale (as described earlier). Roots were cut from the plant at the soil line and placed into envelopes, along with the aboveground shoot material. Shoots and roots were placed into the dehydrator for 48 hours, before obtaining dry weights. Samples of root were then placed into Petri dishes and covered with distilled water. The presence or absence of chlamydospore chains of *T. basicola* were established by observing the root material with a dissecting microscope.

In summer 2009/2010, cotton was planted across the whole trial. Stand counts and severity of Black root rot disease were determined approximately 6-8 weeks after germination. Stand counts were calculated as the average number of plants established in 1 meter transects in each tier in each treatment. After disease severity was rated, roots were then cut from the plant at the soil line and placed into envelopes, along with the aboveground shoot material. Shoots and roots were placed into the dehydrator for 48 hours, before obtaining dry weights. Cotton growth was maintained, cut out early, harvested and the trial site prepared for biofumigation crops to be planted in winter.

No soil cores were taken (Winter 2010) due to staff changes and wet field conditions. On the 24/05/2010 Namoi Vetch, Tyrone Chickpea was planted across the trial. Then on the 25/05/2010 Ag-Muster Canola was planted across the trial. The trial came up on rainfall. Weeds were a problem across all the plots. Due to mixed cropping, wet conditions and fertilizer placement less biomass was produced by all the crops. Biofumigation crops were turned in and beds prepared for cotton to be planted in summer 2010.

In summer 2010/2011, cotton was planted across the whole trial and stand counts and Black root rot severity determined as described earlier. Defoliant was applied to the cotton 25th March 2011. Cotton was picked then the stubble slashed. The trial was cultivated and biofumigation crops were planted the end of April. After irrigation, high weed load and imperfect watering up meant the trial was sprayed out the second week of May and treatments were replanted 8th June. Biomass was cut on the 16th September 2011 before the crops were slashed and turned in on the same day. Data from the biomass cuts was recorded (dry matter converted from grams per plant to tonnes per hectare). Where possible soil samples were collected from each plot and used to grow plants in the glasshouse and growth room to assess disease severity and biomass growth. In summer 2011/2012, cotton was planted across all plots and stand counts and b lack root rot severity determined as described earlier.

Statistical Analysis: GenStat (11th Edition) (Payne, Murray, Harding, Baird, & Soutar, 2008) Regular Grid spatial modelling (REML) was used to analyse data separately for average disease severity (ADS), shoot dry weight and root dry weight (where applicable). Statistical significance was assessed and reported at the 5% probability level.

Results:

Average Disease Severity

There was no significant difference in the average disease severity of black root rot on cotton grown following the first year of growing biofumigation crops in winter 2009 (P=0.988), or 2010 (P=0.241). After a third year of biofumigation crops in winter 2011, the average disease severity was approaching significance (P=0.007). ADS was highest in the fallow treatment (6.442) followed by vetch (5.656), then chickpea (5.592) and finally canola (4.923) (Figure 3.1.2).

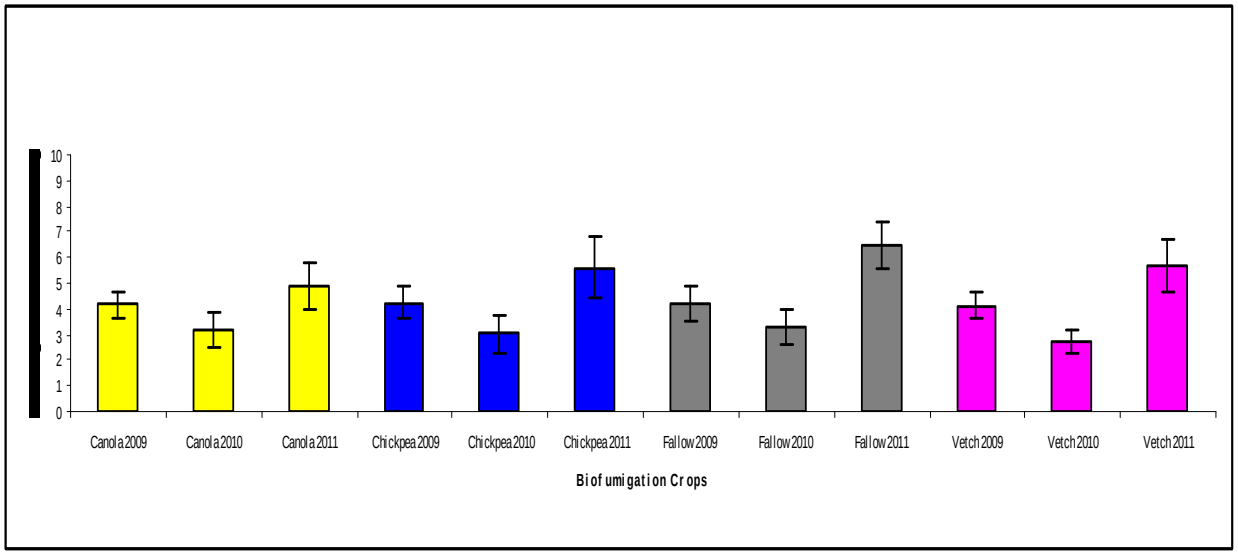


Figure 3.1.2 Effect of consecutive winters growing biofumigation crops: canola, chickpea, fallow and vetch on average disease severity of Black root rot in cotton.

Biomass Assessment

There was no significant difference in shoot dry weights between treatments (P=0.156) however there was a significant difference (P<0.001) in the shoot dry weight (g/plant) after two years of growing biofumigation crops. Figure 3.1.3 shows the increase in biomass per plant for each treatment. The dry matter cuts converted to tonnes per hectare were significantly (P=0.012) higher for canola (3.304) than both chickpea (2.035) and vetch (2.234).

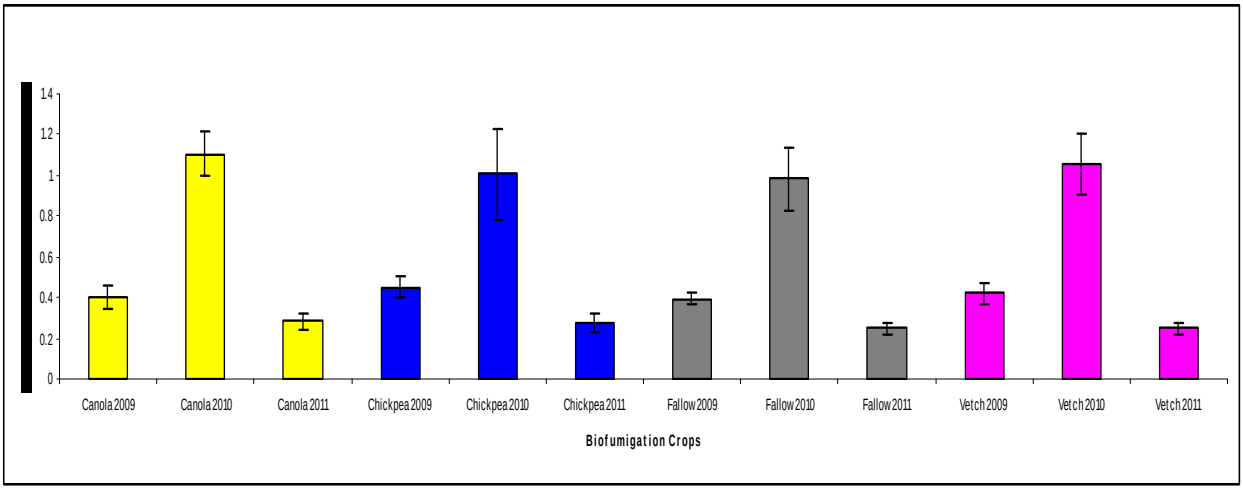


Figure 3.1.3 Effect of two winters growing biofumigation crops: canola, chickpea, fallow and vetch on cotton biomass (dry weight) per plant.

Field results: Stand Count

There was no significant difference in stand counts of cotton grown in any of the treatment plots ($P=0.094$) however there was a significant difference ($P<0.001$) between the first year and second year that biofumigation crops were grown (Figure 3.1.4).

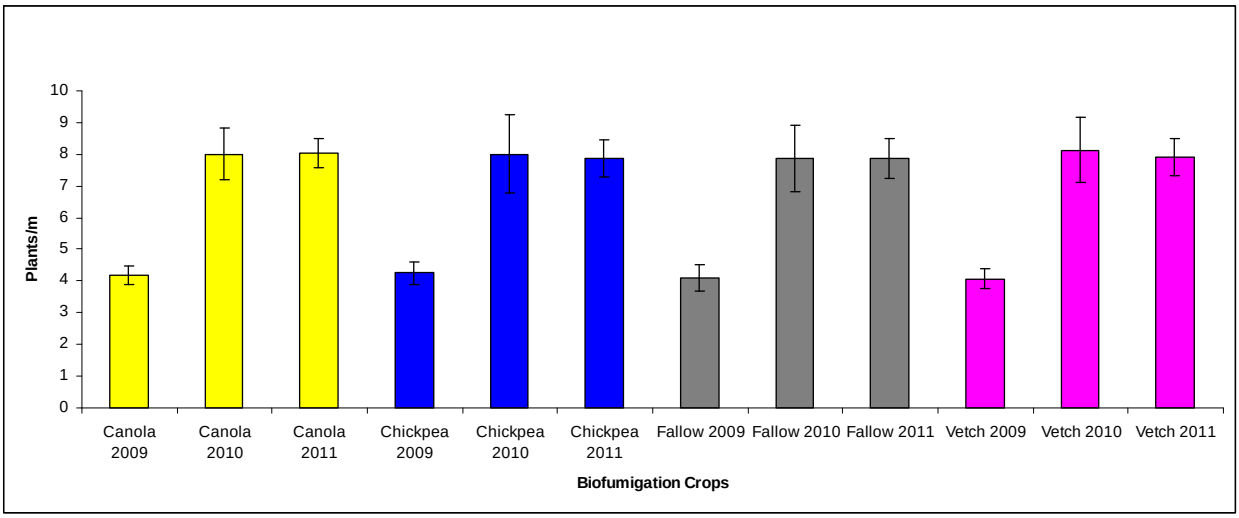


Figure 3.1.4 Effect of two winters growing biofumigation crops: canola, chickpea, fallow and vetch on the stand count per meter of cotton plants

Conclusions:

This trial continues on from the work of Dr Nehl and previous biofumigation experiments. The aim of this trial was to evaluate if biofumigation crops can prevent the increase of black root rot from initial levels of the pathogen in the soil. Dry matter production from the biofumigation crops in 2011 was poor as a result of delays in planting and cool conditions. This trial is in the early stages and the lack of reduction in inoculum levels recorded in this project is similar to the results reported in Nehl's DAN177C report. The low reduction may be as a result of insufficient biofumigation biomass generated during the experiment to have a measurable effect on *T. basicola* spores in the soil. It is important that the long term biofumigation trial continue as previous work (DAN 122C) has demonstrated that biofumigation crops were successful in decreasing the severity of Black root rot and preventing the build-up.

Chapter 4 – Long term rotation

Objective 3 – Continue to evaluate IDM strategies for the control of Black root rot including crop rotation and soil amendments.

Introduction:

Black root rot is difficult to control due to the nature of dormant endospores and chlamydospores, however management practices may aid in reducing severity of this disease. When implementing crop rotation programs in soils affected by Black root rot, it is important to know the host range of *T. basicola*. Crop rotations with non hosts such as cereals can delay, but not prevent the increase in distribution or severity of the disease (Nehl, et al., 2004a). This is achieved by the lack of infection of roots which would normally be followed by the production of propagules, directly preventing major increases in inoculum levels (Reddy & Patrick, 1989). Using legumes (hosts) can increase inoculum levels in soils; however specific methods of managing such legume crops can and has been reported to suppress the disease. Incorporating crops such as vetch can reduce soil populations and incidence of the disease in subsequent cotton crops (Kendig & Rothrock, 1991). Management options for reducing the populations of this pathogen are limited to extended crop rotations, use of fungicide seed treatments, systemic acquired resistance (SAR) chemical inducers, biofumigation and delayed planting dates when soil temperature is warmer (Mondal, Nehl, & Allen, 2005) and planting into raised beds which helps to increase the soil temperature around the seedling and improved drainage. This long term trial evaluates the effect of biofumigation crops in preventing the build up of Black root rot caused by *T. basicola* in a continuous cotton system.

Method and Materials:

The long term rotation trial was set up in Old 2, ACRI in the winter of 2010. This long term rotational trial assessed crop plant species for rotation that may reduce disease development. The experimental design consists of 6 treatments (canola, chickpea, faba bean, oats, wheat and winter fallow/summer cotton), replicated 5 times in a completely randomised design. The trial covered 240 rows (30 plots of 8 rows) (Figure 4.1.1). Soil samples were taken from plots before treatments (rotations) were imposed, with no significant difference (P=0.850) between plots for Black root rot inoculum levels in the soil. Chickpea, canola, faba bean and oats were planted at

the end of May 2010 and bare plots worked the first week of September 2010 in preparation for cotton being planted in October 2010. Due to weed issues the chickpea and faba bean plots were removed in September 2010. Soil samples after rotations were taken to be used in pot experiments to evaluate incidence and severity of Black root rot.

After planting, plants were assessed both in the field and in soil collected from the field and then grown in the glasshouse. Field assessments included the average disease severity of Black root rot, incidence of Verticillium wilt, plant numbers and shoot dry weight. Data collected from plants grown in the glasshouse included average disease severity of Black root rot, shoot and root dry weight.

Summer 2010 cotton was planted in the plots that had previously been fallow. All other plots remained fallow during winter. Summer 2011/2012 a buffer of sunflower was planted in the western 16 rows and cotton (variety 43RRF) was planted in all of the plots. Soil samples were taken from all plots and plants grown in the glasshouse in these soil samples for disease assessment.

Statistical Analysis: GenStat (11th Edition) (Payne, et al., 2008) Regular Grid spatial modelling (REML) was used to analyse data separately for average disease severity (ADS), shoot dry weight and root dry weight (where applicable). Statistical significance was assessed and reported at the 5% probability level.

Figure 3.4.2 Long term rotation field plan cycle.

Results:

Field results: Initial results on commencement of trial showed no significant difference (P=0.303) in the average disease severity in plants assessed in the field for any treatment. Average disease severity was highest following the rotation of canola (3.687), then faba bean (3.303), oats (3.283), wheat (3.203), fallow (3.140) and chickpea (3.070) with a standard error of difference of 0.2761. After 1 cycle of rotation crops grown in winter 2010 and bare fallow in winter 2011, results showed a significant difference (P=0.023) in the average disease severity in plants assessed in the field (Figure 4.1.2). Disease severity was significantly lower in wheat (3.307), fallow (3.433) and oat (3.525) rotations compared to faba bean (3.789), canola (3.820) and chickpea (4.006) with a standard error of difference of 0.2226.

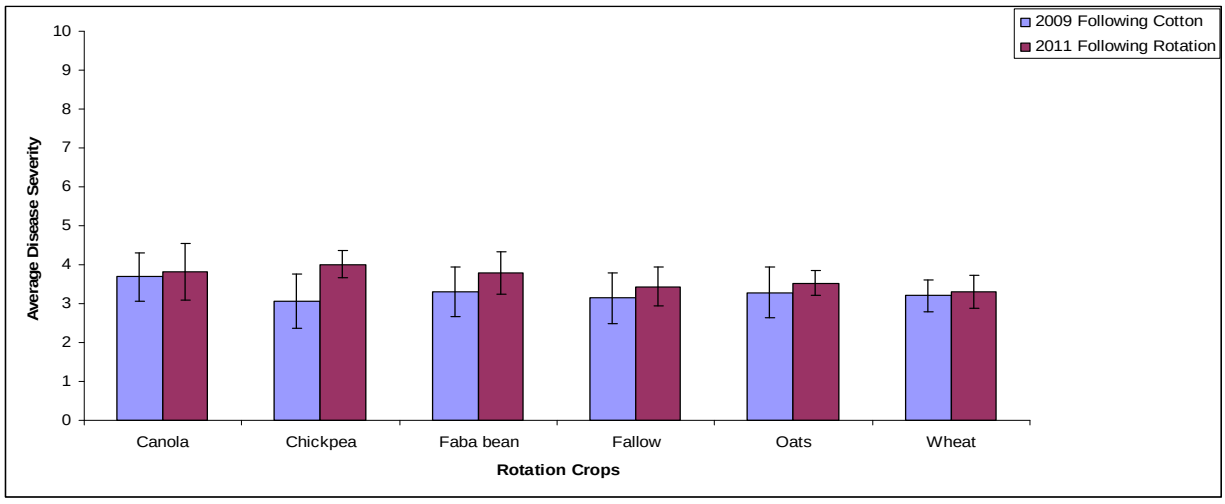


Figure 4.1.2 Average disease severity in cotton plants before and after a winter rotation crop.

There was no significant difference (P=0.460) in the incidence of Verticillium wilt in the initial year of the trial. Disease incidence was highest in plants in plots to be planted with chickpea (24.25), then fallow (21.5), wheat (17.5), faba bean (17.17), oats (15.33) and canola (13.08) with a standard error of difference of 5.962.

No significant difference was found in the number of plants per meter (P=0.973) on commencement of the trial. The highest mean number of plants per meter was found in plots to be rotated with fallow (7.2), chickpea (6.8), faba bean (6.8), canola (6.7), wheat (6.7) and oats (6.7) with a standard error of difference of 0.6627. A significant difference was found in the number of plants per meter (P<0.001) after the rotation crops were grown in winter 2010 and bare fallow in winter 2011 (Figure 4.1.3). The highest mean number of plants per meter was recorded after the fallow rotation (8.7), followed by chickpea (8.4), faba bean (8.2), wheat (7.8), canola (7.6) and oat (7.5) rotations with a standard error of difference of 0.2495.

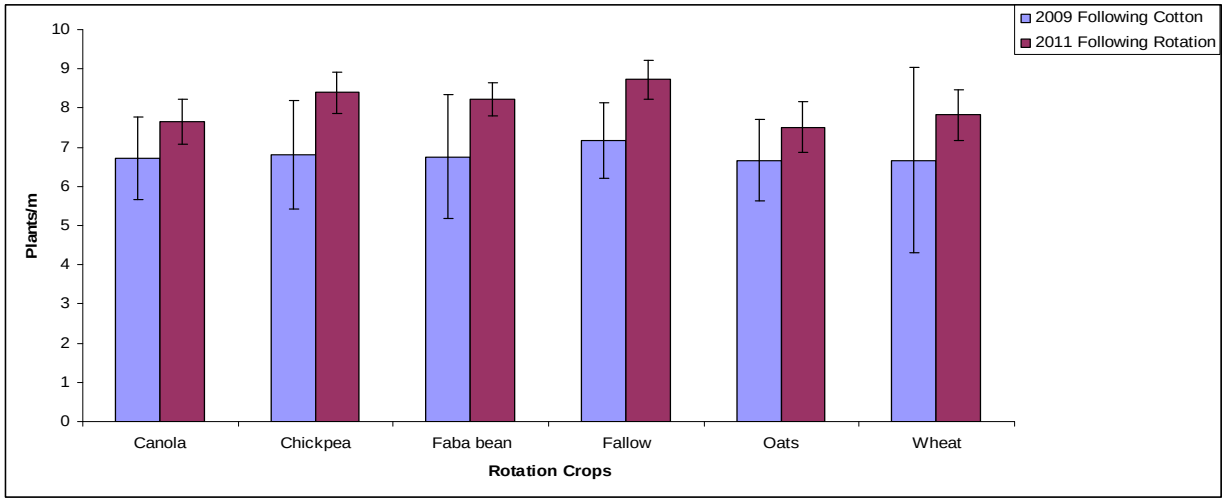


Figure 4.1.3 Average plants per meter before and after a winter rotation crop.

There was no significant difference (P=0.512) in the shoot dry weight per plant upon commencement of the trial. The highest shoot weights were recorded for the plots to be rotated with chickpea (0.3601), then fallow (0.3294), faba bean (0.3146), oats (0.3055), wheat (0.2964)

and canola (0.2827) with a standard error of difference of 0.04157. There was a significant difference (P=0.038) in the shoot dry weight per plant after the rotation crops were grown in winter 2010 and bare fallow in winter 2011 (Figure 4.1.4). The highest shoot weight was recorded following rotations with fallow (0.3899), followed by chickpea (0.3374), wheat (0.3563), oats (0.3543), canola (0.3358) then faba bean (0.3294) with a standard error of 0.01952.

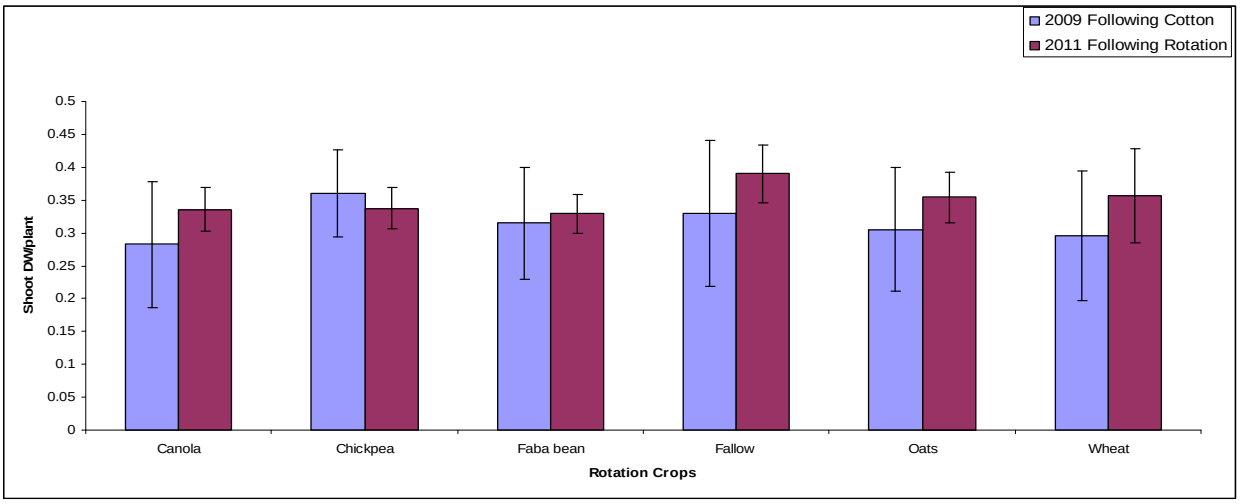


Figure 4.1.4 Average shoot weight per plant before and after a winter rotation crop.

Glasshouse results: There was no significant difference in the average disease severity of Black root rot (P=0.762) of plants grown in the glasshouse from soil collected from the field on commencement of the trial. After the rotation crops were grown in winter 2010 and bare fallow in winter 2011, there was still no significant difference in the average disease severity of black root rot in plants grown from collected soil (P=0.276).

Initially the highest disease severity was recorded in pots from the treatment that was to be followed by the canola rotation (3.021) then oats (2.745), fallow (2.686), faba bean (2.583), wheat (2.540) and chickpea (2.537) rotations with a standard error of difference of 0.3623. After the rotation crops (Figure 4.1.5), the disease was most severe in soil that had the fallow rotation (6.970), followed by wheat (6.750), chickpea (6.170), canola (5.960), oats (5.590) and faba bean (5.035) with a standard error of difference of 0.8922.

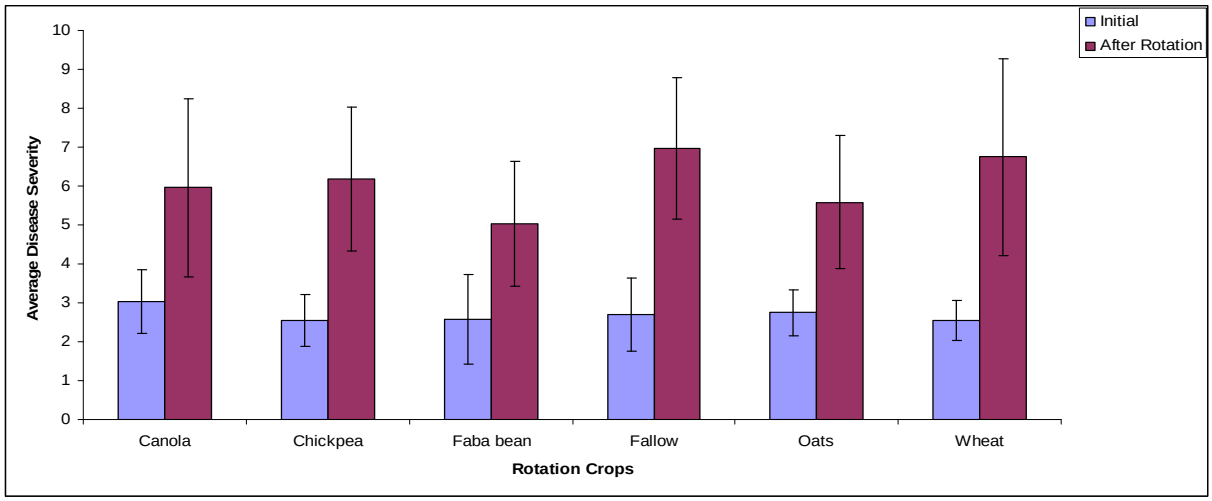


Figure 4.1.5 Average disease severity in cotton plants before and after a winter rotation crop.

There was no difference in the shoot dry weight (P=0.470) from the plants grown in soil collected initially from the field. Shoot dry weights for the different rotations were canola (0.1730), wheat (0.1487), oats (0.1478), fallow (0.1458), chickpea (0.1433) and faba bean (0.1350), with a standard error of difference of 0.01875. After rotation crops (Figure 4.1.6) the shoot dry weight were chickpea (0.1705), wheat (0.1696), canola (0.1671), faba bean (0.1586), fallow (0.1575) and oats (0.1456) with a standard error of difference of 0.01286.

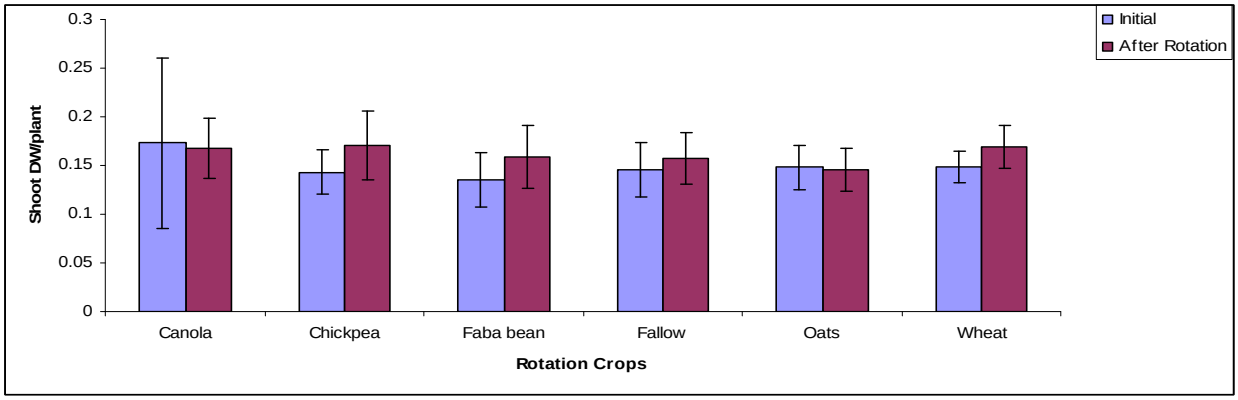


Figure 4.1.6 Average shoot weight per plant before and after a winter rotation crop.

There was no difference in the root dry weights of these plants grown in soil collected initially (P=0.677) or after rotation crops (P=0.316). Initially the highest root weight was recorded from soil collected from that were to be rotated with canola (0.02641), then oats (0.02303), chickpea (0.02087), fallow (0.02006), wheat (0.02000), and faba bean (0.01929), with a standard error of difference of 0.004778. After the rotation crops (Figure 4.1.7) the highest root weight recorded

was in the chickpea rotation (0.03165), followed by canola (0.03025), wheat (0.02990), faba bean (0.02735), fallow (0.02659) and oats (0.02597), with a standard error of difference of 0.002942.

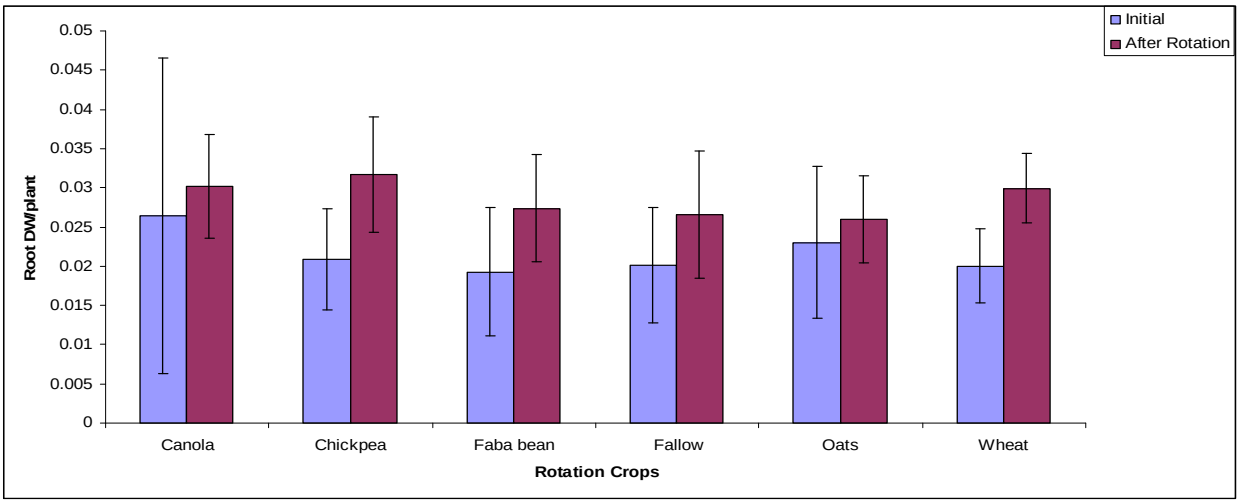


Figure 4.1.7 Average shoot weight per plant before and after a winter rotation crop.

Conclusions:

This rotation trial will continue to be monitored and results gathered up to and including 2012/13 season. Growing conditions, lack of water and/or inability to water at appropriate times, along with weed control contributed to problems with this trial. Refined methods for communication with farm staff and organisational tasks have been implemented in order to improve the outcome from this trial. Further work still needs to be done in this area to ensure growers have the latest information on possible management strategies for disease minimisation.

Chapter 5 – Mycorrhizal colonisation

Objective 4 – Continue to investigate the effect of long bare fallows on mycorrhizal colonisation of cotton.

Introduction:

Mycorrhizal fungi, also known as arbuscular mycorrhizal (AM) fungi, or formally vesicular-arbuscular mycorrhizal (VAM) fungi form a beneficial relationship with the roots of most agronomically important plants, including cotton. Arbuscular mycorrhizal fungi (AMF) colonise the root cortex of mycotrophic plants, forming mycorrhizas which transport phosphorus and other nutrients to the plant in exchange for photosynthate. The fungus benefits from sugars supplied by the host plant and in turn the plant receives improved nutrition. Cotton is highly dependent on VAM for the uptake of these nutrients, and a lack of these beneficial fungi can result in slowed seedling growth (D. Nehl, Allen, & Anderson, 2008). If compatible plants are absent for long periods, it was thought that AMF populations in the soil would decline and the mycorrhizal development may be reduced and consequent nutritional deficiencies (phosphorus and zinc). This disorder was known as the long fallow disorder (Nehl, Allen, Mondal, & Lonergan, 2004c).

The long droughts experienced by many regions led to concerns that mycorrhizal fungi survival being reduced after long periods of fallow. The long term trial was set up to test the hypothesis that VAM levels in soil are reduced in long bare fallows compared to cropped systems.

Method and Materials:

Field site: The long term trial was set up in Field 4, Australian Cotton Research Institute (ACRI), Narrabri, in the summer 2005/2006 cotton season to investigate the impact of cropping versus long bare fallow on inoculum levels in the soil (Figure 5.1). The trial site was 4.6 ha, consisting of 10 plots with a total of 80 rows, each 148.6 m long. The rows were divided in half lengthways to form 2 tiers (within each plot) with tier 1 being towards the head ditch and tier 2 being towards the tail ditch (Table 5.1). In the first year (2005/2006) all rows in each plot were

planted to sunflower. The experiment consisted of 2 treatments: bare fallow and crop rotations sown each winter and summer cotton growing season. For each season, rows 1-8, 17-24, 41-48, 57-64 and 73-80 were bare fallow while rows 9-16, 25-32, 33-40, 49-56 and 65-72 were planted to the rotating crops (Figure 5.2). Cotton planted early, late and both with the addition of Bion was planted in summer 2006/2007. After this a single planting date of cotton was carried out. The long term effects of treatments commenced in winter 2009. Soil cores were collected across all plots and sown to cotton in the glasshouse for assessment of VAM colonisation and Black root rot severity and associated stand counts and shoot weight.

Summer 2010/2011 cotton season had a wet start and continued rain throughout the early growing season resulted in no Black root rot assessment of plants in the field as plants had outgrown the symptoms by the time conditions dried up. Consequently, VAM colonisation and Black root rot severity was assessed from plants growing from seed sown into soil collected from each treatment plot and grown in a temperature and light controlled growth room and glasshouse.

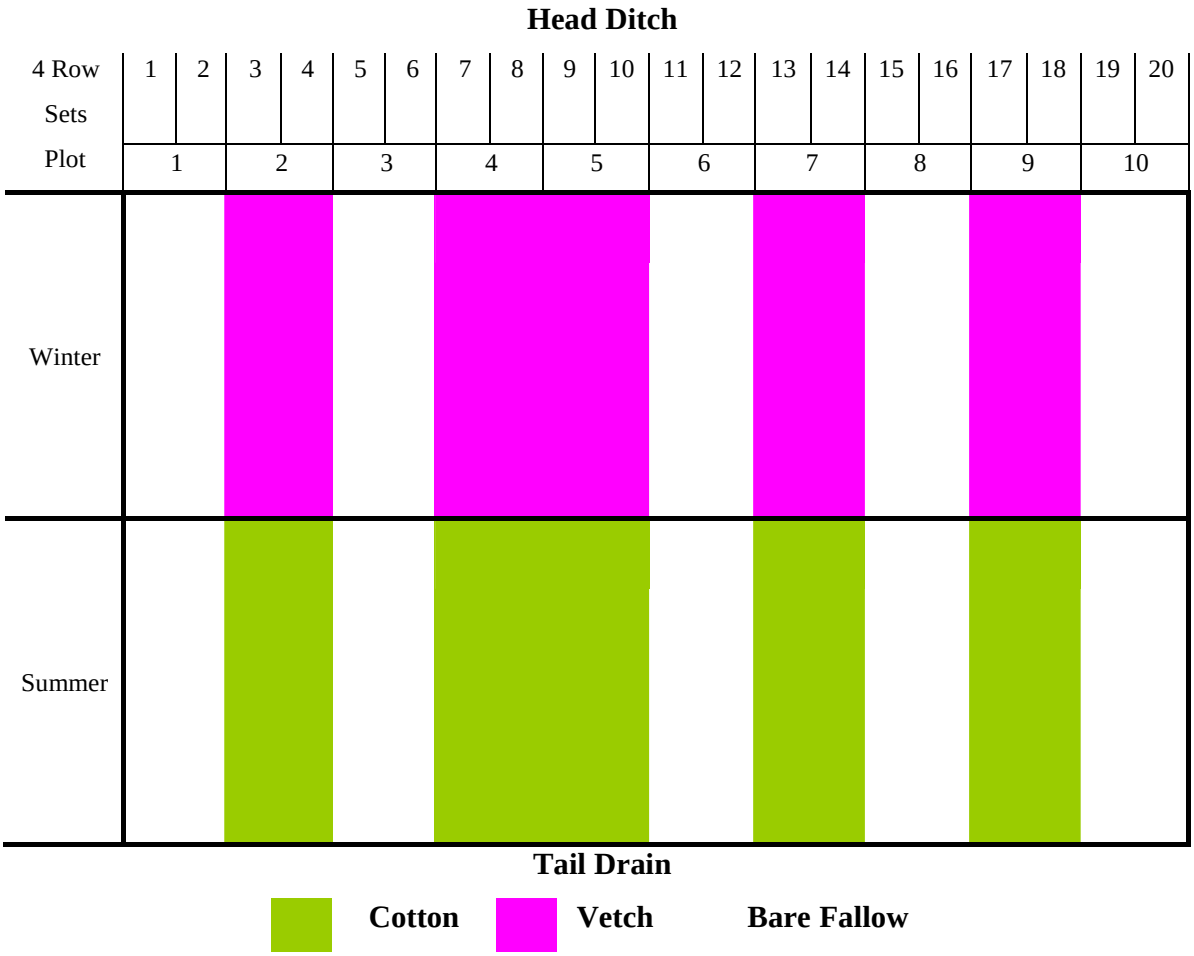
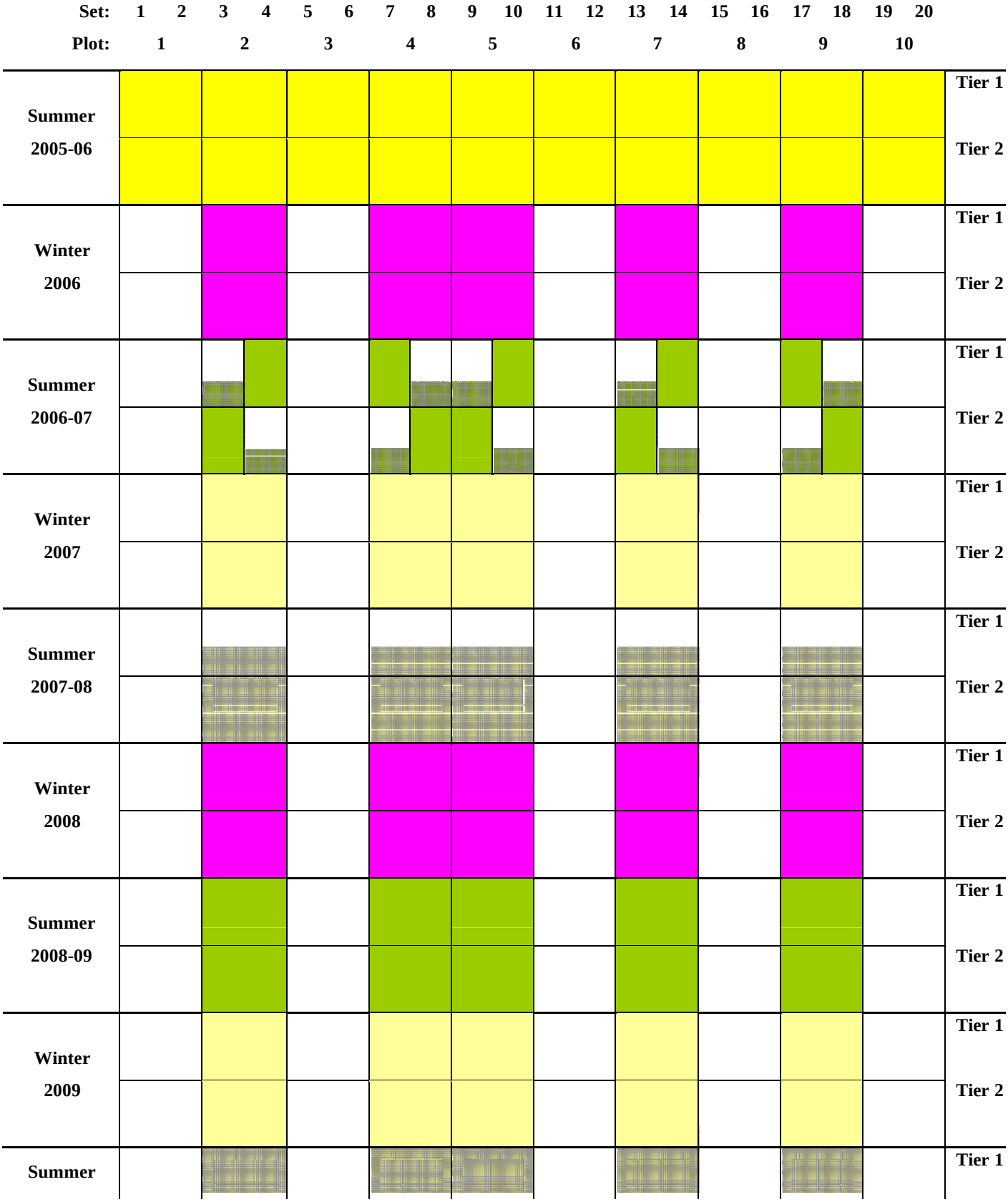

Head Ditch
4 Row
Sets
Plot
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
1 2 3 4 5 6 7 8 9 10
Winter
Summer
Tail Drain
Cotton
Vetch
Bare Fallow

Figure 5.1 Layout of the long term VAM trial in Field 4, ACRI.



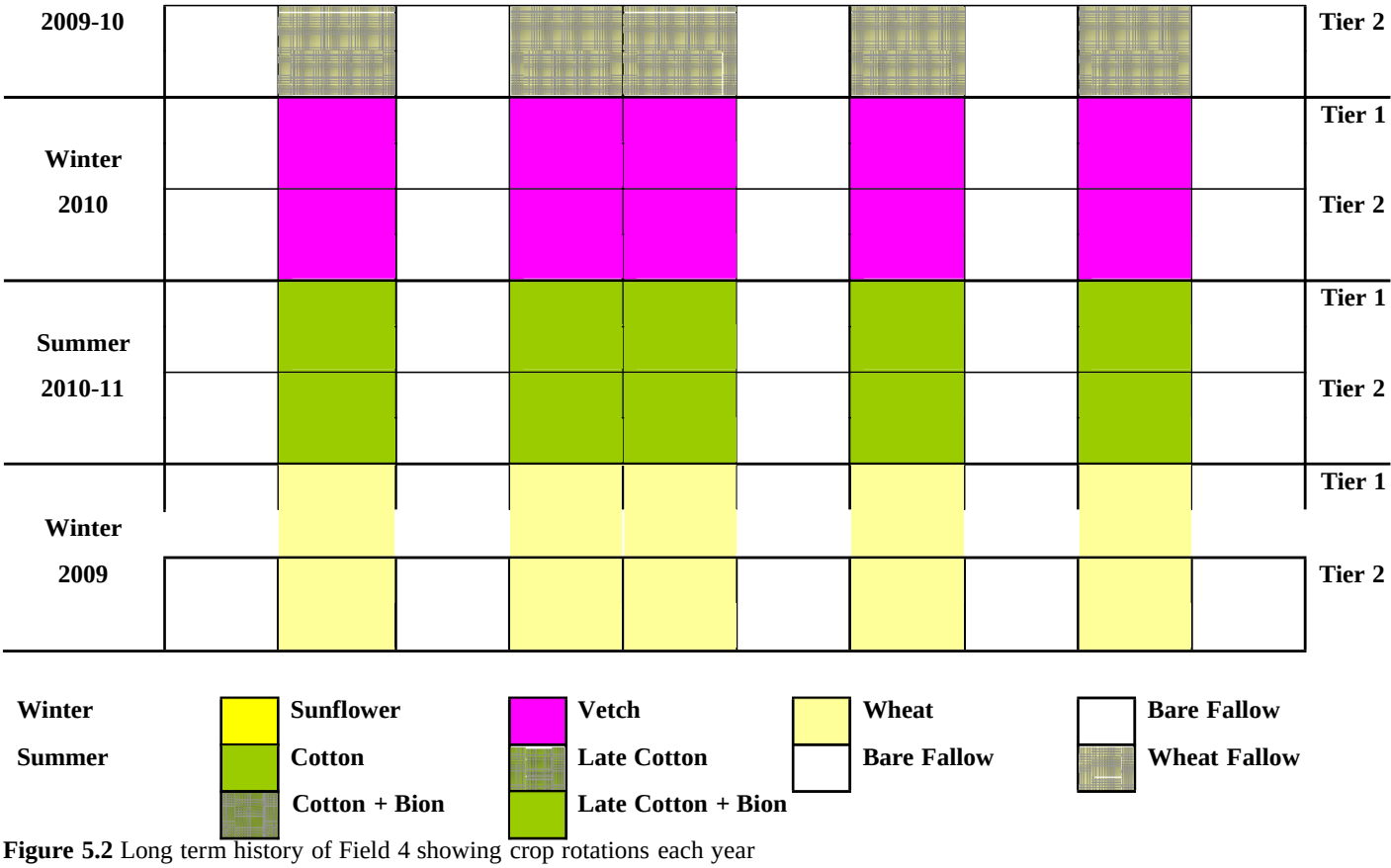


Figure 5.2 Long term history of Field 4 showing crop rotations each year

VAM assessment:

Whole plants were gently removed from pots and washed under tap water to remove soil and debris. Roots were cut from the plant and placed in staining tubes, with tubes being stacked into a stainless steel basket. Empty staining tubes were used to fill the basket when there were less than 30 samples. The basket was placed in a glass dish and 10% KOH (w/v) was added ensuring all the roots were covered by washing the roots to the bottom of the tubes with a stream of tap water before adding KOH. The glass dish was placed into a water bath for 1 hour at 90°C. The KOH was removed from the root samples by washing with running tap water, before leaving the basket to stand in distilled water for a few minutes at room temperature. The water was removed and replaced with 1 L of 2% HCL (necessary for dye to fix to fungal structures) and left for at least 5 minutes at room temperature, ensuring all the roots were well covered. The HCL was removed and replaced with a staining solution: acidic glycerol (500 mL glycerol, 475 mL H_2O, 25 mL 25 HCL) containing 0.05% trypan blue.

Once roots were completely submerged in the stain, they were placed in a water bath for 20 minutes at 90°C. The staining solution was drained. Excess staining solution was washed from the samples and tubes using tap water. The roots were covered with acidic glycerol for de-staining and returned to the 90°C water bath for 30 minutes (or alternatively left overnight at room temperature). The basket containing the vials was tapped vigorously against the side of sink to move the roots to one side of the staining tubes. The roots were washed into vials using a wash bottle containing acidic glycerol. Each vial had approximately 10 mL of acidic glycerol with the root samples.

The vial containing the roots and acidic glycerol were poured into a 90 mm Petri dish with grid lines marked on the bottom of the dish to form approximately 5 mm squares. These gridlines were used as a mechanism for the systematic selection of observational points. Colonisation was assessed by a modified method described by Giovannetti & Mosse (1980). Infection was then measured by recording the number of root/gridline intersects at 100 random points for each plant.

Black root rot assessment:

The severity of black rot disease was assessed approximately 3 to 5 weeks after emergence. Soil was loosened around the base of plants in the field using an asparagus knife. Ten plants were lifted from the soil and the tap roots examined for symptoms of Black root rot and rated from 0 to 10 for each (Nehl, et al., 2004a). The number of cotton plants per 10 m was counted in rows 3, 4, 5 and 6 of each plot and above-ground plant material separated from root material, placed in paper bags and dehydrated for 2 days to obtain dry shoot weights (g/plant).

For glasshouse and growth room assessments, soil was taken from the 10 treatments and placed into 20 grapevine tubes lined with patty cake liners with 2 pots per treatment. A total of 12 seeds (Sicot 43 BRF) were placed on the soil surface and topped with a mix of sand/vermiculite (1:1 ratio). Tubes were water individually by placing round take away containers under each tube and placed in a growth room, set at 12 hours light and a maximum temperature of 22^{o}C, 12 hours dark and a minimum temperature of 18^{o}C. After 1 day, the sand mix was dampened down and containers watered to the top again. Tubes were

then water every couple of days. Three weeks after emergence, dry shoot biomass and disease severity was assessed as described above.

A modified TbCEN medium method described by Specht and Griffin (1985) and Chittaranjan and Punja (1993) was used to determine the inoculum levels of *T. basicola* per gram dry soil collected from fallow and crop plots. *T. basicola* were isolated using this semi-selective medium TB-CEN, which suppresses other fungi and bacteria. Two soil samples were taken from mid distance in each plot. In short, 10 g of sub-sampled soil was suspended in 0.1% distilled water agar (DWA), providing a 1 in 10 soil dilution. Using a mechanical shaker, the samples were shaken vigorously for approximately 10 minutes. One mL of soil suspension was pipetted into each Petri plate (90 mm), after which 25 mL of molten medium was dispensed using sterile tubing attached to a peristaltic pump, using a swirling motion to mix soil and medium whilst dispensing. Five plates were used for each soil collection and 5 containing no soil as controls. Plates were incubated at 23^{o}C under continuous darkness and inspected after 7 to 10 days for the presence of colonies growing on the medium. Counts were adjusted to colony forming units (CFU) per gram of soil by multiplying the number of colonies by the dilution factor of 10.

Statistical analysis:

Completely randomised designs were used for all glasshouse, growth room and field experiments. Results from field experiments were analysed using analysis of variance with spatial analysis (ASREML) applied with planned comparisons of treatments. Liner and nonlinear regression models were fitted to experiments with comparisons of symptoms and other parameters.

Results & Discussion:

VAM assessment:

The Diseases of Cotton IX by Anderson et al. (2010) reported winter 2009 assessments showed no significant difference in VAM levels between cropped and bare soil, indicating that three years of bare fallow was not associated with a reduction in colonisation of cotton roots by VAM fungi. These findings were consistent with Diseases of Cotton VIII final report (Nehl, 2007) in which VAM colonisation occurred in soils that had undergone bare fallows for up to four

seasons or more. The bioassays of VAM inoculum in soil and studies of cotton root colonisation in crops within the field indicated arbuscular mycorrhizal fungi are capable of surviving in soils that undergo bare fallows for many seasons.

The monitoring of the trial continued through 2010/2011 and 2011/2012 cotton seasons. Field assessments of Black root rot were unable to be carried out in 2010/2011 season due to very wet conditions early in the season. Soil was collected in March 2012 and a glasshouse pot experiment was undertaken. Average disease severity from Black root rot and VAM results were not available at the time of this collating this report.

Conclusions:

This long term experiment to date has shown no significant difference in arbuscular mycorrhizal fungi (VAM) levels in plants growing in soil collected from long term bare fallow or cropped soil. These findings were consistent with Diseases of Cotton VIII final report (Nehl, 2007) in which VAM colonisation occurred in soils that had undergone bare fallows for up to four seasons or more. The bioassays of VAM inoculum in soil and studies of cotton root colonisation in crops within the field indicate arbuscular mycorrhizal fungi are capable of surviving in soils that undergo bare fallows for many seasons.

Previous work done by Nehl et al (2004c) on mycorrhizal fungi in long bare fallows reported after 18 months long bare fallow colonisation of cotton by AMF was no lower than with any other crop. In this same report another experiment looked at the effect of nurse crops being grown after 15 months bare fallow at a rain-fed commercial cotton farm. The nurse crops included wheat and chickpea. Again there was no difference in colonisation of cotton by AMF following the nurse crops or long bare fallow. This is consistent with the findings of (Nehl, Allen, Mondal, & Lonergan, 2004b).

Chapter 6 – Pathogen isolates

Objective 5 – Culture collection of isolates of *Verticillium dahliae, Thielaviopsis basicola* and *Fusarium oxysporum vasinfectum* collected.

Introduction:

Samples were collected from infected plants on commercial cotton farms during the 2008/2009, 2009/2010, 2010/2011 and 2011/2012 cotton seasons during the disease surveys. Pathogens causing diseases such as Black root rot, Alternaria, Verticillium wilt, Fusarium wilt, Rhizoctonia, Pythium, sudden wilt and boll rots were isolated and subcultured. Clean single strain isolates have been preserved in long term storage. Information for each isolate includes: GPS coordinates, location, time of sampling and crop history. In total there are 233 single strain isolates in the long term culture collection (Table 6.1).

Table 6.1 Single strain isolates in the long term culture collection.

Pathogen in long term storage	Total
Verticillium dahliae	44
Fusarium oxysporum vasinfectum	52
Alternaria	97
Thielaviopsis basicola	18
Drechslera	4
Rhizoctonia	2
Pythium	1
Alternaria macrospora	1
Curvularia	1
Sudden wilt	7
Mould	2
Unknown	4
	233

Method and Materials:

From this culture collection isolates were used to evaluate the survival of pathogens in different soil types under a range of environmental conditions throughout the NSW. The average disease severity of Black root rot of plants grown in soil collected from four different cotton fields from two cotton regions with the highest levels of Black root rot; the Namoi and Macquarie Valley was evaluated. Soil was collected from different geographical locations known to have naturally high levels of Black root rot. Those being: Namoi (ACRI Old 2) and (Warilea Field 3), Macquarie (Dulla Dulla Field 1) and (Dulla Dulla Field 3).

Soil from each location was potted up into 5 (117x170 mm Black plastic pots with seven 12x15 mm holes in the side at the base) pots. Each pot had 25 (Sicot43BRF) seeds planted before being covered with a sand/vermiculite/potting (1:1:1 mix) and pressed down. Round pot saucers were paced under each pot to facilitate watering from the base. Two experiments were done in the glasshouse and growth room at low and high temperatures. The air temperature was set in the glasshouse for 15-25^{o}C and this was not adjusted before doing a second experiment. Air temperature in the growth room were 17-21^{o}C and 20-25^{o}C range. The average disease severity was assessed in each pot on the 0-10 scale.

Results:

Average disease severity almost doubled after the second experiment in the same soil in both the glasshouse and growth room pot experiments. In order of highest to lowest average disease severity was Dulla Dulla 1 with 9.14, ACRI Old 2 6.39, Dulla Dulla 3 2.82 and Warilea Field 3 2.10 after the second experiment in the glasshouse (Figure 6.1). Similar results were also recorded for the pot experiments in the growth room with average disease severity being: Dulla Dulla 1 with 6.1, Dulla Dulla 3 4.8, ACRI Old 2 4.7 and Warilea Field 3 the lower of 2.7 (Figure 6.2).

Average disease severity almost doubled after the second experiment for both the second glasshouse and growth room trials. The increase in each case may be attributed to inoculum multiplying after the first round of plants were grown in the pots. These experiments were used

as pilot studies for future glasshouse and growth room trials. Future experiments include the isolating the *T. basicola* pathogen from each of these soil types and multiplying up inoculum levels in order to artificially inoculate pasteurised soil from each region. This will allow us to make comparisons of the pathogenicity of each isolate and evaluate if soil type has any effect on disease severity.

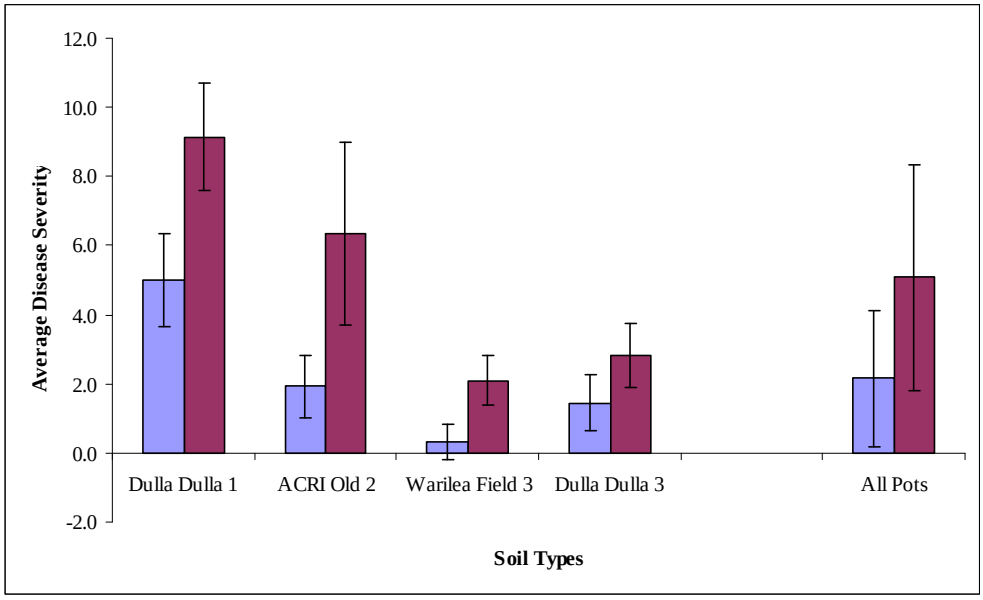


Figure 6.1 Average Black root rot severity in glasshouse pot trials with naturally infected soil collected from different locations in NSW. Temperature range for the glasshouse was 15-25^{o}C.

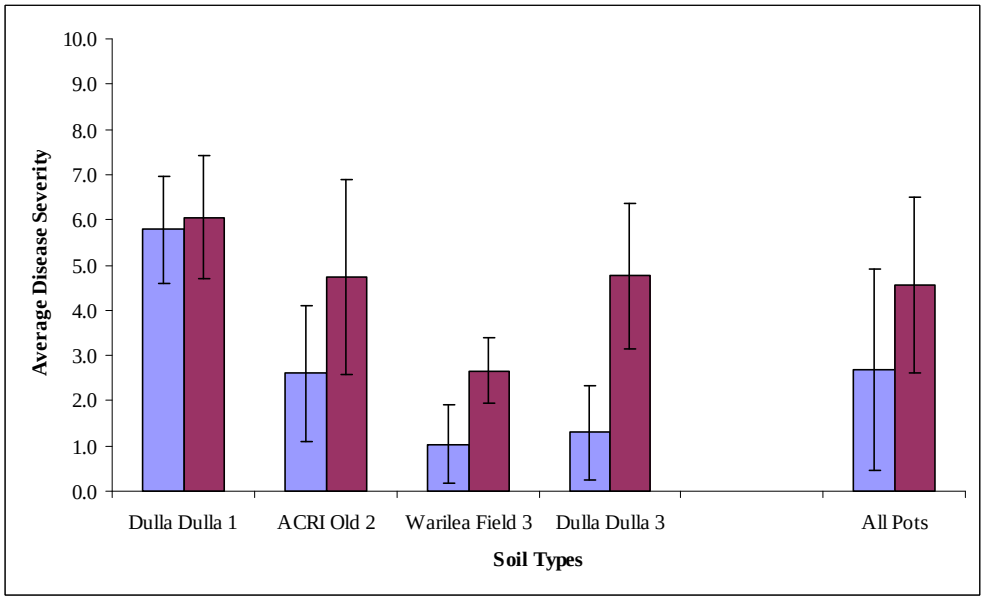


Figure 6.2 Average Black root rot severity in growth room pot trials with naturally infected soil collected from different locations in NSW. The solid bars indicate temperature range of the first experiment of 17-21^{o}C, while the temperature range for the checked bars was 20-25^{o}C.

Conclusions:

The single strain culture collection is an important collection of the pathogens collected from commercial cotton farms in Australia. To date there are 233, however this collection will continue to be built on each year of the disease surveys. This collection will have to be maintained to ensure that future studies on pathogenicity and morphological studies can be conducted. Future work includes re-isolating these strains and trialing several long term storage techniques.

Chapter 7 – Possible interaction between Verticillium wilt and black root rot

Objective 6 – Investigate Verticillium dahliae interaction with black root rot.

Introduction:

Long term trends in disease survey results have shown many fields with a high incidence of Verticillium wilt at the end of the season also have had a high incidence of Black root rot at the beginning of the season. The objective of this research is to investigate the interaction or not between Verticillium and Black root rot. This objective is only partially complete at this stage. The long term data set collected from disease surveys has allowed fields with significantly high incidence (%) of Verticillium wilt and Black root rot to be identified. These are listed in Table

6.1.1. This information will be used in future research to investigate the possible interaction between *Verticillium dahliae* and *Thelaviopsis basicola*.

Table 6.1.1 Fields with an incidence >40% for both Verticillium wilt and Black root rot.

Farm Field	Valley	Season	Black root rot (%)	Verticillium wilt (%)
Redbank32	Gwydir	2001-2002	78	42.5
Trevallyn07	Namoi	2001-2002	60	46
Auscottn21	Namoi	2002-2003	90	68
Togo76-1	Namoi	2007-2008	63.5	54
Lammermoor02	Namoi	2007-2008	84	89.5
Togo76-2	Namoi	2008-2009	67	58.5
Currawidgen23	Namoi	2008-2009	78	49
Togo76-2	Namoi	2009-2010	95.5	85
Warilea02	Namoi	2009-2010	100	42.5

Method and Materials:

Methods for proposed glasshouse and field experimentation are currently being developed. One proposed experiment is taking soil cores to gather basic information on inoculum levels in the soil profile within cotton beds and furrows. Fields with known histories of Black root rot and Verticillium wilt will be selected for sampling. Soil cores will be collected at different depths in the planting line and also at increments between beds. This will provide a profile on where the inoculum is in relation to the planting line on permanent beds. Soil sores will be taken at bed preparation, day of planting, 21, 28 and 35 days after planting. This will be repeated at the end of the season.

Another proposed experiment is a completely randomised pot trial consisting of 4 treatments and 12 replicates was set up in a glasshouse. The treatments to be included will be plants growing in pasteurised soil that was inoculated with *T. basicola*, pasteurised soil inoculated with *V. dahliae*, pasteurised soil inoculated with both *T. basicola* and *V. dahliae* and pasteurised soil as the control. Soil will be collected from Dulla Dulla to be pasteurised. The soil to be stored in a sterile bin for 4 days before 10" pots is filled. A total of 5 seeds will be planted into each pot. After emergence each pot will be stripped to 1 seedling per pot. The incidence of Black root rot will be assessed on all plants by carefully removing from pots, roots washed under running water. Black root rot incidence calculated as the percentage of roots with distinctive blackened colour. The severity of the disease assessed on a scale of 0 to 10.

After the plants are assessed for black root rot they will be transplanted back into their original pots and maintained until maturity. The incidence of Verticillium wilt will then be assessed by cutting the stem of each plant and noting the distinctive brown discolouration of the vascular tissue. Samples of the stem will be collected and isolated in the laboratory for confirmation of the disease.

Results:

Results will be reported during 2012/13 season. An extension has been granted to this project and this objective will continue to be worked on in collaboration with Stephen Allen (CSD). Proposed experiments and methodology will also continue to be evaluated.

Chapter 8 – Seed rots

Objective 7 – Investigate bacterial seed rots identifying the pathogen(s) and potential means of infection.

As a result of staff changes there was a substantial period where there was no project leader (pending my recruitment). In order to best meet this milestone I negotiated with Dr Moazzem Khan (Research Scientist) with Department of Employment, Economic Development and Innovation (DEEDI) to collaborate on this milestone. The pathology unit collected boll samples during the 2011/2012 disease surveys and these were sent to Dr Khan pathogen identification and means of infection identified. Results for this milestone were incomplete at the time of preparing this report.

Chapter 9 – Bacterial blight screening

Objective 8 – Re-establish Australia's capacity to screen for exotic races of bacterial blight.

I made several contacts in the USA prior to leaving for Texas on a DAFF scholarship. The objective of the trip was to gain firsthand experience with Hyper-virulent bacterial blight and Texas root rot in order to write the National Protocols should an incursion occur in Australia. Overseas contacts included Dr Peggy Thaxton, Fred Bourland, Richard Percy and James Frelichowski (curator of the US Cotton Germplasm Collection). I provided James with all the information on the 10 differential lines that I wanted to import into Australia. Unfortunately USDA only had 5 of the 10 lines, those being Acala 44, Stoneville 20, Mebane B-1, Gregg and Empire B4. USDA-ARS National Plant Germplasm System has also been contacted about the missing lines. Dr Peggy Thaxton was able to supply the missing 5 lines (Stoneville 2B-S9, 1-10B, 20-3, 101-102B and DPxP4) from Mississippi State University.

The seeds were imported (March 2012) by NSW Primary Industries under the import permit number IP 11015674. The differential lines are currently being grown for one generation in an AQIS approved facility for Post Entry Quarantine by Agri-Science Queensland, a service of the Department of Employment, Economic Development and Innovation.

Outcomes:

Outcome 1. Disease Surveys – increased industry preparedness to deal with biosecurity threats of exotic diseases through early detection. The disease surveys serve as a benchmark for the industry, growers and research. Long term quantitative information gathered on incidence and severity also illustrates trends in disease and adoption of management practices.

Outcome 2. Seed Treatment Trials - Novel and existing fungal seed treatments were evaluated each year for effectiveness on reducing seedling mortality due to pathogens such as Rhizoctonia and Pythium. Dynasty Complete remains the most consistent seed treatment to date.

Outcome 3. The development of National Diagnostic Protocols for Hypervirulent bacterial blight and Texas root rots pathogens. These protocols will be implemented should an incursion occur within Australia. The biosecurity profile of Australian Cotton Research Institute (ACRI) and NSW DPI has been enhanced with the cotton industry and with the Australian Department Agriculture, Fisheries and Forestry (DAFF). This is of benefit for industry biosecurity preparedness.

Outcome 4. Australia's capacity to screen Hypervirulent bacterial blight re-established with the importation of bacterial blight differentials in 2012. The differential set of cotton lines imported in 2012 for the screening of exotic races of bacterial blight included: Acala 44, Stoneville 20, Mebane B-1, Gregg, Empire B4, Stoneville 2B-S9, 1-10B, 20-3, 101-102B and DPxP4. This is of benefit for industry biosecurity preparedness.

Publications arising from the project:

Allen, S. J., Anderson, C. M. T., Lehane, J., Lonergan, P. A., Scheikowski, L. J., & Smith, L. J. (2010). Cotton Pathology Survey 2009/10 *Cotton Pest Management Guide 2010-11* (Vol. 2010-11, pp. 156): Cotton CRC Development and Delivery Team.

Allen, S., Smith, L., Scheikowski, L., & Kirkby, K. (2011). Fusarium Wilt Update. *Disease*, 2011, from http://www.cottoncrc.org.au/content/Industry/Publications/Disease__Microbiology/Fusarium_Wilt_of_Cotton.aspx

Allen, S. J., Kirkby, K. A., Lehane, J., Lonergan, P. A., Cooper, B. M., & Smith, L. J. (2011). Cotton Pathology 2010-2011, from http://www.cottoncrc.org.au/content/Industry/Publications/Disease__Microbiology/Disease_Surveys/2010_-_2011.aspx and http://www.csd.net.au/asset/send/2467/inline/original/

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Kirkby, K. A., Allen, S., & Lonergan, P. (pending 2012). *An Update on Verticillium Wilt in Australia*. Paper presented at the Beltwide Cotton Conference.

Executive Summary

"Diseases of Cotton X" aimed to increase sustainability of the Australian cotton industry through comprehensive disease surveillance. Continued collaborative research, communication and education will enable better understanding of pathogen biology and ultimately improve integrated disease management strategies. The three year project resulted in several outcomes with direct consequences for the cotton industry.

Important outcomes for the Diseases of Cotton X project include: incorporating surveillance for exotic diseases into the biannual disease surveys has increased the industries preparedness to deal with biosecurity threats of exotic diseases through early detection. Evaluating existing and novel fungicide treatments each season ensures growers have access to the latest information on seed treatments available. This information helps the industry to make decisions on seed treatments in order to decrease seedling mortality.

National diagnostic protocols have been developed for Texas root rot and hypervirulent bacterial blight and are currently being reviewed by DAFF. These protocols will aid the industry in rapid diagnosis using standard procedures should an incursion occur. Australia capacity to screen for exotic races of bacterial blight has been re-established through the importation of 10 differential cotton lines in 2012. The lines included: Acala 44, Stoneville 20, Mebane B-1, Gregg, Empire B4, Stoneville 2B-S9, 1-10B, 20-3, 101-102B and DPxP4.

The "Symptoms of diseases and disorders of cotton in Australia" was updated in 2012, titled "Cotton Symptoms Guide – the guide to symptoms of diseases and disorders in Australian cotton". The pathology unit worked in collaboration with Steve Allen (CSD) and the extension staff from Cotton Delivery and Development Team, particularly Susan Maas. Updated images were supplied and many drafts of the book were reviewed. The industry benefits from having an excellent resource full of images, a symptoms key, looks like section and information on how to send a sample for confirmation.

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Appendix A: *National Diagnostic Protocol Hypervirulent Bacterial Blight*

Hyper-virulent Bacterial Blight

Xanthomonas axonopodis pv. *malvacearum*

Diagnostic Scholarship Report

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Department of Agriculture, Fisheries and Forestry
Office of the Australian Chief Plant Protection Officer

NSW GOVERNMENT Primary Industries

1. Introduction

Bacterial blight of cotton (*Gossypium hirsutum* and *G. barbadense*) is caused by the gram-negative aerobic non spore-forming rod-shaped bacterium *Xanthomonas axonopodis* pv. *malvacearum* (Kado, 2010). Bacterial Blight is a major disease of cotton worldwide causing up to 70% yield losses during severe epidemics (Kirkpatrick & Rothrock, 2001). The pathogen is host-specific to cotton (*Gossypium hirsutum* (upland cotton), *G. barbadense* (pima cotton), *G. herbaceum*) although it can form endophytic infections in secondary hosts such as *Ceiba pentandra* (Kapok tree), *Jatropha curcas* (Barbados nut), *Thurberia thespesioides* (Wild cotton plant) and *Lochnera pusilla* (Tiny Periwinkle).

Bacterial blight has been found in all cotton growing regions of the world. Historically more than one race can be found in a given region. In order to minimise the effect of genotype-environment interactions, Hunter et al. (1968) developed a set of host differentials to determine races of bacterial blight. Nineteen races of the pathogen are recognised in the USA based on the response of these differentials to inoculation with the bacterium (Hillcocks, 1992d). Each differential cultivar contains specific resistance genes so each race is a homogeneous group with the ability to overcome a specific set of resistance genes. Race 18 is the most widespread and aggressive among the races, being the predominant pathogen in the USA, and until recently, in Australia (Allen, 1991). Most modern cotton cultivars in Australia are now resistant to Race 18 and all other recognised races.

In Africa, several strains have evolved with the ability to overcome resistance in all 10 differential cotton cultivars. These isolates are herein designated as Hyper-virulent (HV) strains and can overcome the major resistance genes *B2*, *B3*, *B4*, *B6* and *B7* and combinations thereof found in USA cultivars. Several strains have been collected including the isolates HV1, HV3, HV7, Chad and the Sudan.

1.1 Potential Distribution in Australia

Bacterial blight of cotton was first recorded in Australia in 1923 with races 2 to 5, 7, 9, 10 and 18 reported between 1974 and 1983 (Allen, 1991). If HV strains were to arrive in Australia, the

disease has potential to occur in all cotton growing regions. The HV1 strain is of particular concern to the Australian cotton industry since it is not known if the pathogen will overcome the resistance genes in Australian cotton cultivars. Consequently it is vital to ensure the capacity to differentiate between and correctly identify recognised races and HV strains should an outbreak of bacterial blight occur in Australia in the future.

1.2 Transmission

Bacterial blight is seed-borne, being carried over between generations in and on the seed. The pathogen may over-winter in the soil on un-decomposed plant debris including leaves, bracts and petioles (for up to 4 months) but more commonly it survives internally and externally on seed for up to 2 years (Kirkpatrick & Rothrock, 2001). The study by Alexander (2009) showed the pathogen survived on acid delinted seed.

2. Taxonomic information and synonyms

The bacterial blight pathogen was initially named *Pseudomonas malvacearum* by (E. F. Smith, 1901) who later referred to it as *Bacterium malvacearum* (E. F. Smith, 1920). Following that, Dowson (1939) reclassified it as *Xanthomonas malvaacearum* (E.F. Smith) Dow then *Xanthomonas campestris* pv. *malvacearum* (E.F. Smith) Dow (*Xcm*) (Dye, et al., 1980). The name now generally accepted is *Xanthomonas axonopodis* pv. *malvacearum* (Kado, 2010).

2.1 Classification

KINGDOM	Bacteria
PHYLUM	Proteobacteria
CLASS	Gammaproteobacteria
ORDER	Xanthomonadales
FAMILY	Xanthomonadaceae
GENUS	*Xanthomonas*
SPECIES	*axonopodis*
SUBSPECIES	*malvacearum*

3. Detection

Symptoms of bacterial infection generally show 2 weeks after inoculation. Characteristic symptoms used for detection include symptoms of water soaked lesions commencing on the underside of the leaves and is visible on the upper surface of leaves, water soaked lesions on the bolls and or bracts.

3.1 Parts of the plant(s) on which it may be found

- Cotyledons
- Leaves
- Stem
- Bracts
- Bolls
- Seed
-

3.2 Likely occurrence associated with developmental stages of the host(s), climatic conditions and seasonality

The disease is found in almost every country where cotton is grown today. The pathogen survives better in dry environments and in the USA this may be a more frequent problem due to overwinter survival. Bacterial blight requires rainfall to cause the disease with higher temperatures being favourable for disease development, so hot dry weather coupled with a few sporadic rain events can start epidemics. Consequently it is less important in countries that experience hot, dry weather for most of the cotton growing season (Hillcocks, 1992d). Once a seedling is aboveground, it may become infected as a result of secondary inoculum spread from other infected plants or debris through wind and/or rainfall or irrigation runoff.

3.3 Symptoms

Cotyledons: Small dark green 'water-soaked' lesions either circular or irregular in shape that penetrate all the way through the leaf, being visible on the under and upper surface of the leaf. Cotyledons may become distorted in susceptible cultivars. Under favourable conditions the infection spreads down the petiole to the stem, resulting in stunting or even death of the seedling.

Leaves: Angular leaf spots (ALS) usually 2-5mm appear water-soaked and penetrate all the way through the leaf, being more visible on the underside of leaves. Lesions that occur only on the upper surface are not bacterial blight. The ALS become dark brown to black as they dry out, becoming distorted and necrotic before finally shedding. Lesions extending along the main vein are known as vein blight may occur with or without ALS. Vascular tissue may be invaded by the bacterium, indicated by roughly circular chlorotic patches about 1-3 cm in diameter surrounded by smaller angular spots.

Angular leaf spots that go all the way through the leaf on a cotton plant infected with bacterial blight (Image courtesy Jason Woodward, Texas).

Stem: In the most severe manifestation the infection moves down to the stem, indicated by sooty black lesions that may completely girdle the stem. This is referred to as blackarm and may lead to stems breaking under windy conditions or under the weight of developing bolls.

Bracts: flower buds and young bolls may shed once symptoms of bacterial blight appear.

Bolls: round 'water-soaked' spots 2-5mm in diameter and up to 10mm or more in susceptible hosts become dark brown with age. Lesions may be numerous and clustered close to the suture or the base of the boll under the epicalyx. In severe cases lesions penetrate the boll walls resulting in internal rot usually in the presence of insect damage. Internal rot causes the lint to be stained yellow leading to surface contamination from the bacteria.

Water soaked lesion present on a cotton boll infected with bacterial blight.

Seeds: internal infection of the seed may enter through the micropyle or introduced by the stainer bug piercing the seed while feeding. The pathogen has been known to survive acid delinting.

3.4 Developmental stages:

Atkinson (1891) gave the names angular leaf spot (ALS), blackarm and bacterial boll rot to the various stages of bacterial blight disease, however during 1901-1905, E. F Smith demonstrated that these symptoms were caused by the same pathogen (E. F. Smith, 1920).

3.5 Sampling procedures critical for detection methods and diagnostic procedures:

It is important that growers, agronomists, consultants and pathologists continue to check the health of the plants in the field. These checks should be carried out throughout the growing season. Early detection of this exotic disease will allow control measures to be put in place.

- Look for plants that have lesions and or signs of defoliation
- Examine the under and upper surface of the leaves for the presence of angular water soaked lesions that go all the way through the leaf
- Examine the bolls for water soaked lesions
- If the plant has the lesions described above record the field name and location (GPS coordinates), photograph then remove the whole plant and place in a labeled bag and seal

- Contact the NSW Primary Industries cotton pathologist (02) 67992474
- Bag the sample inside another labeled plastic bag and post to the Cotton Pathologist, Department of Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390

4. Identification

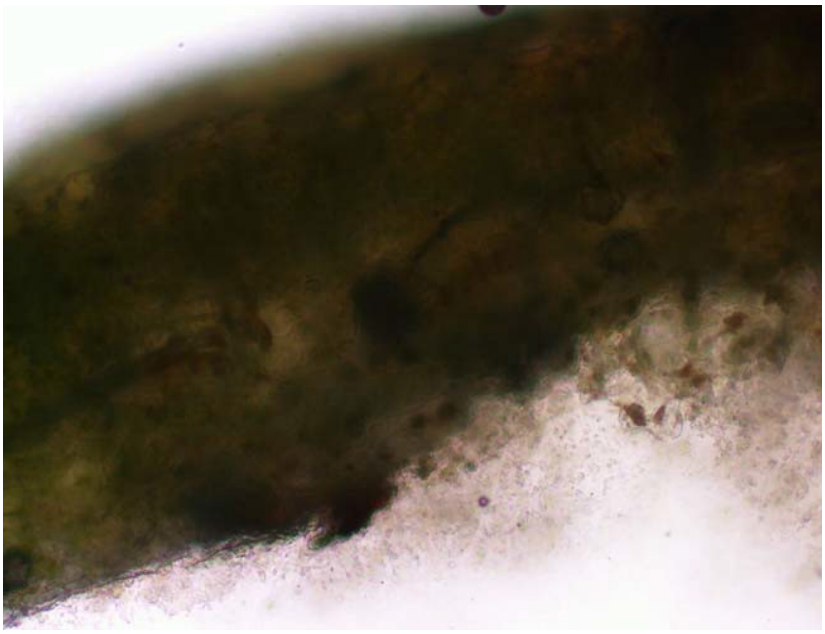
Bacterial blight on susceptible host plants when angular water soaked lesions that penetrate (visible on both bottom and top) of leaves are present. Race of pathogen may be determined by the scratching the surface of each differential host line leaves with a sterile toothpick that has been dipped into an isolated culture of bacteria and examining the response of each host line (see 4.1 Methodology based on response to host differential set). The pathogen may be isolated on potato carrot dextrose agar (PCDA) media and identified by shiny yellow bacterial colonies (see 4.2 Methodology based on morphological and cultural characteristics).

4.1 Methodology based on morphological and cultural characteristics

Examine the whole plant and look for small angular and irregular shaped lesions on the under and upper side of leaves, making note that the pathogen causes lesions that go all the way through the leaf.

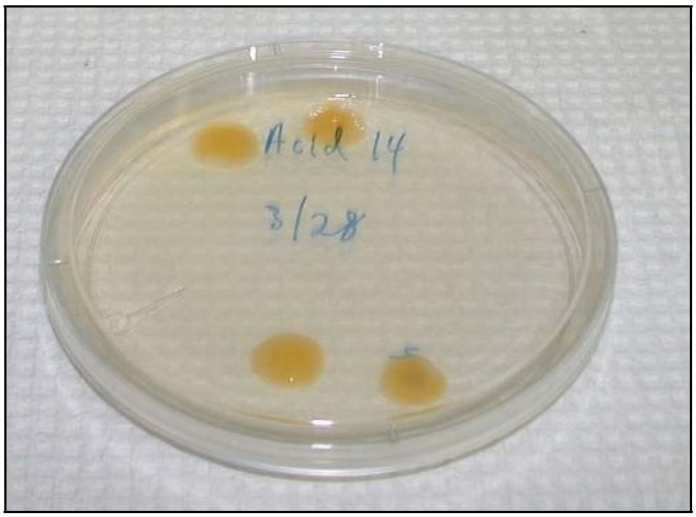
Bacterial stream test and initial isolation of bacteria from infected host tissue

- Fill a small beaker with 70% ethanol
- Place a ring spatula, spoon spatula, tweezers and a scalpel in the ethanol
- Surfaces sterilize the infected leaves by spraying with 70% ethanol and quickly blot the leaf dry with paper towel
- Place 1-5 µl of sterile water in a labeled Petri plate then cover with lid
- Cut out a small amount of diseased tissue (2mm x 4 mm) from the leaf using tweezers and a scalpel and place the tissue on a drop of water on a microscope slide. Cover with a coverslip and view under the microscope looking for bacteria streaming from the cut section of the tissue



Bacteria streaming from infected cotton tissue

- To isolate the bacteria, cut out another small amount of diseased tissue (2 mm x 4 mm) from the leaf using tweezers and a scalpel and place in the drop of water in Petri dish and cover with the lid. Leave the plate for 5-10 minutes
- Dip the spoon end of a spatula in a beaker of 70% ethanol then flame
- Using the back of the spoon, crush the tissue in the water drop
- Flame the inoculation loop and dip into water/sample
- Streak the bacteria onto potato carrot dextrose agar (PCDA). For agar recipe see Table 4.1.1. Ensure agar surface is dry before use as the bacterium is motile in surface moisture and discrete colonies will not form
- Incubate at 30^{o}C for 24 to 48 hours (no light necessary). Colonies visible to the naked eye after only 24 hours are unlikely to be the pathogen. Bacterial blight colonies are slimy and yellow in color. To obtain pure cultures of the yellow bacterium, subculture these colonies onto the potato carrot dextrose agar with peptone and yeast extract (Bird, 1966) (Table 4.1.1) and incubate for 48-72 hours



Isolated bacterial colonies grown on potato carrot dextrose agar

Table 4.1.1 Potato carrot dextrose agar with peptone and yeast extract recipe from Bird (1966).

Ingredient	Per 1L
MgSo4	0.3 gms
CaCO3	0.2 gms
Agar	10 gms
Potato Dextrose Agar (Commercial)*	40 gms
Peptone	2.5 gms
Carrot Juice **	15 ml
Distilled H2O	1 L
Yeast extract	0.5 gms

* Difco

** Commercial canned carrot juice (everyday brand)

4.2 Methodology based on response of the scratch test to host differential set

- The minimum number of plants per differential line (Table 4.1.1) to be screened should be no less than 10-20. A recent variety of cotton that is known to be resistant to bacterial blight race 18 should also be included in the screening process
- Fill tube pots with clean field soil
- Press seed onto the soil surface and top with sand. Water plants as needed
- Germinate plants in a temperature controlled growth room set at 27^{o}C. Maintain plants in growth room until 2 cotyledons have emerged

Plants at ideal stage for inoculation

- With a sterile toothpick make a small cross on the under surface of the cotyledon

Underside of cotyledon scratched with a toothpick

- On the same cotyledon take another sterile toothpick and immerse the tip in the cultured bacterial isolate before making another larger cross on the cotyledon
- Repeat this for all the lines to be screened
- Place inoculated plants in a humidity chamber for 24 hours, aiming for 100% relative humidity
- Return plants to the temperature controlled growth room

- Access the plants 14 days after inoculation for the presence of necrotic tissue around the area of inoculation and/or water soaked lesions on the lower surface of the cotyledons. There should be no signs of infection around the area of the sterile scratch test

Underside of cotyledon showing symptoms of infection

- To determine the race, record the pathogenicity differences between the differential lines and refer to Table 4.2.2

Table 4.2.1 Bacterial blight pathogen responses to host differentials adapted from Hunter et al (1968). *A recent variety that has shown resistance to bacterial blight should always be included in the screening.

	Race																		
Host Differentials	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Acala 44	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stoneville 2B	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-
Stoneville 20	-	+	-	-	-	-	-	+	+	+	-	+	-	+	+	+	+	+	-
Mebane B-1	-	-	-	-	-	+	+	+	-	+	-	-	-	-	-	+	-	+	-
1-10B	-	-	+	-	+	+	+	+	-	+	-	-	-	+	+	-	-	+	+
20-Mar	-	-	-	+	+	-	+	-	+	+	-	-	-	+	-	+	+	+	+
101-102B	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gregg	-	-				+	+	+		+	+	+		+				+	+
Empire B4	-	-				-	-	-		-	-	-		-				+	-
DPX P4	-	-				-	-	-		-	-	-		-				+	-
Resistant variety*																			

5. Contact points for further information

The Cotton Pathology Group of NSW Primary Industries located at Australian Cotton Research Institute (ACRI), Narrabri is the preferred diagnostic laboratory to process plants suspected of Hypervirulent Bacterial Blight. This laboratory has developed expertise in the detection and diagnosis of this disease through visiting Texas, USA where the disease is prevalent. Photos should be taken of suspected plants and sent as soon as possible to the laboratory, along with the location (GPS) of suspected infection and contact information.

- Karen Kirkby, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 992454, Fax 0267 991503, Email karen.kirkby@dpi.nsw.gov.au
- Peter Lonergan, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 99 1531, Fax 0267 991503, Email perter.lonergan@dpi.nsw.gov.au
- Dr. Terry Wheeler, Texas AgriLife Research, 1102 East FM 1294, Lubbock, 79403, Texas. Email ta-wheeler@tamu.edu
- Dr. Thomas Isakeit, Texas A&M University, College Station, Texas. Email t-isakeit@tamu.edu

All samples that test positive for an exotic disease will be sent to Dr Alison Seyb (Plant Pathologist) Private Bag 4008, Narellan, NSW, 2567 for independent confirmation.

6. Acknowledgements

The authors wish to thank Dr. Jason Woodward from Texas AgriLife Extension and Dr. Robert Wright from Texas Tech and Texas AgriLife Research for their assistance with this protocol. The methods of identification used in this protocol have been used with success by staff at both AgriLife Research and Texas Tech for years.

7. References

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8. Appendices (as appropriate)

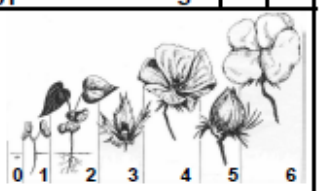
8.1 Sample form for detection of bacterial blight during early season cotton disease surveys.

Code	Date	Deg	Min
		South	
Farm	Field	East	
		Surveyed by:	

	G/S	No True Leaves	Std	BRR (0-10)									
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert Wilt	Pr	Ab
Exotic Fusarium Wilt	Pr	Ab	Hyperviralent Blight	Pr	Ab

Comments:



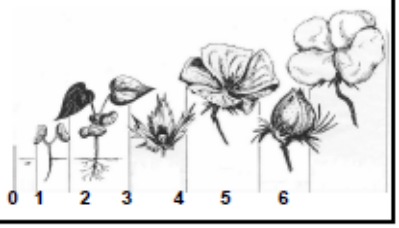
8.2 Sample form for detection of bacterial blight during late season cotton disease surveys

Code	Date		Degrees	Minutes
		South		
FarmField		East		
	G/S		Surveyed by:	

	Fov	Vd	Am%	PBR	OBR	SW	Hor	LM	Bchy	CBT
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
Total										

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert	Pr	Ab
Exotic Fov	Pr	Ab	Hyperviralent BBt	Pr	Ab

Comments:



Appendix B: National Diagnostic Protocol Texas Root Rot

Texas Root Rot

Phymatotrichopsis omnivore

Diagnostic Scholarship Report

Prepared by:

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Department of Agriculture, Fisheries and Forestry
Office of the Australian Chief Plant Protection Officer

NSW GOVERNMENT Primary Industries

1. Introduction

Texas Root Rot (TRR) also known as Cotton Root Rot (TRR) is caused by the soil-borne ascomycete *Phymatotrichopsis omnivora* (Shear) Hennebert (Marek, Hansen, Romanish, & Thorn, 2009). TRR is native to south-western United States and Mexico where it is a devastating pathogen of cotton and alfalfa. The pathogen is found in Mexico, Venezuela, Brazil, and Libya and south-western states of USA including Arizona, Arkansas, California, Louisiana, Nervada, New Mexico, Oklahoma, Texas and Utah (CAB International, 2007) EPPO). This fungus does not cause disease in monocotyledonous plants; however it has potential to impact a huge range of dicotyledonous crops in Australia.

P. omnivora has a broad host range of over 2000 species of dicotyledonous plants including 107 economically important agricultural and horticultural crops, several tree species and ornamental plants (CAB International, 2007); (Blank, 1953). The major hosts and some other hosts are listed below. The major host is cotton including *Gossypium herbaceum, G. hirsutum,* and *G. barbadense*, Alfafa/Lucerne (*Medicago sativa*). TRR also affects nut crops including Almond (*Prunus dulcis*), Pecan (*Carya illinoinensis*), Walnut (*Juglans regia*); Apple (*Malus domestica*) and other *Rosaceae* including European Pear (*Pyrus communis*) and Peach (*Prunus persica*); Fig (*Ficus carica*); *Vitis vinifera* (grapevine); Sugarbeet (*Beta vulgaris var. saccharifera*); legumes including Peanut (*Arachis hypogaea*), Soyabean (*Glycine max*) and various beans (*Phaseolus* spp.); Okra (*Abelmoschus esculentus*) and other Malvaceae including Kennaf (*Hibiscus cannabinus*); Parsley (*Petroselinum crispum*) and other *Umbelliferae*; trees including Pine (*Pinus spp*.), Poplar (*Populus spp*.), Elm (*Ulmus spp*.), and Willow (*Salix spp*.) and Black Locust (*Robinia pseudoacacia*).

As of 2009, eight industry bodies (Apple and Pear Australia Ltd, AUSVEG, Citrus Australia Ltd, Cotton Australia Ltd, Grains Council of Australia, Nursery and Garden Industry Australia, Summer Fruit Australia Ltd, and Winemakers Federation of Australia) have agreed to cost sharing arrangements for *P. omnivora* under the Emergency Plant Pest Response Deed (EPPRD). Moreover, both the cotton and cherry industries consider *P. omnivora* to be a high priority pest (HPP) that could devastate production if it were to become established in Australia. Climate change is likely to increase the potential distribution of the fungus in Australia and thereby increase the risk posed by this fungus to Australian primary industries.

The rate of disease onset differs between plant species. Extensive root decay can often take up to two seasons in tree and fruit crops (CAB International). However disease onset is sudden in vegetables and field crops. The fungus colonises the tap root, causing rapid decay and girdling of the outer periderm and cortical tissues and blocking the flow of water through the vascular cambium (Padil Toolbox). Plants consequently become chlorotic, wilt and then die.

Eradication options for the disease rely on the destruction of infected plant parts and extensive soil fumigation. The fungus will survive for long periods at depths of up to 2.4m (Lyda & Kenerley, 1993). Rapid diagnosis is therefore important to avoid spread of the pathogen through the soil. The diagnosis of TRR is relatively straight forward due to the distinctive morphological characteristics of the pathogen. Diagnosis may be based on identification of symptoms *in planta* along with pathogen morphology of isolated cultures. Diagnosis of TRR based on these techniques should take no longer than three weeks and should be confirmed by at least two laboratories.

1.1 Potential Distribution in Australia

The fungus will survive in alkaline (pH 7.2-8.0) calcareous (calcium carbonate >1%) non-sodic vertisols that occur in regions where the annual mean air temperature is greater than 15^{o}C (Percy, 1983). The average annual temperature across the cotton growing regions of Australia is > 15.6^{o}C. Cotton is usually grown in heavy cracking, mid to high pH vertisols that can be calcareous. Percy (1983) states that the fungus will not survive in soils where sodicity levels are greater than 2-3 meq/100g. The majority of irrigated soils have a sodium content of approximately 2.7 meq/100g soil. The conducive soil types coupled with transcontinental transport of plants and soil (Percy, 1983) indicate there is a distinct possibility for *P. omnivorum* to survive in the cotton growing regions of NSW and QLD, especially in areas of lower sodicity.

1.2 Transmission

The fungus proliferates in the field by the elongation of hyphal strands through the soil from plant to plant. Hyphal strands usually originate from a single focus of sclerotia or from a colonised plant, disseminating the infection from root to root. Thus patches of dead and dying plants appear to grow through a given season. Long range transmission can only occur when sclerotia and/or colonized plant parts are moved in soil and other media (eg. potting mix) or on contaminated machinery.

2. Taxonomic information and synonyms

Phymatotrichopsis omnivora (Shear) Hennebert was previously assumed to be a member of the Basidiomycota with possible teleomorphs *Sistotrema brinkmanii* and *Phanerochaete omnivora*. However, phylogenetic analyses of the nuclear small- and large-subunit ribosomal DNA and subunit 2 of RNA polymerase II from multiple isolates have shown this to be incorrect. *Phymatotrichopsis omnivora* is an anamorphic member of the Ascomycota for which no teleomorph has been described (Marek, et al., 2009). Synonyms for *Phymatotrichopsis omnivora* include *Phymatotrichum omnivorum* Duggar, *Ozonium omnivorum* Shear, *Ozonium auricomum* Link and *Hydnum omnivorum* Shear.

2.1 Classification

KINGDOM	Fungi
PHYLUM	Ascomycota
CLASS	Pezizomycetes
ORDER	Pezizales
FAMILY	Rhizinaceae
GENUS	*Phymatotrichopsis*
SPECIES	*omnivora*

3. Detection

Characteristic symptoms used for detection include wilting or dead plants, hyphae on the stem in the interface region between the above and below ground region of the stem.

3.1 Parts of the plant(s) on which it may be found

- Taproots
- Stem

3.2 Likely occurrence associated with the developmental stages of the host(s), climatic conditions and seasonality

P. omnivora has a low dispersal potential occurring particularly on heavy calcareous soils. The disease is not readily spread, persisting at certain locations where soil conditions are favourable. Depth of sclerotial placement is an important factor in symptom expression in the field; however the rate of fungal growth increases rapidly once soil temperatures exceed 22^{o}C (Rush, Gerik, & Lyda, 1984). Following a rain event mid-season, plants may begin to die in as little as 14 days.

3.3 Symptoms

The disease appears in patches in the field. The first symptoms of the disease are sudden wilting of plants with or without chlorosis of the leaves during the summer. The foliage droops, turns brown and may remain hanging on the branches for a few days before dropping of. At this stage there the roots and lower section of the stem are covered with a network of yellow fungal strands visible to the naked eye. Discolouration can be seen inside the stem when cut in cross sections. Under high moisture conditions there may be sclerotia (brown to black in colour) on the surface of roots. Occasionally in cotton fields, on the ground near dying plants, the conidial stage develops as a creamy yellow spore mat.

3.4 Developmental stages

Mycelium strands formed on the roots of infected plants radiate outwards through the soil until it contacts new host roots. The hyphal strands grow intra- and intercellularly, penetrating the endodermis and xylem tissue. As the disease progresses the dead roots are extensively colonized by mycelium strands.

3.5 Sampling procedures critical for detection methods and diagnostic procedures

- Look for plants that are just starting to wilt. The leaves appear wilted but they are not yet dried up. Preferable to inspect fields in morning before the plants wilt from lack of water. Look in areas of the field where plants have been dead for some time

- Dig or pull up the wilted plants. Check to see if some or the entire top of the root is rotted by scraping the root with fingernail or knife. Roots of infected plants will be discoloured (brown, but not white). Check the lateral roots (also cut lower root in half, check for any discoloration (not white). Sample plants with rotted roots
- Using clippers, cut a sample that is 7-10 cm above the root. Cut off root 7 cm below soil line. Keep just the upper root-lower stem portion (about12 cm)
- If the plant has the symptoms described above record the field name and location (GPS coordinates), photograph then remove the whole plant and place in a labeled bag and seal
- Contact the NSW Primary Industries cotton pathologist (02) 67992474
- Bag the sample inside another labeled plastic bag and post to the Cotton Pathologist, Department of Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390

4. Identification

P. omnivora can be easily identified by observation of its distinctive morphology. The species is characterized by distinctive branching acicular cruciform hyphal (200µm in diameter) emanating that is not known in any other species of fungus. Conidia may be unicellular, hyaline, globose or ovate (4.8 to 5.5µm in diameter).

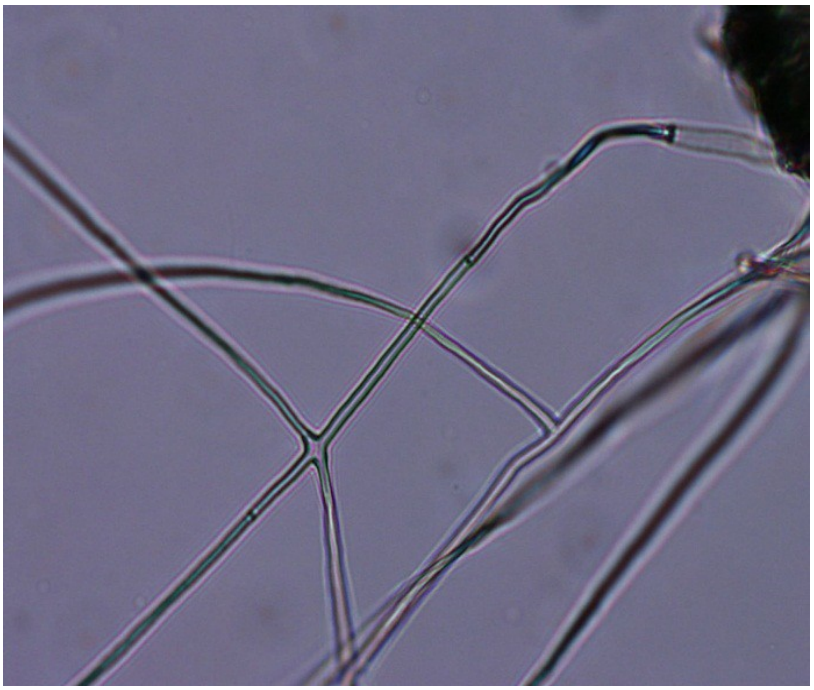
4.1 Methodology based on symptoms of plants infected with of *P. omnivora*

Infected plants may first show signs of sudden wilt. Plants have minimal root system. Brown to black vascular discoloration is seen in the stem.

4.2 Methodology based on morphology of *P. omnivora*

- Once at the lab place a drop of deionized water onto a microscope slide
- Using tweezers remove some of the brown/yellow mycelium from the root sample and place on the drop of water on the microscope slide
- Cover with a cover slip

- Examine under a compound microscope, looking for the characteristic hyphal formation of cruciform hyphae



4.3 Initial isolation of fungi from infected tissue

- Put on rubber gloves
- Remove lateral roots from the samples with clippers and discard
- Wash sample under running water. Remove soil with fingers or brush
- Copiously spray middle 10 cm of root/stem section with 70% Ethanol until it runs off
- Blot dry with clean paper towel and place on clean paper towel
- Hold stem at top end. Dip scalpel in alcohol and flame it. Use the scalpel to peel outer part of stem, peeling away from you
- Peel about 1/2 way around stem
- Look for browning. Peel more and deeper
- Peel close to root/stem interface OR look for where root is browner than the rest
- Peel and cut out a piece (3 x 3 mm) at the brown area that is about 1mm thick
- Place piece on Petri plate of 1/5 strength potato dextrose agar with streptomycin, about 60 mm from edge of the plate. Push the piece firmly onto the agar

- Repeat and put 3 additional pieces on plate. Take pieces from the same area of root or from nearby
- Cover with lid and place in a plastic bag
- Incubate at 30^{o}C for 4 days

5. Contact points for further information

The Cotton Pathology Group of NSW Primary Industries located at the Australian Cotton Research Institute (ACRI), Narrabri is the preferred diagnostic laboratory to process plants suspected of Texas or Cotton Root Rot. This laboratory has developed expertise in the detection and diagnosis of this disease through visiting Texas, USA where the disease is prevalent. Photos should be taken of suspected plants and sent as soon as possible to the laboratory, along with the location (GPS) of suspected infection and contact information.

- Karen Kirkby, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 992454, Fax 0267 991503, Email karen.kirkby@dpi.nsw.gov.au
- Peter Lonergan, Department Primary Industries, Locked Bag 1000, Narrabri, NSW, 2390, Australia. Phone 0267 99 1531, Fax 0267 991503, Email perter.lonergan@dpi.nsw.gov.au
- Dr. Terry Wheeler, Texas AgriLife Research, 1102 East FM 1294, Lubbock, 79403, Texas. Email ta-wheeler@tamu.edu
- Dr. Thomas Isakeit, Texas A&M University, College Station, Texas. Email t-isakeit@tamu.edu

All samples that test positive for an exotic disease will be sent to Dr Alison Seyb (Plant Pathologist) Private Bag 4008, Narellan, NSW, 2567 for independent confirmation.

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8. Appendices (as appropriate)

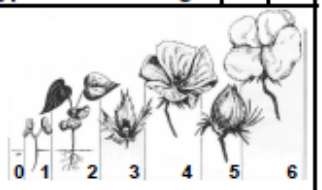
8.1 Sample form for detection of Texas root rot during early season cotton disease surveys

Code		Date		Deg		Min	
				South			
Farm		Field		East			
				Surveyed by:			

	G/S	No True Leaves	Std	BRR (0-10)									
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert Wilt	Pr	Ab
Exotic Fusarium Wilt	Pr	Ab	Hyperviralent Blight	Pr	Ab

Comments:



8.2 Sample form for detection of Texas root rot during late season cotton disease surveys

Code	Date		Degrees	Minutes
		South		
FarmField		East		
	G/S		Surveyed by:	

	Fov	Vd	Am%	PBR	OBR	SW	Hor	LM	Bchy	CBT
1										
2										
3										
4										
5										
6										
7										
8										
9										
10										
Total										

CLCuVirus	Pr	Ab	Texas Root Rot	Pr	Ab
Blue Disease	Pr	Ab	Defoliating Vert	Pr	Ab
Exotic Fov	Pr	Ab	Hyperviralent BBt	Pr	Ab

Comments: