DISEASES UPDATE

Stephen J. Allen
Cotton Seed Distributors Ltd.
Australian Cotton Research Institute
Narrabri

Disease Surveys

Staff of the NSW Department of Primary Industries, Cotton Seed Distributors Ltd. and the Queensland Department of Primary Industries and Fisheries annually survey the distribution, incidence and severity of diseases in commercial cotton crops in all production areas of Eastern Australia. The results of these surveys provide an invaluable guide for planning and evaluating pathology research.

Cotton diseases caused by soil borne pathogens have emerged as significant challenges for growers, consultants and pathologists. This does not indicate 'ruined' soils or impaired soil health. It does indicate that our cotton growing systems provide favourable conditions for the survival and dispersal of soil borne pathogens.

Verticillium wilt - *Verticillium dahliae*

Verticillium wilt continues to be a significant problem, particularly in the Namoi valley of NSW where the mean incidence of the disease continues to reflect the 'V.rank' of the cotton varieties being grown (Figure 1). The NSW DPI survey of 2005-2006 found Verticillium wilt in over 80% of the fields inspected in the Namoi Valley with a mean incidence of 10.1% of plants affected.

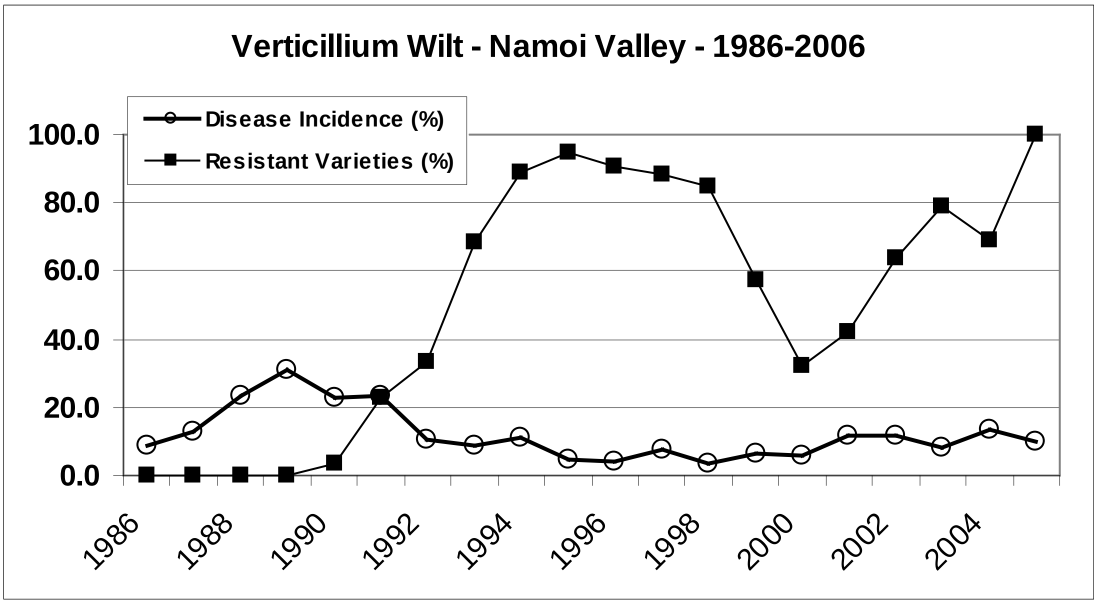

Figure 1. The incidence of Verticillium wilt and the use of resistant varieties (V.rank>90) in commercial cotton crops in the Namoi valley of NSW 1986-2006. (NSW DPI Annual Disease Surveys. Courtesy David Nehl)

Environmental conditions also have a major effect on the incidence and severity of Verticillium wilt. Even the more resistant varieties of cotton appear susceptible following periods of cool weather. However, repeated use of the more resistant varieties results in a decline in the incidence of Verticillium wilt.

Black root rot - *Thielaviopsis basicola*

Black root rot has now been confirmed as present in all cotton production regions in Eastern Australia. The disease is favoured by cool conditions early in the season and is therefore more prevalent in the southern areas. The NSW DPI 2005-2006 survey reported black root rot in 37 of the 43 fields inspected in the Namoi, Macquarie, Lachlan and Murrumbidgee Valleys with the mean incidence in the Namoi and Macquarie Valleys being 54% and 29% respectively (Figure 2). It is possible that the drought-enforced fallows and long fallows in the Macquarie valley have contributed to the decline in mean incidence over the last four seasons.

Black root rot does not kill seedlings. Affected plants are delayed in their development and will 'catch up' at the end of the season provided the environmental conditions allow.

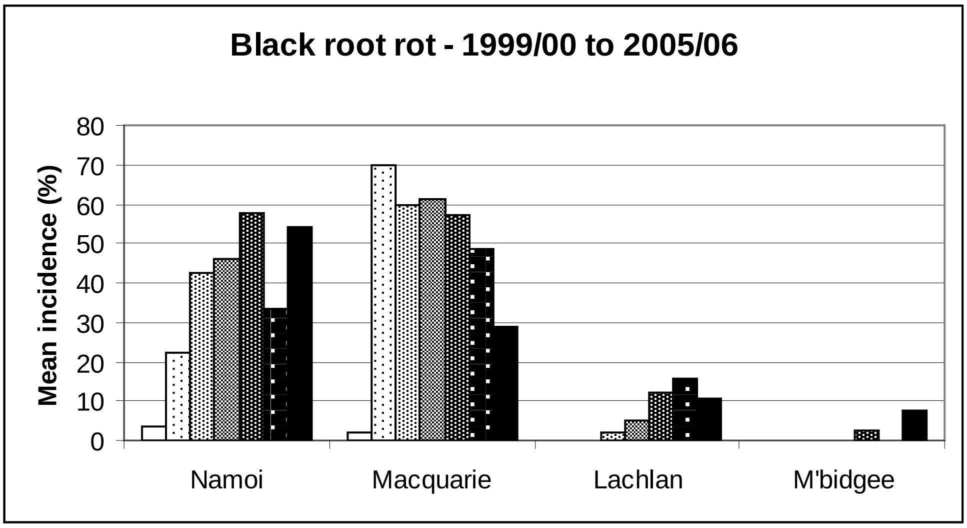

Figure 2. The incidence of black root rot in commercial crops in the Namoi, Macquarie, Lachlan and Murrumbidgee Valleys for each of the last seven seasons. (NSW DPI Annual Disease Surveys. Courtesy David Nehl)

Fusarium wilt - *Fusarium oxysporum* f.sp. *vasinfectum* (Fov)

Fusarium wilt has not yet been reported from the Emerald, Tandou, Lachlan, Murrumbidgee and Lower Namoi production areas. The disease is widely distributed in all other areas. The mean incidence is generally low for all areas except the Darling Downs of Queensland (Figure 3).

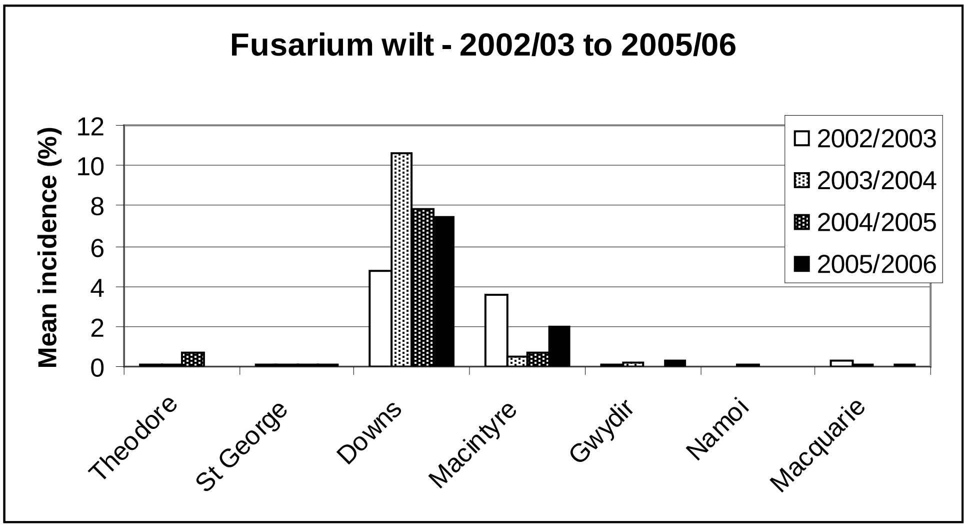

Figure 3. The incidence of Fusarium wilt in commercial crops for each of the last four seasons. (NSW DPI Annual Disease Surveys. Courtesy David Nehl)

Higher levels of resistance in commercial varieties, less favourable environmental conditions and increased attention to farm hygiene may have contributed to a lower incidence in recent seasons. There has also been some reticence on the part of growers to submit samples. Consequently the number of samples received by the QDPI&F Fusarium diagnostic team at Indooroopilly has declined considerably in recent years (Figure 4).

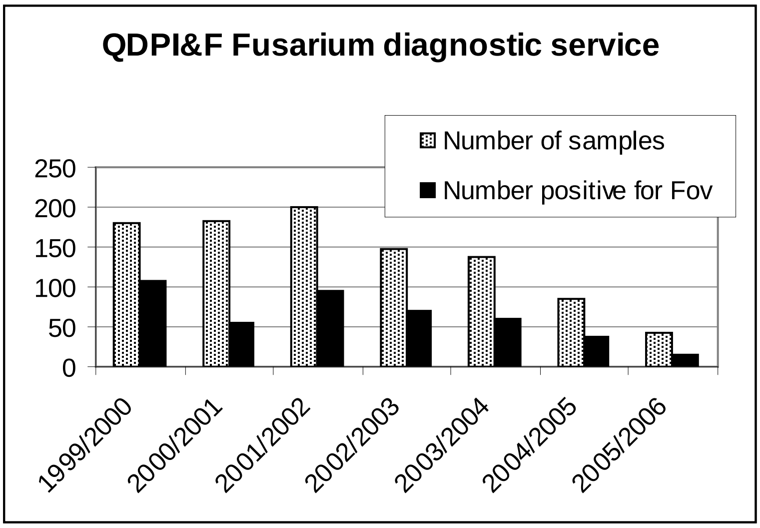

Figure 4. The number of samples submitted and the number of samples that were positive for Fusarium wilt for each season from 1999/2000 to 2005/2006. (Courtesy Linda Smith)

Significant Developments/New Initiatives in Cotton Pathology

The Plant Breeding Teams

Cotton breeders regard resistance to Fusarium as one of the most important selection criteria in their programs. The CSIRO and Deltapine breeding teams continue to produce varieties with better levels of resistance to Fusarium wilt. Varieties with F.ranks in excess of 120 are available in conventional, Bollgard, Roundup Ready and Pima backgrounds. Varieties with V.ranks over 100 are readily available.

The Queensland DPI&F Team

Joe Kochman, Linda Smith, Linda Swan, John Lehane, Dan Carrick and Greg Salmond

A new strain of Fov

A new strain of *Fov* in the Macintyre valley was collected by Dr David Nehl and characterized by QDPI&F, Indooroopilly. The isolate gave negative results for VCG analysis in 2005 but was shown to be pathogenic to cotton in a glasshouse pathogenicity test on Siokra 1-4. This isolate was characterized using two molecular techniques to determine if the isolate belonged to either VCG 01111 or 01112, or was a new strain. A neighbour-joining phylogenetic tree was generated and showed that the unknown *Fo* isolate was genetically different from known Australian isolates. The unknown *Fo* isolate is more similar to VCG 01112 than it is to VCG 01111 and clearly different to all of the overseas isolates tested.

Non-pathogenic Fusarium oxysporum (NPFo)

The literature provides considerable support for the use of non-pathogenic strains of *Fusarium oxysporum* as biological control agents of Fusarium wilts. Isolates of NPFo were therefore recovered from roots and stem of cotton not showing symptoms of disease but growing in diseased fields. Isolates of NPFo recovered from native cotton species by Dr. Bo Wang were also investigated for their biological control potential. A bioassay was developed to assess the biological control potential of these isolates. To date, none of the isolates tested cross-protected cotton seedlings against *Fov*. However, two NPFo isolates significantly increased plant emergence in field soil naturally infested with *Pythium* spp.

Crop rotation trials

Crop rotation trials are in progress. Results highlight the importance of a fallow preceding a cotton crop grown on Fusarium wilt infected soils. Significantly more cotton plants survived until maturity following a bare fallow rotation compared to cotton following either maize, cotton or sorghum crops. Greatest plant death and severity of disease in cotton occurred where soybean and mungbean crops had previously been grown. Residue and organic matter levels may influence field pathogen survival and disease incidence.

Evaluation of germplasm

Some 20 hectares (8000 plots) of field trials were planted near Cecil Plains in November 2005 and harvested in May 2006. These included trials to assess germplasm from Dr Ulloa's USDA program in California, as well as material from the CSIRO and Deltapine breeding programs. Preliminary yield data from some plots of experimental lines were excellent but, most of the USDA lines were very susceptible with selections of germplasm made from only three of the 40 lines.

Role of silicon

In glasshouse trials using field soil naturally infested with *Fov,* applications of Potassium silicate, magnesium silicate (Silvine) and acidified calcium silicate (acidified Wollastonite) have significantly reduced the severity of Fusarium wilt in cultivar Siokra V17. Results from a second glasshouse trial have shown that application of phosphorus at a rate in excess of what is required for plant growth significantly increased severity of Fusarium wilt. Other researchers have shown that the application of phosphorus to soil reduces the availability of silicon from an applied source when phosphorus and silicon are in close proximity. Current investigations seek to determine if phosphorus reduces plant uptake of silicon thereby affecting the ability of the plant to respond effectively to fungal invasion.

A field trial in 2004-2005 showed that soil application of silicon (as potassium silicate and magnesium silicate) increased Si uptake as shown by leaf nutrient analysis but there was no effect on disease severity or cotton seed yield. The threshold level of silicon required for disease control may not have been met in this trial. The trial was repeated in 2005–2006 with both Sicot F-1 and Sicot 189. There were no differences in cotton seed yield between treatments for variety Sicot F-1 (P=0.676) but there was a trend towards treatment differences for Sicot 189 (P=0.060).

The CSIRO Canberra Team

The potential for native Fusarium to give rise to new cotton field pathogens

Bo Wang, Curt Brubaker, Walter Tate, Matt Wood, and Jeremy Burdon

Previous work has shown that the *Fov* genotypes causing Fusarium wilt of cotton in Australia evolved from a lineage of native *Fusarium oxysporum* – Lineage A. Our current project aims to determine 1) the prevalence of Lineage A in cotton fields; 2) the process of virulence evolution among Lineage A isolates; and 3) the competitive interactions among *Fov* genotypes. Surveys across six major cotton growing regions demonstrated that Lineage A occurs in cotton field soils and in uncultivated soils near cotton fields and that some Lineage A isolates are genetically similar to *Fov* infecting cotton plants. In the glasshouse, some Lineage A isolates were able to increase their virulence after ten successive infection--reisolation cycles on susceptible cotton plants, suggesting that they have the potential to become more aggressive over time.

It was also determined that VCGs 11 and 12 differ in the ability to infect cotton plants. While the severity of the wilting symptoms was similar, a VCG 11 isolate infected more plants than a VCG 12 isolate when the plants were challenged with an inoculum that was comprised of equal quantities of both isolates. In addition, a recently detected VCG 11 genotype showed differential preference to tolerant cotton cultivars . The new isolate, I-16, whilst less virulent overall, was more aggressive on the tolerant cultivar, Sicot 189, than it was on the susceptible cultivar, Siokra 1-4. This implies that the interaction between *Fov* genotypes may be influenced by the resistance level of cotton cultivars.

Genetics of Fov resistance in cultivated Gossypium

Augusto Becerra, Bronwyn Matheson, Walter Tate and Curt Brubaker

The purpose of this project is to provide cotton breeders with new tools and knowledge that they can use to maximize the potential of currently identified sources of Fusarium wilt resistance. The focus is on identifying quantitative trait loci (QTLs) contributing to fusarium wilt resistance and their mode of action in cultivated cotton. The QTL genetic analyses of Fusarium wilt resistance will incorporate molecular marker identification so that cotton breeders can more effectively apply the information obtained for marker-assisted selection in fusarium wilt resistant cotton breeding.

The Melbourne Teams

Hexima/University of Melbourne, School of Botany

Robyn Heath, Jillian Hinch, James McKenna, Bruce McGinness, Christina Hall

Field trials to evaluate transgenic cotton lines expressing the anti-fungal NaD1 defensin gene are planned for each of the next three seasons at sites on the Darling Downs of Queensland and in NSW. The trials will target Fusarium wilt, black root rot and Verticillium wilt. The OGTR license for these trials is expected to be issued in late August. A study of the infection process of Fov has been completed.

Hexima/La Trobe University, Department of Biochemistry

Marilyn Anderson, Fung Lay, Sophie Cole, Kate Carroll, Phuong Nguyen, Nicole van der Weerden.

This research group are focused on the mechanism of action of defensins, the isolation and analysis of antimicrobial molecules and the production and targeting of defensins in plants.

The NSW DPI Team

David Nehl, Susannah Driessen, Chris Anderson, Peter Lonergan, Tracey Mor.

There was no lack of mycorrhizal colonisation of cotton after three and four years of bare fallow at Bourke. Following three years without cotton, the incidence of black root rot in a field at Bourke decreased from 70% of plants (2001-02 season) to 2% of plants (2005-06 season).

Different seedling pathogens vary in dominance from field to field and year to year; the fungicide treatment Dynasty™ is consistently performing as well as the standard fungicides. In cooler regions of NSW, application of the fungicide azoxystrobin has potential to improve stand establishment, if the seedling pathogens affected by this fungicide are active.

Black root rot

Delaying sowing as late as possible can decrease the period of exposure of cotton to conditions that favour black root rot and seedling disease, leading to substantial decreases in disease severity. There was no effect of pre-plant and at-planting herbicides on the severity of black root rot of cotton.

Field experiments confirmed the capacity of Bion®, applied with the standard seed coatings, to activate resistance against black root rot. A comparison of the effectiveness of Bion® in all experiments conducted by Cotton Seed Distributors and NSW DPI in the 2005-06 season suggests that the resistance response induced by Bion® is more effective when disease severity is moderate and less effective when disease severity is high.

Observations of the relationship between soil type and the incidence of black root rot were commenced in a field at Warren. In one area of the field (lighter soil) the incidence of black root rot was negligible, despite severe symptoms elsewhere in the field (heavier soil). These observations in 2006 matched similar observations in 1999 at the same site, indicating that the pathogen had failed to establish in the lighter soil after a prolonged presence at high levels in adjacent areas of heavier soil. The suppressive nature of this soil is being investigated.

Fusarium wilt – Black root rot interaction

The results of in-field pot experiments using soil naturally infested with the Fusarium wilt pathogen and added inoculum of the black root rot pathogen indicated that there is no interaction between Fusarium wilt and black root rot.

Fusarium symptoms

Appearances can be deceiving. Looking at the plants from the outside is not always a good indicator of how Fusarium wilt is affecting plants on the inside. The appearance of the external symptoms of Fusarium wilt may lag well behind the progress of internal infection by the pathogen. External symptoms may either not appear at all or not appear until later in the season.

Delayed sowing and Trash management and Fusarium wilt

Climatic conditions in November are usually warmer than in October and therefore delayed sowing can be an effective tool for reducing the incidence of Fusarium wilt. Transgenic cotton varieties with high early retention may enable yield potential to be maintained, with delayed sowing. Retaining trash on the surface for as long as possible before incorporation OR burning cotton stubble, can also be effective in reducing the incidence of Fusarium wilt in the subsequent cotton crop.

Dispersal of Fusarium oxysporum

An estimated 20,000 colony forming units of *F. oxysporum* per litre were detected in irrigation water collected as it entered the tail drain. Inoculum levels reduced with passage of that water through the return system and storage dam. *F. oxysporum* also moved around the farm in mud adhering to floating trash (estimated 160 million colony forming units per kg trash). Minimizing tailwater and the elimination of trash from the return system is paramount to reducing the spread of Fusarium wilt around the farm.

The UNE Team

Lily Pereg-Gerk, David Backhouse, Margaret Katz, Jason Moulynox, Joelle Coumans-Moens, Samiya Al Jaaidi.

Black root rot pathogenicity and biocontrol

"We are analysing factors involved in pathogenicity of *T. basicola* towards cotton using molecular methods, including genetics and proteomics. We have managed so far to genetically transform *T. basicola* and construct a few mutants that show reduced pathogenicity towards cotton. We are planning to use this method to construct a larger library of mutants with reduced pathogenicity. We also established the conditions for extracting and mapping of both cotton and *T. basicola* proteins, a tool which can be used in identifying plant and fungal proteins involved in pathogenicity. Molecular tools established, combined with the study of the interaction of the wild-type fungus and its mutants with cotton, could lead to recognition of plant factors which are involved in *T. basicola* pathogenicity and to targeted breeding of resistant plants. Another initiative is the project on biocontrol of black root rot using bacteria, proteins and decoy plants/crops that suppress the disease."

The CSD Team

Stephen Allen, Greg McNamara

Rotation with cereals

Winter cereals such as wheat, oats and barley are the preferred rotation crops for over 80% of Australian cotton growers and cereal residues appear to have a significant impact on the epidemiology of Fusarium wilt of cotton. Results of a previous study showed that there was a significant increase in the incidence of Fusarium wilt in the subsequent cotton crop after either a wheat or barley rotation when compared to a long bare fallow. The use of long, bare fallows is not considered to be an economic or practical option and therefore the management of cereal residues is an important issue. The results of two trials near Brookstead suggest that cereal residues should be burnt, baled or incorporated into the soil as soon as possible after harvest to minimize the saprophytic build-up of the pathogen. It is possible that incorporation into the soil might not be as effective under dry seasonal conditions.

Repeated use of high F.rank varieties

Soil bioassays of inoculum levels after three seasons and disease assessments after three and four seasons have shown that repeated use of Sicot F-1 has resulted in a decline in inoculum levels and disease incidence while repeated use of Sicot 189 has contributed to an increase in inoculum levels and increasing disease incidence.

Re-establishment after summer flooding

A field in the Macintyre valley was flooded during the summer of 2000/2001 and Fusarium wilt was almost eradicated. The re-establishment of the disease in the field has been monitored with each crop of cotton grown in the field. After two crops of cotton, a barley fallow and two further crops of cotton Fusarium wilt was detected at 56% of the 63 assessment points and 9.8% of plants were affected. The results of this study highlight the impact of repeated crops of cotton, a barley rotation, the use of varieties with an F.rank over 100 and a hot season on epidemic development.

Effectiveness of Integrated Disease Management

The incidence of Fusarium wilt of cotton was monitored in a field near Boggabilla in 1997/98 (20%), 1998/99 (52 %), 1999/00 (41%), 2002/03 (43%). The grower imposed a crop rotation, delayed planting until 21st October, 2005, planted Sicot F-1 which has an F.rank of 209(18) and used the Bion seed treatment. The incidence of Fusarium wilt in the 2005/06 cotton crop was 5%.

Bion seed treatment

28 large scale trials were established under an APVMA permit to evaluate the efficacy of Bion seed treatment for the control of black root rot, Fusarium wilt and Verticillium wilt of cotton. Despite seasonal conditions that did not favour the pathogens the Bion seed treatment significantly reduced black root rot severity in 10 out of 15 trials and seedling death caused by Fusarium wilt in 4 out of 6 trials. Seed cotton yields were substantially increased in 8 out of 19 trials. The Bion seed treatment appeared to have no effect on Verticillium wilt.

Other field trials

Other field trials in the 2005/06 season investigated the effects of fruit load, plant density, foliar Zinc applications, mineral rock dust and Trichoderma biocontrol formulations on the incidence of Fusarium wilt.

Integrated Disease Management

'Integrated Disease Management' involves the selection and application of a harmonious range of disease control strategies that minimize losses and maximize returns. Each of the disease control strategies by itself is not able to provide adequate control. However, when several such strategies are used in combination then acceptable control is achieved.

Integrated Disease Management for Verticillium wilt

Choose varieties with V.ranks over 100
Manage for earliness
Avoid late season irrigations
Incorporate cotton residues soon after harvest
Rotate with non-hosts such as cereals or sorghum
Control alternative weed hosts
Minimize your tailwater
Always practice good farm hygiene

Integrated Disease Management for Black root rot

Choose varieties that can 'catch up'
Use Bion seed treatment
Prepare beds well ('high and firm' not 'low and loose'!)
Pre-irrigate and/or plant into moisture
Delay planting if possible
Rotate with non-hosts for up to 3 years
Avoid legumes and control weeds
Effective biofumigation with vetch or mustard
Minimize your tailwater
Always practice good farm hygiene
Summer flooding if possible

Integrated Disease Management for Fusarium wilt

Plant a high F.rank variety with Bion seed treatment
Delay planting to the end of October
Avoid cultivating with knives
Retain cotton residues on the surface for 60 days
Bare fallow rotation is best
If using a cereal rotation then burn, bury or bale cereal residues ASAP
Minimize your tailwater
Always practice good farm hygiene
Summer flooding if possible

Acknowledgements

I acknowledge the input of my fellow pathologists who have volunteered their contributions. Without financial support from the CRDC, CRC and CSD and willing cooperation from numerous cotton growers and consultants none of this work would have been possible.