Cotton Catchment Communities CRC

FINAL REPORT

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Diseases of Cotton IX

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Part 1 - Summary Details

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Contents

Background

Cotton in Australia is affected by a range of diseases that if not managed appropriately threaten sustainable cotton production. Cotton in Australia is also threatened by a suite of exotic plant pests and by potential new diseases that may evolve from native pathogen populations. Ongoing surveillance and management of disease is therefore a crucial tool in ensuring sustainable cotton production long into the future. The "Diseases of Cotton" projects have run since 1984 and have played a key role in monitoring the emergence and decline of cotton diseases over time, often following the adoption of new technologies. Consequently, the data gathered through these projects has helped the industry assess the effectiveness of its R&D investment in cotton breeding and integrated disease management. The research outlined in this report adds to the important long-term data set of disease severity and incidence collected throughout other Diseases of Cotton projects. In-depth molecular studies highlight previously unknown variation in some seedling pathogen populations that may explain increased seedling mortality in southern NSW. And results of long-term experiments at the Australian Cotton Research Institute continue to build a better picture of the effectiveness or otherwise of potential integrated disease management strategies over time.

Objective 1

Monitor the distribution and importance of all diseases in cotton and identify environmental and cultural factors influencing the emergence or re-emergence of disease threats.

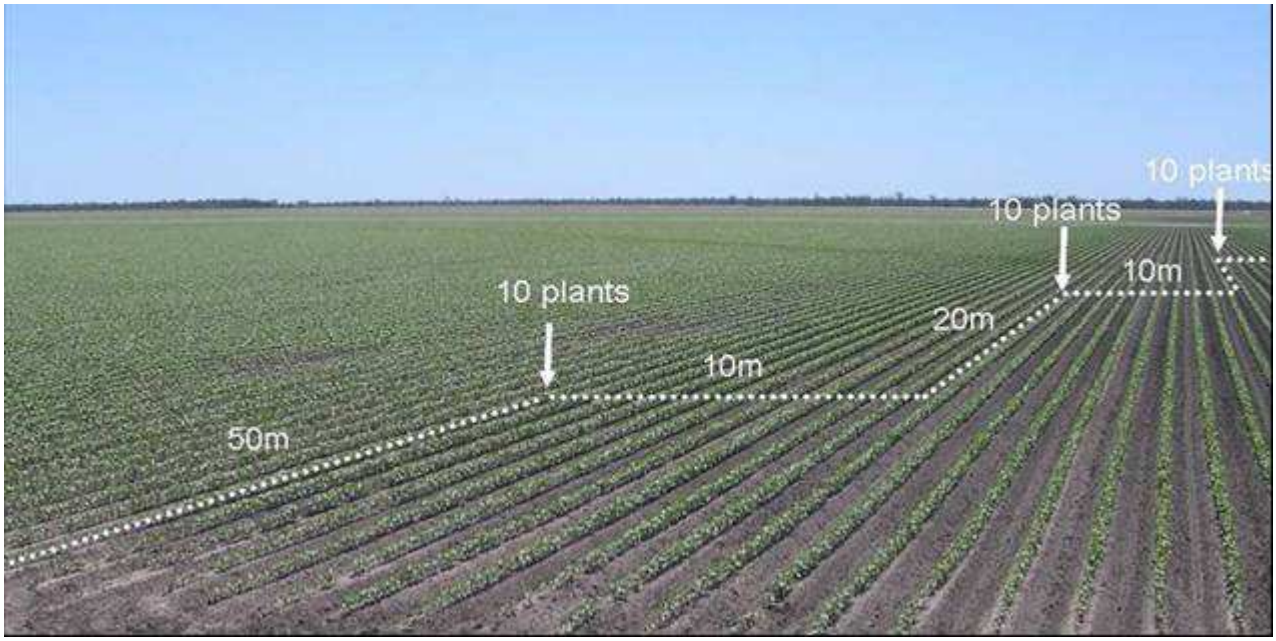

Figure 1. Disease survey transect during early season disease surveys. 100 plants are surveyed along each transect and 2 transects completed in each field.

Introduction and Materials and Methods

This objective relates to the bi-annual cotton disease surveys. Early and late season surveys were conducted in every year of the project as reported in six-monthly reports completing the 27th year of data collection for cotton diseases in NSW. No new disease threats were detected over the course of the project. However several known diseases continue to threaten sustainable cotton production. An exotic insect pest *Solenopsis* mealybug was detected in QLD but was not found to be present in NSW.

Commercial cotton crops in fields sampled from all cotton growing areas of NSW were inspected during November and March of each season allowing for assessment of disease incidence and severity at early and late stages of crop development. The incidence of disease was assessed at points determined using a step-point method across two transects (each 100 m long) in each field (Figure 1). Disease incidence was based on symptoms, with confirmation of the presence of some pathogens (e.g. the black root rot fungus, using a field microscope), and other pathogens with laboratory methods (e.g. Fusarium, Verticillium wilt and boll rots). The progress of Fusarium wilt will be monitored in transects of fields with known infestations.

1.1 Seedling Mortality

Figure 1.1. Seedlings in a field near Boggabilla killed by the Fusarium wilt fungus and seedling 'damping off' caused by *Rhizoctonia solani*.

Seedling mortality is a simple measure of a complex problem. Seedling mortality continues to fluctuate from year to year. Such fluctuations are largely influenced by the pathogens present in soil, insect feeding, and various abiotic factors including soil temperature and water content, soil crusting and bed preparation. Despite the complex nature of factors affecting seedling mortality, there appear to be long term trends from periods of higher mortality to periods of lower mortality in each valley (Figure 1.1.1). Figure 1.1.2 outlines the long term average seedling mortality across NSW cotton growing regions from 1989/90 – 2009/10. Notably, there is a clear and significant increase in seedling mortality from northern to southern NSW. This correlates well (R square = 0.93) with the long term (>10 years) average minimum temperature in October for each valley (data courtesy of Bureau of Meteorology) (Figure 1.1.3).

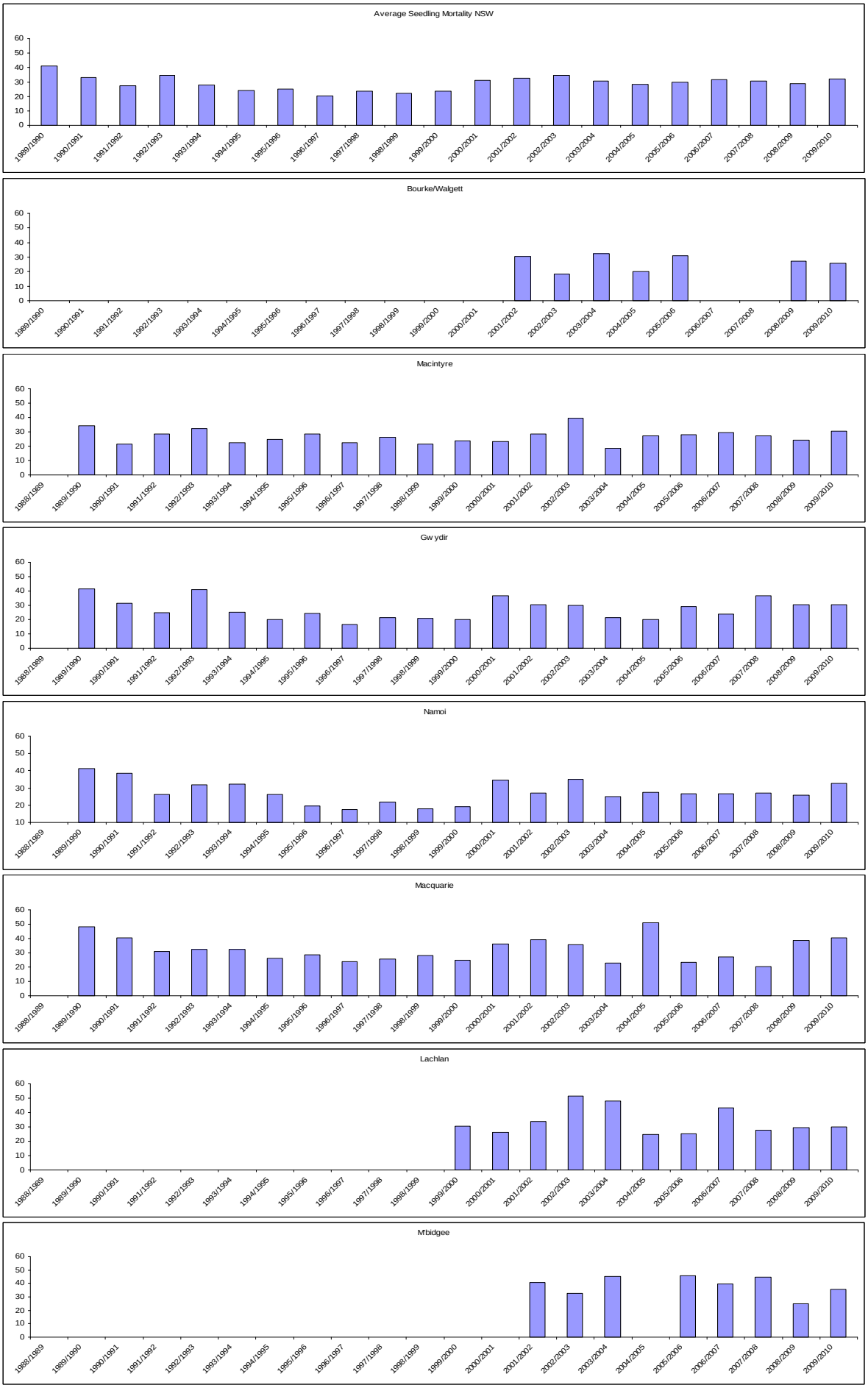

Figure 1.1.1. Long term trends in average (%) seedling mortality across each cotton growing region in NSW. Note trends in higher seedling mortality in the southern regions (Lachlan/Murrumbidgee) and long term switches from periods of lower to periods of higher seedling mortality.

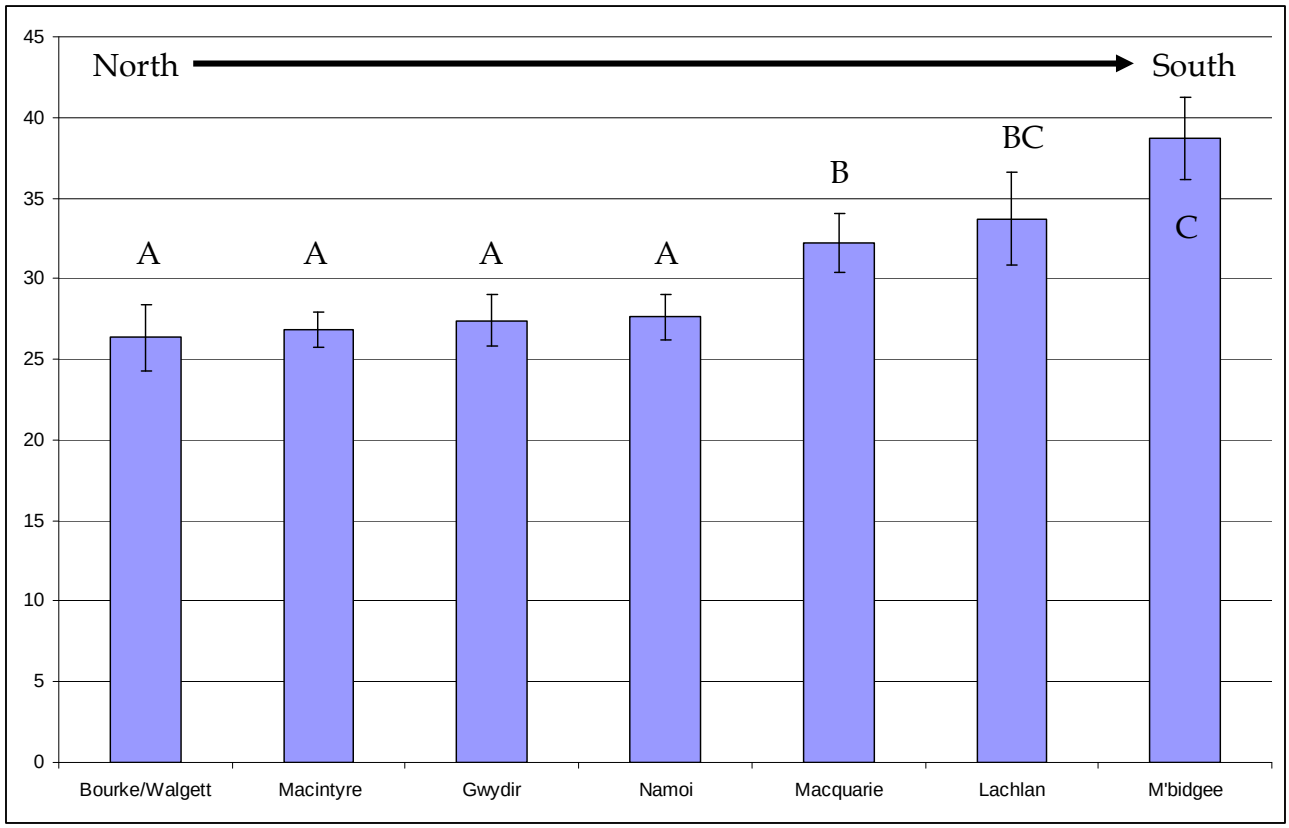

Figure 1.1.2. Mean (standard error) seedling mortality for all years of data collected across each valley. Note seedling mortality increases south of the Namoi. Significant differences indicated by letters (P<0.001).

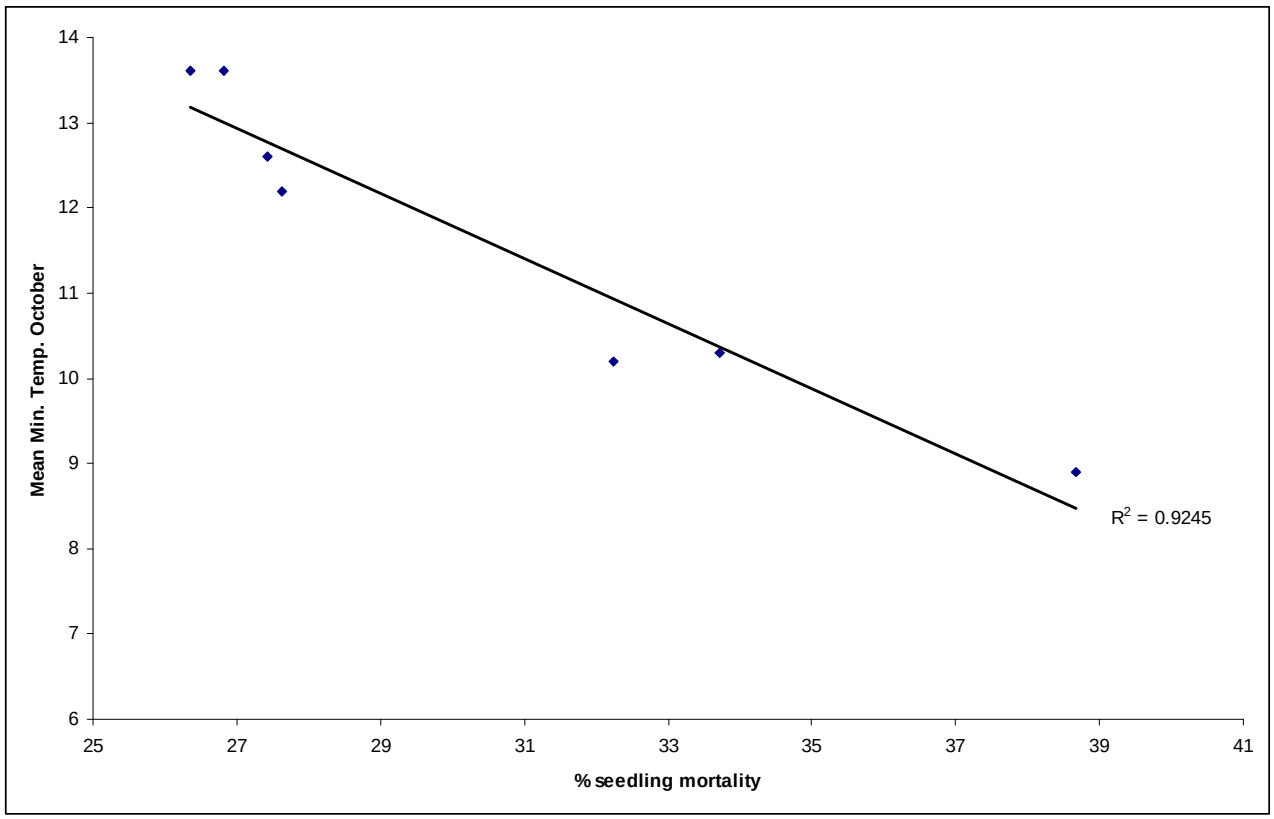

Figure 1.1.3. Seedling mortality increases as mean minimum temperature in October decreases (R square=0.93). Data points left to right: Bourke, Macintyre, Gwydir, Namoi, Macquarie, Lachlan, Murrumbidgee valleys.

2007/8

A total of 36 fields were surveyed across the Macintyre, Gwydir, Namoi, Macquarie, Lachlan and Murrumbidgee valleys due to reduced plantings on account of the drought and staff changes. 7200 plants were sampled during the early season survey with an average of 3.8 nodes per plant. Seedling mortality averaged 31% across the state (Macintyre 27%, Gwydir 36%, Namoi 27%, 20%, Lachlan 27%, Murrumbidgee 45%). The low number of fields surveyed per valley (between 2 and 13 fields) complicated direct comparisons of seedling mortality between valleys. However seedling mortality in the Murrumbidgee valley was substantially higher than all other valleys, reflecting the deleterious impact of cool early season conditions on seedling survival in southern NSW.

2008/9

Increased planting of cotton coupled with the addition of new survey farms in the Lachlan, Murrumbidgee, Macquarie and Gwydir valleys saw the size of the survey increase from 36 fields in 2007/8 to 73 fields in 2008/9. 14600 plants were sampled during the survey. On average, 3.1 nodes were counted per plant. Seedling mortality averaged 29% across the state (Bourke/Walgett 27%, Macintyre 24%, Gwydir 30%, Namoi 26%, Macquarie 39%, Lachlan 29%, Murrumbidgee 25%). Stand establishment in the Murrumbidgee improved greatly compared to 2007 and this probably reflects a warmer start to the season and the addition of new fields that have not previously grown many crops of cotton and therefore have lower levels of *Rhizoctonia* and *Pythium*. Seedling mortality was highest in the Macquarie Valley. The seedling pathogen *Rhizoctonia solani* was observed on plants in 100% of fields.

Wire worm damage was observed in 30% of fields across the state (Bourke/Walgett 13%, Macintyre 20%, Gwydir 57%, Namoi 6%, Macquarie 27%, Lachlan 29%, Murrumbidgee 72%) and was particularly common under cereal residue (Figure 1.1.4). Wireworm, when uncontrolled, can have an impact on stand equivalent to that caused by seedling pathogens.

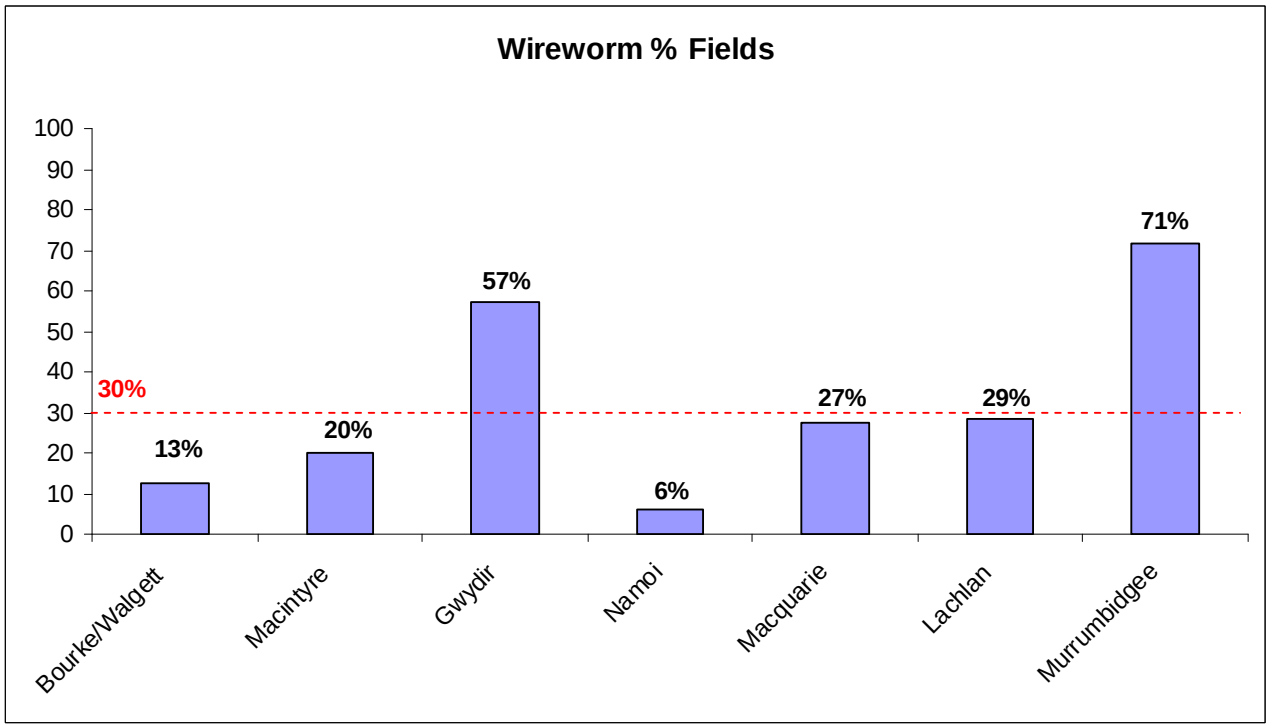

Figure 1.1.4. Evidence of wireworm damage to plant stand (% fields) in the 2008/9 season.

Many abiotic factors also affect stand establishment and seedling development. Stunting and poor root development associated with chemical damage was detected in 22% of fields across the state (Bourke/Walgett 25%, Macintyre 0%, Gwydir 21%, Namoi 31%, Macquarie 9%, Lachlan 57%, Murrumbidgee 14%). Chemical damage can occur when fertilizer is applied too close to the seed and when rain washes herbicide into the planting slot soon after sowing. Chemical burn may predispose seedlings to attack from pathogens. Hormone damage was also observed on seedlings in the Gwydir valley and at Bourke (6% of fields in the state).

2009/10

Surveys were completed in 82 fields across NSW in the Macintyre (12 fields), Gwydir (11 fields), Namoi (20 fields), Macquarie (12 fields), Lachlan and Murrumbidgee Valleys (16 fields) and the Bourke/Walgett region (11 fields). The surveys began later than usual due to cool conditions in October that delayed sowing in many regions until mid to late October. Significant stand loss was observed in areas where cotton was sown in September.

NSW average seedling mortality increased from 29% in 2008/9 to 32% in 2009/10. The Macquarie valley again recorded the highest level of seedling mortality at 40%. Seedling mortality was also high in the Murrumbidgee (36%) where many crops were subject to cool conditions in September/October. In one field near Griffith, plants did not emerge until 4 weeks after sowing. Substantial increases in average seedling mortality occurred in the Murrumbidgee (25-36%), Namoi (26-33%), and Macintyre (24-31%) this season compared to 2008/9. This was a combined impact of cool temperatures in October favouring seedling disease and record high temperatures in November causing rapid loss of soil moisture under seedling cotton and also favouring wireworm. Stunted seedlings, especially those affected by black root rot, were adversely affected by the heat and loss of soil moisture in November 2009.

1.2 **Black Root Rot**

Figure 1.2. A clump of seedlings affected by black root rot adjacent to an unaffected seedling, and discolouration on the roots of the same seedlings when removed from the soil.

Black root rot caused by the soil borne fungus *Thielaviopsis basicola* can stunt and delay plant development (Figure 1.2). The disease is favoured by cool conditions, with plants outgrowing the pathogen when temperatures increase. The pathogen was first detected on survey farms in the Namoi valley in the early 90's and has since spread to all cotton growing regions of NSW. The disease is most prevalent in the Namoi and Macquarie valleys where incidence can reach up to 100% of plants in a single field in some seasons. Disease incidence (% fields) peaked between 2000 and 2004 across NSW after which the number of fields planted to cotton fell causing an apparent fall in disease incidence across the state (Figure 1.2.3). In the Namoi however, disease incidence has risen and has generally remained high over the last decade (Figure 1.2.3). Disease incidence in the Namoi is probably a reflection of the true disease spread across other cotton growing regions of NSW. Consequently, it should be expected that disease incidence will rise sharply as water allocations improve and the area sown to cotton increases in 2010/11 and beyond. While average incidence and severity may appear insignificant, black root rot can be locally severe (Table 1). There is strong evidence that the disease continues to increase rapidly within fields in southern NSW (Figure 1.2.2). In one field in the Murrumbidgee Valley disease incidence and severity has almost tripled in three years (Figure 1.2.2). The disease may also progress more quickly under overhead irrigation systems (Figure 1.2.1). Black root rot should remain a high priority for disease research in NSW.

Table 1. The severity of black root rot in fields recording high incidences of the disease in NSW.

REGION	SEASON	INCIDENCE (%)	SEVERITY (/10)
Macintyre	2009/10	100	5.38
Macquarie	2008/09	99.5	9.46
Lachlan	2008/09	98.5	8.78
Namoi	2008/09	93	6.05
Namoi	2009/10	100	7.47
Namoi	2009/10	100	6.05
Namoi	2009/10	100	3.22
Namoi	2009/10	98.5	4.43
Namoi	2009/10	95.5	5.91
Namoi	2009/10	95.5	4.74

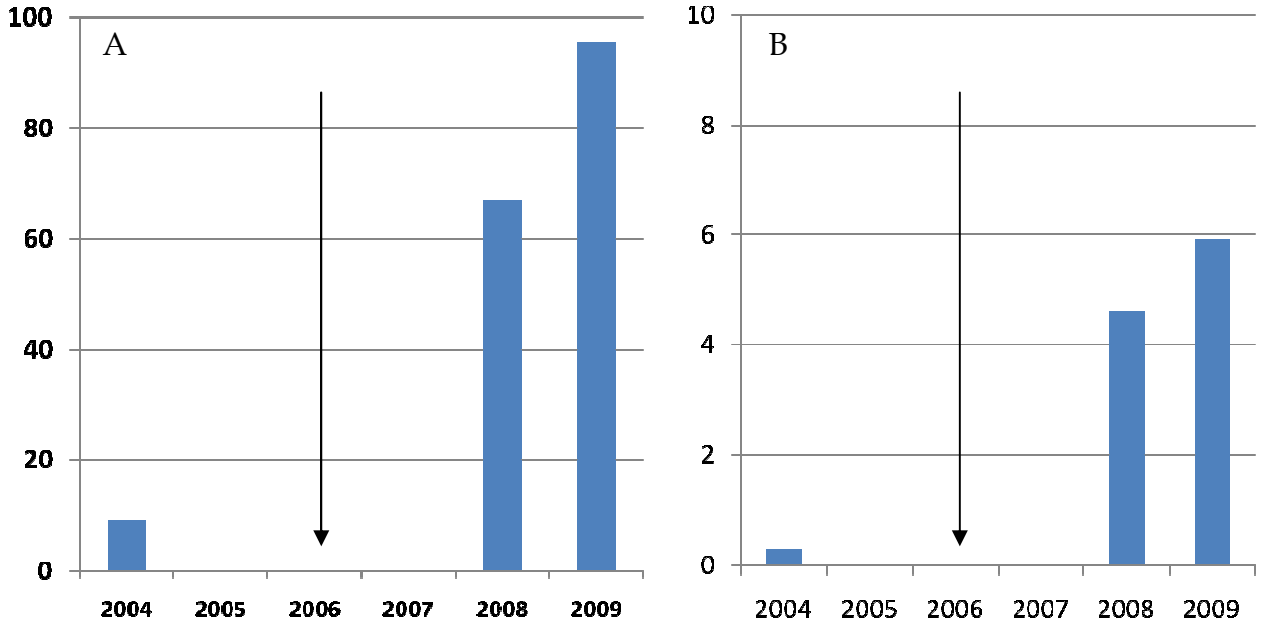

Figure 1.2.1. Incidence (A) and severity (B) (on a scale of 1-10) of black root rot in a field near Narrabri. Lateral move irrigation was installed in the field in 2006 as indicated by arrows (Graphs drawn by Dr Stephen Allen, data collected by C. Anderson).

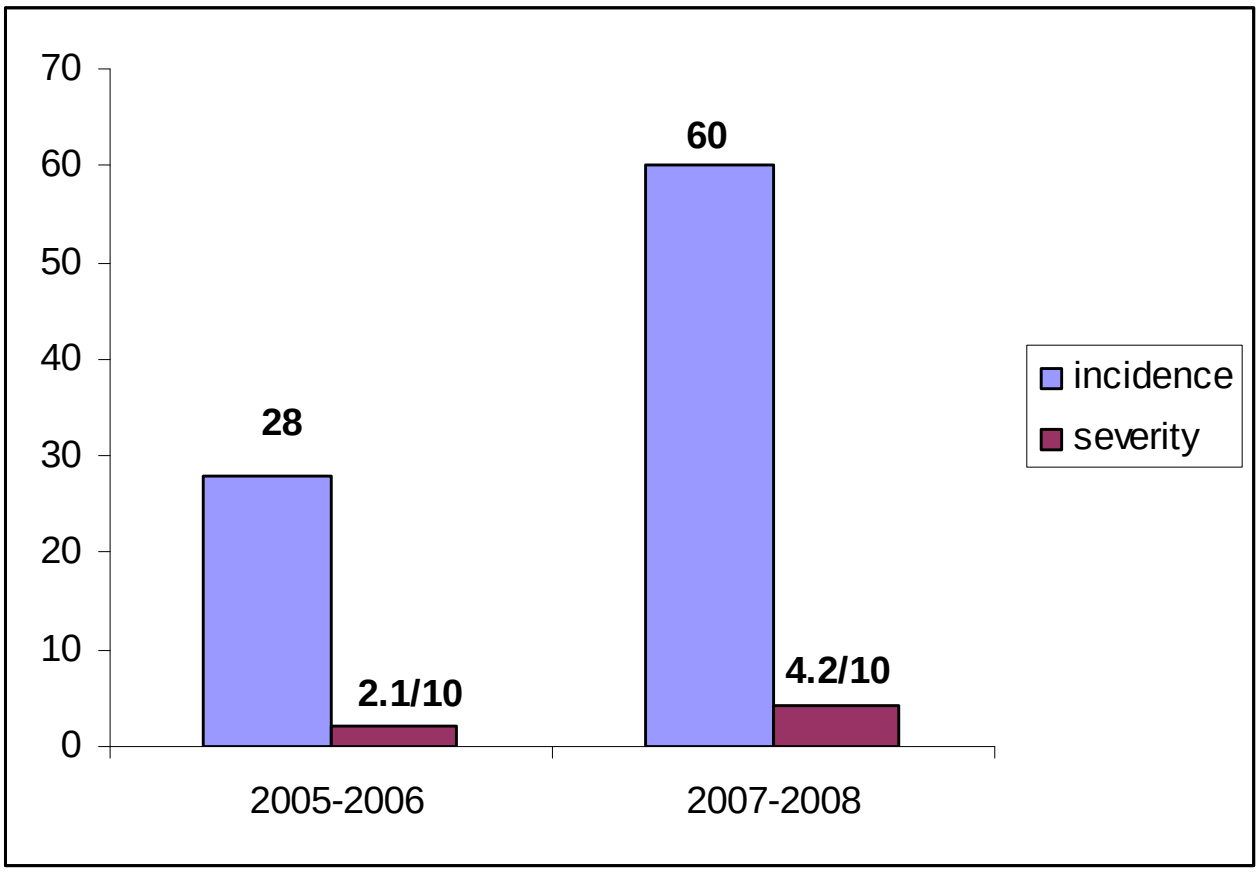

Figure 1.2.2. Increase in both incidence (% plants) and severity of black root rot in a field in the Murrumbidgee Valley over three years.

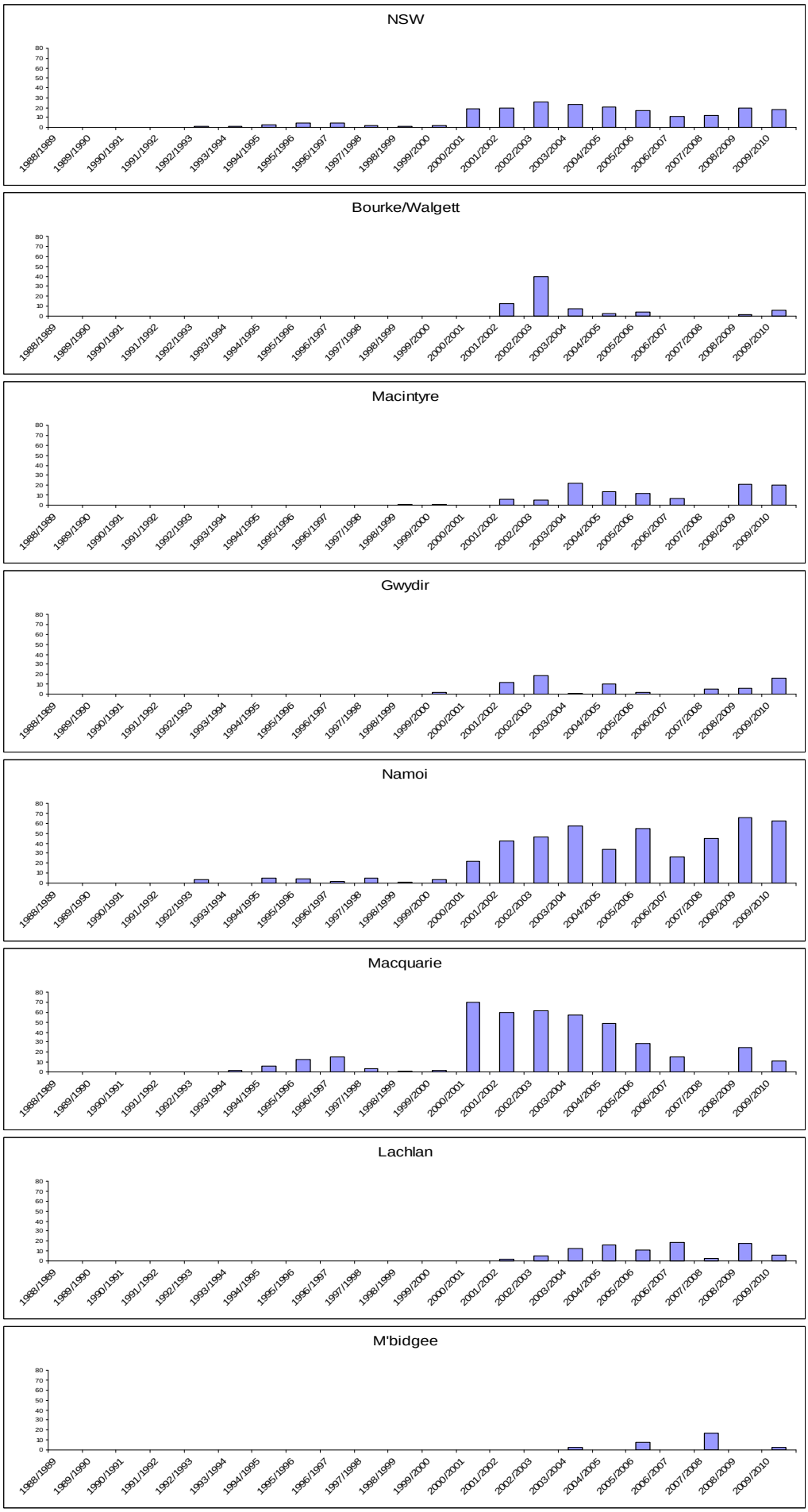

Figure 1.2.3. Long term incidence (% fields) of black root rot in each cotton growing region of NSW.

2007/8

Black root rot was observed in 50% of fields surveyed and 20% of plants in the 2007/8 season (Figure 1.2.4). While the severity of black root rot averaged out across all infected plants to be 1 on a 1-10 scale, the disease was locally severe (Figure 1.2.5). The disease was most commonly observed in the Namoi (100% fields and 45% of plants), followed by the Murrumbidgee valley (60% fields, 17% of plants), and the Lachlan, indicating the need to continue monitoring of black root rot in the southern areas of NSW (Figure 1.2.4). Disease severity was also highest in the Namoi (2.3/10) and Murrumbidgee valleys (1.2/10) (Figure 1.2.5) reflecting a higher number of severely infested fields.

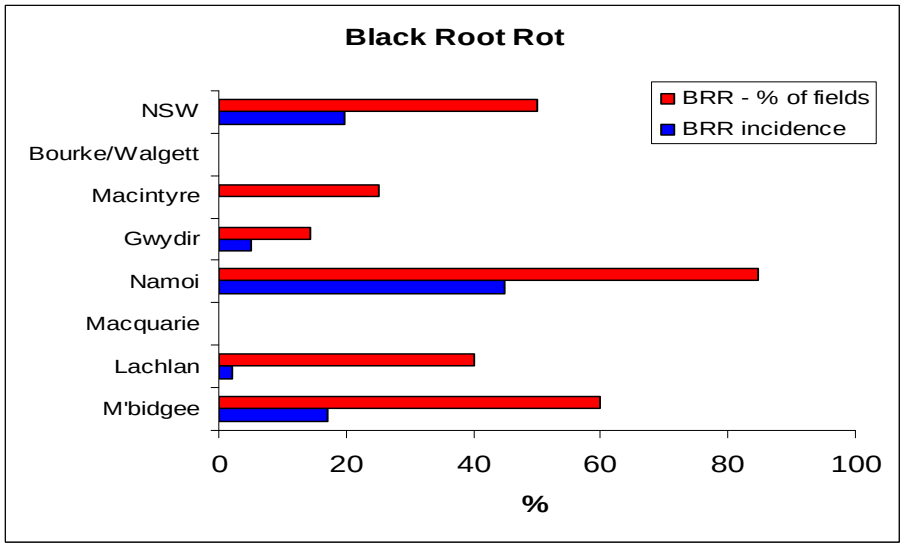

Figure 1.2.4. Incidence (% fields and % plants) of black root rot in the 2007/8 season.

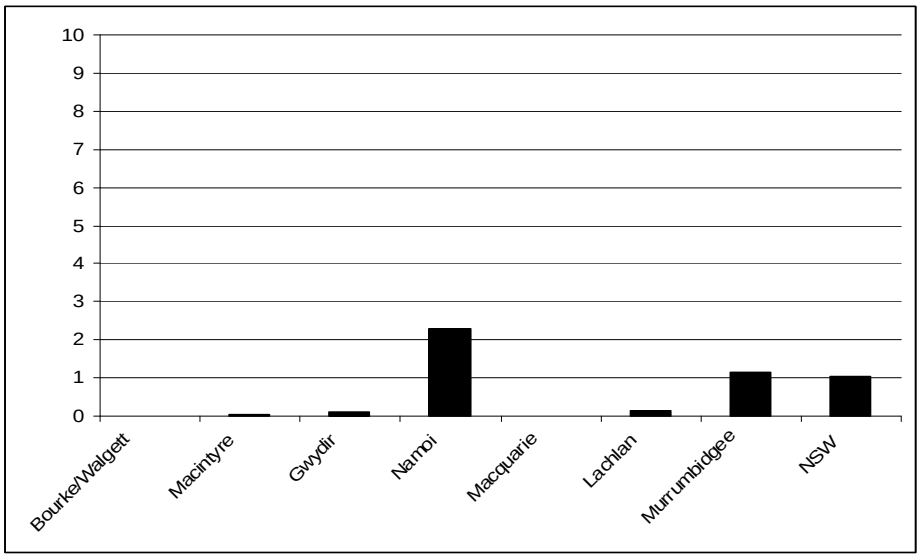

Figure 1.2.5. Severity (scale of 1-10) of black root rot in the 2007/8 season.

2008/9

Black root rot was observed in 52% of fields across the state (Bourke/Walgett 13%, Macintyre 60%, Gwydir 29%, Namoi 100%, Macquarie 64%, Lachlan 57%, Murrumbidgee 0%) (Figure 1.2.6). The addition of relatively new cotton fields in the Murrumbidgee masked the presence of black root rot, which was recorded in 60% of fields and on 17% of plants in the previous season. The severity of black root rot was higher across the state at 1.4/10 (Figure 1.2.7), some fields in the Namoi and Macquarie valleys were particularly severe with 100% of plants severely affected. The Namoi yet again recorded the highest

severity of black root rot at 4/10 reflecting historical build up of the pathogen (Figure 1.2.7).

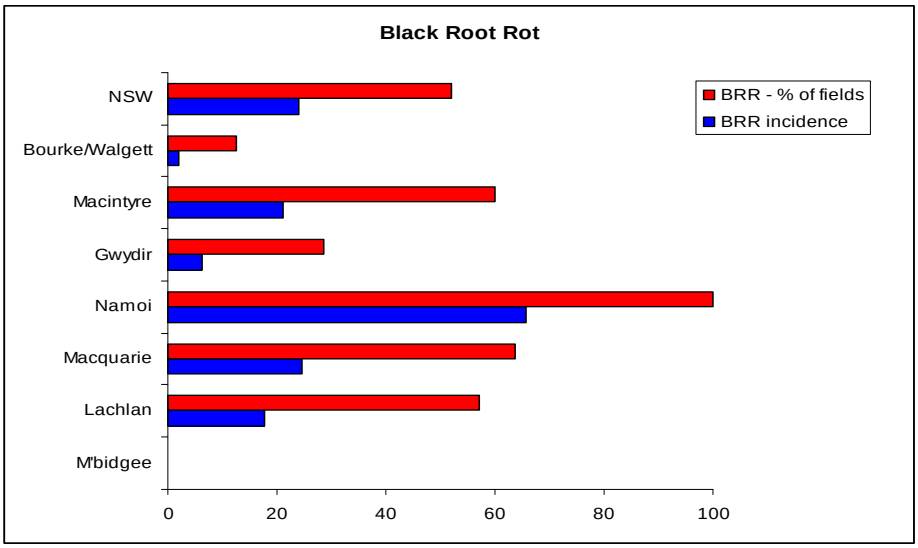

Figure 1.2.6. Incidence (% fields and % plants) of black root rot in the 2008/9 season.

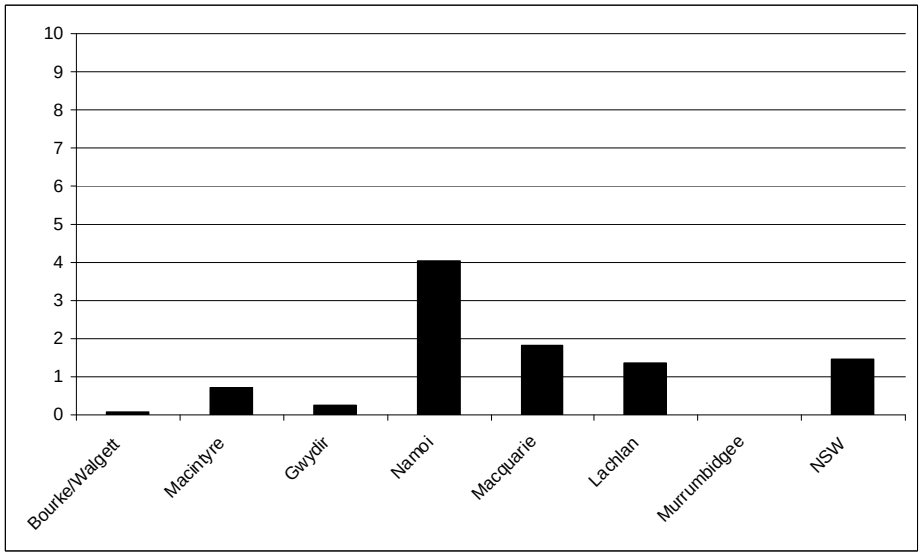

Figure 1.2.7. Severity (scale of 1-10) of black root rot in the 2008/9 season.

2009/10

Black root rot was detected on 18% of plants and in 54% of fields surveyed in 2009/10 across NSW. Disease severity fell from 1.4/10 in 2008/9 to 1/10 in 2009/10 (Figure 1.2.9). Highest levels of the disease were again detected in the Namoi where 63% of plants were infected by the pathogen and this was similar to last season (Figure 1.2.8). Disease severity was also highest in the Namoi (3.1/10), although this was lower than last season (4/10) (Figure 1.2.9). The disease was also prevalent in the Macintyre and Gwydir regions where many crops were sown into fields with a history of the disease under cool conditions in September/October. Disease incidence and severity were lower this year compared to last season due to record high temperatures in November and generally later sowing across the industry to avoid cool conditions in September/October. This is an encouraging result that reflects adoption of integrated disease management guidelines.

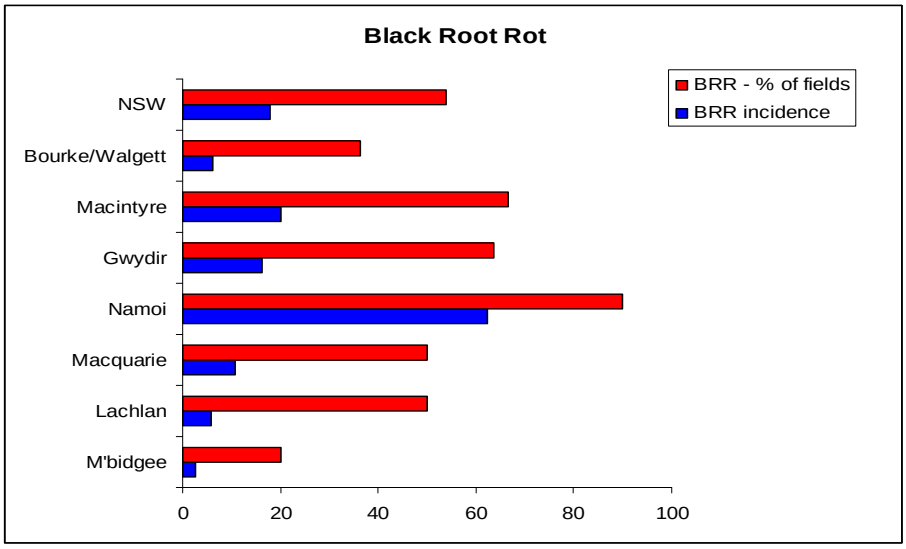

Figure 1.2.8. Incidence (% fields and % plants) of black root rot in the 2009/10 season.

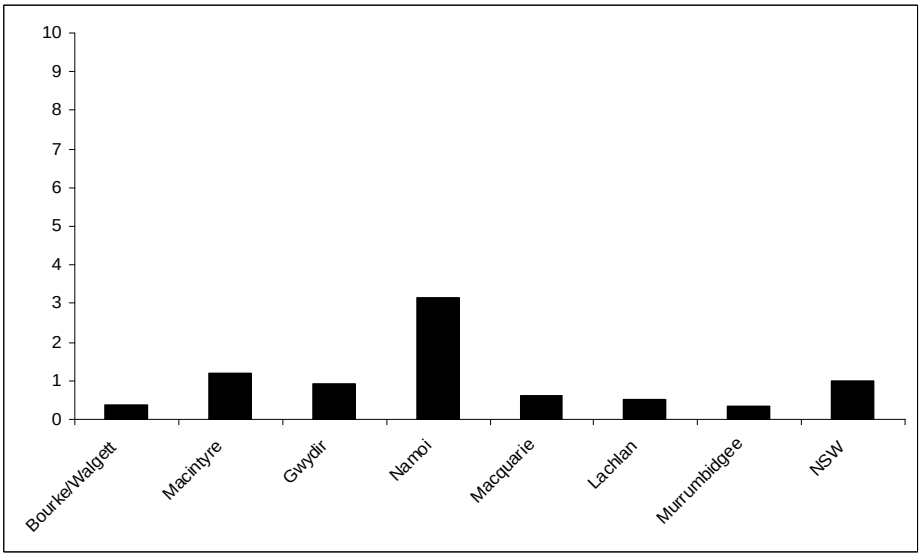

Figure 1.2.9. Severity (scale of 1-10) of black root rot in the 2009/10 season.

1.3 Vascular Wilts – Verticillium and Fusarium Wilt

Figure 1.3. Early season stand loss caused by Fusarium wilt on a farm near Boggabilla.

Fusarium and Verticillium wilt continue to be present at high levels in some fields within NSW. Verticillium wilt has been detected in all cotton growing regions of NSW where Fusarium wilt has not yet been detected in the Lachlan and Murrumbidgee valleys.
During this project, there were two additional detections of Fusarium wilt; one in the Gwydir Valley and one in the Namoi near Maules Creek (Figure 1.3). One of these detections was made during the annual disease surveys and the other was reported to Plant Pathologist Dr Stephen Allen. The total number of reported cases of this disease has now reached 83 (Figure 1.3.1).

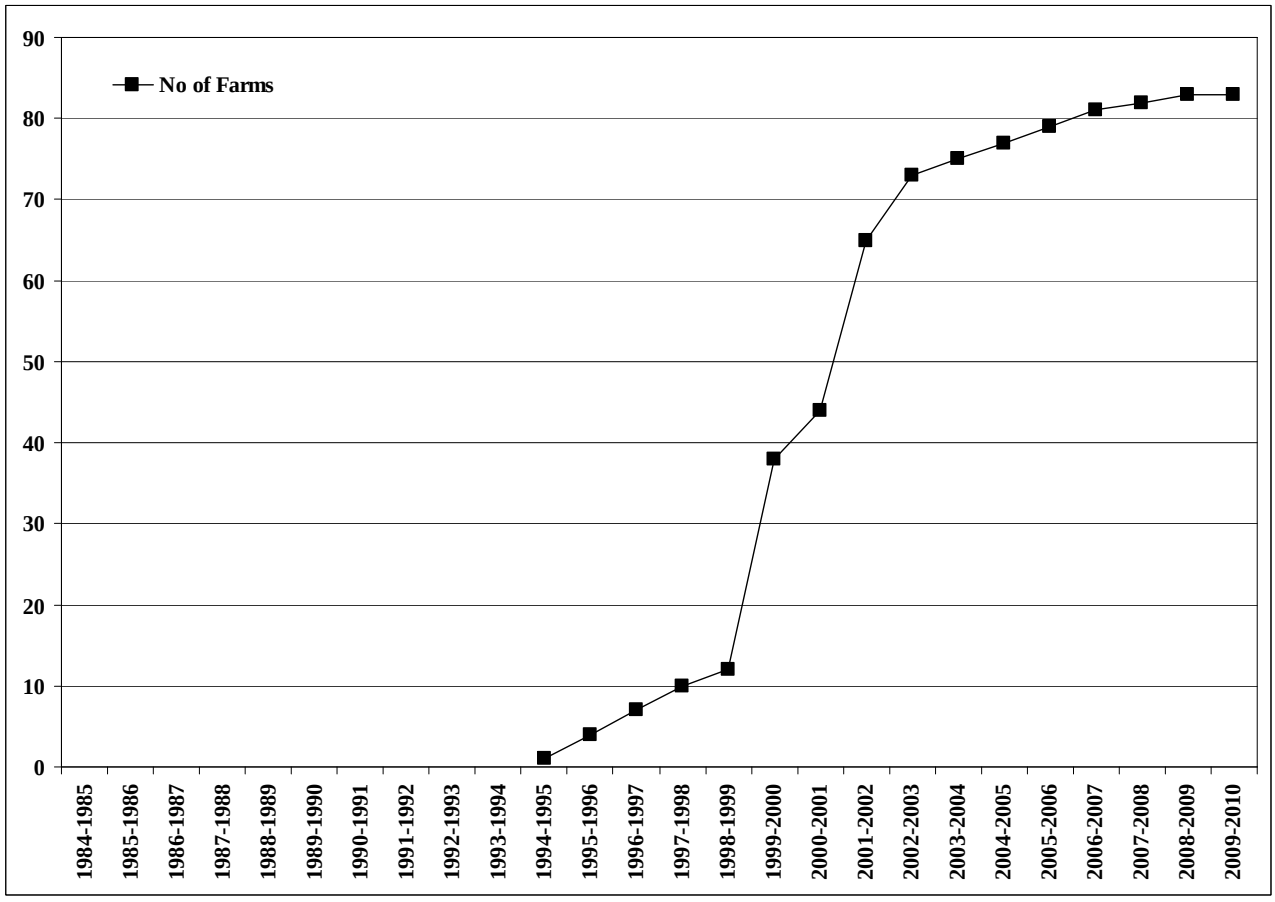

Figure 1.3.1. Number of reported cases of Fusarium wilt in NSW.

There has been an apparent slowdown in the detection and reporting of Fusarium wilt over the past 8 years (Figure 1.3.1). The power of the surveys to detect new cases of this disease has fallen due to the presence of the disease on 100% of survey farms in the worst affected regions (Macintyre and Gwydir valleys). Moreover, with the widespread adoption of resistant varieties (Figure 1.3.2), new outbreaks of Fusarium wilt are likely to be small and may go unnoticed, and this in turn affects the likelihood that farmers will report new outbreaks. Known strains of Fusarium wilt do not cause the large scale crop damage associated with this disease in the past, except in fields with a long history of the disease. Despite this, new strains of the disease continue to evolve and monitoring of known and new strains will remain important to protect the industry from future outbreaks.

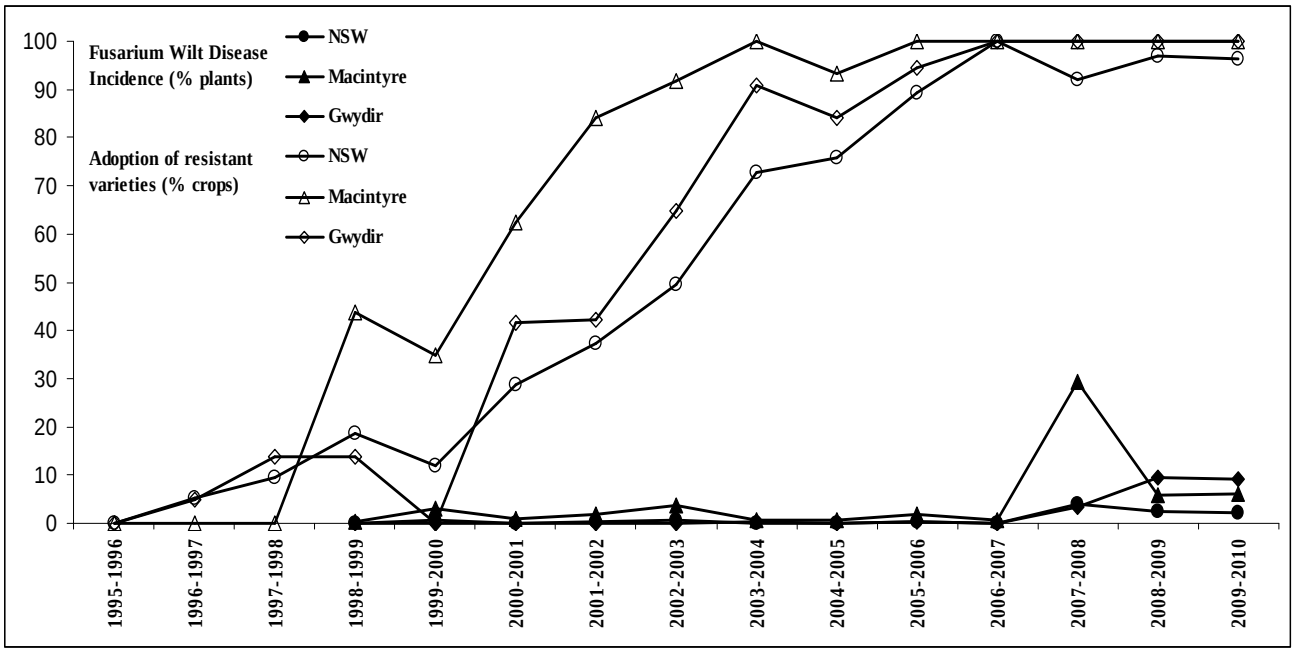

Figure 1.3.2. Comparison of the incidence of Fusarium wilt in NSW with the adoption of resistant varieties.

Adoption of resistant varieties has also been of key importance in the management of Verticillium wilt. Since the adoption of resistant varieties in the early '90s, the incidence of Verticillium wilt has remained relatively low with one notable exception (Figure 1.3.3).

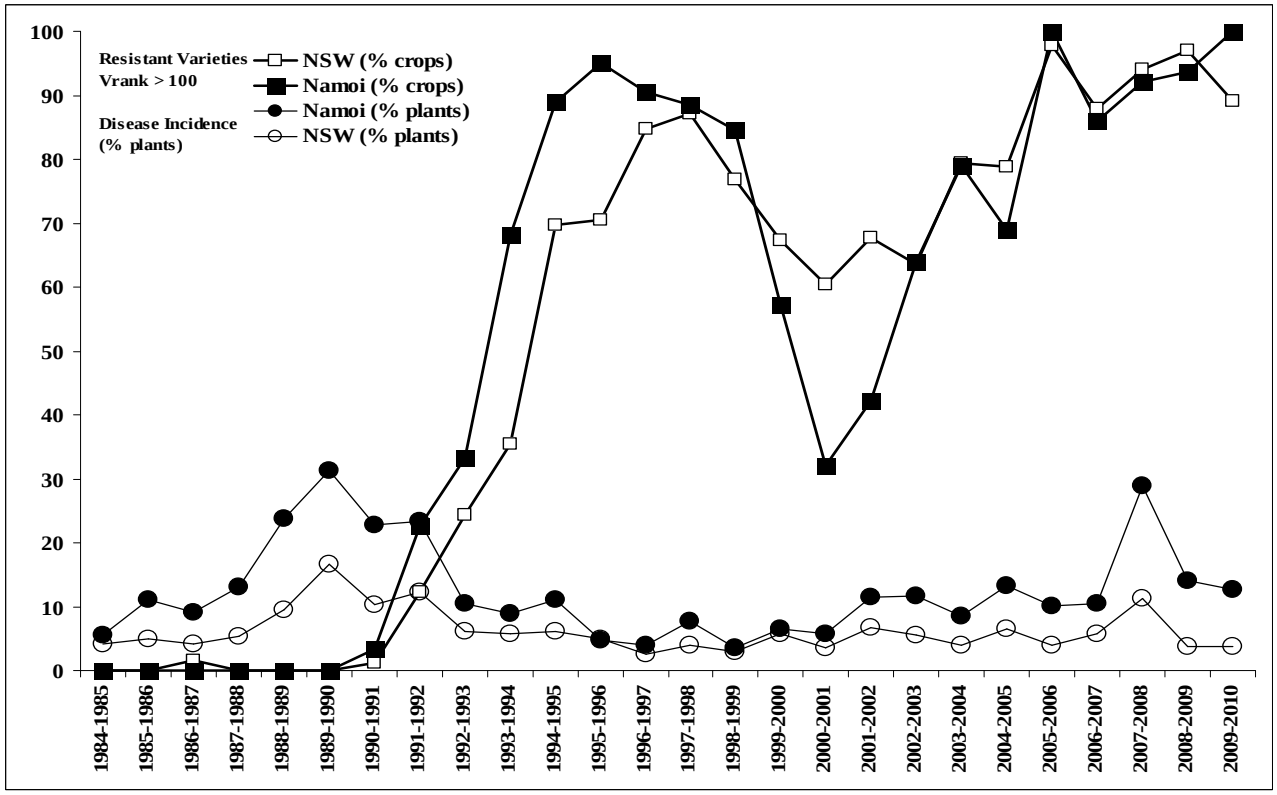

Figure 1.3.3. Comparison of the incidence of Verticillium wilt in NSW compared to the adoption of resistant varieties.

The incidence of Verticillium wilt across NSW jumped from 4.9% plants infected in 2006/7 to 11.2% in 2007/8, a 15 year high (Figure 1.3.3). This jump was even more pronounced in the Namoi valley where disease incidence was recorded at 28.9% of plants (Figure 1.3.3). In some fields this disease was recorded in over 98% of plants, and appeared to kill plants

from the top down. Plant death has not previously been associated with Verticillium wilt in Australia. Given the sharp increase in incidence and severity, it will be important to closely monitor this disease over the coming seasons. This peak in disease incidence and severity was not associated with a drop in the adoption of resistant varieties (Figure 1.3.3) and so is likely due to prolonged cool wet conditions. Varietal resistance to Verticillium wilt is temperature dependent. Resistance breaks down under prolonged cool weather conditions. This emphasises the importance of complementing varietal choice with good integrated disease management practices for Verticillium wilt in fields with a history of the disease.

Annual Disease Progress

2007/8

Verticillium wilt was detected in 51.4% of fields and 11.2% plants across NSW (Table 2). As previously mentioned, this was the highest recorded incidence of Verticillium wilt in over 15 years. Verticillium wilt was detected in 100%, 57% and 20% of fields in the Namoi (28.9% plants infected), Gwydir (2.2% plants infected) and Murrumbidgee (0.1% plants infected) valleys respectively (Table 2). Verticillium wilt was not detected in the Macintyre, Lachlan or Macquarie valleys. Interestingly, one plant was found in the Murrumbidgee valley, within a field which had not previously been used to produce cotton. The pathogen was probably already present in the soil before the field was developed for irrigation, surviving in weed species like Bathurst Burr.

Fusarium wilt was detected in 17.1% of fields and 4% of plants across NSW (Table 2). Fusarium wilt was detected in 57% and 50% of fields in the Gwydir (3.4% plants infected) and Macintyre (29.3% plants infected) valleys respectively (Table 2). The spike in plant infections in the Macintyre may have been caused by the generally cool wet seasonal conditions. This increase in disease incidence was also observed in Field E2 near Boggabilla (Figure 1.3.5). One new record of the disease was found in the Gwydir valley. Fusarium wilt was not detected in the Namoi, Lachlan, Murrumbidgee or Macquarie valleys.

2008/9

Verticillium wilt was detected in 40% of fields and 3.8% plants across NSW (Table 2). The disease was present in: 94% fields and 14% of plants in the Namoi; 50% fields and 2.6% plants in the Macintyre; 28.6% fields and 0.9% plants in the Gwydir; 27.3% fields and 0.8% plants in the Macquarie; 25% fields and 0.7% plants at Bourke (Table 2). The disease was not detected in the Lachlan and Murrumbidgee valleys. The disease continues to be common in the Namoi valley.

Fusarium wilt was detected in 19.2% fields and 2.6% plants across NSW (Table 2). The disease was present in the Gwydir in 57% fields and 9.4% plants and the Macintyre in 60% fields and 5.7% plants (Table 2). The disease was not detected in any other valleys. One new case was detected in the Namoi valley near Maules Creek bringing the total number of reported cases to 83 farms.

2009/10

Verticillium wilt was again common in the Namoi valley where it was detected in 80% of fields and 13% of plants (Table 2). On average, the disease was present in 36% of fields and 2.4% plants in NSW (Table 2).

Fusarium wilt was detected in the Macintyre (58% fields, 6% plants) and Gwydir (46% fields, 9% plants) valleys (Table 2). This was similar to last season and is probably explained by improvements in varietal F rank and dry warm conditions in November that were unfavourable for infection of plants by the Fusarium wilt fungus. No new detections of Fusarium wilt were made in the 2009/10 season.

Table 2. Incidence (% fields and % plants) of Fusarium and Verticillium Wilt in 2007/8, 2008/9 and 2009/10.

	Fusarium Wilt (% fields)				Fusarium Wilt (% plants)		
	2007/8	2008/9	2009/10		2007/8	2008/9	2009/10
M'bidgee	0.0	0.0	0.0	M'bidgee	0.0	0.0	0.0
Lachlan	0.0	0.0	0.0	Lachlan	0.0	0.0	0.0
Macquarie	0.0	0.0	0.0	Macquarie	0.0	0.0	0.0
Namoi	0.0	0.0	0.0	Namoi	0.0	0.0	0.0
Gwydir	57.1	57.1	45.5	Gwydir	3.4	9.4	9.3
Macintyre	50.0	60.0	58.3	Macintyre	29.3	5.7	6.3
Bourke/Walgett	no data	0.0	0.0	Bourke/Walgett	no data	0.0	0.0
NSW	**17.1**	**19.2**	**14.6**	**NSW**	**4.0**	**2.6**	**2.2**
	Verticillium Wilt (% fields)				**Verticillium Wilt (% plants)**		
	2007/8	**2008/9**	**2009/10**		**2007/8**	**2008/9**	**2009/10**
M'bidgee	20.0	0.0	0.0	M'bidgee	0.1	0.0	0.0
Lachlan	0.0	0.0	16.7	Lachlan	0.0	0.0	0.3
Macquarie	0.0	27.3	25.0	Macquarie	0.0	0.8	0.2
Namoi	100.0	93.8	80.0	Namoi	28.9	14.0	12.7
Gwydir	57.1	28.6	54.5	Gwydir	2.2	0.9	2.0
Macintyre	0.0	50.0	41.7	Macintyre	0.0	2.6	1.5
Bourke/Walgett	no data	25.0	36.4	Bourke/Walgett	no data	0.7	0.3
NSW	**51.4**	**40.0**	**36.0**	**NSW**	**11.2**	**3.8**	**2.4**

Fusarium Transects

Industry and Investment NSW has been monitoring the progress of Fusarium wilt across several fields known to have a history of the disease for over 10 years. Many of these fields have not been sown to cotton for several years due to drought. Three fields were sown during the Diseases of Cotton IX project. Fusarium transects were completed on two fields near Boggabilla: field 7 (Figure 1.3.4) and field E2 (Figure 1.3.5).

Field 7 near Boggabilla in the Macintyre Valley was previously assessed in the 2006 season as part of the Diseases of Cotton VIII project (Figure 1.3.4). The disease appeared to have spread across the field between the 2004 and 2006 (Figure 1.3.4). Disease levels appeared similar in 2008 indicating that the observed increase in 2006 was real and not an artefact of the timing of assessment. In addition, a more detailed study of disease spread in Field 7 was completed in 2008. Three field length transects were completed, one each at 50m, 100m and 150m from the tail drain, along which 10 plants were assessed every 10m. These transects indicate that the disease is relatively evenly spread across the lower end of the field but increases in incidence closer to the tail drain (Figure 1.3.6). Disease incidence appears higher on the southern end (right hand side) of the field. Therefore several transects were completed across this section of the field at distances of 50m, 100m, 150m, 200m, 250m, and 350m from the tail drain (Figure 1.3.7). These transects again show that disease incidence is highest near the tail drain as is expected as a result of inoculum build up in tail water. Repetition of these high intensity transects across Field 7 should be continued into the next project.

Field E2 was assessed in 2008 and again in 2010. An historical high disease incidence of 19% was recorded in 2008 and this is likely due to disease-conducive seasonal conditions

(Figure 1.3.5). A similar jump in disease incidence (% plants) was recorded across the Macintyre during this season (Table 2). Disease levels had fallen to 7% in 2009/10 reflecting a much warmer and drier start to the season.

Interestingly, the disease has been present in both fields 7 and E2 for a similar period, however the incidence of infected plants in field 7 is much higher than in field E2, possibly reflecting the impact of soil type and cultural practice on the build up of this pathogen. The level of Fusarium wilt in field 7 has not increased substantially since 2006. However, the level in E2 fluctuated in the same period suggesting a much higher soil-borne inoculum in field 7. Inoculum levels in E2 may have been diluted during construction of a water storage on the north end of the field.

Field 1 near Carroll in the Upper Namoi Valley was previously assessed in the 2006/7 season (Figure 1.3.8). This field has a few small patches of Fusarium wilt and so provides an ideal scenario for observation of disease build-up and spread over time. It appears that the disease spread within this field has been limited to very low levels potentially through the growth of resistant cotton varieties and other unknown factors like suppressive soil (although this is not substantiated by experimental results).

Figure 1.3.4. The incidence of Fusarium wilt across Field 7 near Boggabilla.

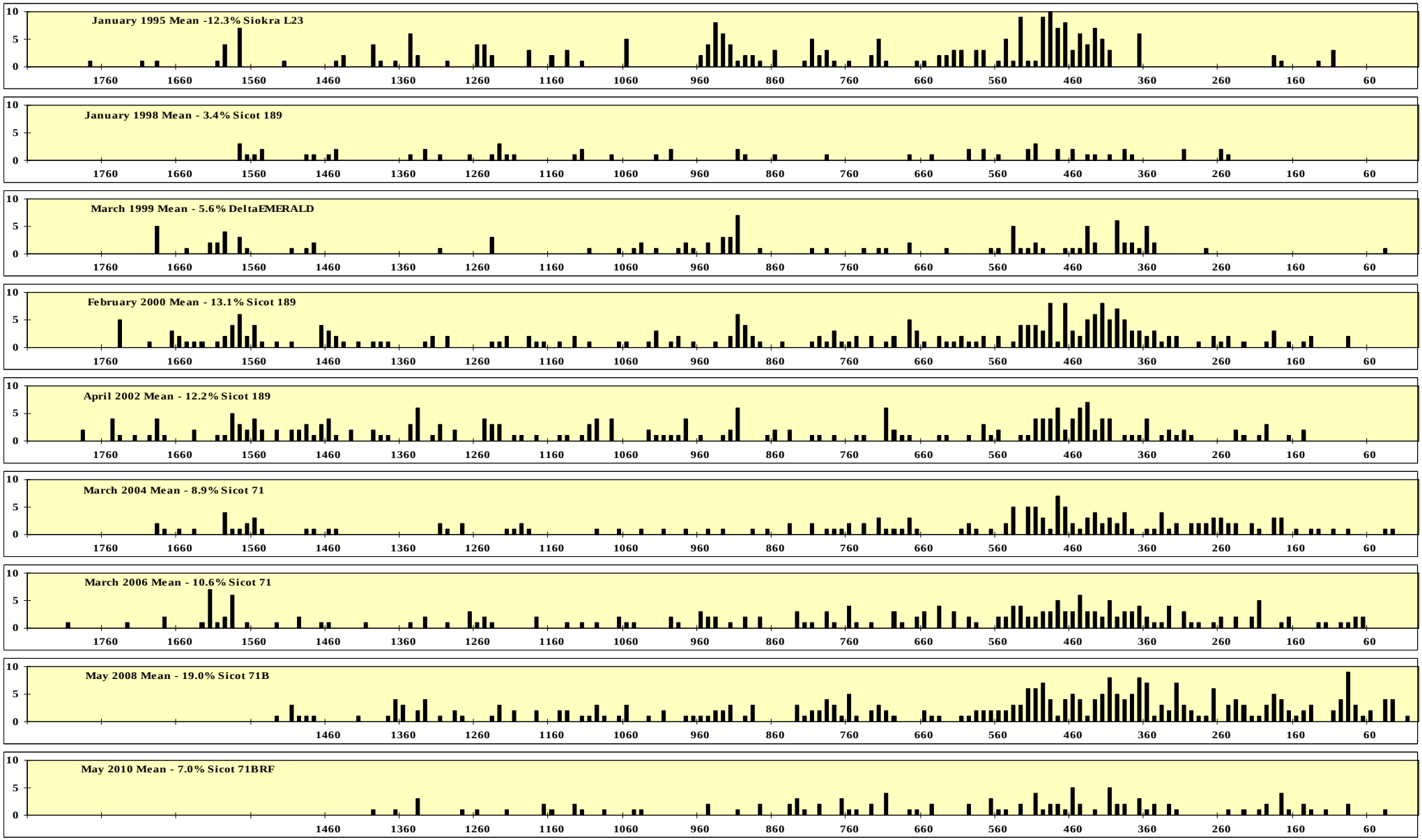

Figure 1.3.5. The incidence of Fusarium wilt across Field E2 near Boggabilla.

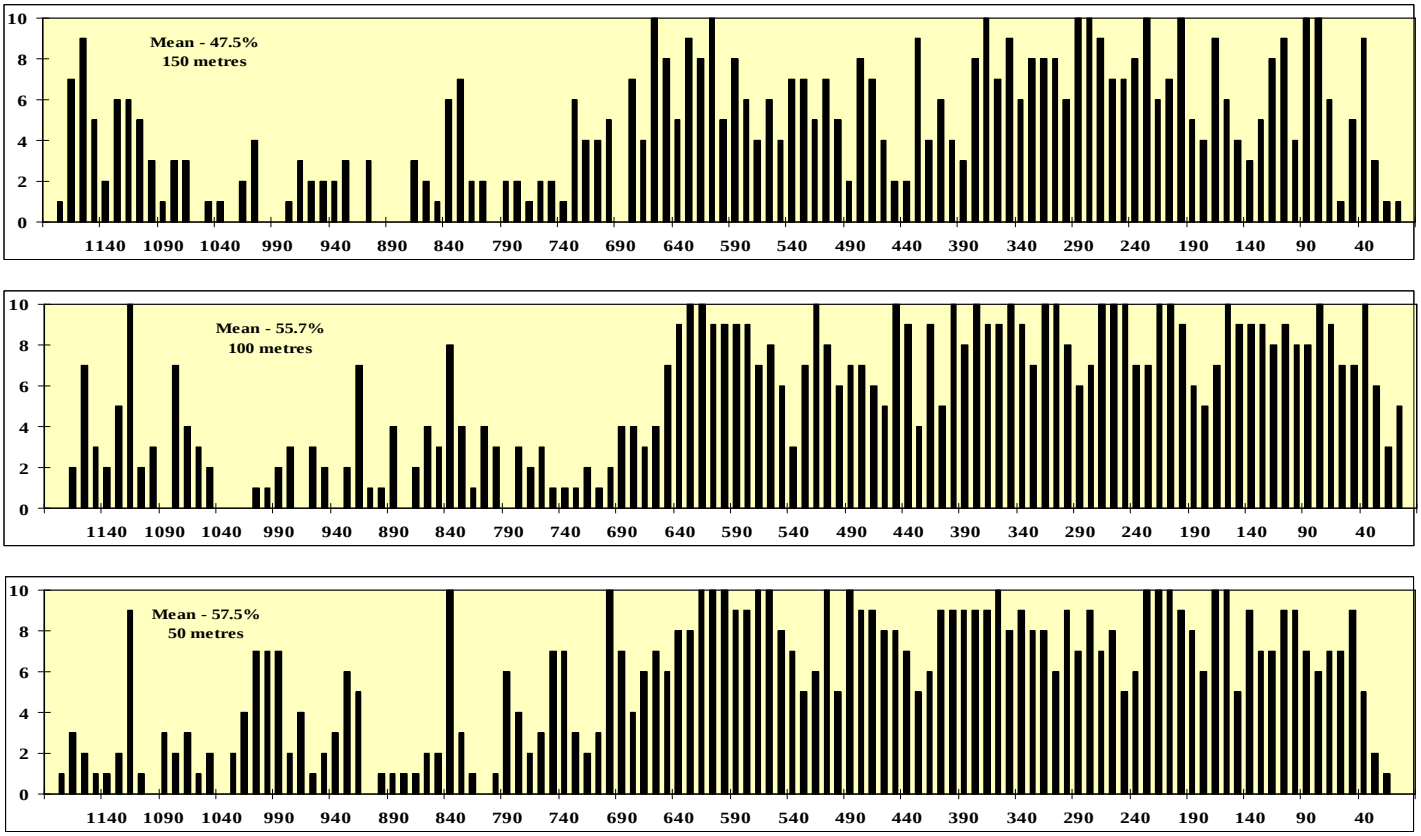

Figure 1.3.6. Incidence of Fusarium wilt at 50m, 100m and 150m from the tail drain of field 7 near Boggabilla.

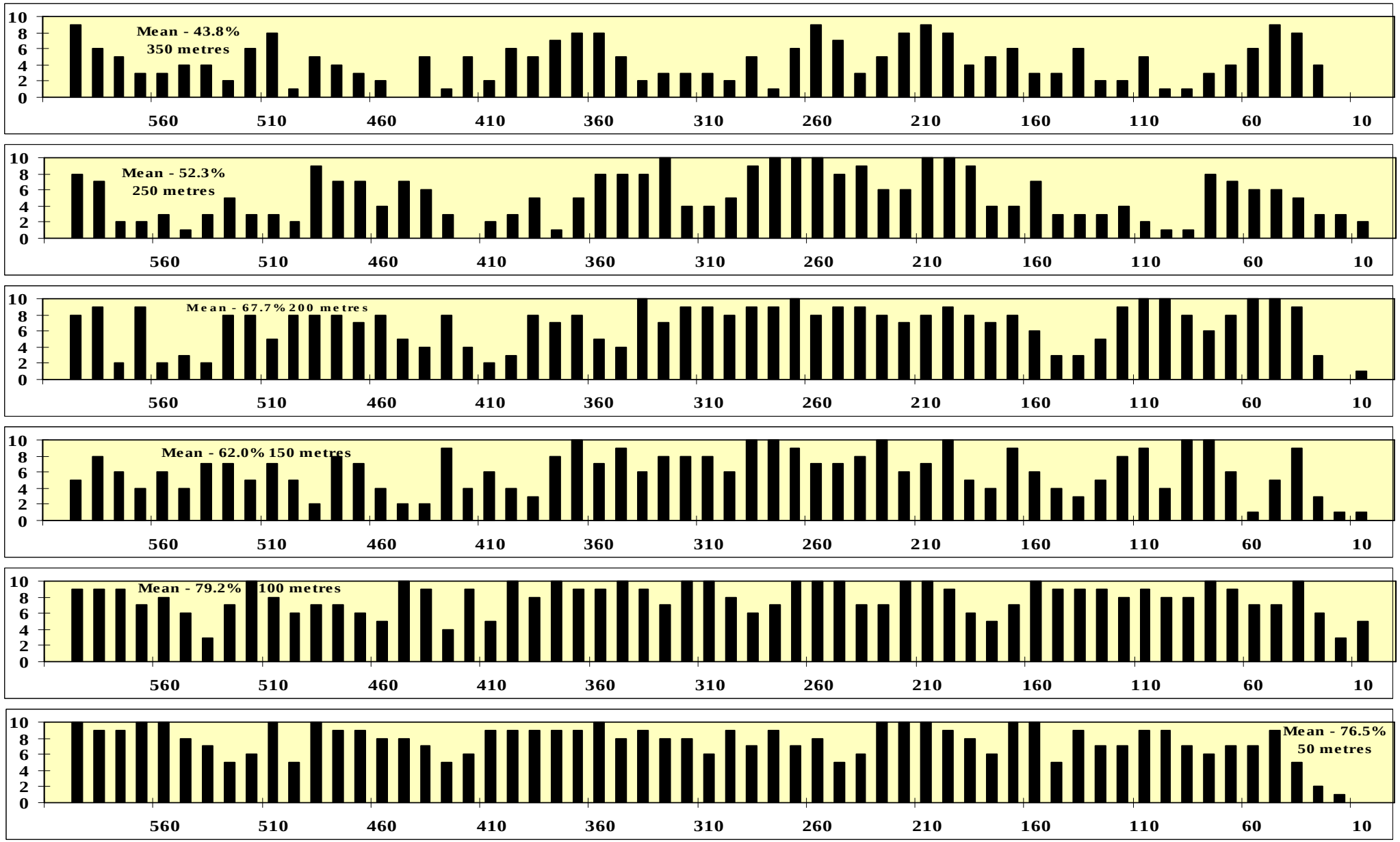

Figure 1.3.7. Distribution of Fusarium wilt in the southern end of Field 7 near Boggabilla at 50, 100, 150, 200, 250, 350m from the tail drain.

Figure 1.3.8. Spread of Fusarium wilt in Field 1 near Carroll, NSW.

1.4 Boll Rots and Alternaria Leaf Spot

Figure 1.4. Phytophthora boll rot (left) and sporangia of *Phytophthora* spp. on lint (right).

Boll rots are caused by a suite of fungi including *Fusarium*, *Colletotrichum* and *Phytophthora* spp. (Figure 1.4). Phytophthora boll rot is associated with heavy rainfall and flooding during boll fill which transports the organisms from the soil to the boll enabling infection to occur. Alternaria leaf spot is caused by the fungi *Alternaria macrospora* and *Alternaria alternata* which multiply on and survive in trash. Alternaria leaf spot was widely observed on almost all crops in each season at low levels, but poses no threat to sustainable cotton production. The *Alternaria* fungi occasionally cause boll rots when lesions form on the outer carpal wall causing locules to fuse. Spores of this fungus are produced en mass after defoliation, and are easily airborne contributing to community health problems including asthma and hay fever.

Boll rots were recorded at higher than average levels in the 2008/9 and 2009/10 seasons. Similar levels have not been recorded since 1999/2000 and 2000/01 when rainfall events in some valleys affected the overall % boll rots in the state. The high level of boll rots in 2009/10 was unprecedented with records being set for NSW (9.6%), Bourke/Walgett, Macintyre, Gwydir, Namoi, Lachlan and Murrumbidgee valleys (Table 3, Figure 1.4.1).

Table 3. Incidence of boll rots in all cotton growing regions of NSW from 1993/4 to 2009/10

	Boll rots (incidence)						
	Bourke/Walgett	**Macintyre**	**Gwydir**	**Namoi**	**Macquarie**	**Lachlan**	**M'bidgee**
1993/1994	1.8	3.8	0.8	0.7	0.3	*	*
1994/1995	1.7	2.5	3.1	0.3	0.5	*	*
1995/1996	0.6	1.9	0.8	0.2	0.0	*	*
1996/1997	4.1	4.0	1.6	0.9	0.5	*	*
1997/1998	0.5	0.3	2.3	2.0	0.9	*	*
1998/1999	0.5	2.6	2.3	1.1	1.1	*	*
1999/2000	7.8	0.9	1.8	4.6	12.7	0.0	*
2000/2001	1.1	1.6	11.1	3.4	0.9	0.5	*
2001/2002	0.2	0.8	0.4	0.3	0.9	0.1	0.2
2002/2003	0.0	0.3	0.3	0.3	0.1	0.04	0.0
2003/2004	0.3	1.1	1.1	2.1	0.4	0.0	0.0
2004/2005	0.01	0.7	0.2	0.2	0.0	0.1	0.0
2005/2006	0.0	2.1	1.5	0.8	0.2	0.2	0.0
2006/2007	*	0.6	1.3	0.4	0.1	0.0	0.0
2007/2008	*	0.1	1.0	1.5	0.1	0.1	0.1
2008/2009	3.1	4.7	6.2	1.8	0.4	0.4	0.1
2009/2010	8.0	17.2	13.0	7.1	8.2	6.0	8.0

The spike in % boll rots across the state was likely caused by persistent higher than average rainfall in December, January and February. When averaged across all NSW cotton growing regions, total precipitation over summer in 2009/10 was substantially higher (approx. 100ml) than in 2008/9 (Figure 1.4.2). There were also more rainy days over summer in 2009/10 compared to 2008/9 indicating prolonged wet periods (Figure 1.4.2).
Isolation of boll rot fungi in the laboratory found very high levels of *Phytophthora* spp. in 2008/9 and 2009/10. *Phytophthora spp*. were recovered from 61% of fields in 2008/9 and 82% of fields in 2009/10 indicating that *Phytophthora spp*. continue to be the main agents of boll rot in NSW. Several cases of fungal boll rots associated with *Helicoverpa* feeding were also observed in 2009/10. Insect damage allows many relatively benign fungi to colonise and kill wounded bolls. Tight lock appears to be an increasingly common phenomenon that is purportedly caused by an insect vectored bacterium. Further research is required to reduce the impact of this disease on yield.

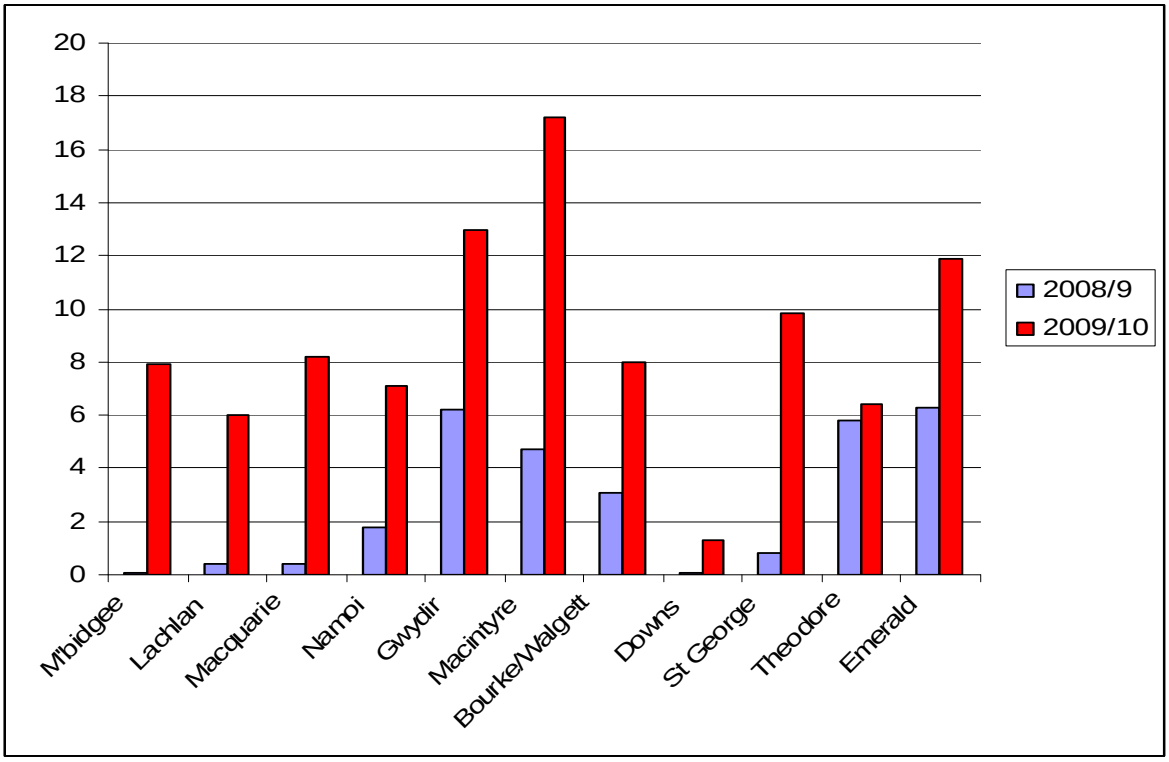

Figure 1.4.1. Comparison of boll rots in the 2008/9 and 2009/10 season.

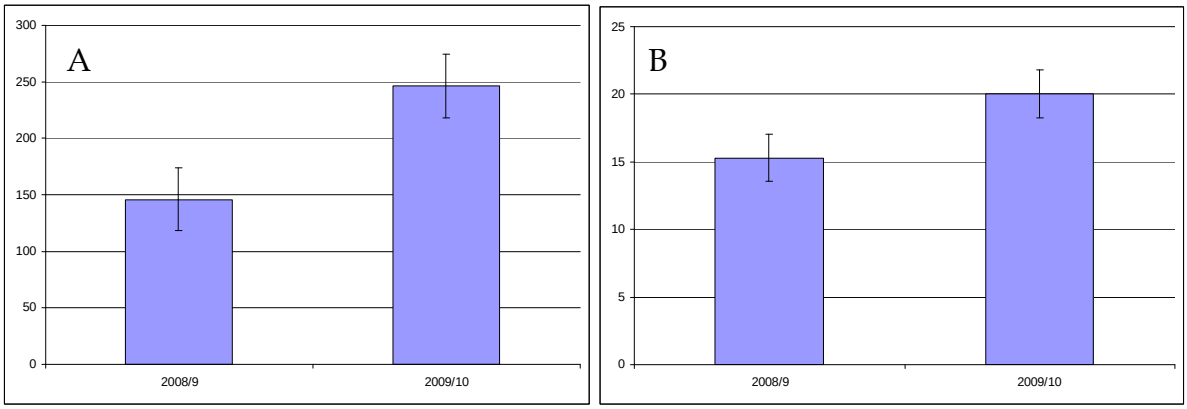

Figure 1.4.2. A) Mean (standard error) summer rainfall across NSW cotton growing regions in 2008/9 and 2009/10 and B) Mean (standard error) number of rainy days during summer across NSW cotton growing regions in 2008/9 and 2009/10.

1.5 Hormone Damage

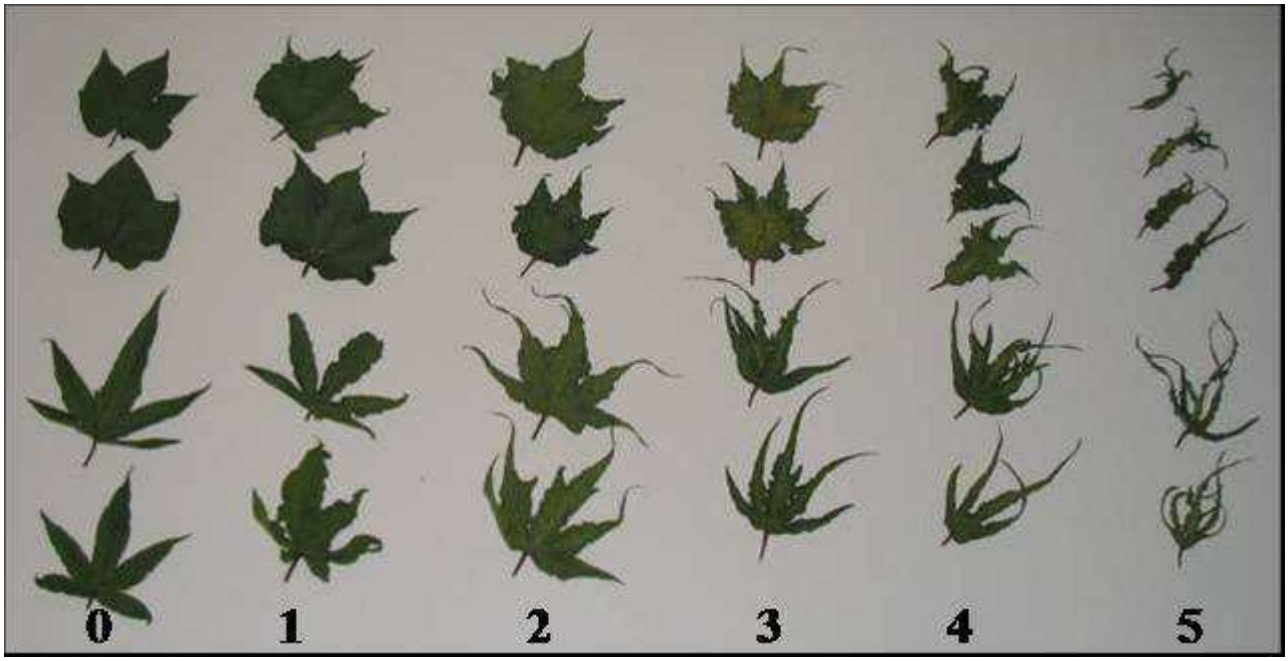

Figure 1.5. Scale used for assessment of severity of hormone damage.

Herbicide spray drift has increasingly become a problem for sustainable cotton production throughout the industry. Damage to crops following exposure to 2,4-D phenoxy herbicide drift (hormone) has been noted in disease surveys for several years. Following widespread severe damage across the industry in 2007/8, the incidence and severity of hormone damage was quantified in the 2008/9 season using a severity index (Figure 1.5). The position of damage in the canopy was noted, along with the incidence of affected plants (Table 4).

Table 4. Incidence (% Fields, % Plants) and severity of hormone damage through the canopy in the 2008/9 season in all cotton growing regions of NSW.

	Incidence		Location of Damage		
	% Fields	% Plants	Low	Middle	Top
Murrumbidgee	42.9	8.9	0.22	0.27	0.55
Lachlan	14.3	0.3	0.20	0.20	0.20
Macquarie	72.7	21.5	0.03	0.39	1.38
Namoi	43.8	18.7	0.46	1.45	2.02
Gwydir	78.6	6.3	0.07	0.26	0.41
Macintyre	60.0	5.0	0.08	0.30	0.22
Bourke/Walgett	37.5	4.1	0.00	0.05	0.63

10.5% of plants in NSW were damaged by hormone drift. Most plants were damaged in the Macquarie Valley (21.5%) followed by the Namoi (18.7%), Murrumbidgee (8.9%), Gwydir (6.3%), Macintyre (5.0%), Bourke (4.1%) and Lachlan (0.3%) regions (Table 4). Damage was usually most severe in the upper canopy (Figure 1.5.1).

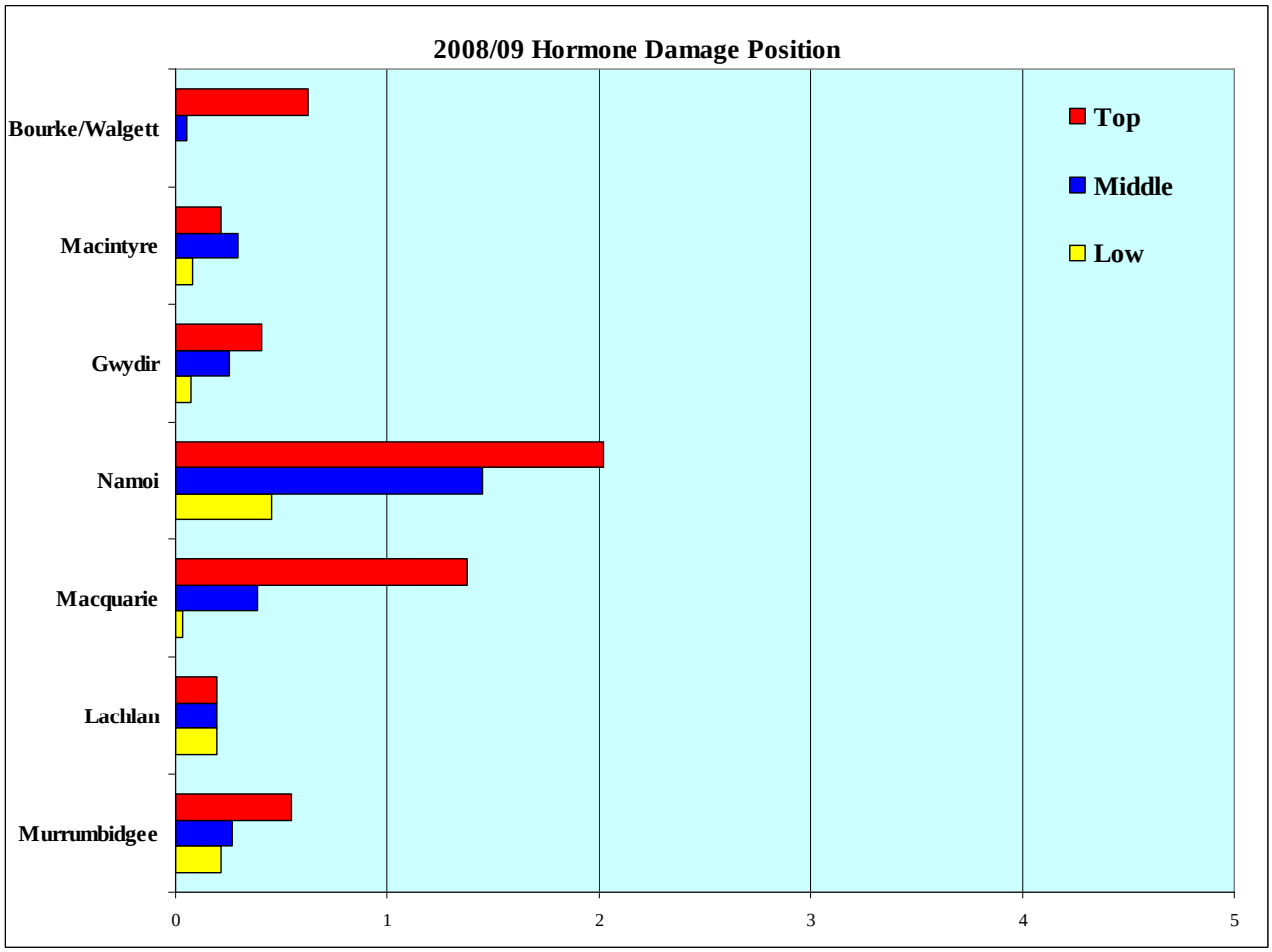

Figure 1.5.1. Severity (scale of 1-5) of hormone damage through the canopy in each cotton growing region of NSW in the 2008/9 season.

Data display a strong correlation (Rsquare 89%) between incidence and severity in the top canopy (Figure 1.5.2). That is, when incidence is high, hormone damage tends to be more severe.

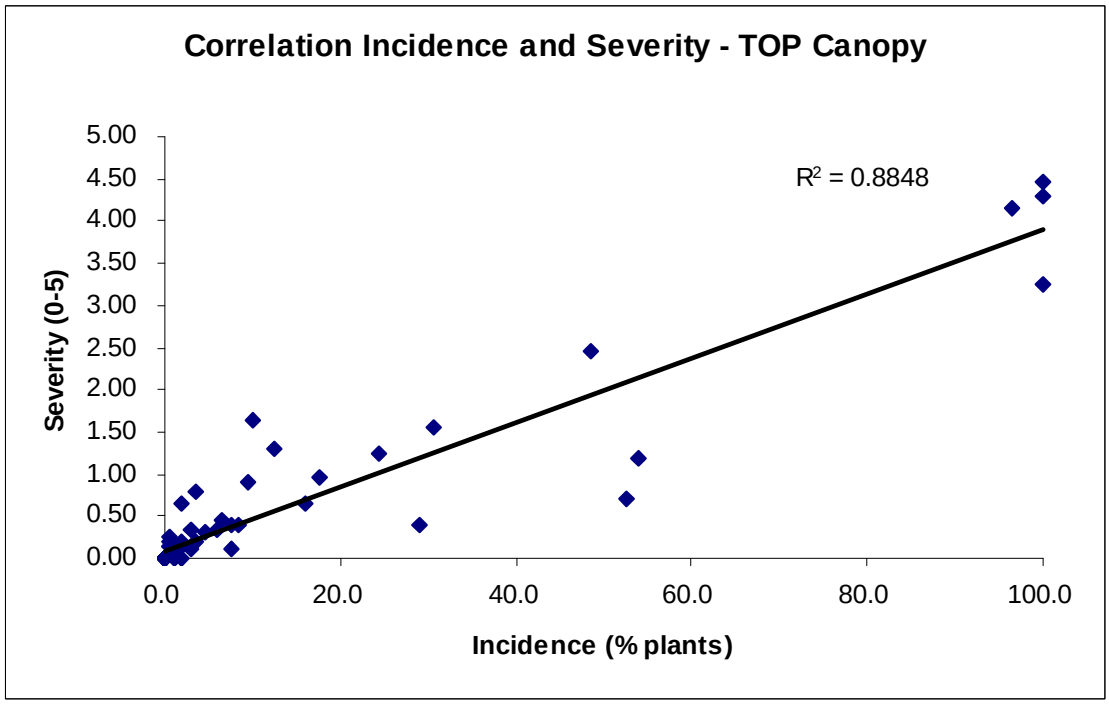

Figure 1.5.2. Correlation between hormone damage severity in the TOP canopy and % plants affected in a field.

The strength of the correlation weakens in the mid (Rsquare 72%) and low (Rsquare 47%) canopy. Damage in the lower and mid canopy indicate early exposure to 2,4-D herbicide as symptoms take time to develop after initial exposure. Severe damage in the top canopy coupled with high incidence across a field indicates repeated exposures to 2,4-D herbicide. Therefore, we can conclude that fields which recorded both high incidence and severity were repeatedly exposed to 2,4-D herbicide over the early part of the cotton season, probably at high doses. Fields where incidence is high but severity low were exposed later in the season and/or at a much lower dose. Substantially less hormone damage was recorded in 2009/10 with only 5.4% of plants affected in the Bourke/Walgett regions, 3.2% in the Gwydir, 2.5% in the Lachlan, and 1.0% in the Namoi (Figure 1.5.3). This reflects the efforts of Cotton Australia to improve industry awareness and practice.

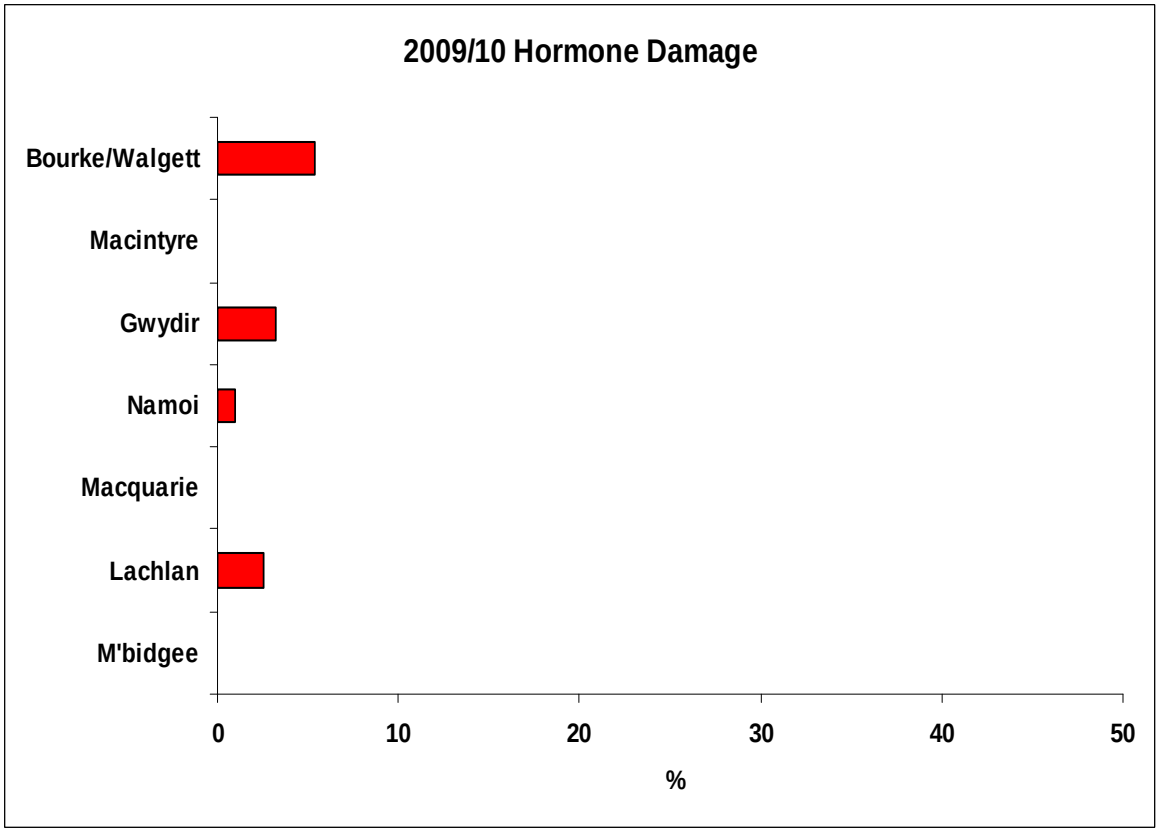

Figure 1.5.3. Incidence (% plants) affected by hormone damage in the 2009/10 season.

1.6 Emergency Plant Pests

Figure 1.6. Solenopsis mealybug clustered on a cotton stem. Photo courtesy of DEEDI.

The exotic Solenopsis mealybug (Figure 1.6) was detected in QLD in 2009/10 sparking a huge effort by industry and government to reduce the risk of the pest being moved into other parts of the industry. Evidence of mealybug infestation was observed for during the late season cotton disease surveys in NSW. No evidence was found to indicate that the pest had reached NSW cotton growing regions. This is good news for NSW growers as Solenopsis mealybug can cause severe crop damage if not managed effectively. Several other important emergency plant diseases were included in the early and late season cotton disease surveys including Texas Root Rot, Bacterial Blight, Cotton Leaf Curl Disease, Blue Disease, exotic strains of Fusarium wilt and defoliating strains of Verticillium wilt. No evidence of these diseases was found in NSW in 2007/8, 2008/9 and 2009/10. The collection of absence data for exotic plant pests is becoming increasingly important as a means of demonstrating evidence of pest absence. Improvements could be made in the way surveys are conducted for plant viruses across the industry. It would be pertinent to run an industry wide plant virus survey for Cotton Leaf Curl Disease, Blue Disease and other viruses including Tobacco Streak Virus and Cotton Bunchy Top each season. Many viruses are not naturally evident at the times that early and late disease surveys are conducted.

The prevalence of volunteer (ratoon) cotton was assessed during early season surveys in 2009/10. Volunteer cotton can harbour insect pests and plant viruses like Cotton Bunchy Top. The incidence of ratoon cotton on survey farms in NSW was very low (2 out of 82 farms). However, high levels of self sown volunteer cotton were noted during the late season survey (Figures 1.6.1, 1.6.2). Growers should continue to be alert to the presence of

new pests and diseases and practice good farm biosecurity, including the control of volunteer cotton, to prevent the build up and spread of pests throughout the industry.

Figure 1.6.1. A thicket of volunteer cotton on the edge of a dam.

Figure 1.6.2. Volunteer cotton out of control in a natural area adjacent to fields.

Conclusions

Seedling Disease

- Cool wet conditions in September and October are associated with high levels of seedling mortality.
- There is a clear trend towards higher seedling mortality in the southern valleys of NSW and this is strongly correlated to long-term average minimum temperatures in October.
- There appear to be long-term trends between periods of higher seedling mortality and periods of lower seedling mortality and these may reflect early season conditions and planting date.
- Abiotic factors including chemical damage and wireworm can play a significant role in seedling mortality and stand loss if not managed effectively.

Black Root Rot

- Continues to increase in incidence and severity in the southern valleys.
- Continues to be severe in the Namoi and Macquarie valleys.
- May be more severe under overhead irrigation.
- True spread of the disease may be masked by drought enforced fallows in recent years.
- Black root rot should be flagged as a major incentive for growers in the south to practice farm hygiene.

Fusarium Wilt

- Fusarium wilt has now been reported on 83 farms in NSW.
- Improved varietal resistance has lead to an overall reduction in the severity of symptoms associated with Fusarium wilt. However, incidence (% plants) may still be high especially when climatic conditions favour infection.
- Three strains of the pathogen are present in NSW. Continued monitoring is required to ensure that known and new strains of the pathogen do not overcome varietal resistance.
- Farm hygiene is crucial in minimising the spread of known and new strains of the Fusarium wilt fungus.

Verticillium Wilt

- Varietal resistance to Verticillium wilt is temperature dependent. Resistance breaks down under prolonged cool weather conditions.
- Integrated disease management should be focussed on fields with a history of this disease to minimise the impact of resistance-breakdown during cool seasons.
- Farm hygiene is crucial in preventing further spread of Verticillium wilt.

Boll Rots and Alternaria Leaf Spot

- Summer rainfall in 2009/10 lead to the highest recorded incidence of boll rot in NSW history.
- *Phytophthora* spp. were the most commonly isolated organisms from diseased bolls.
- Tight lock associated with insect feeding appears to be an increasing problem.
- Alternaria leaf spot is common and has little impact on crop health, but may have significant impacts on human health. Severe *Alternaria* can be associated with boll rots when lesions cause locules to fuse together.

Hormone Damage

- There is a strong correlation between high incidence and high severity of hormone damage in the upper canopy suggesting that the most severely affected fields are exposed to repeated doses of chemical throughout the season.
- The sharp decline in incidence and severity of hormone damage in 2009/10 may reflect the efforts of Cotton Australia in promoting responsible usage patterns and awareness of spray drift damage.

Emergency Plant Pests

- There is no evidence of Solenopsis mealybug in NSW.
- There continues to be no evidence of Texas Root Rot, Bacterial Blight, Cotton Leaf Curl Disease, Blue Disease, exotic strains of Fusarium wilt or defoliating strains of Verticillium wilt in NSW.
- Industry awareness of biosecurity threats is crucial in preventing and/or successfully eradicating incursions of exotic plant pathogens.
- Farm hygiene coupled with on-farm biosecurity is a first stop in bolstering farm and therefore whole of industry biosecurity preparedness.

Objective 2

Investigate the suite of pathogens that attack cotton seedlings across NSW and continue to evaluate IDM strategies for seedling disease.

Figure 2. Cotton seedlings affected by black root rot in a field near Hillston in the 2009/10 season.

2.1 Seed Treatment Fungicide Trials

Introduction and Materials and Methods

Seedling disease caused by a suite of fungi including *Rhizoctonia solani* and *Pythium* spp. is the single most expensive disease related problem in the Australia cotton industry. Millions of dollars are spent each season on seed treatment fungicides and it is important that the effectiveness of current fungicides and potential new fungicides is continually tested. Several fungicide seed treatments, including the industry standard DynastyCST™ and its components were applied to black seed with a carrier at varying rates. Black seed + carier was used as a control in all experiments, and the insecticide Cruiser™ was tested as a control for wireworm in 2009/10. Treated seed was sown with a cone seeder with assistance from CSIRO Plant Industry at several sites across NSW. Completely randomised block designs were used in all experiments to account for intra-field variation in pathogen populations. At least 10 replicate 10-12m plots were sown for each seed treatment. Seedling survival was assessed by stand count at 3-4 weeks after sowing.

Results

Seed Treatment Trials 2007/8

An experiment was run at ACRI to compare the effect of 1) seed treatment with the fungicides Apron, PCNB, QAP or DynastyCST™, and 2) sowing date, on seedling mortality/stand establishment. Seeds were sown on 2/10/07 and 16/10/07. A control of untreated seed was sown on both dates. There was no difference in seedling mortality between seed treatments in cotton sown on 16/10/07. However, in cotton sown on 2/10/07, seedling mortality was significantly lower ($P<0.02$) where seed had been treated with Apron, QAP or DynastyCST™, compared to untreated seed and seed treated with PCNB. This is a clear indication that *Pythium* spp. were the dominant seedling pathogens in the field on that date. The lack of differentiation between seed treatments in the later sowing indicates low disease pressure. Thus in 2007/8 at ACRI, seedling disease could be controlled by either sowing early with a fungicide seed dressing, or sowing late irrespective of the fungicide seed dressing. We continue to recommend that growers sow treated seed, later.

Seed Treatment Trials 2008/9

16 fungicide seed treatment combinations were tested against seedling disease in fields at Hillston, Narrabri and Mungindi. DynastyCST™, its component fungicides and two experimental fungicides, SYN524 from Syngenta Crop Protection and an experimental product from Bayer Crop Sciences were tested for efficacy against seedling disease. All products were applied at the commercial rate unless specified. The treatments were as follows:

1. Untreated seed
2. Dynasty CST
3. Fludioxonil (component of Dynasty CST)
4. Azoxystrobin (component of Dynasty CST)
5. Metalaxyl (component of Dynasty CST)
6. Syngenta Rate 1 (5g a.i. per 100kg seed)
7. Syngenta Rate 2 (10g a.i. per 100kg seed)
8. Syngenta Rate 3 (20g a.i. per 100kg seed)
9. Syngenta Rate 1 + Dynasty CST
10. Syngenta Rate 2 + Dynasty CST
11. Syngenta Rate 3 + Dynasty CST
12. Bayer Rate 1 (5g a.i. per 100kg seed)
13. Bayer Rate 2 (7.5g a.i. per 100kg seed)
14. Bayer Rate 3 (10g a.i. per 100kg seed)
15. Bayer Rate 4 (20g a.i. per 100kg seed)
16. Bayer Rate 5 (30g a.i. per 100kg seed)

Treatments were replicated 10 times at Narrabri and Mungindi, and 15 times at Hillston. An additional treatment of planting date was included at Narrabri.

At Narrabri, the experiment was sown on 1/10/08 and 14/10/08. Fungicide treatments did not improve stand when sown on the 14/10/08 indicating that seedling disease pressure

can be avoided by delaying sowing. In contrast, there was a significant difference between treatments in the stand of cotton sown on the 1/10/08 (Figure 2.1.1). Stand was highest in all plots treated with DynastyCST™ followed by Apron and Azoxystrobin indicating that the dominant pathogen was *Pythium* spp (Figure 2.1.1). All other treatments including Fludioxonil were not different from the untreated control.

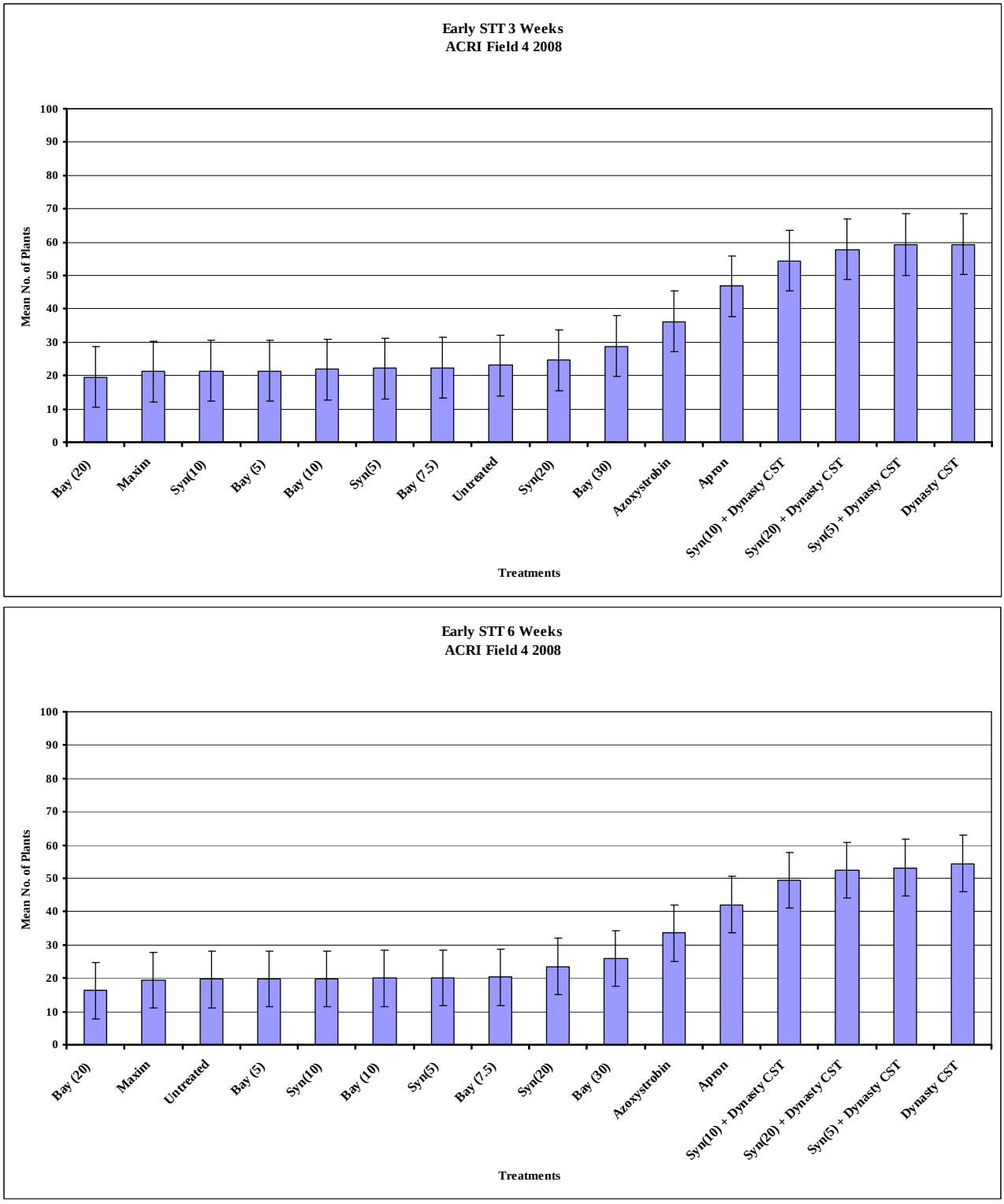

Figure 2.1.1. Mean (standard error) survival of seedlings following seed treatment with various fungicides at Narrabri (ACRI). Seed sown on 1 October 2008. Stand counts at 3 and 6 weeks after sowing. Difference between treatments significant at 3 and 6 weeks (P<0.001).

At Hillston, poorest stand was observed in untreated plots and plots treated with Apron and Fludioxonil, indicating that *Rhizoctonia solani* was the dominant pathogen.

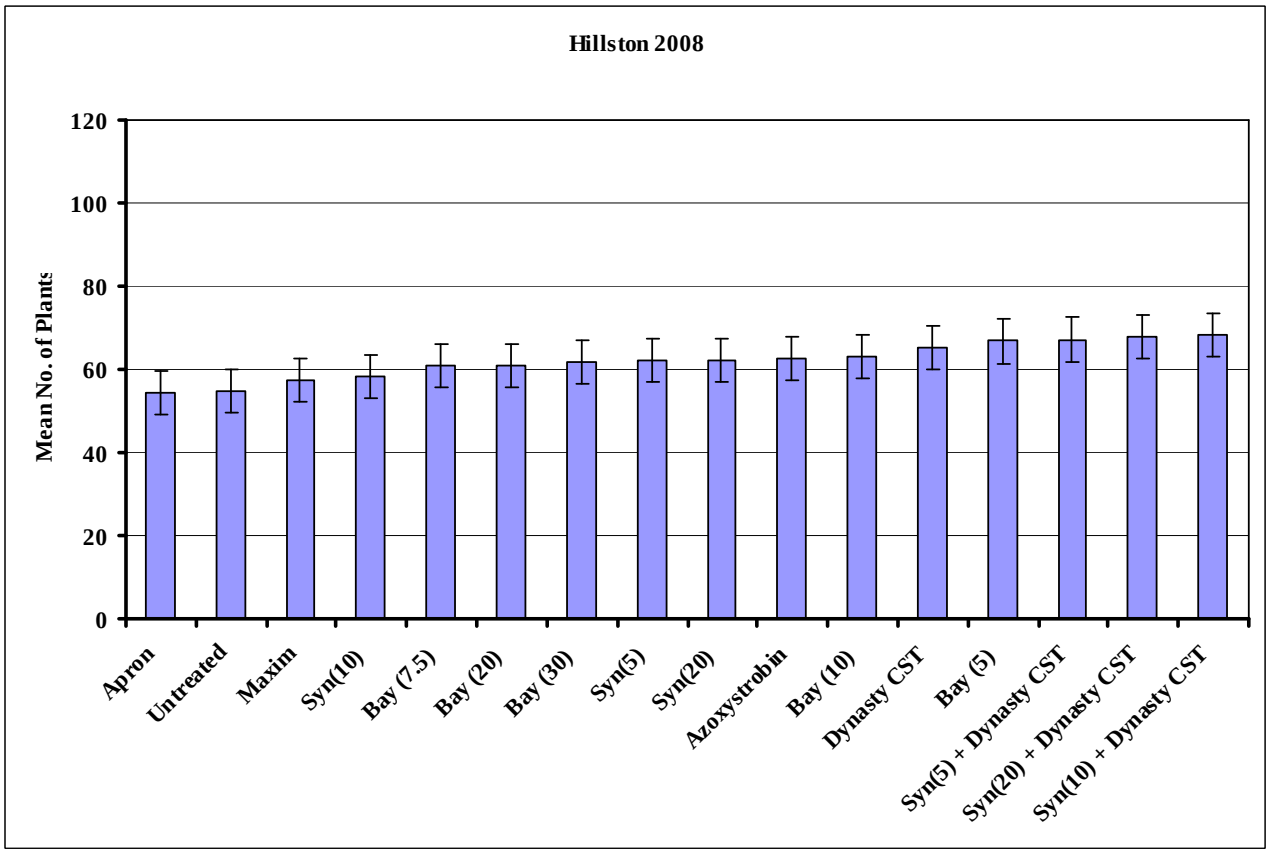

Figure 2.1.2. Mean (standard error) survival of seedlings following seed treatment with various fungicides at Hillston. Stand counts at 6 weeks after sowing. Difference between treatments significant (P<0.001).

A significant (P<0.001) difference was observed between treatments (Figure 2.1.2). Stand was also reduced by wireworm damage and this may have masked the extent to which fungicides preserved plant stand. Stand was again highest in plots treated with DynastyCST™. The Bayer chemical, at 5g a.i. per 100kg seed appeared to be equally effective to DynastyCST™, although this may be an artefact of wireworm damage (Figure 2.1.2).

At Mungindi, plots treated with Fludioxonil contained the lowest stand, followed by plots treated with Apron and untreated plots indicating that *Rhizoctonia solani* was the dominant pathogen (Figure 2.1.3). Highest stand was observed in all plots treated with Dynasty CST including combined treatments of Dynasty CST and the Syngenta compound. Differences were significant (P<0.001). The Bayer compound performed better than the control indicating activity against *R. solani*. This fungicide may be a candidate for trial as an in furrow spray.

Fludioxonil was ineffective at all sites. Fludioxonil is purported to have activity against *Fusarium* spp. When studied in laboratory pathogenicity assays, *Fusarium* spp. including the Fusarium wilt fungus do not grow as quickly and are not as aggressive as *Pythium* spp. and *R. solani*. Therefore, Fludioxonil may have little effect in the field as *Fusarium* spp. are usually only a small component of the seedling disease complex.

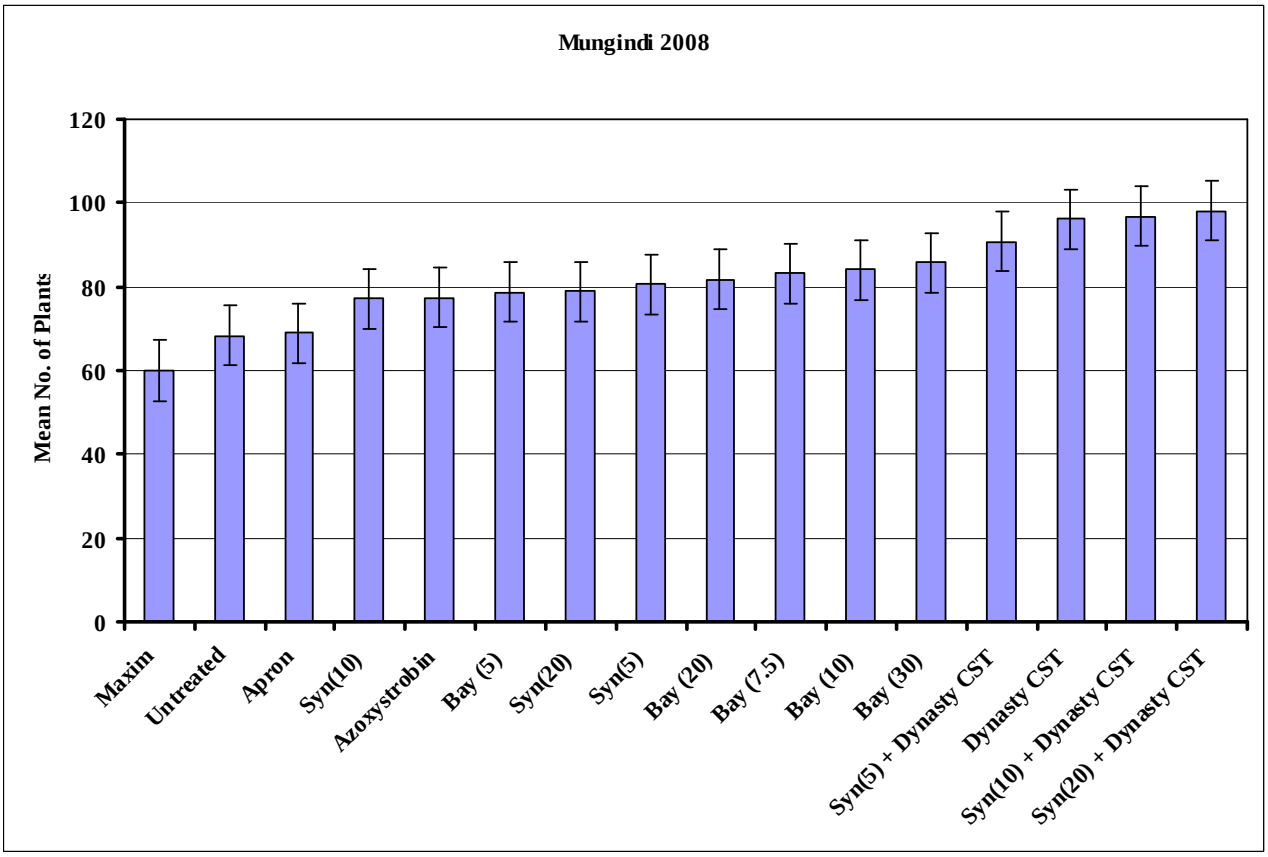

Figure 2.1.3. Mean (standard error) survival of seedlings following seed treatment with various fungicides at Mungindi. Stand counts at 6 weeks after sowing. Difference between treatments significant (P<0.001).

Seed Treatment Trials 2009/10

15 fungicide seed treatment combinations were tested against seedling disease in fields at Hillston, Trangie, Narrabri and Mungindi. Cotton was treated with the following fungicide combinations:

1. PCNB (active against *Rhizoctonia*)
2. Dynasty CST
3. Dynasty Plus (a new combination of SYN524 and DynastyCST)
4. Fludioxonil
5. Apron (metalaxyl)
6. Azoxystrobin
7. Syn524
8. Fludioxonil+Apron
9. Fludioxonil+Azoxystrobin
10. Fludioxonil+Syn524
11. Apron+Azoxystrobin
12. Apron+Syn524
13. Azoxystrobin+Syn524
14. Cruiser
15. Untreated

Narrabri

At Narrabri, cotton was sown on the 28th of September and again on the 15th of October. Both plantings were assessed three and six weeks after sowing. All fungicide seed treatments except Azoxystrobin alone significantly reduced seedling mortality compared to the untreated control in early planted cotton (Figure 2.1.4).

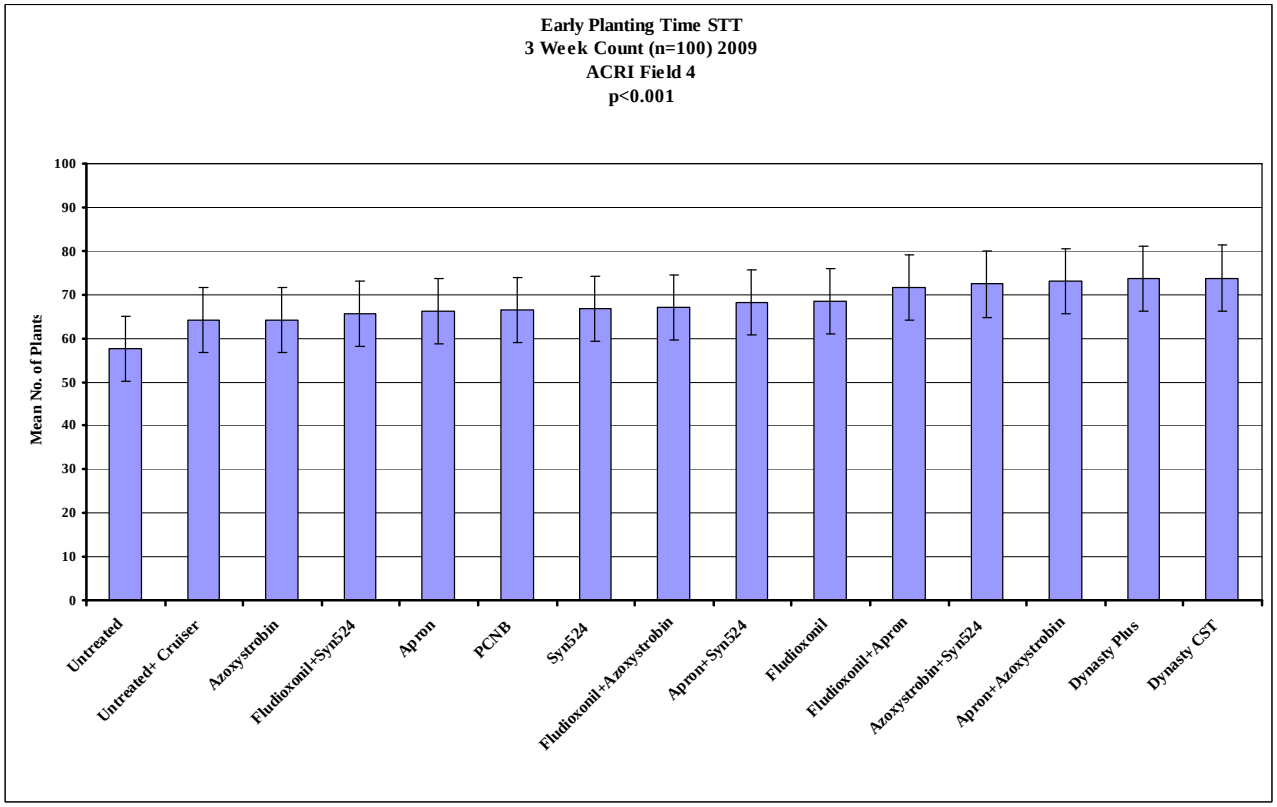

Figure 2.1.4. Mean (standard error) survival of early sown seedlings following seed treatment with various fungicides at Narrabri. Stand counts at 3 weeks after sowing. Difference between treatments significant (P<0.001).

Dynasty CST, Dynasty Plus, Apron+Azoxystrobin, Azoxystrobin+Syn524, and Fludioxonil+Apron significantly reduced seedling mortality compared to both the untreated control and the cruiser control. These results indicate that no one seedling pathogen was dominant at ACRI. It is more likely that *Rhizoctonia*, *Pythium* and other fungi were active in killing seedlings during the cool conditions in October. The late trial was sown on 15th October and assessed at three and six weeks after sowing. Seedling mortality was much lower across that trial at 13%. No significant difference was detected between treatments at 3 weeks after sowing indicating that fungicide seed treatment had no effect on stand in the late sown cotton. This reflects warmer conditions in late October and November that did not favour seedling disease.

Mungindi

At Mungindi, seedling mortality averaged 25% across the trial. Only Dynasty CST was significantly (P=0.002) higher than the untreated control (Figure 2.1.5). Dynasty CST was also significantly higher than Fludioxonil and Fludioxonil+Azoxystrobin which were not different from the untreated control. Differences between other treatments were difficult to determine due to significant row (P<0.001) and tier (P=0.048) effects.

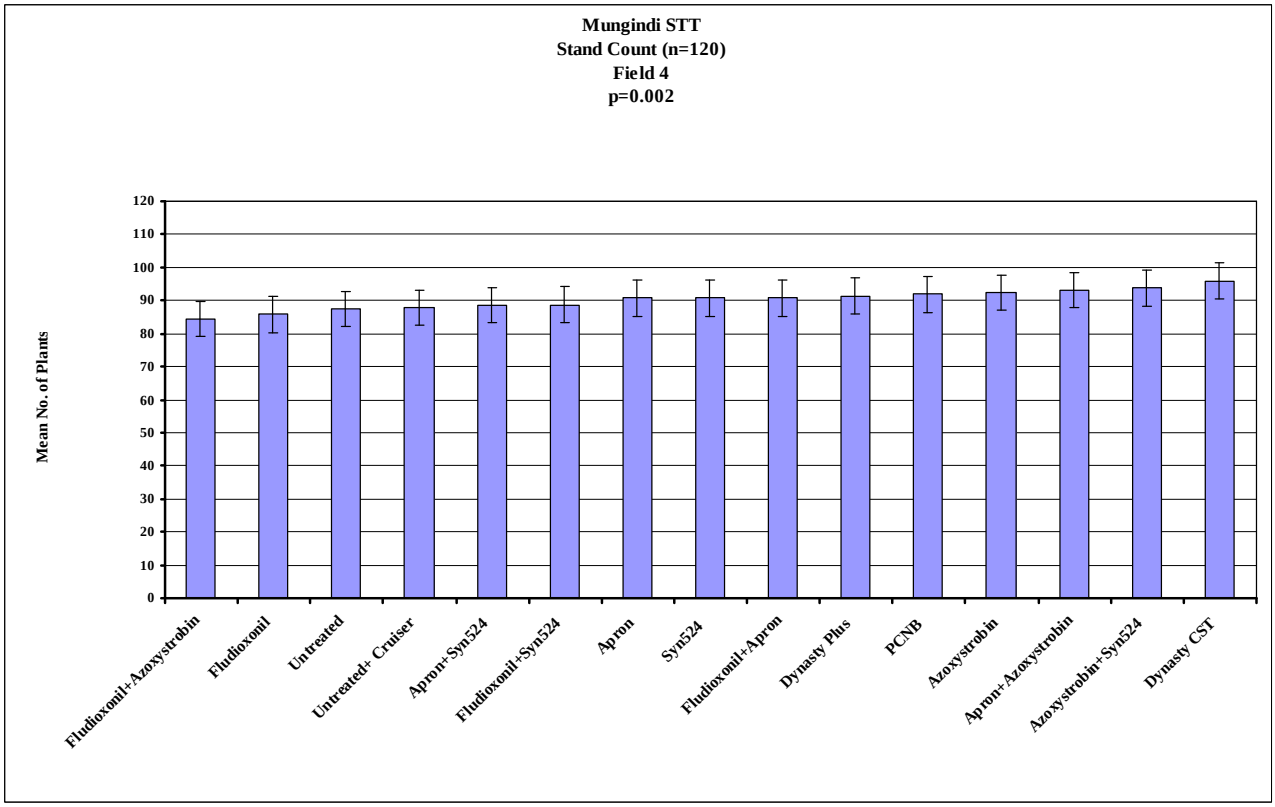

Figure 2.1.5. Mean (standard error) survival of seedlings following seed treatment with various fungicides at Mungindi. Stand counts at 3 weeks after sowing. Difference between treatments significant (P=0.002).

Hillston

At Hillston seedling mortality averaged 31% across the trial. Treatments were replicated 10 times in 10m plots. Seedling mortality in plots treated with Syn524, Fludioxonil, and Fludioxonil+Syn524 was not different from the control of untreated seed (Figure 2.1.6). Plots treated with cruiser had significantly (P<0.001) higher stands compared to the control (Figure 2.1.6). Plots treated with Dynasty and Azoxystrobin had stands that were equivalent to plots treated with cruiser indicating an interaction between filamentous fungi (eg. *Fusarium* spp.) and wireworm. Most treatments including Apron were also effective indicating some impact from *Pythium* spp.

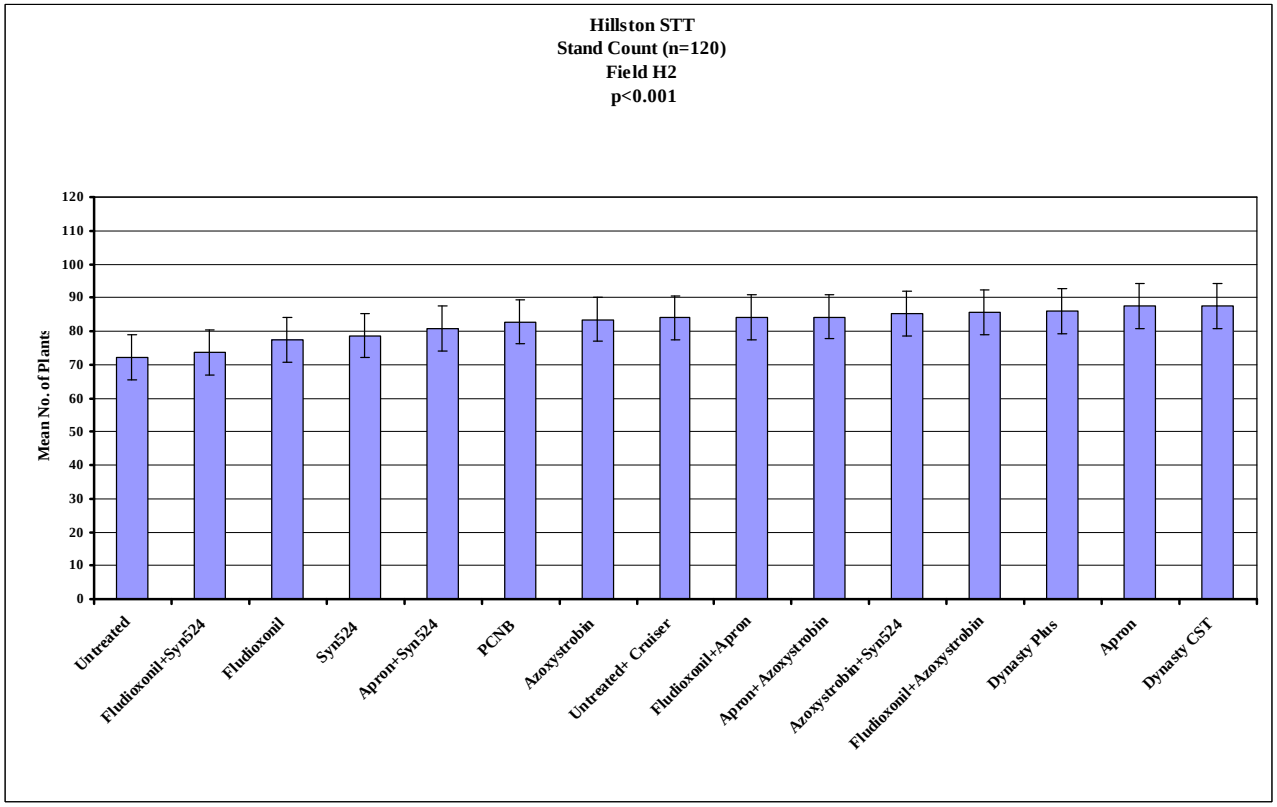

Figure 2.1.6. Mean (standard error) survival of seedlings following seed treatment with various fungicides at Hillston. Stand counts at 3 weeks after sowing. Difference between treatments significant (P<0.001).

Trangie

At Trangie seedling mortality averaged 37% across the trial. Treatments were replicated 8 times in 10m plots. Most fungicide combinations with activity against *Pythium* reduced seedling mortality compared to the two controls (Figure 2.1.7). Syn524 and Fludioxonil did not reduce seedling mortality in this experiment indicating ineffectiveness against *Pythium* spp.

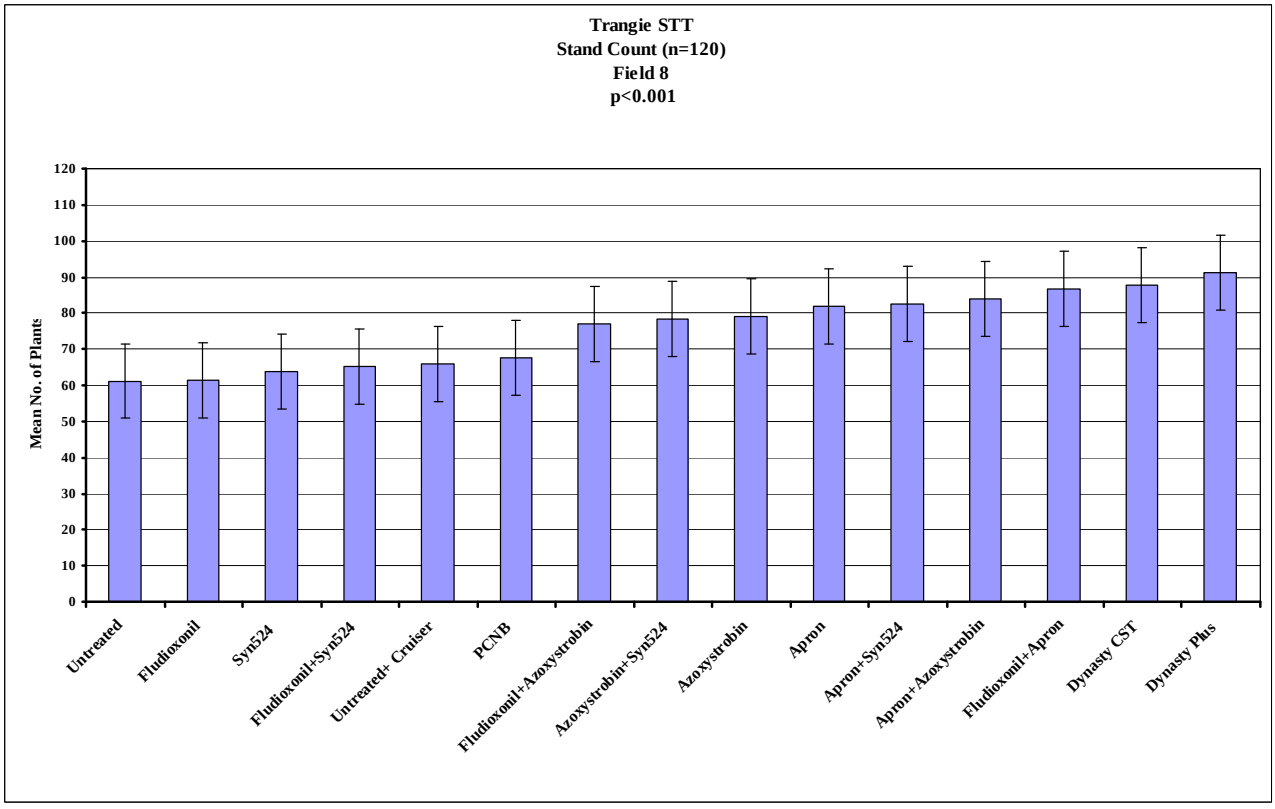

Figure 2.1.7. Mean (standard error) survival of seedlings following seed treatment with various fungicides at Trangie. Stand counts at 3 weeks after sowing. Difference between treatments significant (P<0.001).

Conclusions

- Seedling mortality is consistently higher in southern NSW, however seed sown in September and early October in northern NSW is likely to suffer equivalent rates of mortality.
- Sow seed treated with fungicides and insecticide to minimise the risk of stand loss due to seedling pathogens and wireworm.
- Where possible, delay sowing to avoid cool early season conditions that favour seedling mortality.
- Dynasty CST continues to be the most effective fungicide combination.
- Fludioxonil alone often has no impact on seedling mortality and could be considered for removal from cotton seed treatments.
- Pathogen pressure varies between sites and is strongly influenced by temperature and soil moisture. Cool temperatures in October
- Cereal stubble can also be associated with higher levels of stand loss caused by wireworm and additional precautions (eg. cruiser seed treatment) should be taken when sowing into cereal stubble.

2.2 Seed treatment fungicides for black root rot

Introduction and Materials and Methods

The Australian cotton industry is currently experiencing an epidemic of black root rot caused by the soil borne fungus *Thielaviopsis basicola*. At present there is no effective seed treatment fungicide for this disease and other chemical treatments including Bion® do not give consistent control. The disease appears to be spreading quickly in the southern valleys of NSW where cool early season conditions favour infection of plants. Recent work in the United States has indicated that the fungicide Myclobutanil may be an effective seed treatment for black root rot and may improve the efficacy of Bion®. The fungicide Banrot® is used to control this disease in glasshouse crops but has not been trialled in cotton. The fungicide Baytan Plus® has also shown some activity against black root rot in previous field trials. Bion®, Myclobutanil, Banrot® and Baytan Plus® were screened in pot experiments for their ability to reduce the severity of black root rot on cotton seedlings in naturally infested soil. They were also trialled alone and in combination with one another as seed treatments for black root rot in a pot experiment and in three field experiments. All pot experiments were conducted in controlled glasshouse conditions at Narrabri using naturally infested soil, and each treatment combination replicated at lest six times, with 15 seeds sown per pot.

Results

Bion and Myclobutanil

Bion (0.6g and 1.0g/100kg seed) and Myclobutanil (42g and 60g/100kg seed) were applied as seed treatments alone and in all possible combinations. A control of untreated seed was used. Seed was sown into natural soil infested with the black root rot fungus. Each treatment was replicated in six pots with 15 seeds per pot. Plants were assessed for disease at 4 weeks after sowing. Severity of black root rot was reduced (Figure 2.2.1) by both Bion ($P<0.001$) and Myclobutanil ($P<0.001$), however there was no significant interaction between Bion and Myclobutanil ($P=0.178$) when applied in combination (Figure 2.2.1). Neither Bion or Myclobutanil had a significant effect on shoot or root weight.

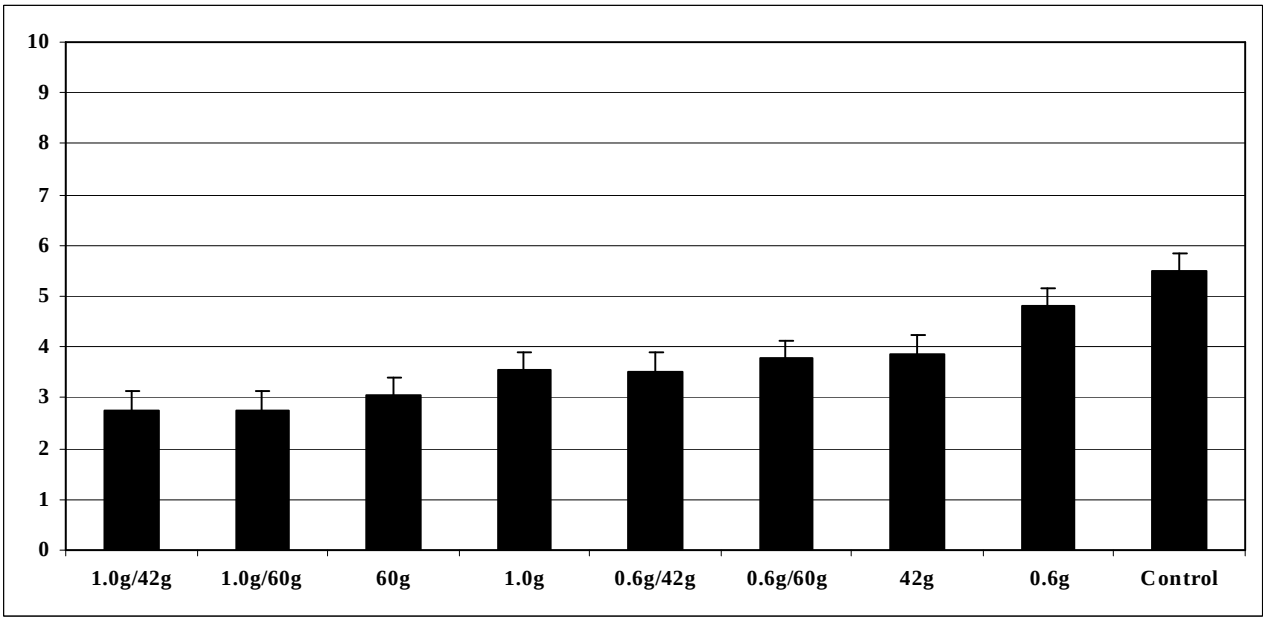

Figure 2.2.1 Mean(standard error) severity of black root rot in cotton treated with Bion (0.6g and 1.0g/100kg seed) and Myclobutanil (42g and 60g/100kg seed) and combinations thereof.

Banrot

Banrot was applied as a cotton seed treatment at 264, 26, 2.6g/kg seed. Controls of 1) untreated seed and 2) the use of a Banrot soil drench at the commercial rate were also used. All rates of Banrot reduced the severity of black root rot compared to the untreated control (Figure 2.2.2). Banrot at 2.6g/kg seed was the most effective rate for reducing the severity of black root rot and was also associated with the highest root and shoot growth (Figures 2.2.3, 2.2.4).

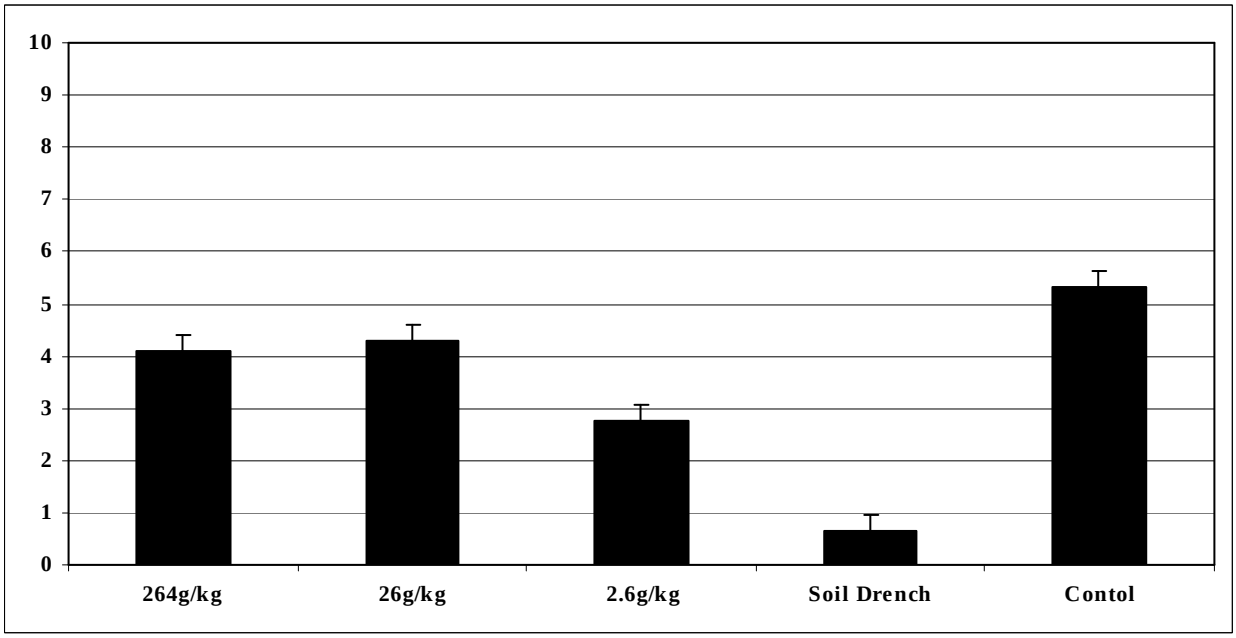

Figure 2.2.2. Mean (standard error) black root rot severity in plants treated with varying rates of Banrot. Differences are significant (P<0.001).

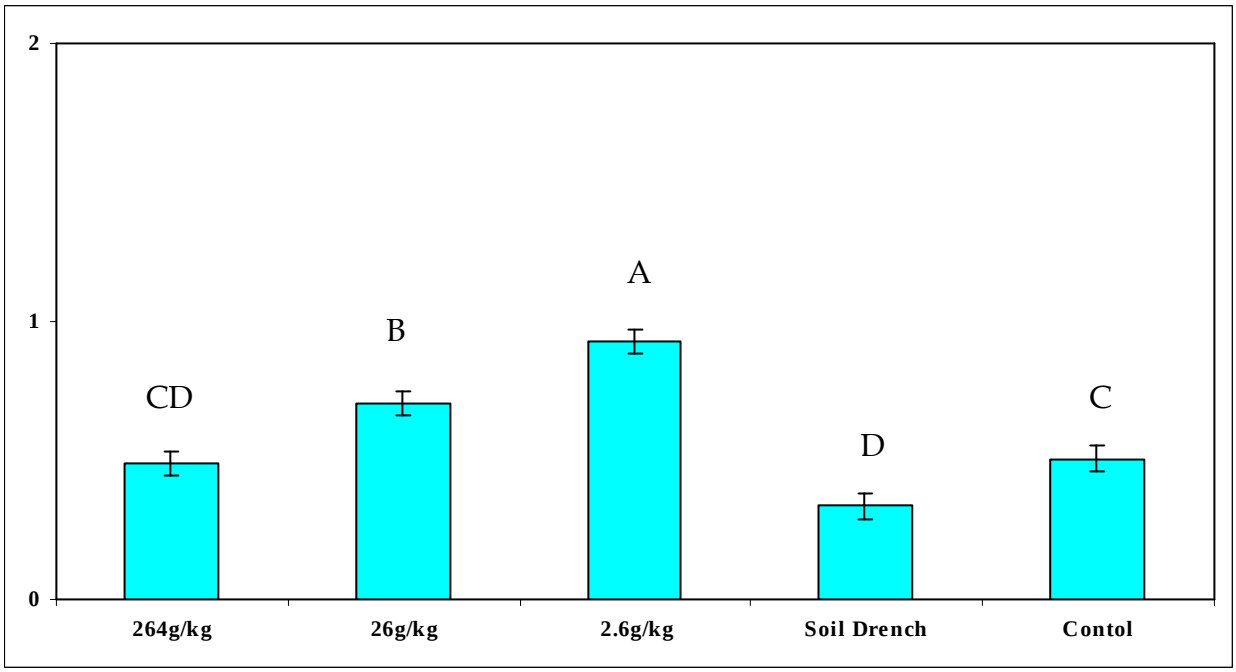

Figure 2.2.3. Mean (standard error) shoot weight (g/plant) in plants treated with varying rates of Banrot. Differences are significant (P<0.001) as indicated by letters A-D.

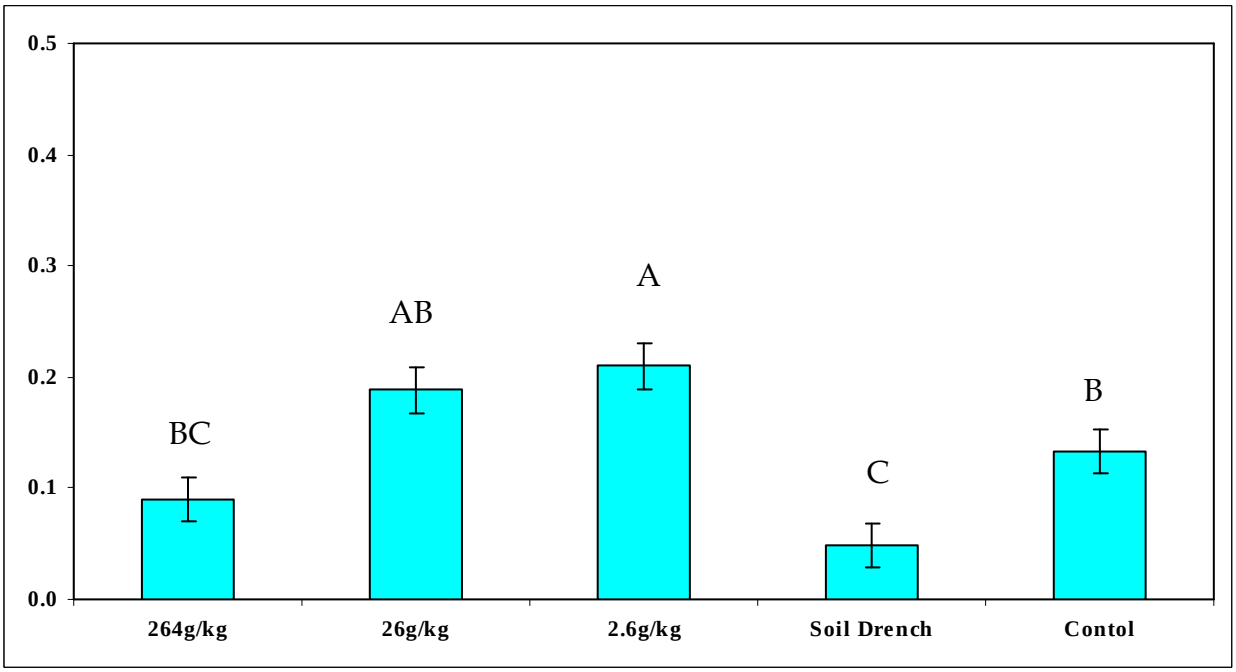

Figure 2.2.4. Mean (standard error) root weight (g/plant) in plants treated with varying rates of Banrot. Differences are significant (P<0.001) as indicated by letters A-C.

Baytan Plus

Baytan Plus was applied as a cotton seed treatment at 50, 100, 150 and 200g/100kg seed. All rates of Baytan Plus significantly (P<0.001) reduced black root rot severity by 50% (Figure 2.2.5). Shoot and root weight did not differ between treatments or the control.

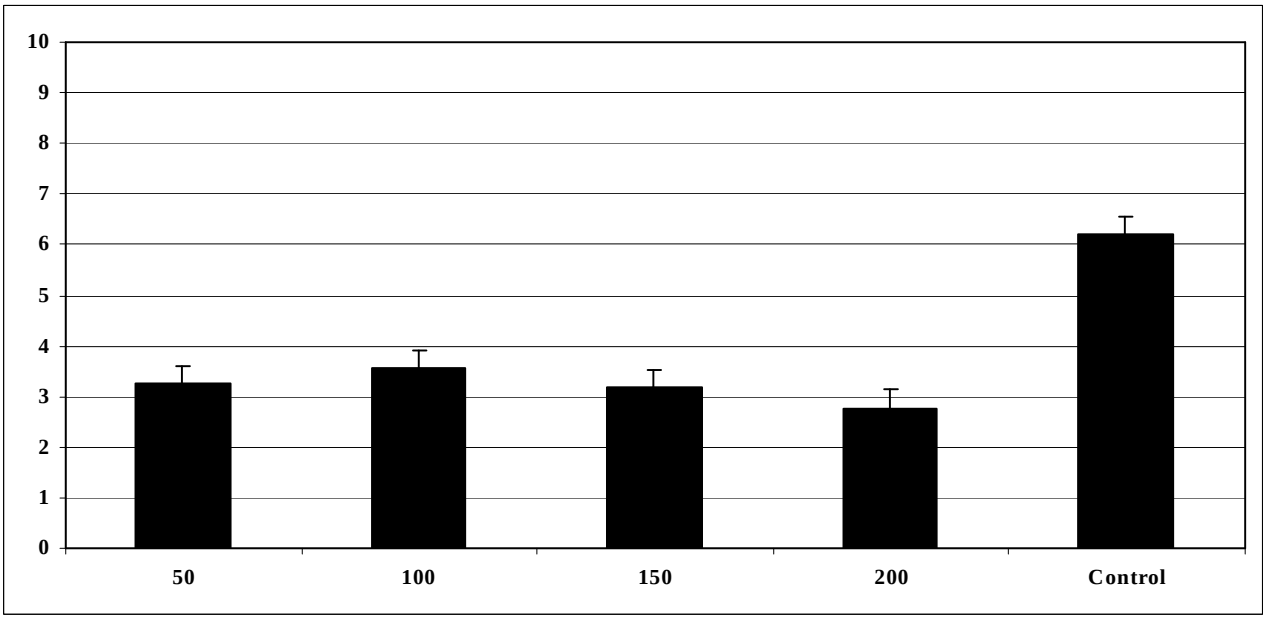

Figure 2.2.5. Mean(standard error) severity of black root rot in cotton treated with Baytan Plus at 50, 100, 150, and 200g/kg seed .

Fungicide Combinations

All fungicides tested above were active in reducing the severity of black root rot of cotton. Therefore an additional pot trial was established along with three large scale field experiments. Seed was treated with each fungicide alone and in several combinations at the most effective rate as indicated in the previous experiments. Treatments were as follows: Banrot, Myclobutanil, Baytan Plus, Bion, Banrot*Myclobutanil, Banrot*Baytan Plus, Banrot*Bion, Myclobutanil*Baytan Plus, Myclobutanil*Bion, Baytan Plus*Bion, Banrot*Myclobutanil*Baytan Plus, Banrot*Myclobutanil*Baytan Plus*Bion, and a control treatment of Dynasty.

Banrot, Banrot*Myclobutanil*Baytan Plus*Bion, Baytan Plus*Bion, Myclobutanil*Baytan Plus, Banrot*Bion, Myclobutanil*Bion, and Banrot*Myclobutanil*Baytan all significantly reduced the severity of black root rot compared to the control of Dynasty in pots (Figure 2.2.6).

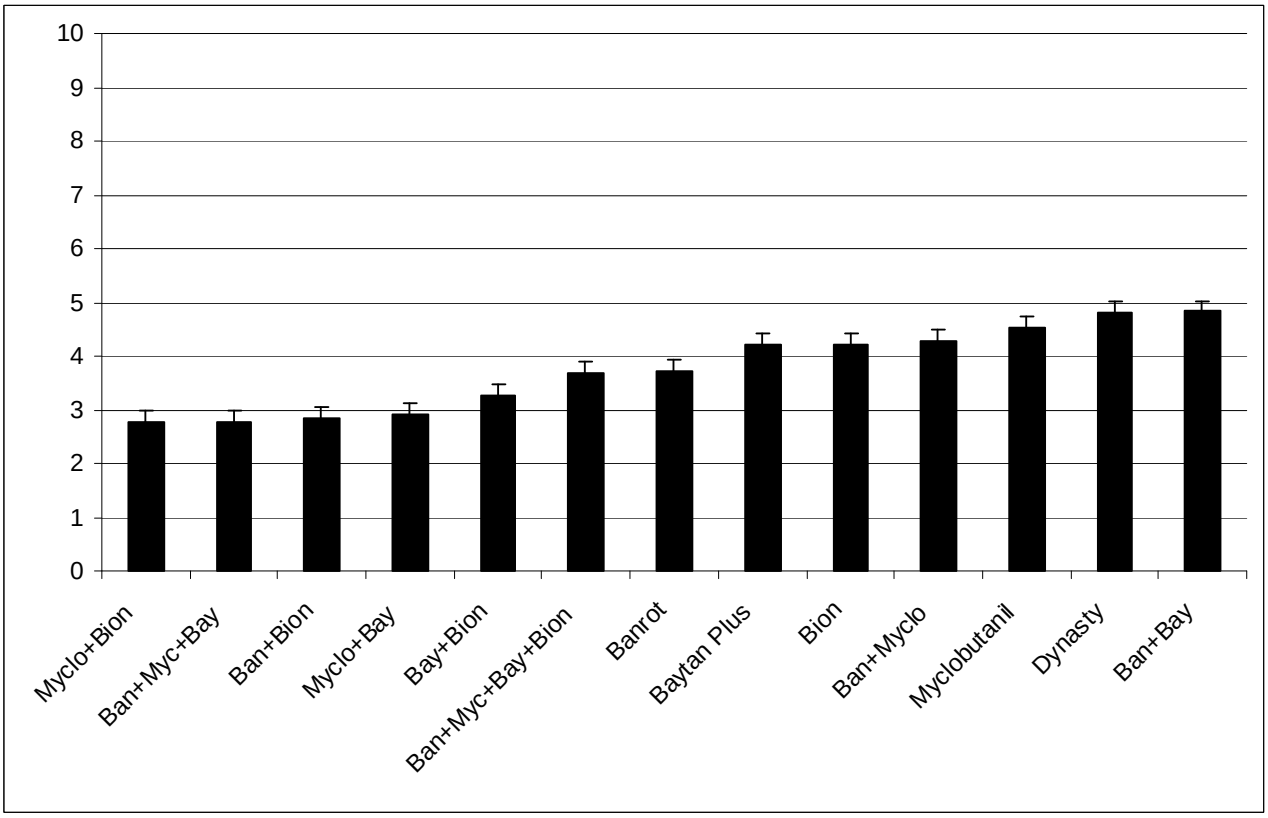

Figure 2.2.6. Mean (standard error) severity of black root rot of cotton grown in pots and treated with various fungcides.

Only low levels of disease were detected in field experiments and this is probably due to the unseasonably hot conditions in November. Consequently there were no significant differences in black root rot severity between treatments at any sites. Stand and shoot weight were also assessed in all experiments and no significant differences were detected.

Novel Fungicides from Syngenta

Eight fungicides were supplied by Syngenta Crop Protection for screening against black root rot. A large scale pot experiment was set up to test each chemical at three rates in two types of soil: a red crusting soil from the Macquarie valley and a brown cracking clay from the Namoi. In addition, Bion was tried at three rates in isolation and at the commercial rate in combination with Myclobutanil and SYN524B. Three controls consisted of 1) Dynasty, 2) Dynasty + Bion at the commercial rate and 3) black seed. Each treatment was replicated in 8 pots.

Both soil type and fungicide treatment had significant effects on the severity of black root rot. Disease severity was higher ($P<0.001$) in the red crusting soil (7.4/10) compared to the brown cracking clay (4.8/10). Fungicide efficacy varied between soil type (Figure 2.2.7). The untreated seed is indicated in black (Figure 2.2.7). In both soils, the only fungicide combination that reduced black root rot compared to the untreated seed was Dynasty+Bion at the commercial rate, indicating that none of the fungicides tested in the experiment could provide a better level of control than Bion in combination with Dynasty. Interactions between fungicide and soil type were not observed.

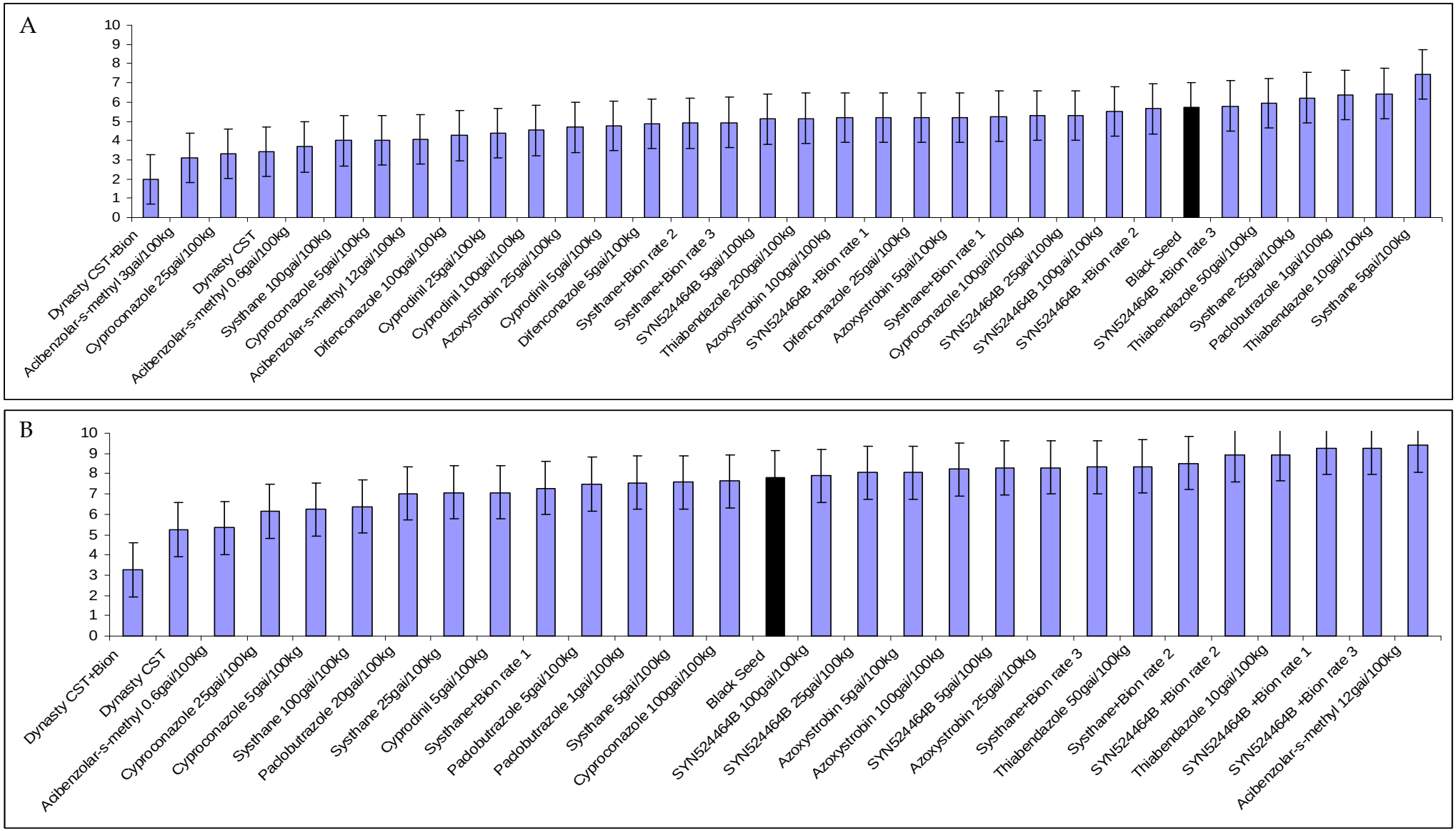

Figure 2.2.7. Severity of black root rot in cotton treated with a range of fungicides in soil from the Namoi (A) and Macquarie (B) valleys. Results were not obtained for all fungicides in each soil type due to stand loss. Severity of black root rot was significantly affected by fungicide in both soils (P<0.001).

Conclusions

- Fungicide efficacy varies between experiments.
- Bion is the only seed treatment available for black root rot and continues to provide best control of the disease when tested against other chemical treatments.
- Banrot is used in horticulture to control this disease and appears to be effective as a cotton seed treatment. Further research is required.
- Banrot, Baytan Plus, and Myclobutanil show promise as potential novel seed treatment funcides but require field validation over several seasons.
- Growers should continue to use Bion in combination with Dynasty as a seed treatment, especially when sowing into fields with high levels of black root rot.

2.3 Experiments and studies on the biology of *Rhizoctonia solani* in cotton contributing towards the doctorate of the principal researcher.

The following experiments and studies were undertaken as part of Objective 2 of the Diseases of Cotton IX project and also contribute towards the doctoral studies of Chris Anderson through the University of Sydney. Mr Anderson has now completed two years equivalent of full time study (two years part time and one year full time enrolment). Mr Anderson left the Diseases of Cotton project to take up a position within the NSW Plant Biosecurity Unit in March 2010 and has returned to part time studies. The University of Sydney timetable requires a final submission date of March 2014. A copy of the completed PhD thesis will be provided to the Cotton R&D Corporation. The aim of the PhD thesis is to investigate the fungal pathogens that are associated with seedling disease in cotton with a focus on *Rhizoctonia solani,* one of the most aggressive pathogens which continues to cause widespread stand loss despite fungicide seed treatments.

Figure 2.3. Setting up an experiment to "bait" fungi from soils collected across NSW.

2.3A Isolation of potential pathogens from across NSW.

Potential seedling pathogens were collected from across NSW, from 1) diseased seedlings in the field and 2) from glasshouse bioassays (Figure 2.3) of soil collected during the DAN190 project in 200/7 and the early season cotton disease surveys in 2007/8 and 2008/9. Fungi were isolated from symptomatic seedlings on water agar. The border region between symptomatic and healthy tissue was excised from cotton hypocotyls, surface disinfested in 2/3 Bleach (4% W/V Hypochlorite) and 1/3 70% EtOH for 30 seconds and rinsed in sterile distilled water. The disfinfested hypocotyl was then incubated on water agar at 23C for 48 hours. Slower growing fungi like *Thielaviopsis basicola* were avoided by the fast incubation time and non-selective medium. *T. basicola* does not cause stand loss and so was not collected as part of this study. *Pythium* and *Rhizoctonia* like fungi usually grew out of the tissue within 24 hours. *Fusarium* and other fungi were slower growing and were only collected when not overgrown by *Pythium* or *Rhizoctonia*. All fungi were grown through a layer of potato dextrose agar (PDA) for an additional 24 hours to remove any bacteria or mites, and then subcultured onto PDA in preparation for storage. A total of 229 fungi representing all *Rhizoctonia* like fungi and representative isolates of *Fusarium*, *Pythium* and other fungi were stored in sterile distilled water, oil and carnation leaf agar.

The most commonly isolated fungi were *Fusarium* spp. Over 150 isolates of *Fusarium* like fungi were collected with most placed in long term storage on carnation leaf agar and a representative sample of 56 isolates entered into the culture collection. Three species of *Fusarium* were identified: *F. oxysporum*, *F*. equiseti and *F. compactum*. Many *Fusarium* like fungi failed to produce macrocondia and so could not be identified by conventional methods. 42 isolates of Pythium were collected and stored in the collection in water and oil (Table 5). These have not been identified to species level but are expected to represent *P. ultimum* and *P. vexans* which are known to affect cotton. 76 isolates of *Rhizoctonia* like fungi including *Ceratobasidium* were isolated from across NSW with no *Rhizoctonia* collected in soils from the Murrumbidgee valley. 18 isolates of *Macrophomina phaeseolina* were collected, with half of these collected in the Murrumbidgee valley. Six other groups of fungi were collected (Table 5). All fungi remain in storage at Orange Agricultural Institute and are being used in ongoing studies.

Table 5. Genera of fungi associated with diseased cotton seedlings collected across NSW

Genus/species	Valley
Ceratobasidium sp. (Bi-nucleate *Rhizoctonia*)	Bourke (6) Namoi (1)
Chaetomium sp.	Gwydir (2) Namoi (2)
Fusarium compactum	Bourke (8)
Fusarium equiseti	Bourke (8) Murrumbidgee (1)
Fusarium oxysporum	Gwydir (7) Macintyre (7) Macquarie (1) Murrumbidgee (1)
Fusarium oxysporum f.sp. vasinfectum	Macintyre (4) Macquarie (4) CSIRO Black Mountain
Fusarium sp.	Bourke (6) Gwydir (3) Macintyre (1) Murrumbidgee (1) Namoi (2)
Macrophomina phaeseolina	Bourke (1) Gwydir (1) Lachlan (1) Macquarie (3) Murrumbidgee (9) Namoi (2)
Mortierella hyalina	Lachlan (1)
Paecilomyces lilacinus	Gwydir (1)
Phialophora sp.	Lachlan (5)
Pythium sp.	Bourke (4) Gwydir (1) Macintyre (6) Murrumbidgee (6) Namoi (5) Lachlan (16) Macintyre (3) Tandou (1)
Rhizoctonia solani	Bourke (20) Gwydir (10) Lachlan (2) Macintyre (8) Macquarie (11) Namoi (15) Tandou (3)
Sclerotium rolfsii	Emerald (1) ** Macintyre (1)
Thielavia sp.	Namoi (1)
Unknown	Gwydir (3) Macintyre (1) Macquarie (1)

**** collected by Dr Stephen Allen during 2008/9 early season disease surveys in QLD**

2.3B Characterisation of *Rhizoctonia* like isolates

Nuclear Number

Rhizoctonia like fungi were characterised by first counting the number of nuclei in each cell. Cells were fixed in paraformaldehyde and stained with the fluorescent DAPI stain (Figure 2.3.1). Groups within the genus *Rhizoctonia* are uni-nucleate, bi-nucleate or multinucleate, and nuclear number is the first step used to identify taxonomic groups. Nuclei in 20 cells from three cultures of each isolate (total 60 cells) were counted under ultra violet light.

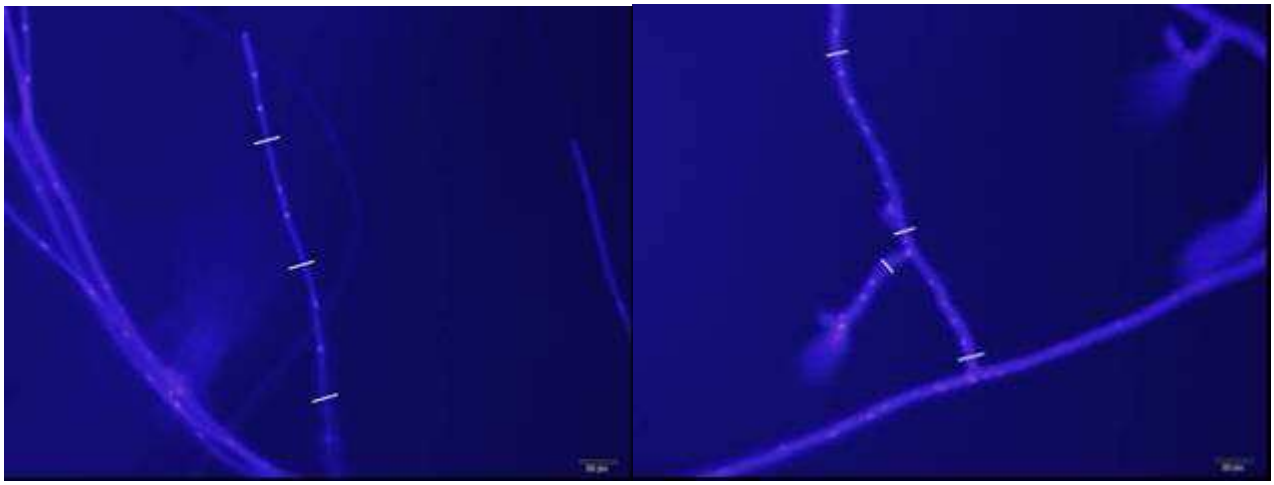

Figure 2.3.1. DAPI fluorescent staining of cell nuclei in *Rhizoctonia* spp. A Binucleate *Rhizoctonia* (*Ceratobasidium*) on the left hand side and a multinucleate fungus (*Rhizoctonia solani*) on the right hand side.

The majority of isolates were multinucleate (Figure 2.3.2) and so were described as *Rhizoctonia solani*. A small group was binucleate indicating that these fungi belong to the *Ceratobasidium* complex which do not usually cause disease on cotton. There was a large degree of variation in nuclear number among *R. solani*, suggesting the presence of at least 3 taxonomic groups, although this data also includes tester isolates and isolates obtained from the NSW Plant Pathology Herbarium that do not originate from cotton soils.

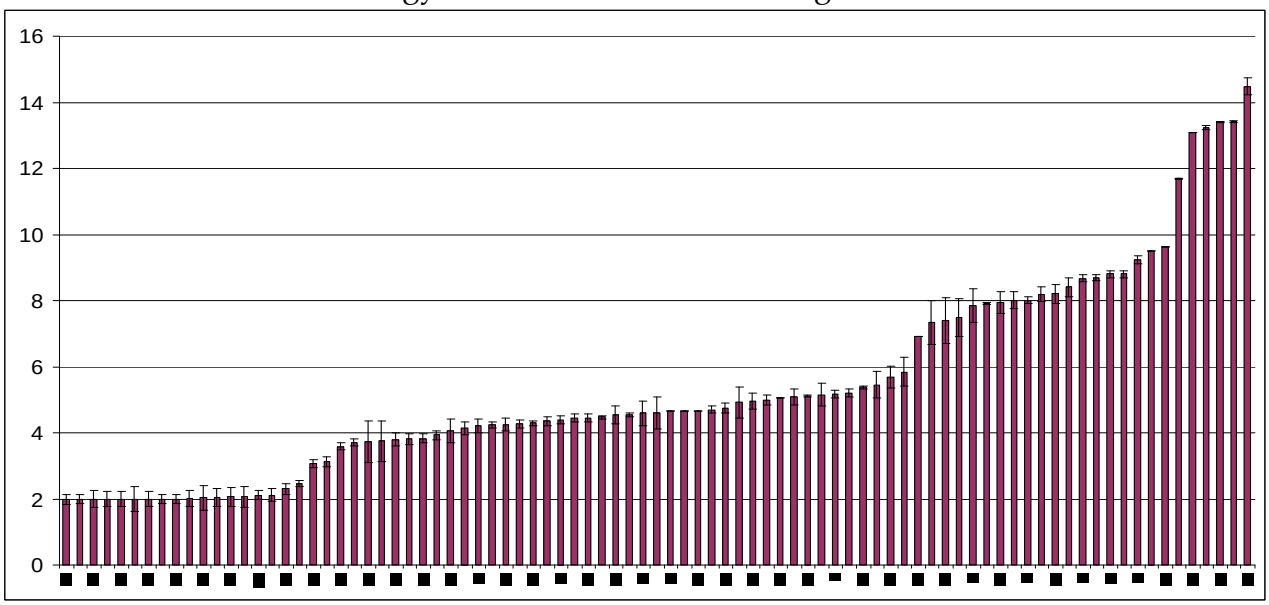

Figure 2.3.2. Mean (standard error) nuclear number in *Rhizoctonia* like fungi.

2.3C Molecular Phylogeny- how do isolates relate to each other and to other isolates from overseas on cotton and other hosts?

Rhizoctonia is a large genus and is often divided up into smaller groups by various physiological and morphological methods. The method of nuclear count mentioned above is one way of determining which isolates belong to the species *R. solani*. *R. solani* is then often split up into smaller groups called subgroups, based on the ability on a culture from one subgroup to fuse cells with a culture from another subgroup. This process is called anastomosis and these subgroups are called anastomosis groups (AGs). Anastomosis groups are then split further into subgroups based on multiple methods including growth rate, physiology and genetics.

AGs can be readily identified by sequencing the internal transcribed spacer (ITS) region of fungal DNA. In some instances, the tedious process of molecular cloning is required because each isolate has more than one ITS sequence. Interestingly, this appears to be common among isolates of AG4 subgroup HGIII, but not subgroup HGI. In this study, 56 isolates have been sequenced to date and sequences indicate that the isolates represent three groups that correspond to AGs. Sequences of three isolates of AG2 (representing subgroups 2-1 and 2-2), two isolates of AG3, and 36 isolates of AG4 (representing subgroups HGI and HGIII) from cotton were obtained. Ten isolates remain to be cloned and sequenced. AG2-1 is sometimes recovered from cotton overseas. AG2-2 and AG3 have not previously been associated with cotton and therefore are new records.

Full phylogenetic trees (diagrams that indicate how closely one isolate is related to another) have been completed for AG2 and AG3 isolates (Figures 2.3.3, 2.3.4 – Australian cotton sequences are highlighted in yellow), but not for AG4. AG2 isolates collected from cotton in Australia were compared to isolates from other hosts in Australia and overseas, however ITS sequence could only distinguish the existence of subgroup 2-1 and 2-2 and did not indicate any similarities between sequences were based on geography or host plant. Analysis of ITS sequences from AG3 isolates suggests that at least three lineages of AG3 have evolved. AG3 on Tobacco appears to be the oldest lineage. Isolates on cotton appear closely related to those on potato indicating a possible evolutionary jump from Solanaceae to Malvaceae hosts. Interestingly AG2-1 is also known to be a pathogen of potato and so host testing of AG2, AG3, and AG4 will be completed on potato and compared to pathogenicity on cotton.

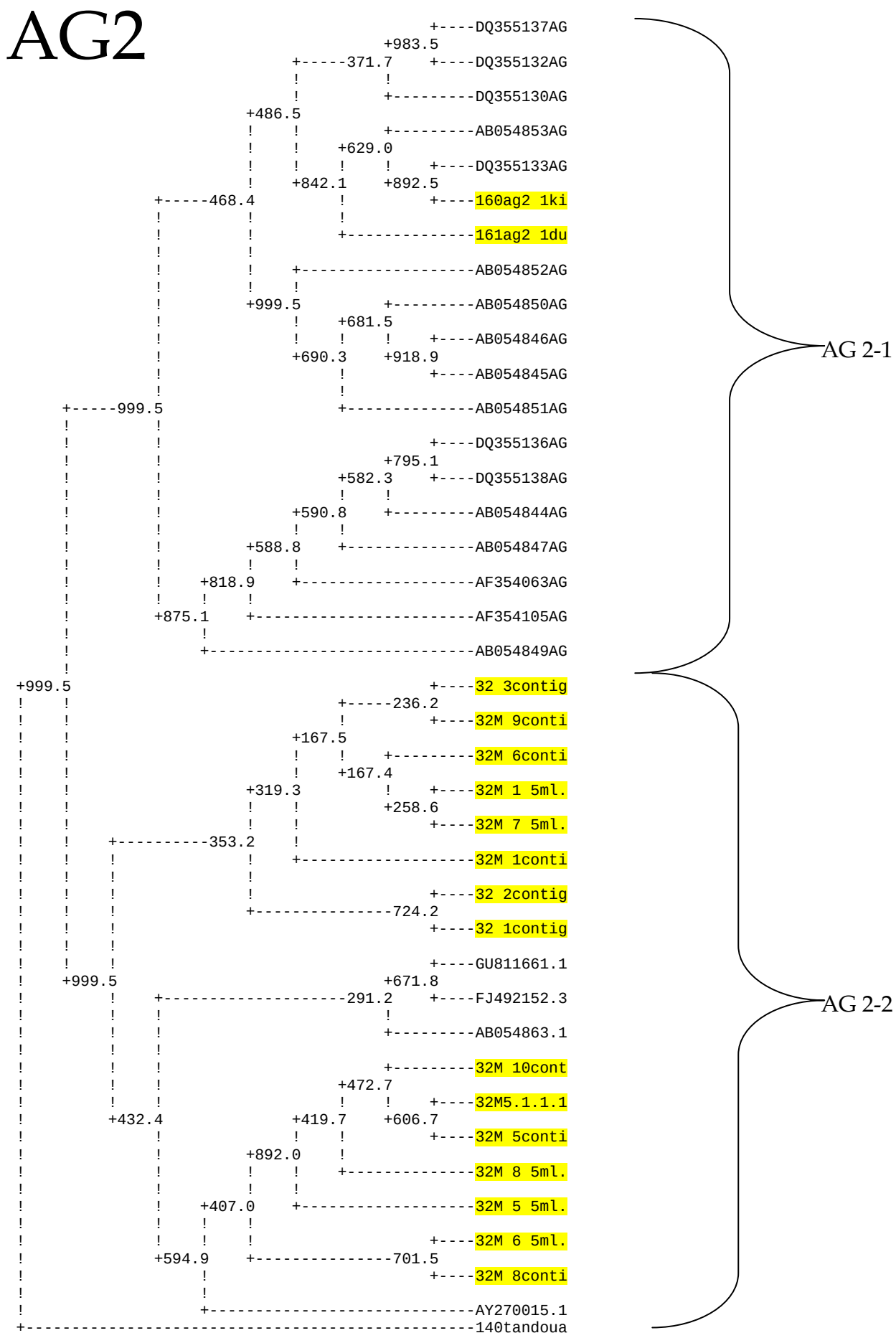

Figure 2.3.3. Relatedness of AG2 isolates collected from cotton and isolates from different hosts in Australia and overseas. Numbers at intervals are bootstrap values based on 1000 replicates.

AG3

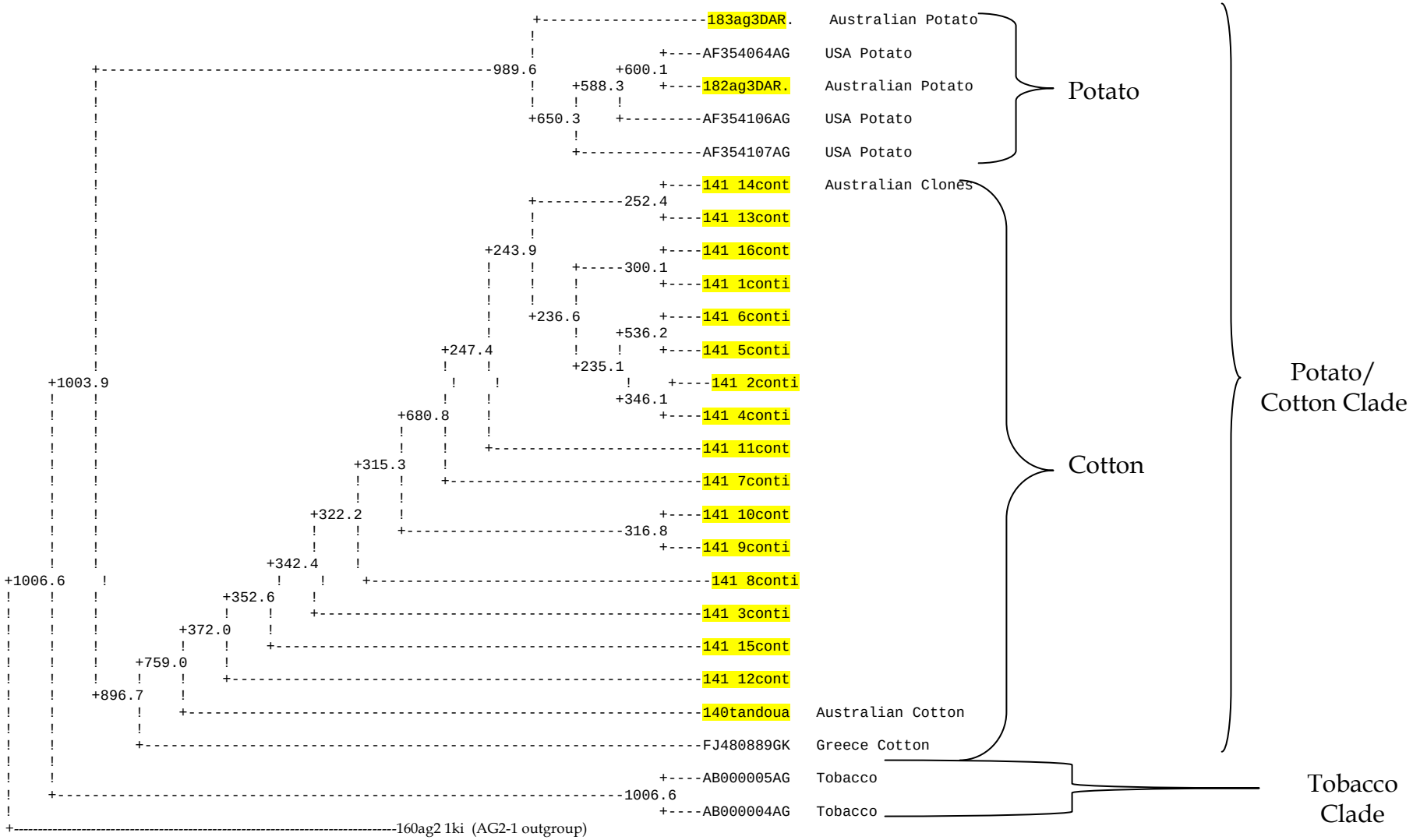

Figure 2.3.4. Relatedness of AG3 isolates collected from tobacco, potato and cotton. Numbers at intervals are bootstrap values based on 1000 replicates.

2.3D Growth Rates

Isolates of *R. solani* and *Ceratobasidium* were grown on potato dextrose agar at 15, 23 and 30C for 72 hours. Three incubators were utilised, with each incubator being run at each temperature once. This allowed each isolate to be grown at each temperature in each incubator. There was considerable variation in growth rate between isolates at different temperatures. For example, AG3 isolates appear to grow faster 15C when compared to other isolates at 23C and 30C (Figure 2.3.5). This probably reflects the evolution of these isolates in cooler climatic regions compared to the more common AG4 which is widespread in warm cotton growing regions. Isolate 32 (AG2) grew at a significantly faster rate than isolate 29 (AG4) indicating that is belongs to subgroup 2IIIB of AG2.

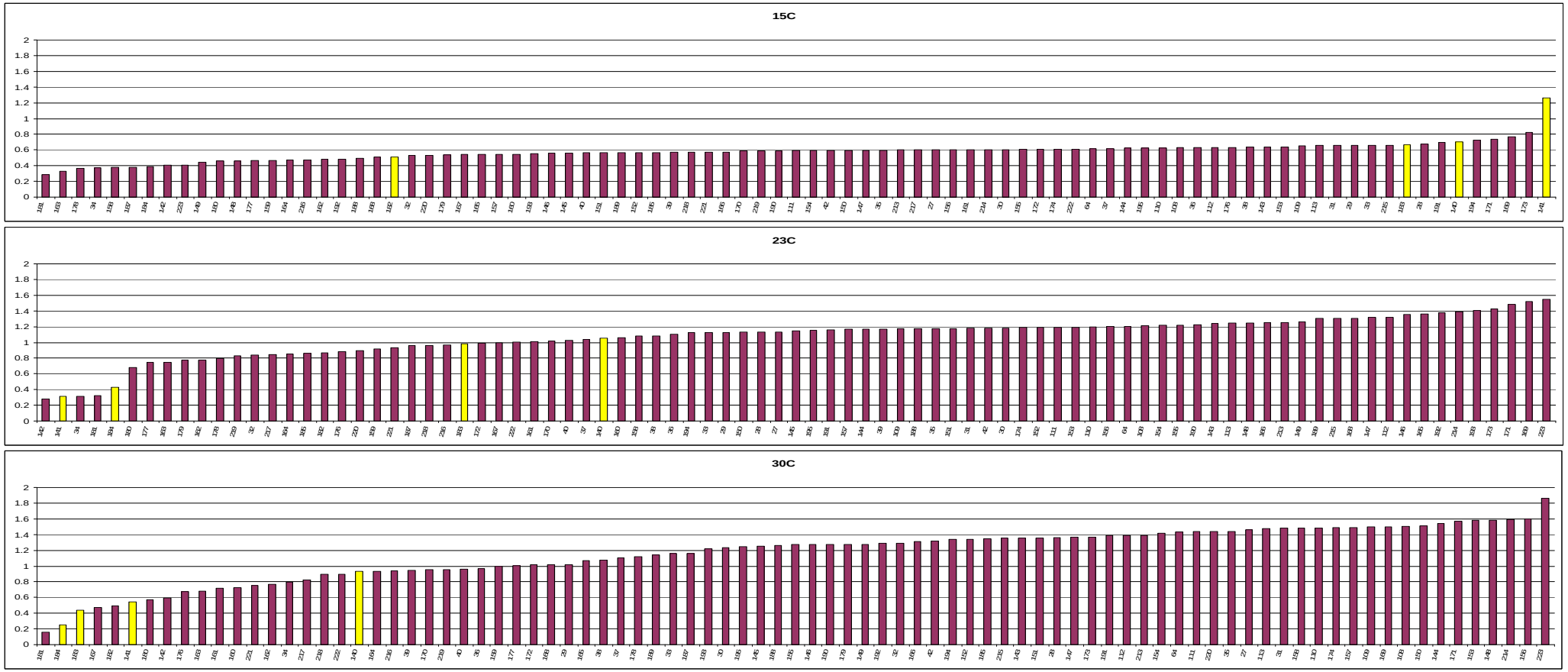

Figure 2.3.5. Growth rates (mm/hr) of *Rhizoctonia* spp. at 15, 23 and 30C. Note that isolates belonging to AG3 (highlighted yellow) collected from cotton grow much faster at 15C than at 23C and 30C compared to other *Rhizoctonia*.

2.3E Variation in fungicide tolerance in *Rhizoctonia solani*

The effectiveness of the fungicide PCNB was tested in a small pilot study *in vitro* against five strains of the seedling pathogen *Rhizoctonia solani* isolated from diseased seedlings from Moree, Boggabilla, Warren and Narrabri. The growth (mm/hr) of each isolate was measured on 1/10 PDA with or without PCNB as Terrachlor (120mg/L). This rate is an approximate equivalent of the recommended rate 5kg/Ha. Growth rate was determined over 72 hours and compared using Analysis of Variance. PCNB significantly reduced the growth rate of each isolate ($P<0.001$). However, there was a significant difference in the extent to which growth rate was reduced between isolates ($P<0.001$). The isolate from Warren did not grow on the PCNB amended media, whereas all other isolates grew at reduced rates (approx. 20% of normal growth rate). This is an important observation as it indicates the existence of significant variation between populations of *R. solani* from cotton fields in NSW. While variation in fungicide tolerance is considered an important indicator of genetic variation among population of *R. solani*, this line of investigation was not pursued to date. As it has been identified that two subgroups of AG4 (HGI and HGIII) are the most widespread seedling pathogens in the *R. solani* complex, an experiment may be run to test for variation in fungicide tolerance between these two subgroups if time permits.

2.3F Study of colony colour after growth at different temperatures.

Cultures grown during the growth rate experiment above were also characterised using Munsell's Book of Colour. This enabled the identification of isolates that produce large numbers of dark brown highly melanised survival structures called sclerotia. These structures help fungi survive for long periods in unfavourable environmental conditions (say between cotton crops). As expected, growth habits and colour differed at different temperatures within and between isolates. For example, isolate 36 from Boggabilla produced sclerotia and grew slowly at 15C, growing significantly faster at 23 and 30C ($P=0.01$) without producing sclerotia indicating preference for warmer temperatures (Figure 2.3.6). Isolate 34 from Moree did not grow at 15C, grew slowly at 23C and grew fastest at 30C (Figure 2.3.6). Only a subsample of results is presented here due to the large number of isolates examined. The production of sclerotia will also be quantified by counts in the same cultures and compared with pathogenicity results on cotton to determine if there is a relationship between pathogenicity and production of sclerotia at different temperatures.

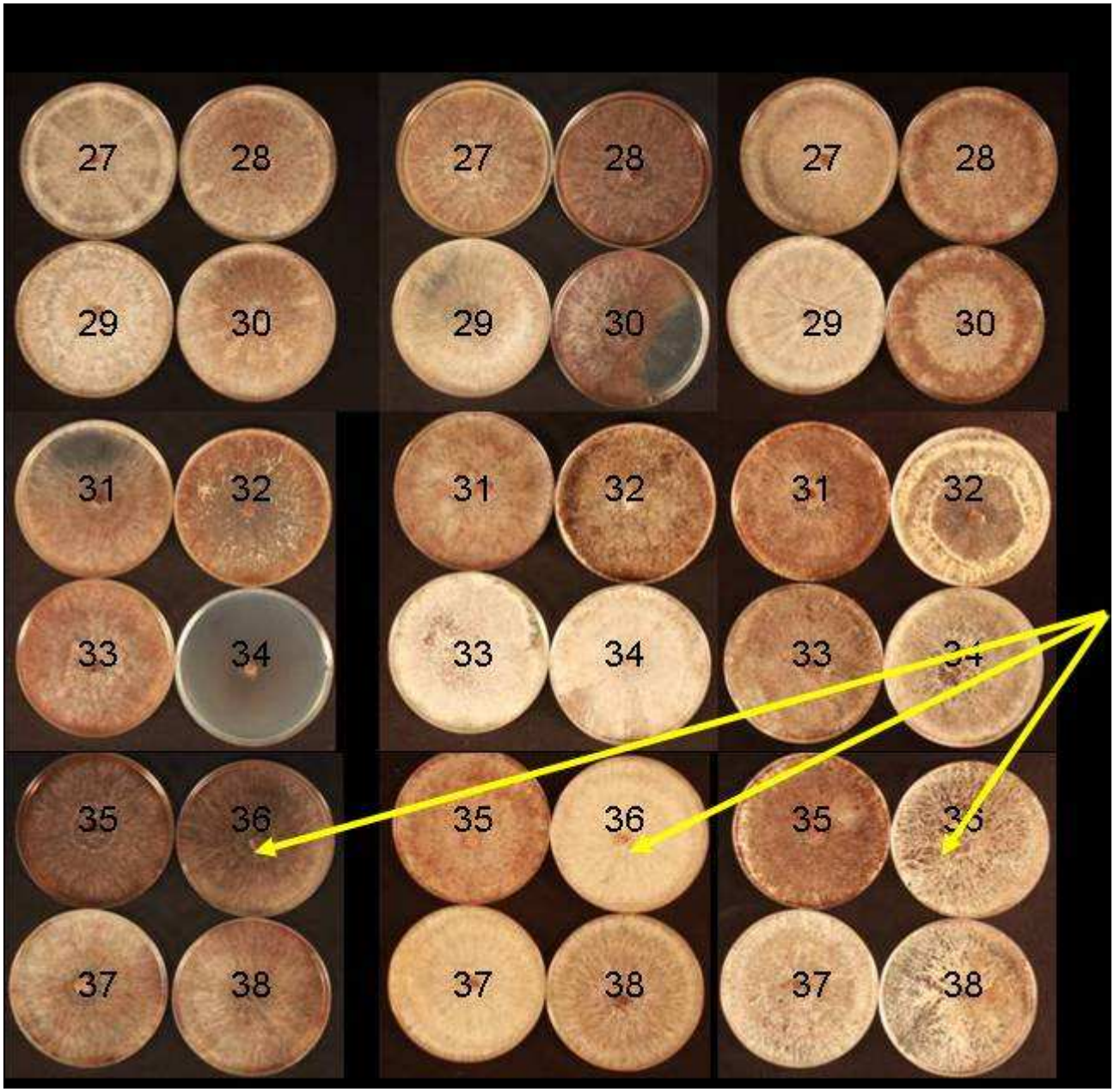

Figure 2.3.6. Colony morphology and colour of isolates 27-38 at 15, 23 and 30C.

2.3G *In vitro* (lab based) pathogenicity assay of fungi isolated from cotton

Fungi collected during soil baiting assays and disease surveys were assessed in the laboratory for ability to cause disease or kill cotton seedlings. Seed of Sicot 71 was surface disinfested in bleach (4% hypochlorite), rinsed in sterile distilled water and placed into sterile glass bottles containing 5mL of 2% DIFCO water agar and allowed to germinate for 7 days at 23C in the dark. Germinated seedlings were then inoculated with a 5x5mm plug of 7 day old PDA culture (Figure 2.3.7). Plants were grown in the incubator for 7 days at 23C under fluorescent light for 12hrs per day. Disease severity was assessed at 7 days after inoculation on a scale of 0-2 where 0 represents no disease, 1 represents disease and 2 represents plant death. Data is presented as an average of the score of each bottle (Figure 2.3.8). An average of 0 indicates the isolate is non-pathogenic. An average between 0 and 2 indicates capacity to cause disease without consistently killing seedlings. An average of 2 indicates capacity to cause disease and consistently kill seedlings in this bioassay.

Figure 2.3.7. Germinated seedlings growing in 2% water agar following inoculation with an agar plug.

Rhizoctonia spp. were the most pathogenic fungi isolated from cotton seedlings in NSW. AG2-1, 2-2IIIB, and 3 caused disease on seedlings in this assay representing a first record of AG3 and AG 2-2 causing disease on cotton. Evidence of host specificity was observed with isolates of AG2-2 and AG3 from other hosts unable to cause levels of disease equivalent to isolates from cotton. Most AG4 isolates tested could consistently kill seedlings. There was variation in pathogenicity among *Pythium*, *Macrophomina* and *Fusarium* isolates. Most *Fusarium* and *Macrophomina* isolates were only mildly pathogenic. Both isolates of *Sclerotium* consistently killed seedlings whereas several *Pythium* isolates could kill seedlings.

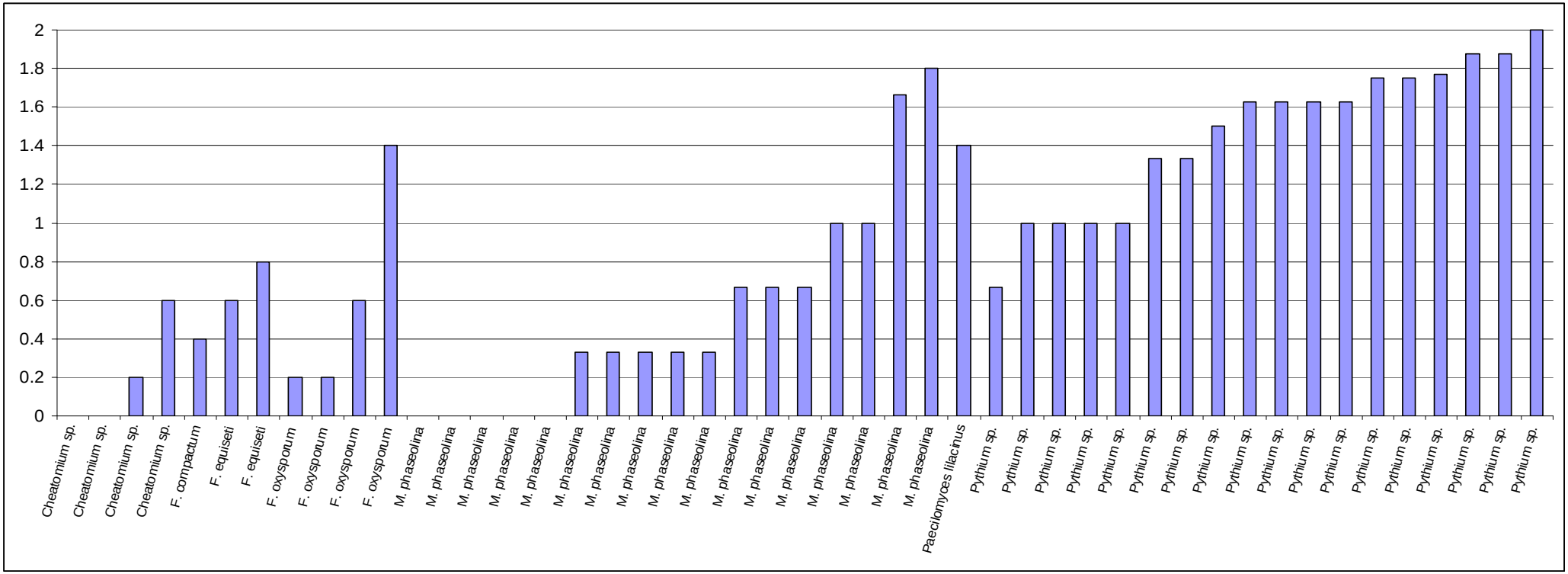

Figure 2.3.8. Average pathogenicity of fungi isolated from cotton tested in vitro. Continued on next page.

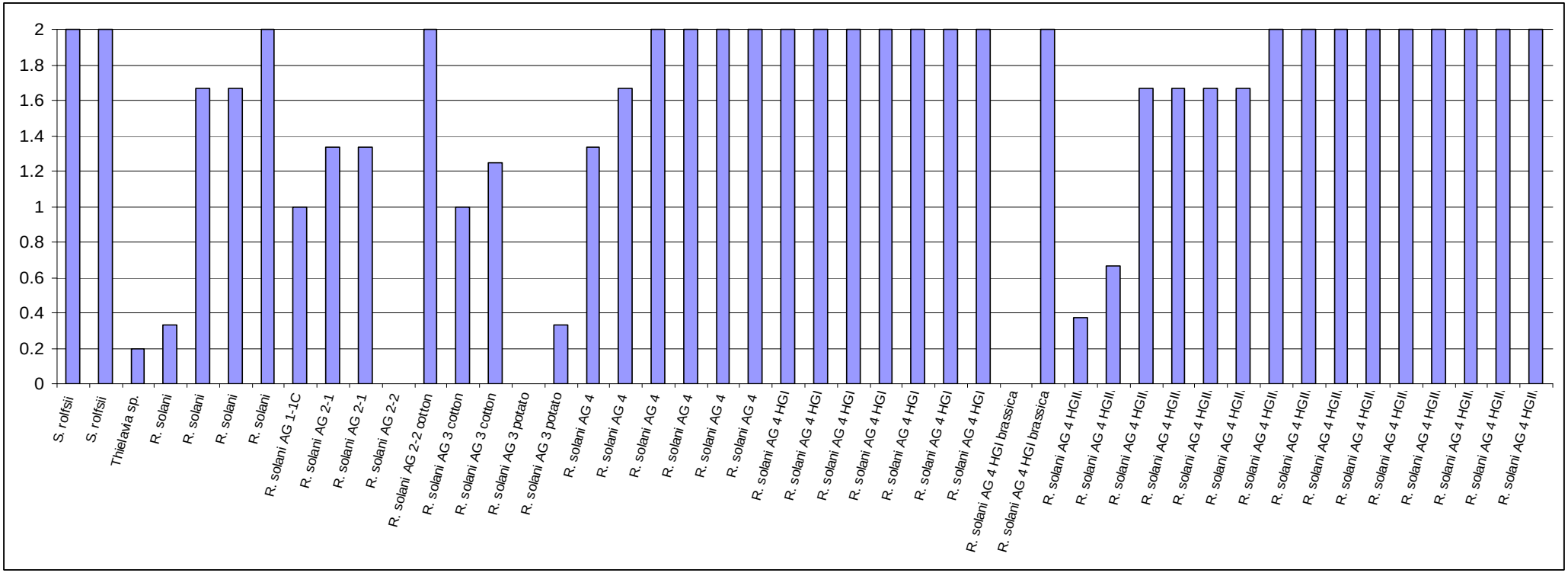

Figure 2.3.8. Average pathogenicity of fungi isolated from cotton tested in vitro. Continued on next page.

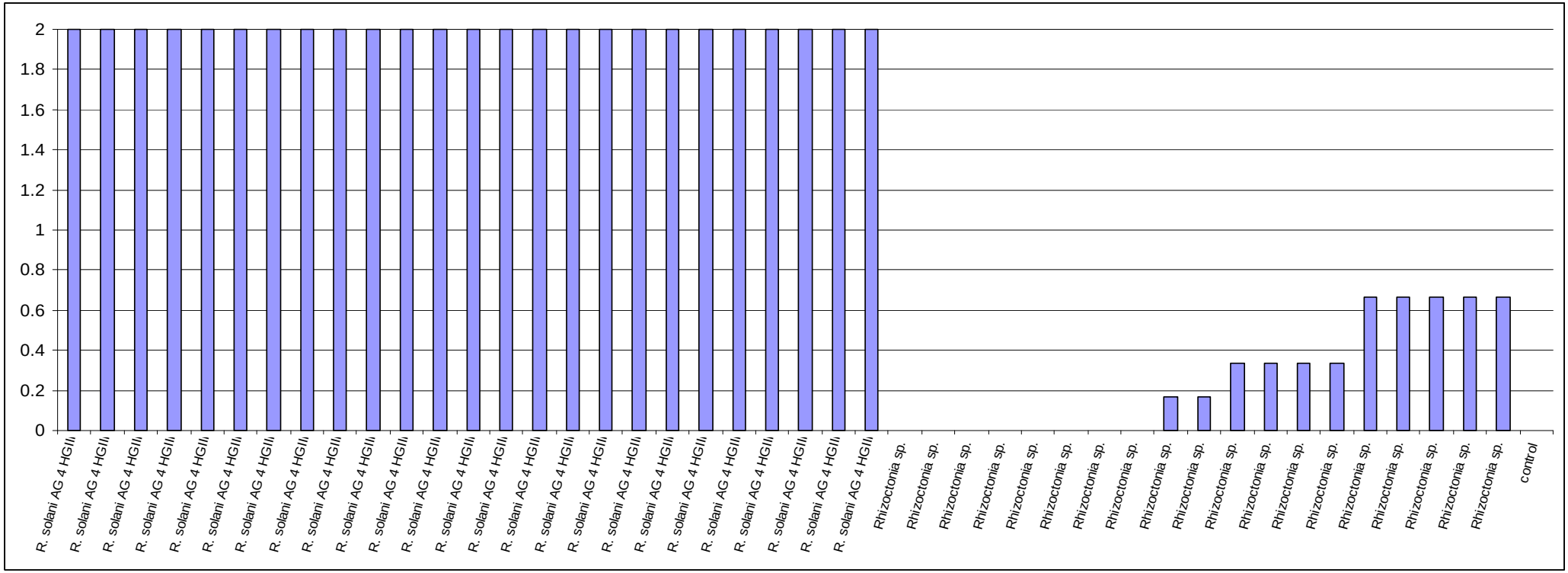

Figure 2.3.8. Average pathogenicity of fungi isolated from cotton tested in vitro.

Each fungus assayed here *in vitro* will also be tested in a glasshouse based pot experiment. All of the *Rhizoctonia* isolates tested here have been screened in isolation of other fungi in a large glasshouse based pot experiment aimed at accurately differentiating pathogenicity among isolates of *Rhizoctonia*. The *Rhizoctonia* isolates were trialled in isolation to other fungi due to a lack of glasshouse space and impracticality of experiment size if all fungi were tested together.

2.3H *In planta* (pot based) bioassay of pathogenicity of *Rhizoctonia* isolated from cotton

All *Rhizoctonia* isolated from cotton and isolates from other hosts sourced from the NSW Plant Pathology Herbarium and other sources were tested for pathogenicity towards cotton at 23C in a large pot experiment. 87 isolates and a control of water agar were screened in 10cm black pots under fluorescent lights on a 12hr day/night cycle. Only two replicate pots could be screened per isolate per run of the experiment due to bench space restrictions. Therefore the experiment was run three times. The third iteration is in progress, so data from two runs (4 replicate pots per isolate) is presented here.

Amgrow Black Label Seed Raising Mix was steamed twice for 40 minutes at 60C and allowed to rest for 7 days before use. Each pot was inoculated with one 7 day old culture of *Rhizoctonia* on water agar (15mL per 9cm Petri dish). The culture was buried in the centre of the pot and 9 surface sterilised seeds were sown at 30mm depth with a wooden dibber. Pots were watered every third day and assessed for stand at 14 and 21 days after sowing (DAS). Severity of collar rot was assessed at 21 DAS using a rating system similar to that used in the in vitro assay: 0 = no lesion, 1 = lesions but not collared, 2= lesions and collared and 3 = dead (Figure 2.3.9). Roots were also collected for assessment of root dry weight.

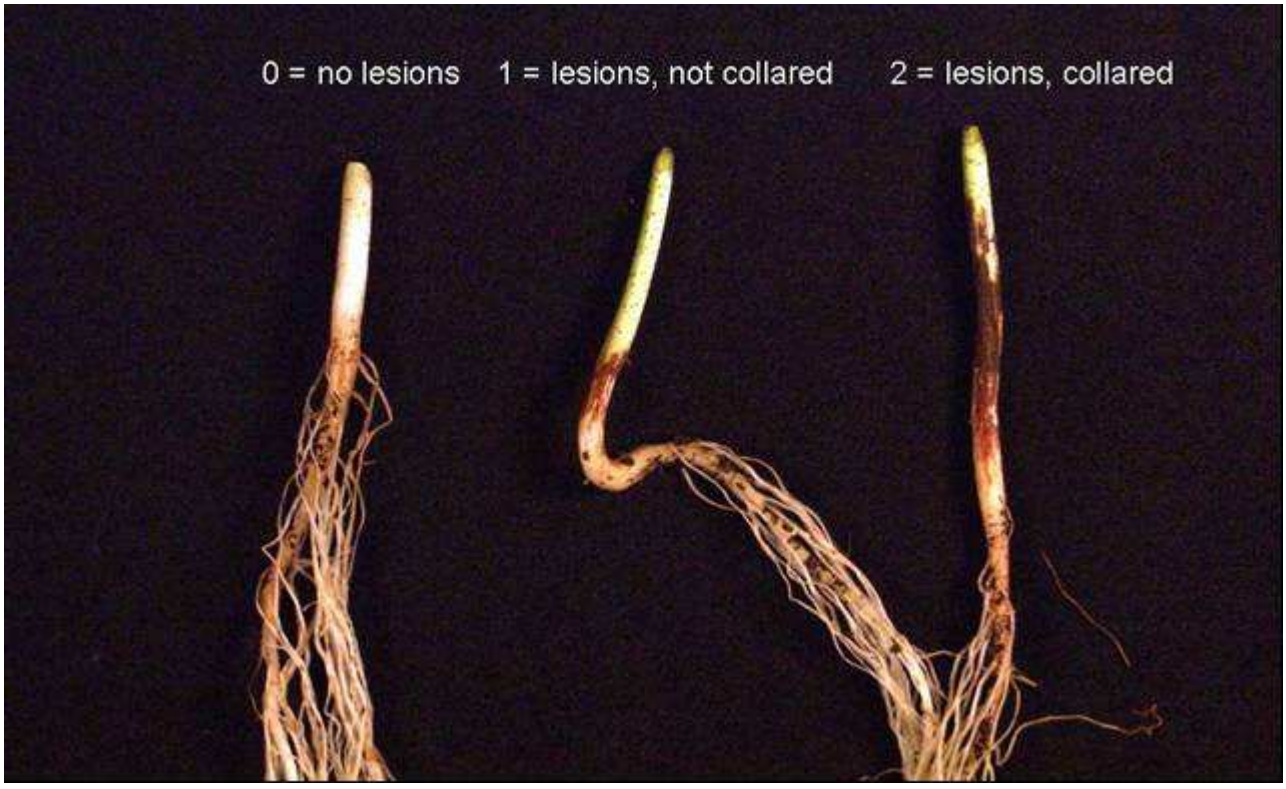

Figure 2.3.9. Disease rating system for the glasshouse bioassay of *Rhizoctonia* isolates. Note 3 = all plants dead.

There was a significant difference ($P<0.001$) in stand at 21DAS among isolates of *Rhizoctonia*. However, only two isolates, isolate 29 and 192, both AG4 isolates, consistently killed all cotton seedlings by 21DAS. AG 3 and AG2-2 did not cause stand loss (Figure 2.3.10). However AG2-1 caused an intermediate level of stand loss and was also associated with an intermediate level of disease severity (Figure 2.3.11) indicating that while all groups of *Rhizoctonia* could cause disease in the in vitro assay, only AG2-1 and AG4 could

cause disease in a pot assay where conditions were less favourable for disease development. It is possibly that only AG4 and AG2-1 can cause disease in the field. Interestingly, root mass (dry weight) was not affected by inoculation with *Rhizoctonia* indicating that only the collar region at the soil surface becomes diseased and that root development remains largely unaffected during the infection process. Results in this glasshouse assay show a strong correlation between disease severity and stand loss (Figure 2.3.12) indicating that isolates capable on average of collaring cotton seedlings usually kill those seedlings in this assay. That is to say, cotton seedlings did not recover after a lesion had completely collared the seedling.

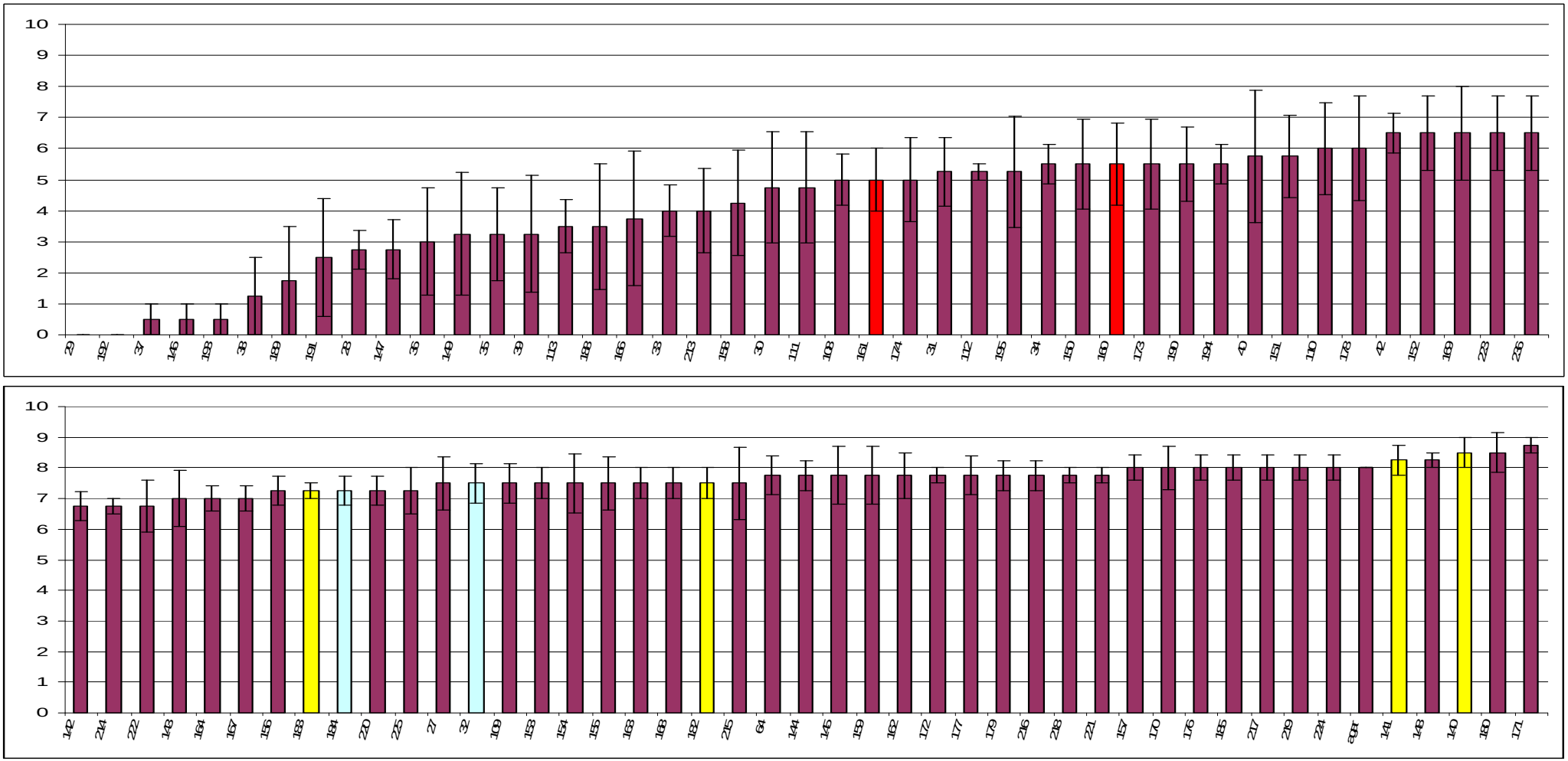

Figure 2.3.10. Mean (standard error) plant stand at 21 days after sowing. AG 2-1 indicated in red, AG3 in yellow and AG2-2 white.

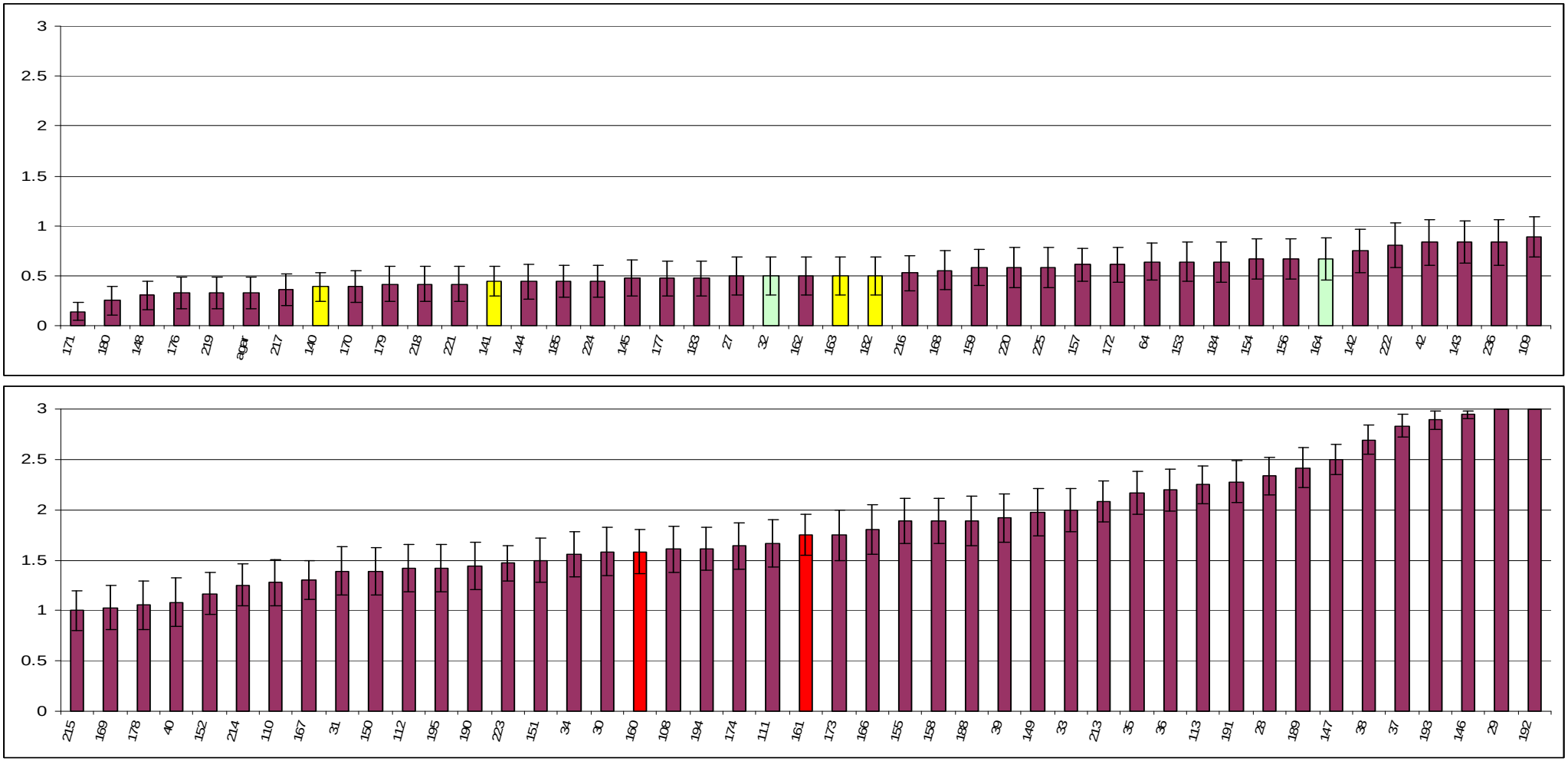

Figure 2.3.11. Mean (standard error) disease severity (score of 0-3) at 21 days after sowing. AG 2-1 indicated in red, AG3 in yellow and AG2-2 white.

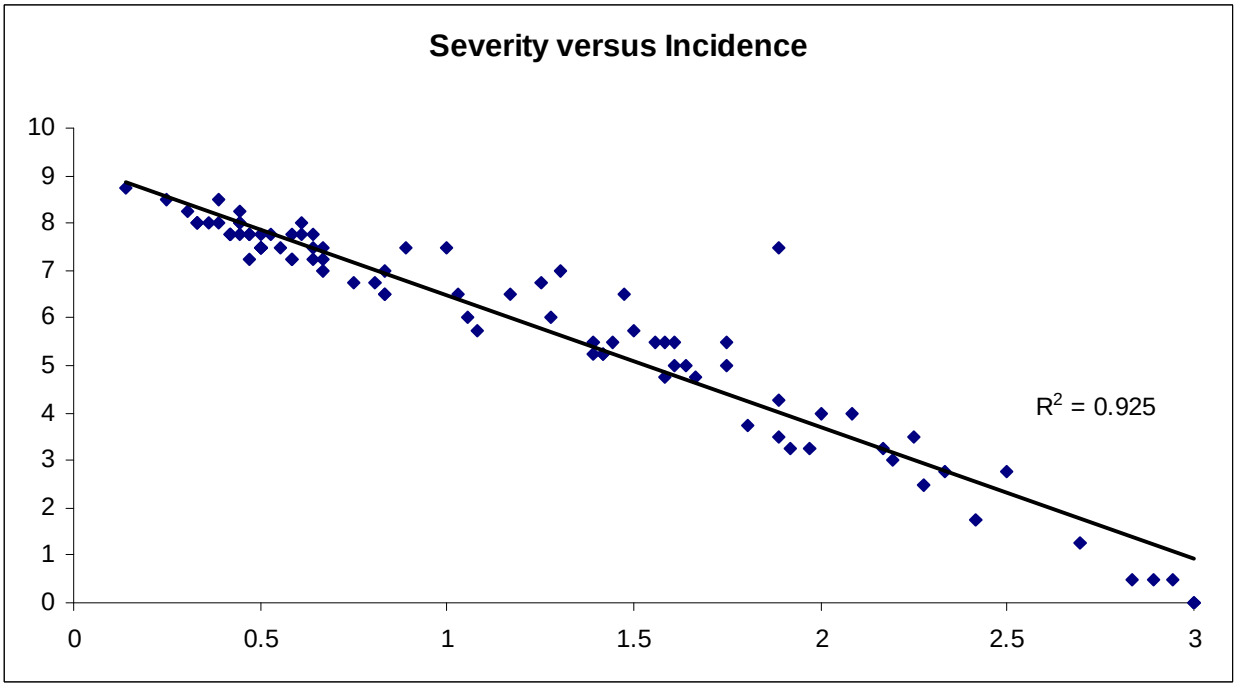

Figure 2.3.12. Correlation of average disease severity rating (x axis) and plant stand (y axis) in pots inoculated with *Rhizoctonia solani* (Rsquare = 0.93)**.**

Conclusions

- *Fusarium* including *F. oxysporum*, *F. equiseti* and *F. compactum* is the most commonly isolated fungus associated with seedling disease in NSW, although most *Fusarium* species including the Fusarium wilt fungus do not cause severe disease and are likely to be opportunistic pathogens that invade seedlings that have been colonised by *Rhizoctonia* or *Pythium*.
- *Pythium* and *Rhizoctonia* are the most aggressive and widespread seedling pathogens in NSW.
- At least three variants of *R. solani* persist in cotton fields in NSW and can invade cotton seedlings.
- At least two of these variants can cause disease and probably contribute to stand loss in the field (AG4 and AG2-1).
- AG2-2 and AG3 are recorded on cotton for the first time.
- Other fungi including *Macrophomina phaseolina* and *Sclerotium rolfsii* occur sporadically in NSW cotton fields and may contribute to some damping off.
- Bi-nucleate *Rhizoctonia* also persist in NSW cotton fields but do not cause disease.
- There is substantial variation in growth rate and morphology among isolates growing at different temperatures with AG3 isolates favoured by cooler conditions and AG4 isolates favoured by warmer conditions and this probably reflects why AG3 is not more widespread on cotton throughout the world.
- There is evidence to suggest that AG3 isolates that occur in cotton in Australia and Greece belong to a cotton lineage within the AG3 subgroup of *R. solani* that may have evolved from AG3 populations on Solanaceae plants like potato.
- There may be evidence of fungicide tolerance in some populations of *Rhizoctonia* but this requires further investigation.

Remaining work

The principal researcher is continuing PhD studies part time with a final submission date of March 2014. The following objectives remain to be completed:

- Complete characterisation of hyphal morphology and sclerotia production.
- Complete experimental testing of anastomosis groups to confirm ITS sequence data.
- Complete final round of *Rhizoctonia* pathogenicity bioassay.
- Complete the glasshouse based pathogenicity assay of fungi other than *Rhizoctonia*.
- Complete host testing of AG 2, 3 and 4 isolates collected from cotton on potato, cauliflower, and other hosts as suggested molecular phylogeny.
- Complete ITS sequencing of 9 tester isolates and *Ceratobasidium* spp. and analyse the full phylogeny of AG4.

The CDRC and the CCC CRC will be provided with a copy of the final thesis when complete.

Objective 3

Continue long-term field experiments on the role of black root rot, mycorrhizal fungi (VAM) and other soil organisms in soil ecosystem function, including collaborative links to other projects.

3A. Long Term Wheat Rotation for black root rot

A long term experiment to test the impact of wheat rotation on black root rot was established at the Australian Cotton Research Institute at Narrabri in the 2004/5 season. This hypothesis was based on anecdotal evidence from a trial in the Macquarie valley in which the severity of black root rot appeared to have been reduced after three years of wheat. Treatments included rotations of WWWWWC, WWWWCC, WCWCWC, WWCWWC and WWWCCC where W is wheat and C is cotton (Figure 3.1). Black root rot and Verticillium wilt were assessed along two and three transect lines respectively in each year the experiment was run where plots were sown to cotton. Soil cores were also taken periodically to assay pathogen levels in the soil with selective media and by glasshouse bioassay.

Results in the sixth year of the experiment when all plots were sown to cotton were not clear.

Severity assessed in the field yielded a statistically significant difference in black root rot levels between treatment and this was not affected by the position of the transect in the field indicating that the result was caused by the rotation (Figure 3). However the levels detected in each treatment were low (<4/10) and the differences detected are unlikely to be biologically meaningful. The result indicates that only the WWWWCC rotation was able to reduce black root rot severity compared to the other treatments with the exception of WWWCCC. However WWWCCC did not differ from any treatments. The results also show strong plant growth in the WWWWCC and WWWCCC treatments (Figure 3.2). This may appear to suggest that the improved plant growth and reduced black root rot severity in these treatments were linked. However the hypothesis that rotation with wheat reduces the severity of black root rot cannot be substantiated by these results as treatments with longer wheat rotations had higher levels of black root rot. Therefore another factor must be involved. Both of these treatments had come out of a cotton rotation, whereas the remaining treatments had come out of a wheat rotation. Nutgrass levels in the WWWWCC and WWWCCC plots were higher than in other treatments (based on observation during assessment). Consequently stand was gappy and plants grew larger during the same period of time compared to plants in wheat plots. In addition, a herbicide for control of nutgrass had been applied to the wheat plots, and this appeared to cause some damage to the roots of cotton seedlings sown into these plots resulting in smaller plants and potentially causing some root browning. The larger plants in the WWWWCC and WWWCCC plots had sloughed off much of the black root rot infection which was already at a low level across the trial. Therefore no conclusion could be drawn from the statistically significant difference in severity observed in the WWWWCC treatment. In order to overcome the effect of nutgrass, soil cores were taken across the plots and planted to cotton in the glasshouse. Plants were grown for four weeks and then assessed for tap root discolouration. No significant difference was detected in

black root rot levels between treatments or between transect lines, and the level of black root rot was again very low. Therefore we can conclude that none of the rotations of wheat and cotton trialled in this experiment had any affect on black root rot severity. This is not surprising given the ability of this fungus to form highly resistant survival spores that can survive long periods in the absence of a suitable host. If the hypothesis was to be retested, it would be better to compare consecutive years of cotton cropping rather than various wheat rotations. For example, CCCCCC, CCCCWC, CCCWWC, CCWWWC, and CWWWWC where C is cotton and W is wheat. The design of the current experiment confused comparison between some treatments. A simpler experimental design like the one mentioned above may be more useful in detecting the effect of rotating out of cotton with wheat, however there remains no experimental evidence to support claims that this will lead to lower levels of black root rot.

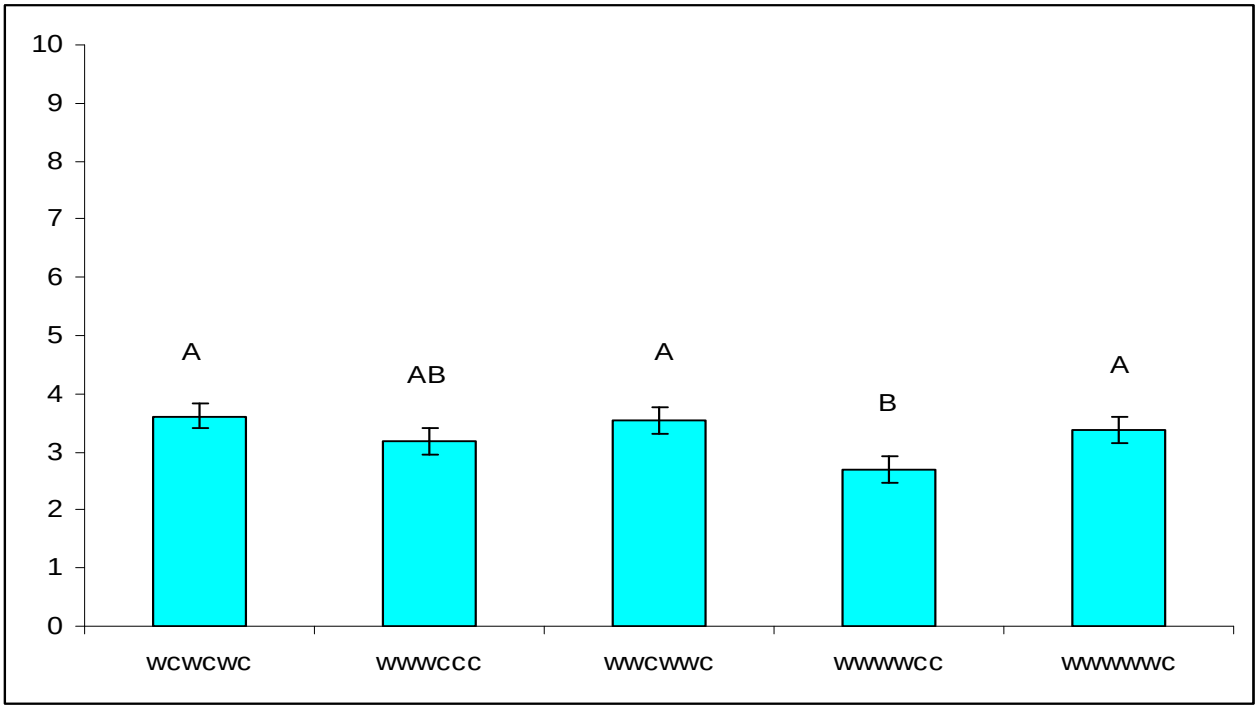

Figure 3. Mean (standard error) severity of black root rot (1-10) in field plots following various long term wheat rotations. Significant differences indicated by letters (P=0.001).

Lowest levels of Verticillium wilt were recorded in plots which had grown five wheat crops or two wheat crops, however there was a significant interactive effect between the treatment and the block is was sown into (P=0.013) indicating that the result was probably due to an uneven distribution of inoculum across the trial site resulting from several years of repetitive cotton cropping in sections of the field. This experiment did not support the hypothesis that rotation with wheat can reduce the impact of Verticillium wilt in successive cotton crops. However this has been demonstrated in several trials completed in past projects and should continue to be adopted as an integrated disease management strategy for Verticillium wilt of cotton.

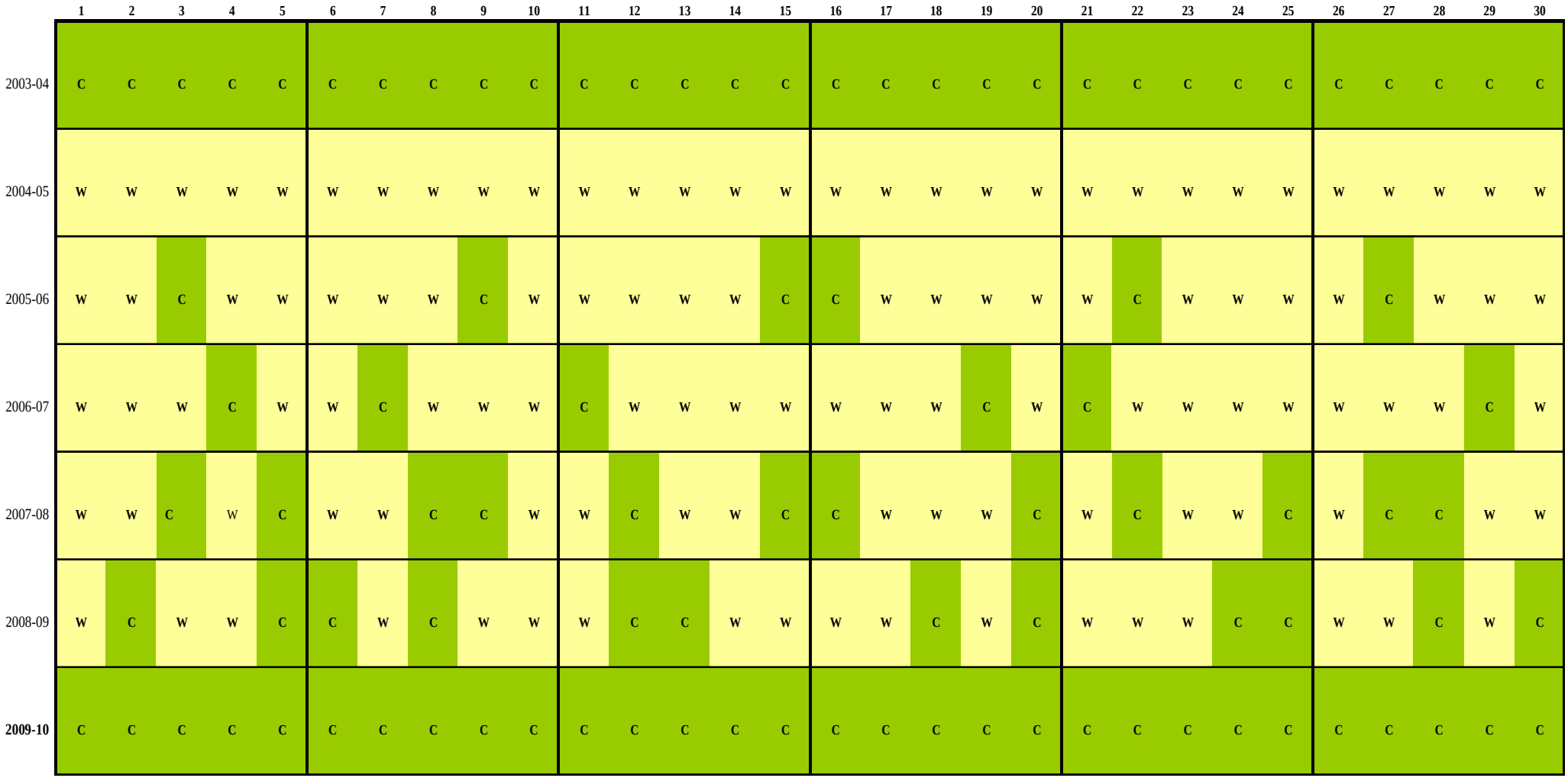

Figure 3.1. Trial layout by year in Field Old2 at ACRI. Note five treatments: WWWWWC, WWWWCC, WCWCWC, WWCWWC and WWWCCC.

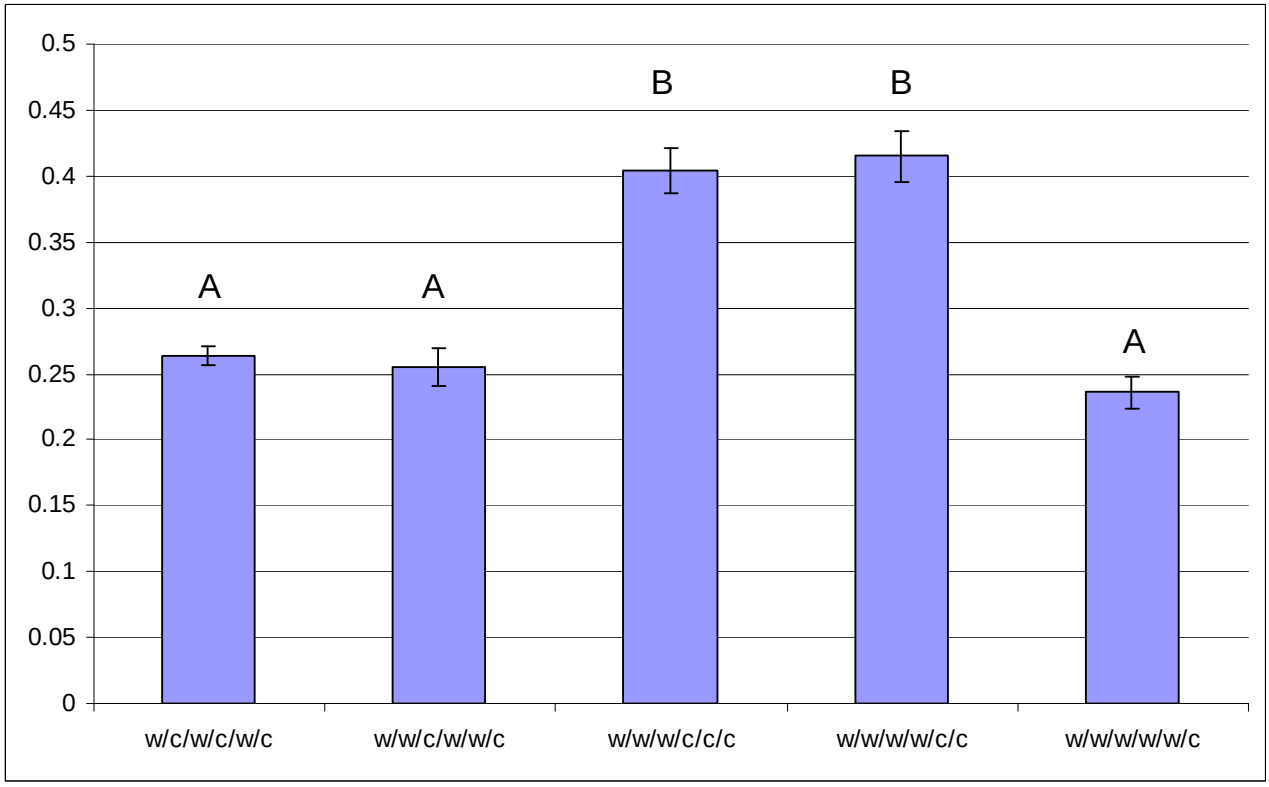

Figure 3.2. Mean (standard error) shoot dry weight of cotton following various long term wheat rotations. Significant differences indicated with letters (P<0.001).

3B. Biofumigation with canola, chickpea and wheat to control black root rot

Biofumigation has been shown to be an effective means of reducing the impact of soil borne diseases. Biofumigation with various crops including canola and wheat has been trialled in Field 4 at ACRI for several years. Experiments carried out as part of the Diseases of Cotton VIII project failed to demonstrate the efficacy of biofumigation with canola and vetch against black root rot primarily due to poor growth of biofumigation crops. However, experiments completed during DAN122C demonstrated that capacity for biofumigation to reduce black root rot. In 2007/8 biofumigation crops were sown but the trial was not assessed due to the departure of the principal researcher Dr David Nehl from the project. In 2008/9 biofumigation crops were not planted in Field 4 due to a weed ecology experiment that was occupying the space used for the biofumigation experiment and which could not be removed in time for sowing of biofumigation crops. An assessment of black root rot levels was completed across the trial by collecting soil cores and planting them to cotton in the glasshouse. Sunflower was then sown across the entire experiment to minimise any impact of the weed ecology experiment on disease levels prior to sowing of biofumigation crops in winter 2009. Black root rot levels were again assessed in soil cores across the experiment after Sunflower. Black root rot levels remained low and did not change following the sunflower crop (Figure 3.3).

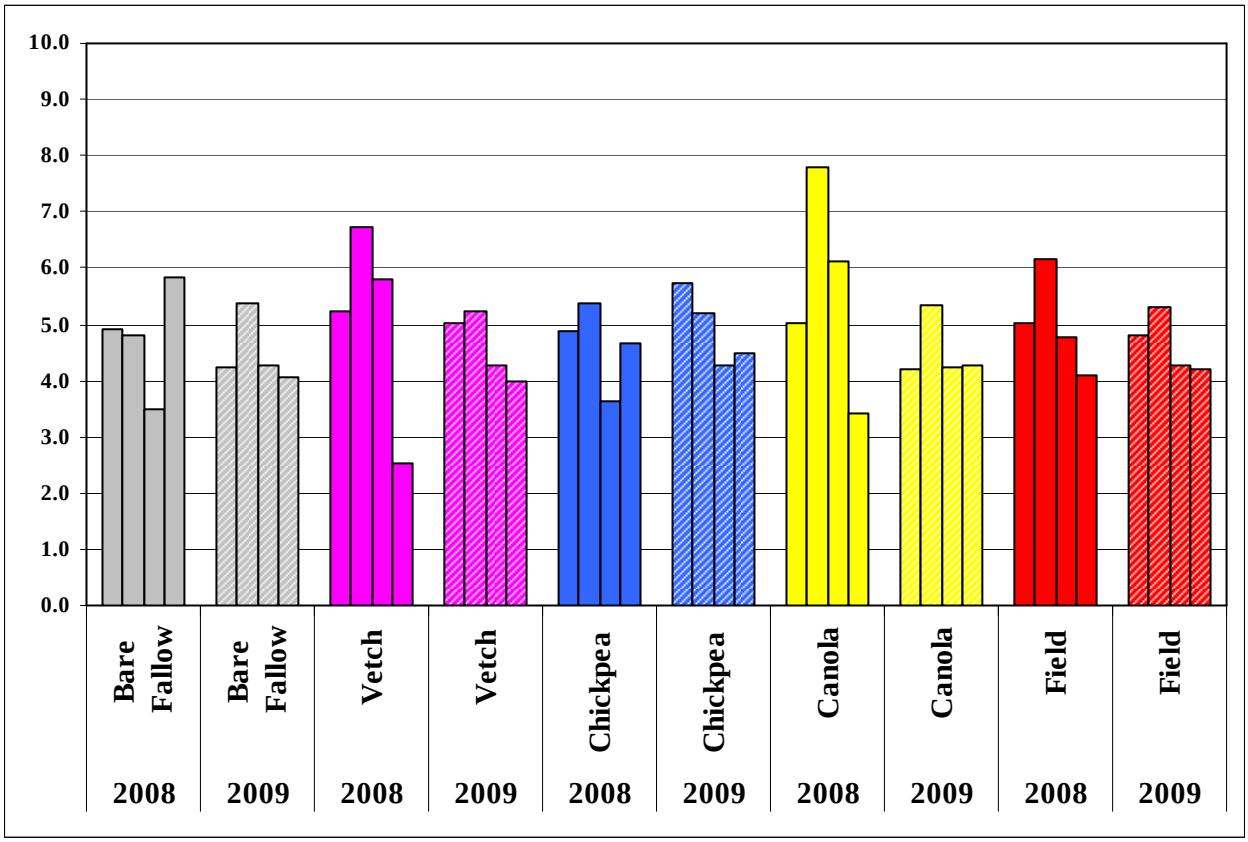

Figure 3.3. Mean black root rot severity in plots utilised for each treatment before sunflower (October 2008) and after sunflower (May 2009).

In 2009/10 plots were sown with vetch, chick pea, canola or left bare (Figure 3.4). Each biofumigation crop was replicated in eight 30m by 16 row plots (Figure 3.4). Biofumigation crops were incorporated on 1st September 2009. Crop biomass was as follows: chick pea 2.4 tonnes/Ha; vetch 3.0 tonnes/Ha; and canola 4.5 tonnes/Ha.

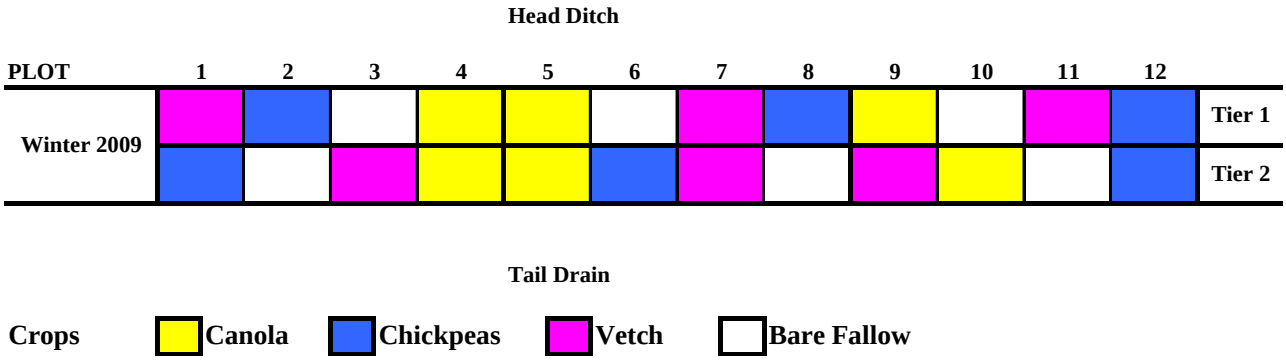

Figure 3.4. Experimental set up of biofumigation plots in Field 4 at ACRI in 2009/10

Cotton was sown on 14th October (six weeks after incorporation). Black root rot severity, stand establishment and plant dry weight were assessed across two transects in each tier on the 17th November. There was no difference in black root rot severity across the trial indicating that biofumigation did not control black root rot this season. Average levels of the disease were low (approx. 4/10) and warm conditions in November did not favour disease development. There was no difference in seedling mortality between any treatments indicating that biofumigation did not reduce or increase seedling mortality compared to the control. However, seedling mortality levels were high across the experiment averaging 65% and this may reflect cool conditions in October and poor

breakdown of incorporated biofumigant crops. Interestingly, the shoot dry weight of cotton sown into plots biofumigated with chick pea was significantly higher than cotton sown into bare fallow plots and plots biofumigated with canola (P=0.045). However there was no difference in shoot dry weight between vetch and chick pea plots. This probably reflects the ability of chick pea and vetch to fix nitrogen and may also indicate allelopathy between canola residues and cotton. Results in the field suggest that warm conditions in November when the trial was assessed masked any benefit from biofumigation in 2009/10.

3C. Long Term Farming Systems Trial

A long term farming systems strip trial operated by Dr Nilantha Hulugalle in Field D1 at ACRI was assessed for stand, black root rot and shoot weight in 2008/9 and 2009/10. There are six treatments and each treatment is replicated three times in 165m long 20row plots. The treatments are as follows:

T1: Cotton-vetch-cotton
T2: Cotton-winter fallow-cotton
T3a: Cotton-wheat-summer and winter fallow-cotton; wheat stubble incorporated
T3b: Summer and winter fallow-cotton-wheat; wheat stubble incorporated
T4a: Cotton-wheat-summer fallow-vetch-cotton; wheat stubble retained as standing stubble
T4b: Summer fallow-vetch-cotton-wheat; wheat stubble retained as standing stubble

In 2008/9 treatments 1,2, 3a and 4a were sown to cotton and assessed for black root rot severity, stand and shoot weight. There were statistically significant differences between treatments in all variables. Black root rot severity was extremely low in all treatments and so differences have no biological significance. Plant stand was highest in treatment 3a (P=0.006) which had been fallowed in the previous summer and winter with incorporation of wheat stubble. Research indicates that seedling mortality can be reduced in fields where crop residues are broken down prior to planting. This is supported here. Shoot dry weight was highest in treatment 4a followed by treatment 2 although there is no indication as to why this was the case. In 2009/10 treatments 1,2,3b and 4b were assessed. Plant stand, black root rot severity and shoot weight did not differ between treatments in 2009/10. Levels of black root rot were consistently low across this trial averaging less than 3/10 which is not biologically significant.

3D. Long term bare fallow and its impact on Mycorrhizal fungi in cotton production systems

Several experiments were run over the course of this project investigating the influence of long bar fallow on mycorrhizal fungi (VAM) in the cotton production system. It is often thought that VAM deficiency should be assumed following periods of long bare fallow. However, research presented here will demonstrate that this is very much dependent upon the field in which cotton is grown.

Long term bare fallow at ACRI

A long term trial has been in place since 2006 in Field 4 at ACRI to examine the impact of cropping versus long bare fallow on inoculum of VAM in the soil (Figure 3.5). The trial was assessed 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. Black root rot levels were extremely low in all plots and did not differ between treatments. VAM levels were not different between cropped and bare soil indicating that three years of bare fallow was not associated with a reduction in the colonisation of cotton roots by VAM fungi. Similar results were reported for the previous VAM trial in Field 4 (see Diseases of Cotton VIII). The trial was assessed again in Winter 2010 and the same result was obtained. This experiment will be continued indefinitely.

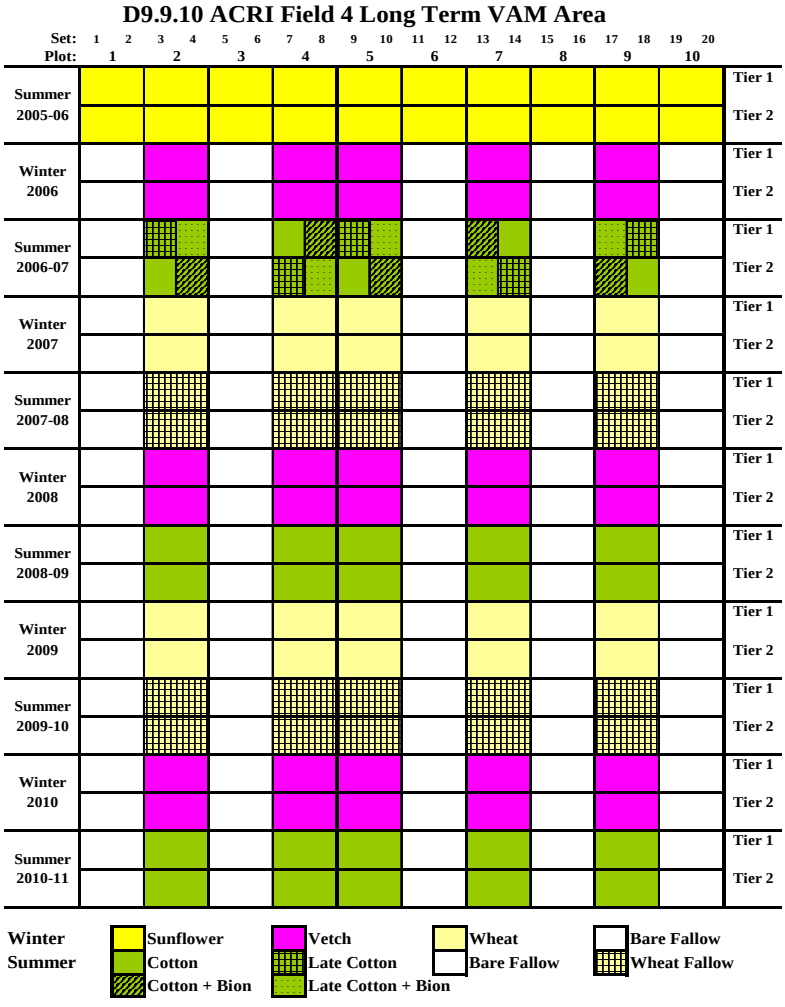

Figure 3.5. Layout of the long term VAM trial in Field 4 at ACRI.

Interaction between black root rot and VAM

An experiment was initiated to test the hypothesis that colonisation of roots by the black root rot fungus reduces colonisation of roots by vesicular arbuscular mycorrhizal (VAM) fungi. Soil was collected from an uncropped area near Narrabri thought to be free of the black root rot fungus. Soil was also collected from an adjacent field known to be infested with the black root rot fungus. Both soils were assayed to determine base levels of VAM inoculum and black root rot in the glasshouse. There was no difference in VAM levels between cropped and uncropped soils. However, there was a significantly higher ($P<0.001$) level of black root rot in cropped soil which was associated with a significantly lower ($P=0.034$) shoot dry weight (Figure 3.6). These results indicate that infection of roots by the black root rot fungus does not reduce the capacity for mycorrhizal fungi to colonise cotton.

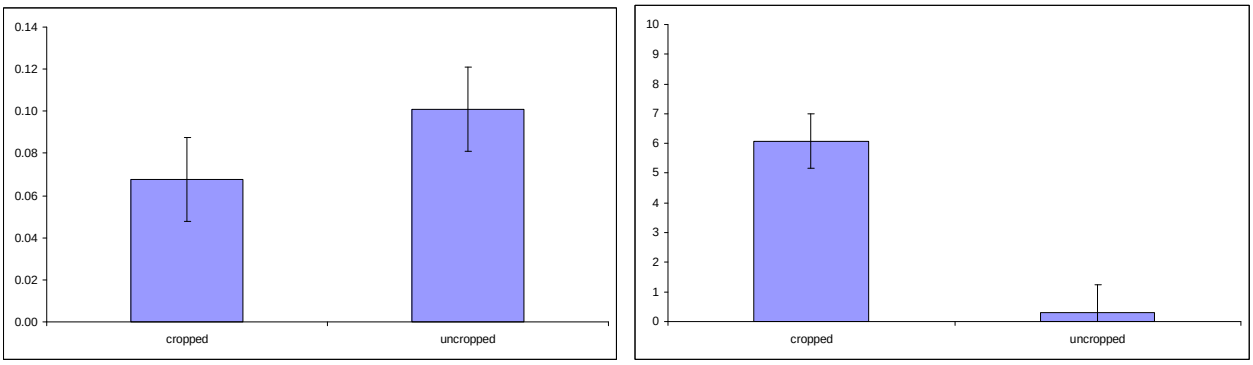

Figure 3.6. Mean (standard error) shoot dry weight (g) and black root rot severity in cropped and uncropped soil.

Managing long fallow disorder in cotton with chick pea

Long fallow disorder occurs when the level of inoculum of mycorrhizal fungi in the soil falls below the level required to promote healthy plant growth. Symptoms of long fallow disorder include stunting and zinc deficiency. Long fallow disorder may be caused by long periods of bare fallow. Nurse crops are sacrificial crops that are used to promote the growth of mycorrhizal fungi in long fallow situations. The crop is grown for a short time before being reincorporated in preparation for the following commercial crop. Legumes can be used as nurse crops and have the additional benefit of nitrogen fixation.

Experiments were established at Lake Tandou, Hillston and Bourke, in fields that were maintained as bare fallows for more than three years. At Lake Tandou, plots were either maintained as bare fallow or sown with chick pea, and each treatment was replicated six times. The experiment was completed in two fields. At Bourke and Hillston, plots were either 1) maintained as bare fallow, 2) sown with chick pea, 3) inoculated with soil from under wheat, and 4) inoculated with soil and sown to chickpea. The additional treatments at Bourke and Hillston will allow comparison between the impact of the chick pea crop on mycorrhizal levels compared to natural cropped soil. In all experiments, chick peas were watered and grown for at least two months. Four soil cores from each plot in each experiment were collected. The experiment at Bourke was sampled twice. Cores were sown with cotton in the glasshouse. Shoots and roots were harvested from each treatment at two, four, six and eight weeks after sowing. Roots were stained to enable counts of mycorrhizal colonisation.

Bourke

There was no difference in %VAM colonisation of cotton at 2,4,6 or 8 weeks after sowing into 1) bare soil, 2) bare soil inoculated with soil from another field, 3) bare soil sown with a sacrificial chick pea crop, and 4) bare soil sown with a chickpea crop and inoculated with soil from an adjacent field (Figure 3.7). Cotton sown into soil from this field did not have long fallow disorder. VAM colonisation was at 40-50% by 8WAS which is normal. Similar results have been noted in long fallow fields on this farm in previous experiemtns (see Diseases of Cotton VIII). The shoot dry weight of cotton sown after "chickpea + soil" was significantly higher than all other treatments (P=0.006) at 6WAS. This may indicate that the rate of growth in "chickpea+soil" plots was slightly faster than in the other plots. However, all other treatments had caught up by 8WAS.

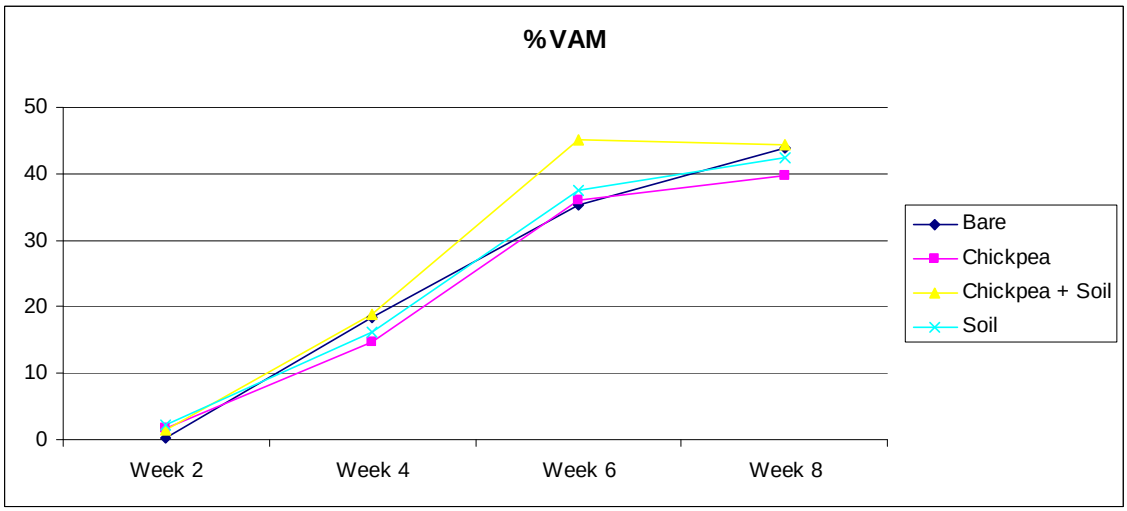

Figure 3.7. No difference in VAM colonisation between treatments over eight weeks.

Tandou

Very low levels of VAM colonisation were detected in each field indicating a deficiency in soil VAM inoculum. After 8 weeks of cotton, VAM levels within the roots did not exceed 20% colonisation in the bare plots (figure 3.8). It is normal to see upward of 50% colonisation in a regularly cropped soil. Colonisation was improved by growing a crop of chick pea before cotton and this was significantly higher in field B4 at all points in time ($P<0.001$). However, over all levels of colonisation were still low at 8 weeks. A sacrificial chick pea crop or a cereal rotation would also assist in restoring the soil VAM potential.

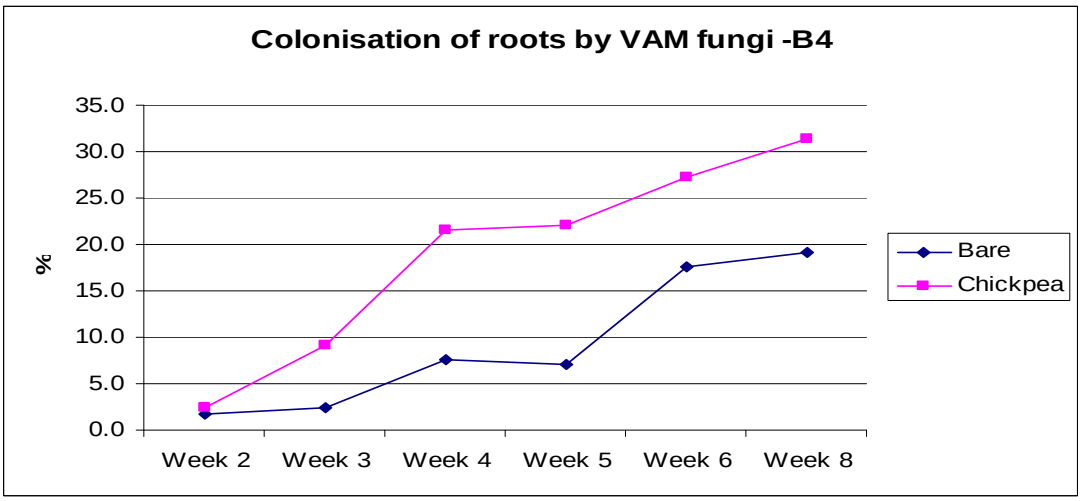

Figure 3.8. VAM colonisation of cotton was increased in soil planted with chick pea.

Hillston

Soil cores were assessed at 2,4,6 and 8 weeks after sowing indicating no difference in VAM colonisation of cotton roots between treatments, and normal levels of VAM colonisation by six weeks (Figure 3.9). This field had been fallowed since 2005, with one failed wheat crop in winter 2007 and did not have a VAM deficiency.

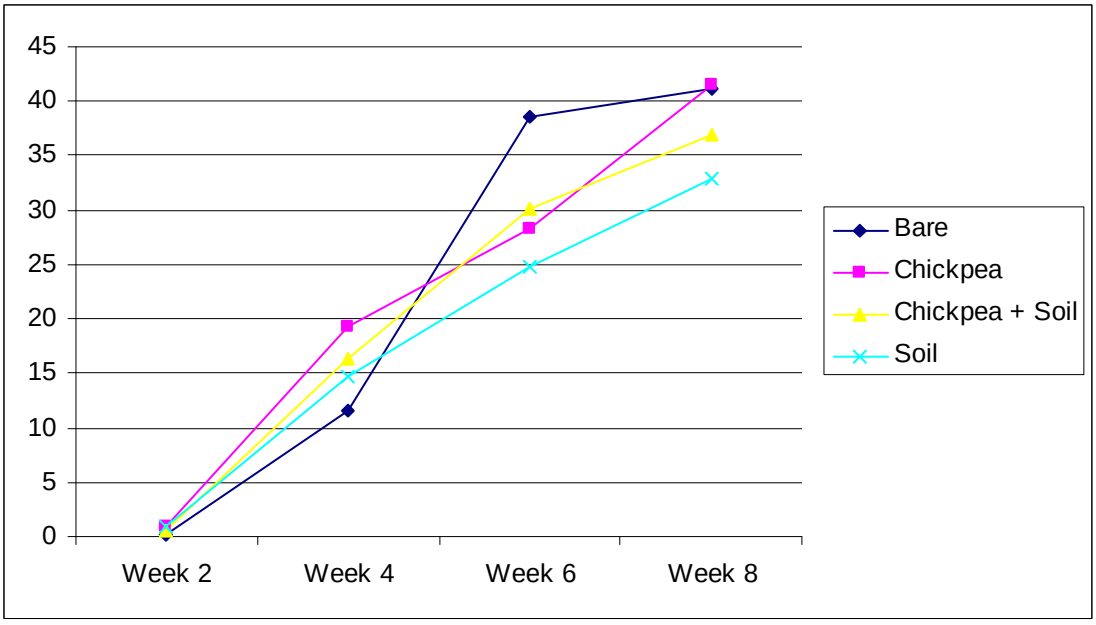

Figure 3.9. No difference in VAM colonisation between treatments over eight weeks.

These results demonstrate the usefulness of sacrificial crops like chickpea in increasing the VAM potential of soil that has been fallowed for long periods. Not all fallowed soils will have VAM deficiency (eg. soils at Bourke in this experiment). So farmers should test their

soils by growing small strips of a VAM sensitive crop like cotton, chickpea or linseed in the soil to be tested and in regularly cropped soil. If there is a difference in growth, then farmers should consider sowing a sacrificial crop like chick pea.

Conclusions

- Rotation out of cotton with wheat did not have an impact on the severity of black root rot in subsequent cotton crops. This hypothesis should be tested further.
- Biofumigation can control black root rot, but the benefits of biofumigation may be masked in seasons that are not conducive to black root rot.
- Seedling mortality is reduced in fields where crop residues are broken down prior to planting.
- VAM colonisation will not necessarily be affected by extended bare fallow. Rather, the impact of bare fallow on VAM colonisation appears to differ between fields.
- Black root rot does not reduce VAM colonisation.
- Growers can replenish VAM inoculum by growing a sacrificial crop like chick pea.
- Growers should test plant growth in fields suspected of being deficient in VAM by growing test strips of chick pea, linseed or cotton and comparing to growth in recently cropped soil.

Research Completed in the first 18 months of CRDC Project DAN190

Chris Anderson was principal researcher on the CRDC funded project DAN190 Survival and Reproduction of the Fusarium Wilt Fungus from July 2006 to November 2007 when he left the project to work on Diseases of Cotton IX replacing Dr David Nehl. The project had no researcher until the appointment of Dr Alison Seyb in March 2008. DAN190 was originally intended to constitute PhD objectives for Mr Anderson who originally intended to study the role of the ecology of the Fusarium wilt fungus. Field experiments could only be completed in the first season of the project, the 2006/7 cotton season as Mr Anderson left the project at the beginning of the 2007/8 cotton season. The focus of the project was also changed substantially 9 months into the project due to the recommendations of the CRDC Disease Research Review panel in 2007. The following experiments were completed in the 2006/7 cotton season.

How does cultivar resistance affect survival and reproduction of the Fusarium wilt fungus?

Field experiments were established to address the impact of high cultivar resistance (F. rank) on the soil population of the Fusarium wilt fungus (Fov). Sicot F1 (high F rank) and Sicot 71 (average rank) were sown in six eight row strips in a field near Boomi, NSW. The population of Fov was determined before and after sowing by analysing soil samples from each plot with a selective medium called Komadas medium which is selective for *Fusarium oxysporum*. Plant survival was also measured in each cultivar. Plant survival was significantly higher in plots sown to Sicot F1 at 76% compared to 23% in plots sown to Sicot 71 ($P<0.001$). While inoculum of *F. oxysporum* increased under both cultivars (Figure 3.10), the increase was significantly reduced under Sicot F1 at 600cfu/g soil compared to Sicot 71 2400cfu/g soil ($P=0.005$). Interestingly there was no difference in initial stand between cultivars suggesting that the Fusarium wilt fungus did not contribute to initial stand loss in that field.

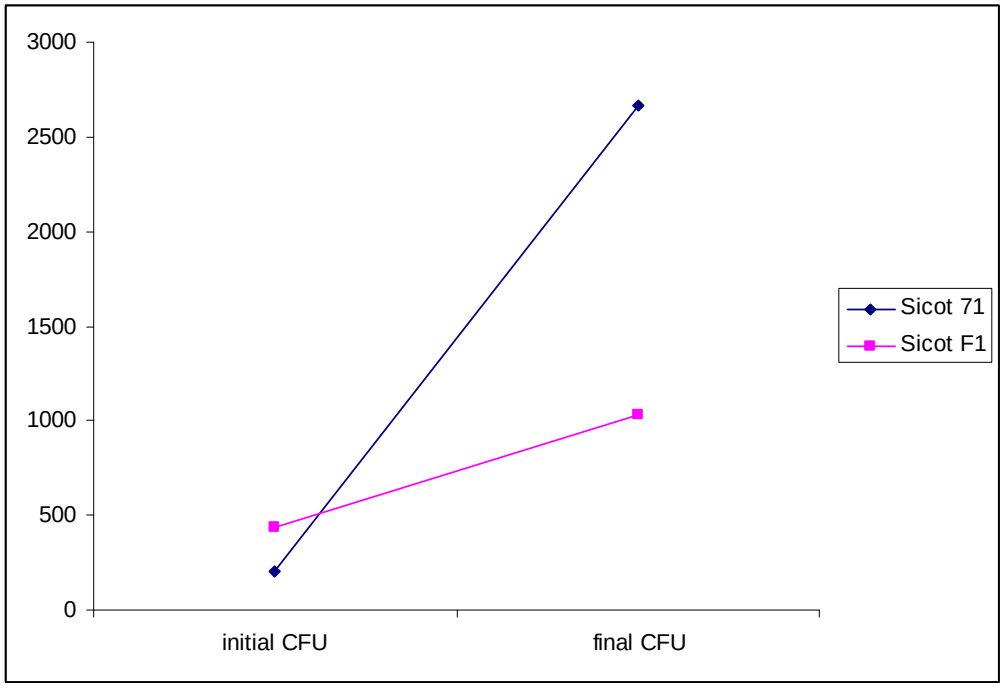

Figure 3.10. Average increase in soil inoculum levels of F. oxysporum.

Impact of systemic acquired resistance on the survival and reproduction of the Fusarium wilt fungus

Three large scale field experiments were conducted near Moree, Goondiwindi and Boomi to assess the effectiveness of acibenzolar-S-methyl (Bion ®) in reducing disease severity in cotton cultivars of varying resistance to Fusarium wilt. The experiment near Goondiwindi was destroyed by hail so data from only two experiments is discussed.

Conventional cotton varieties Sicala 43 (F rank 68), Sicala 45 (F rank 148), 189 (F rank 100) and F1 (F rank 209) were sown in 30m plots each 8 rows wide with or without Bion seed treatment. Each variety was replicated in 12 plots (six with Bion and six untreated). Variety choice significantly improved total survival over the season, with Sicot F1 outperforming all other varieties ($P<0.001$). However seed treatment with Bion did not improve plant survival in any varieties. Varietal choice did not have an impact on seedling survival.

A similar experiment was set up near Moree using transgenic cultivars Sicot 289br (F rank 103), Sicot 43br (118), Sicala 40br (111), Sicala V3br (79) with and without Bion seed treatment. Disease levels were low across the experiment and no significant differences in plant survival were observed at the end of the season.

Impact of climate and systemic acquired resistance on survival and reproduction of Fov

A large outdoor pot trial utilising garbage bins as pots was established at Boggabilla and Trangie to test the impact of climate and systemic acquired resistance on the survival and reproduction of Fov. 28 large garbage bins of soil were collected from a field near Goondiwindi known to contain several strains of Fov. The 28 bins were divided into two groups of 14 bins. One group was set up at Goondiwindi and the other at Trangie. Within each group, the plants in seven bins were treated with Bion® and the rest remained untreated. All bins were sown on the same date and watered simultaneously using automatic watering systems. This experiment would have enabled us to study the impact of both climate and Bion® on the populations of Fov in the soil, and also the impact of climate on the efficacy of Bion® in reducing the severity of Fusarium wilt and suppressing soil populations. However, the group of bins at Goondiwindi were destroyed by hail, and the plants at Trangie suffered salt toxicity due to high salt levels in the irrigation water, so the experiment was disbanded for the 2006/7 season. This experiment was not repeated due to a revision of project objectives.

Trash Management

A strip trial was set up during project DAN190 to test the impact of trash management on Fusarium wilt in the following crop. Cotton stalks were mulched and either incorporated immediately (early incorporation) or left on the surface for six weeks before incorporation (late incorporation). Stand counts were completed on 17 October 2008 and no difference in plant stand was observed between treatments indicating that early and late trash incorporation did not have an impact on seedling survival. Total survival could not be assessed as the trial was mulched immediately after picking over a weekend.

Conclusions

- Resistant cultivars suppress reproduction of the Fusarium wilt fungus and can help slow the build up of the fungus in fields.
- Seed treatment with Bion does not always increase plant survival in fields infested with the Fusarium wilt fungus.
- Trash management does not have an impact on stand establishment and Fov does not appear to be involved in the seedling disease complex.

Outcomes

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

Table 6. Achieved outputs and planned outcomes as identified in the project application

Project Outputs	Expected Science Outcomes	Expected Industry Outcomes	Contribution of Outputs to Outcomes
Quantification of the distribution and severity of diseases in the field.	Understanding of the factors contributing to epidemics.	Quantify the relative importance of disease threats and ensure adequate responses by the cotton industry, communities and government agencies.	Both outcomes were achieved. Data gathered during the annual disease surveys continues to contribute towards understanding of the factors contributing to epidemics For example, the impact of seasonal conditions on varietal resistance for Verticillium wilt was identified as a key factor contributing to an epidemic of the disease during this project. The surveys also continue to quantify the threat posed by endemic and emerging plant pathogens (See results section Objective 1).
Evaluation of new and existing seed treatment fungicides for seedling disease.	Greater understanding of the factors contributing to cotton seedling mortality.	Effective seed treatments for seedling disease with potentially reduced input of fungicide to the environment.	Both outcomes were achieved. The annual seed treatment fungicide trials provide data on the efficacy of current and novel fungicide seed treatments, potentially enabling optimisation of fungicide rates. For example there is evidence to support the removal of Fludioxonil from cotton seed treatments as it is consistently ineffective in seed treatment trials. The trials also regularly expose the dominant seedling pathogen in each field, and/or the influence of insects on seedling mortality eg. wireworm damage.
Evaluation of new and existing seed treatment fungicides for black root rot.	Greater understanding of the factors contributing to cotton seedling mortality and black root rot.	New tools for management of seedling disease in cool production areas.	Both outcomes were achieved. Several fungicides show promise for management of black root rot. These may be deployed in future field trials in cooler regions of NSW. Experiments also demonstrated the efficacy of Bion as seed treatment for black root rot.
Continuation of the long-term biofumigation experiment at the Australian Cotton Research Institute.	Demonstrate the potential for biofumigation as a preventive measure.	Tools for preventive control of black root rot; a demonstration site for extension to growers.	These outcomes were partly achieved. Biofumigation was not demonstrated as a potential preventative measure for black root rot due to seasonal conditions. The biofumigation trial will be continued into the future thus providing an excellent resource for students and growers.
Identification of the pathogens that cause seedling disease in cotton in Australia	Improved knowledge of the suite of pathogens that attack cotton worldwide.	Better ability to target IDM at pathogens.	Both outcomes were achieved. Several previously unknown pathogens were found to attack cotton including some new pathogens and variants of known seedling pathogens. The large variation in the seedling pathogen population reiterates the benefits of a

Project Outputs	Expected Science Outcomes	Expected Industry Outcomes	Contribution of Outputs to Outcomes
			risk mitigation "best-bet" approach to seedling diseases IDM in NSW.
Continuation of a long-term field experiment at ACRI with rotations of 1, 2, 3, 4 and 5 years of wheat, with evaluation of the effects on black root rot and Verticillium wilt.	Understanding of factors affecting the survival of *Thielvaviopsis* and *Verticillium* in soil.	New tools for management of black root rot and other pathogens.	These outcomes were partly achieved. Long term wheat rotation was not effective in reducing levels of *Thielaviopsis* or *Verticillium*. Thus wheat rotation could not be recommended as a new tool for management of black root rot. Survival of both pathogens over time was not influenced by wheat cropping in this experiment.
Continuation of a second long-term experiment investigating the contribution of cotton to VAM, and completion of several experiments testing the effectiveness of sacrificial cropping to improve VAM levels.	Greater understanding of (i) carbon cycling in soil and the persistence of mycorrhizal fungi and their carbon products, and (ii) soil ecology	Identification of the potential for managing soil ecosystems ('soil health'), including VAM fungi and their carbon by-products; contribute to cotton industry carbon audit	These outcomes were partly achieved. Carbon cycling was not investigated. However, results from several experiments indicate that survival of VAM fungi differs between fields and is therefore a function of soil ecology. The project identified a strategy for managing VAM levels in soil.

Technical advances and IP

Please describe any:-

a) technical advances achieved (eg commercially significant developments, patents applied for or granted licenses, etc.);
b) other information developed from research (eg discoveries in methodology, equipment design, etc.); and
c) required changes to the Intellectual Property register.

Not applicable.

Conclusion

Plant diseases at all stages of the cotton crop continue to threaten sustainable cotton production in Australia. The following is a summary of conclusions for each of the major disease threats and control strategies investigated in this project.

Seedling Disease

Cool wet conditions in September and October are associated with high levels of seedling mortality. There is a clear trend towards higher seedling mortality in the southern valleys of NSW and this is strongly correlated to long-term average minimum temperatures in October. There appear to be long-term trends between periods of higher seedling mortality and periods of lower seedling mortality and these may reflect early season conditions and planting date. Abiotic factors including chemical damage and wireworm can play a significant role in seedling mortality and stand loss if not managed effectively. Growers should continue to delay sowing to avoid cool conditions that favour seedling disease, and treat seed with both fungicide and insecticide to minimise the risk of stand loss from seedling disease and wireworm.

Seedling Pathogens in NSW

- *Fusarium* including *F. oxysporum, F. equiseti* and *F. compactum* is the most commonly isolated fungus associated with seedling disease in NSW, although most Fusarium species including the Fusarium wilt fungus do not cause severe disease and are likely to be opportunistic pathogens that invade seedlings that have been colonised by *Rhizoctonia* or *Pythium*.
- *Pythium* and *Rhizoctonia* are the most aggressive and widespread seedling pathogens in NSW.
- At least three variants of *R. solani* persist in cotton fields in NSW and can invade cotton seedlings.
- At least two of these variants can cause disease and probably contribute to stand loss in the field (AG4 and AG2-1).
- AG2-2 and AG3 are recorded on cotton for the first time.
- Other fungi including *Macrophomina phaseolina* and *Sclerotium rolfsii* occur sporadically in NSW cotton fields and may contribute to some damping off.
- Bi-nucleate *Rhizoctonia* also persist in NSW cotton fields but do not cause disease.
- There is substantial variation in growth rate and morphology among isolates growing at different temperatures with AG3 isolates favoured by cooler conditions and AG4 isolates favoured by warmer conditions and this probably reflects why AG3 is not more widespread on cotton throughout the world.
- There is evidence to suggest that AG3 isolates that occur in cotton in Australia and Greece belong to a cotton lineage within the AG3 subgroup of *R. solani* that may have evolved from AG3 populations on Solanaceae plants like potato.
- There may be evidence of fungicide tolerance in some populations of *Rhizoctonia* but this requires further investigation.

Black Root Rot

Black root rot continues to increase in incidence and severity in the southern valleys and is persistently severe in the Namoi and Macquarie valleys. The disease may be more severe under overhead irrigation. True spread of the disease may be masked by drought enforced fallows in recent years and the industry should expect an increase in black root rot severity as fields come back into production. Growers should continue to delay sowing to avoid conditions that favour black root rot and practice good farm hygiene to avoid spreading the pathogen, especially in southern NSW.

Fusarium Wilt

Fusarium wilt has now been reported on 83 farms in NSW. Improved varietal resistance has lead to an overall reduction in the severity of symptoms associated with Fusarium wilt. However, incidence (% plants) may still be high especially when climatic conditions favour infection. Three strains of the pathogen are present in NSW. Continued monitoring is required to ensure that known and new strains of the pathogen do not overcome varietal resistance. Farm hygiene is crucial in minimising the spread of known and new strains of the Fusarium wilt fungus.

Verticillium Wilt

Varietal resistance to Verticillium wilt is temperature dependent. Resistance breaks down under prolonged cool weather conditions. Integrated disease management measures including rotation with wheat and early incorporation of residues should be practiced in fields with a history of this disease to minimise the impact of resistance-breakdown during cool seasons. Farm hygiene is crucial in preventing further spread of Verticillium wilt.

Boll Rots and Alternaria Leaf Spot

Summer rainfall in 2009/10 lead to the highest recorded incidence of boll rot in NSW history. Phytophthora spp. were the most commonly isolated organisms from diseased bolls. Tight lock associated with insect feeding appears to be an increasing problem. Alternaria leaf spot is common and has little impact on crop health, but may have significant impacts on human health. Severe Alternaria can be associated with boll rots when lesions cause locules to fuse together. Growers should continue to manage crops to avoid rank canopy growth.

Hormone Damage

There is a strong correlation between high incidence and high severity of hormone damage in the upper canopy suggesting that the most severely affected fields are exposed to repeated doses of chemical throughout the season. The sharp decline in incidence and severity of hormone damage in 2009/10 may reflect the efforts of Cotton Australia in promoting responsible usage patterns and awareness of spray drift damage.

Emergency Plant Pests

Emergency plant pests continue to threaten sustainable cotton production in Autralia as evidenced by the detection of Solenopsis mealybug in QLD. Extensive surveillance did not detect the pest in NSW cotton fields. There continues to be no evidence of Texas Root Rot, Bacterial Blight, Cotton Leaf Curl Disease, Blue Disease, exotic strains of Fusarium wilt or

defoliating strains of Verticillium wilt in NSW. Industry awareness of biosecurity threats is crucial in preventing and/or successfully eradicating incursions of exotic plant pathogens. On-farm biosecurity and farm hygiene measures underlie whole of industry biosecurity awareness and preparedness.

Seed Treatment Fungicides for Seedling Disease

Seedling mortality is consistently higher in southern NSW, however seed sown in September and early October in northern NSW is likely to suffer equivalent rates of mortality. Dynasty CST continues to be the most effective fungicide combination. Fludioxonil consistently has no impact on seedling mortality and could be removed from current cotton seed treatments. Pathogen pressure varies between fields and therefore a best-bet approach is required to minimise the impact of early season stand loss on yield. Growers should continue to delay sowing to avoid cool conditions that favour seedling disease, and treat seed with both fungicide and insecticide to minimise the risk of stand loss from seedling disease and wireworm.

Seed Treatment Fungicides for Black Root Rot

Banrot, Baytan Plus, and Myclobutanil show promise as potential novel seed treatment fungicides but require field validation over several seasons. Bion is the only seed treatment available for black root rot and continues to provide best control of the disease when tested against other chemical treatments. Growers should continue to use Bion in combination with Dynasty as a seed treatment, especially when sowing into fields with high levels of black root rot.

Crop Rotation and Biofumigation for Black Root Rot

Rotation out of cotton with wheat did not have an impact on the severity of black root rot in subsequent cotton crops. This hypothesis should be tested further. Biofumigation can control black root rot, but the benefits of biofumigation may be masked in seasons that are not conducive to black root rot such as 2008/9. Growers should consider using biofumigation crops in fields with high levels of black root rot.

VAM in Cotton

VAM colonisation will not necessarily be affected by extended bare fallow. Rather, the impact of bare fallow on VAM colonisation appears to differ between fields. Black root rot does not reduce VAM colonisation. Growers can replenish VAM inoculum by growing a sacrificial crop eg. chick pea. Growers should test plant growth in fields suspected of being deficient in VAM by growing test strips of chick pea, linseed or cotton and comparing to growth in recently cropped soil.

Long Term Farming Systems Trial at ACRI

Seedling mortality is reduced in fields where crop residues are broken down prior to planting.

Conclusions of CRDC project DAN190

- Resistant cultivars suppress reproduction of the Fusarium wilt fungus and can help slow the build up of the fungus in fields.
- Seed treatment with Bion does not always increase plant survival in fields infested with the Fusarium wilt fungus.
- Trash management does not have an impact on stand establishment and Fov does not appear to be involved in the seedling disease complex.

Take Home Messages

The following are take home messages for the cotton industry derived from the conclusions outlined above:

- Growers should continue to delay sowing to avoid cool conditions that favour seedling disease, and treat seed with both fungicide and insecticide to minimise the risk of stand loss from seedling disease and wireworm.
- Growers should continue to delay sowing to avoid conditions that favour black root rot and practice good farm hygiene to avoid spreading the pathogen, especially in southern NSW.
- Incidence and severity of black root rot may increase under overhead irrigation, so growers should avoid installing overhead irrigation systems in fields with a history of the disease and take active steps to suppress the disease in overhead irrigated fields.
- Seedling disease is caused by a large and variable group of fungi.
- Varietal resistance and farm hygiene are key tools in continuing to reduce the impact of Verticillium and Fusarium wilt on cotton production. However growers should practice all aspects of integrated disease management for these diseases as varietal resistance can break down in cool seasons.
- Boll rot fungi have the potential to wipe out yield. Always manage the crop to avoid rank growth.
- Industry awareness of biosecurity threats is crucial in preventing and/or successfully eradicating incursions of exotic plant pathogens. On-farm biosecurity and farm hygiene measures underlie whole of industry biosecurity awareness and preparedness.
- Dynasty CST continues to be the most effective fungicide seed treatment for seedling disease.
- Fludioxonil is ineffective in controlling seedling disease and should be considered for removal from current cotton seed treatments.
- Several fungicides have shown promise as seed treatments for black root rot although further research is required. Bion continues to be the most effective seed treatment against black root rot.
- Rotation with wheat does not appear to reduce levels of black root rot.
- Biofumigation is an effective means of reducing the impact of black root rot, although benefits may be masked when conditions do not favour disease.
- Growers should test plant growth in fields suspected of being deficient in VAM by growing test strips of chick pea, linseed or cotton and comparing to growth in recently cropped soil.

These take home messages are a best bet approach to minimising the impacts of cotton diseases on sustainable cotton production and compliment existing Integrated Disease Management guidelines. Adoption of these take home messages will improve industry biosecurity and reduce the risk posed by exotic and endemic threats to Australian cotton production.

Extension Opportunities

Detail a plan for the activities or other steps that may be taken:

(a) to further develop or to exploit the project technology.

Not applicable.

(b) for the future presentation and dissemination of the project outcomes.

Results of the project will be presented at the 2010 CCC CRC Science Forum at the Crossing Theatre Narrabri on October 28 2010.

(c) for future research.

The annual cotton disease surveys should be continued indefinitely as they assist the industry to build a picture of current and emerging disease threats. The development of an effective seed treatment fungicide for black root rot should also be expedited through research.

Publications

A. Conference proceedings and publications

Anderson CMT (2010) Bacterial blight exotic strains. Plant Health Australia Factsheet. http://www.planthealthaustralia.com.au/pidd-docs/Bacterial%20blight%20FS.pdf

Anderson CMT (2010) Texas root rot. Plant Health Australia Factsheet. http://www.planthealthaustralia.com.au/pidd-docs/Texas%20root%20rot%20FS.pdf

Allen SJ, Anderson CMT, Wang B (2008) Current and future trends – a disease update. In 'Proceedings of the 14th Australian Cotton Conference'. Broadbeach, Australia.

Anderson CMT, Lonergan PA (2008) Seedling Disease – getting to the root of the problem. In 'Proceedings of the 14th Australian Cotton Conference'. Broadbeach, Australia.

Anderson CMT (2009) Ecology and taxonomy of *Rhizoctonia solani* from cotton in NSW. CCC CRC Science Forum, Narrabri, Australia

Anderson CMT (2009) Surveillance of endemic and exotic diseases in the cotton industry. FUSCOM workshop. Toowoomba, Australia.

Anderson CMT (2009) Ecology and taxonomy of *Rhizoctonia solani* from cotton in NSW. FUSCOM workshop. Toowoomba, Australia.

Press releases and media coverage

Early and late season disease survey results are released to the media each year through a press release. Press releases were published/aired in through the following outlets:
The Land newspaper
The Narrabri Courier p20 8 January 2009
Ag Today p4 29 January 2009
Area News p10 19 January 2009
NBN Tamworth
2TM Tamworth (interview)
ABC Rural Report 7 January 2009 (interview)
2MO Gunnedah, 2VM Moree
Southern Cross Rural News

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

Anderson CMT (2010) Bacterial blight exotic strains. Plant Health Australia Factsheet. http://www.planthealthaustralia.com.au/pidd-docs/Bacterial%20blight%20FS.pdf

Anderson CMT (2010) Texas root rot. Plant Health Australia Factsheet. http://www.planthealthaustralia.com.au/pidd-docs/Texas%20root%20rot%20FS.pdf

Part 4 – Final Report Executive Summary

The following industry take home messages are a summary of practical findings pertinent to growers and industry personnel:

- Growers should continue to delay sowing to avoid cool conditions that favour seedling disease and black root rot, and treat seed with both fungicide and insecticide to minimise the risk of stand loss from seedling disease and wireworm.
- Incidence and severity of black root rot may increase under overhead irrigation, so growers should avoid installing overhead irrigation systems in fields with a history of the disease and take active steps to suppress the disease in overhead irrigated fields.
- Seedling disease is caused by a large and variable group of fungi.
- Varietal resistance and farm hygiene are key tools in continuing to reduce the impact of Verticillium and Fusarium wilt on cotton production. However growers should practice all aspects of integrated disease management for these diseases as varietal resistance can break down in cool seasons.
- Boll rot fungi have the potential to wipe out yield. Always manage the crop to avoid rank growth.
- Industry awareness of biosecurity threats is crucial in preventing and/or successfully eradicating incursions of exotic plant pathogens. On-farm biosecurity and farm hygiene measures underlie whole of industry biosecurity awareness and preparedness.
- Dynasty CST continues to be the most effective fungicide seed treatment for seedling disease.
- Several fungicides have shown promise as seed treatments for black root rot although further research is required. Bion continues to be the most effective seed treatment against black root rot.
- Rotation with wheat does not appear to reduce levels of black root rot.
- Biofumigation is an effective means of reducing the impact of black root rot, although benefits may be masked when conditions do not favour disease.
- Growers should test plant growth in fields suspected of being deficient in VAM by growing test strips of chick pea, linseed or cotton and comparing to growth in recently cropped soil.

These take home messages are a best bet approach to minimising the impacts of cotton diseases on sustainable cotton production and compliment existing Integrated Disease Management guidelines. Adoption of these take home messages will improve industry biosecurity and reduce the risk posed by exotic and endemic threats to Australian cotton production.