The Fusarium threat – are we making the progress?

Joe Kochman[1, 5, 6], Linda Swan[1, 5], Natalie Moore[2, 5*], Suzy Bentley[4, 5], Wayne O'Neill[2, 5], Anthony Mitchell[1, 5], Neale Obst[1, 5], John Lehane[1, 5], Lisa L.Gulino[4, 5] and Greg Salmond[3, 5]

[1] Farming Systems Institute, DPI, 203 Tor St, Toowoomba, Q 4350.

[2] Farming Systems Institute, DPI, 80 Meiers Rd, Indooroopilly, Q 4068

[3] Farming Systems Institute, DPI, cnr Drayton and Cunningham Sts, Dalby, Q 4405

[4] CRC for Tropical Plant Protection, Molecular Diversity and Diagnostics Research Laboratory, Indooroopilly Research Centre, 80 Meiers Road, Indooroopilly Qld 4068

[5] Australian Cotton CRC. [6] CRC for Tropical Plant Protection

*Dr Moore accepted a position with NSW Agriculture in Grafton in January 2002.

Introduction

Fusarium wilt of cotton was first identified on the Darling Downs in Queensland almost 10 years ago in March 1993. This destructive disease of cotton is caused by a soil-inhabiting fungus, *Fusarium oxysporum* f.sp. *vasinfectum* (*Fov*), and two different strains of the causal pathogen have since been described in Australia (Kochman, 1995; Davis *et al.*, 1996; Kochman, *et al.*, 1998). The two different genotypes (strains) identified amongst the Australian isolates of *Fov* corresponded to Vegetative Compatibility Groups (VCGs) 01111 and 01112. At present, each of these strains appears to be equally capable of causing disease in the current commercial varieties in Australia, but this may not be the case with new varieties in the future. The two Australian genotypes are distinct from all overseas strains of *Fov* and the other species of *Fusarium* examined thus far.

The disease has been found in new areas every season since 1993. During the 2001/2002 season, new recordings of *Fov* were confirmed in the Brewarrina (NSW) district and in Pima cotton at Bourke. Pima cotton was also devastated by the disease in the trials at "Cowan" near Cecil Plains in Queensland. The disease was identified on more farms at Brookstead, Dalby, Goondiwindi, Toobeah, St George and Theodore in Queensland as well as Moree, Bourke, Carroll and Warren in New South Wales. No records of *Fov* have yet been made from the production areas of Emerald in Queensland, Tandou or Hillston in New South Wales or in Western Australia. No new strains of *Fov* have been identified amongst the specimens received to date.

The disease has caused severe losses, particularly in susceptible varieties. Fusarium wilt is considered by many growers, ginners, consultants and other industry personnel to be the most important constraint to sustainable cotton production to have developed in recent years.

An increasing research effort has been mounted to counter the threat of this disease. Currently researchers from many agencies are collaborating in this effort. They include the

Queensland Department of Primary Industries (QDPI), CSIRO, Australian Cotton Cooperative Research Centre (Australian Cotton CRC), the Cooperative Research Centre for Tropical Plant Protection (CRCTPP), New South Wales Agriculture (NSW Ag), Cotton Seed Distributors (CSD) and Deltapine. This paper will discuss some of the problems encountered and progress achieved to date.

Characterisation of *Fov* in Australia

The species *F. oxysporum* includes many plant pathogens that cause wilt diseases in a broad range of agricultural and ornamental plants, as well as saprophytic forms. Morphologically, pathogenic strains of *F. oxysporum* are indistinguishable from saprophytic forms so other techniques have been developed to identify pathogenic strains. Davis *et al.*, 1996, used pathogenicity tests, VCG analyses, aesculin hydrolysis, relationships between growth and temperature and volatile compound production to characterise the *Fov* isolates initially collected from the Darling Downs. The data indicated that the Australian isolates differed to foreign isolates of the fungus.

Subsequently, DNA fingerprinting of genomic DNA, and RFLP and DNA sequence analysis of the ribosomal (r) DNA were used to analyse genetic variation among Australian isolates of *Fov*, and isolates of *Fov* from overseas (Bentley *et al.* 2000). DNA fingerprinting analysis confirmed two different genotypes amongst the Australian isolates of *Fov* that corresponded to VCGs 01111 and 01112. These molecular techniques have been used to thoroughly analyse genetic diversity among Australian and overseas isolates of *Fov*, from the genus to the strain-specific taxon levels. This information is being used in the development of a PCR-based DNA diagnostic system specific for the Australian genotypes of *Fov*.

There has been significant progress in the development of this test. It has been successfully applied to identification of *Fov* directly from plant material, soil, water, and cottonseed, and is now in a validation phase. This test will be a particularly useful tool to monitor the distribution and spread of *Fov* throughout the cotton production areas of Australia, and to evaluate the effectiveness of different agronomic practices (e.g. crop rotation) on the survival of *Fov* in affected fields.

Factors affecting disease development.

A number of factors have been associated with enhanced risk of fusarium wilt development. These include, variety selection, growing conditions, introduction of the pathogen to new areas, presence of particular weeds in fields and some agricultural practices. There are still many aspects that are not well understood and confounding results continue to occur.

Seasonal conditions

Cold shock conditions prevailed throughout many of the cotton growing areas during November 2001, severely stressing seedlings, and resulting in significant seedling death. *Fov* was associated with 30 to 40% of these deaths in Fusarium wilt affected areas with *Rhizoctonia* and *Thielaviopsis* (Black Root Rot) isolated from the other diseased seedlings. These conditions were ideal for *Fov,* which is a stress related pathogen, meaning that cotton plants are often able to resist attack until they become stressed. These conditions were undoubtedly a factor in the number of new recordings this season.

Planting at dates to reduce the risk of cold shock conditions and thus reduce the risk of seedling diseases, including *Fov*, has had some success. The practice of very early planting, particularly on the Downs and in the southern areas, may need to be reviewed in the light of increased seedling disease problems.

Resistant varieties

The identification and selection of resistant germplasm and development of resistant varieties has been a major component of the research effort since the first field trials conducted by QDPI officers showed that there were some very susceptible varieties on the Australian market. Evaluation of varieties and germplasm has continued and expanded from about 100 plots on two hectares in 1994/95 to more than 5000 plots over about 25ha at "Cowan", Graham and Wendy Clapham's property on the Darling Downs, during the 2001/02 season.

There have been a number of sources of resistance identified in germplasm both from overseas and within Australia and these have been used in breeding programs. During 1995/6, seed of 11 wild species of cotton, native to Australia, were obtained by QDPI from Dr Tony Brown from the Centre for Plant Biodiversity, CSIRO in Canberra. These were tested for reaction to *Fov* under controlled conditions in the glasshouse at Indooroopilly. Although some of these wild species were susceptible, plants of seven species including *Gossypium bickii, G. nelsonii, G populifolium, G. pulchellum, G. robinsonii, G. rotundifoium,* and *G. sturtianum*, showed some resistance. This information was passed back to CSIRO and used by Dr Curt Brubaker and his team in Canberra in a breeding program to introduce the resistance from these wild species into cultivated cotton. While there is still much more work to be done before this resistance is available in commercial varieties, Dr Brubaker's team has made considerable progress with accessions evaluated in the QDPI glasshouse at Indooroopilly and more than 150 selections from inter-species crosses being screened in the field trials at "Cowan" last season.

Both cotton breeding programs in Australia (CSIRO and Deltapine) are continuing major efforts to breed for resistance to the Australian strains of *Fov*. The best of the current commercial varieties (Sicot 189 and DeltaEMERALD) have only partial, and not complete resistance. This means that some losses can be expected when growing these varieties on severely affected farms, but not as much as if susceptible varieties are grown.

The issue of disease resistance in all new varieties, including transgenic breeding lines, is of major concern to the industry. There has been good progress made in the selection and development of resistance in varieties, with some new selections showing much higher levels of resistance than the industry standards. In addition, there have been significant improvements in some of the Ingard® varieties. However, we still have a way to go, with results from last season indicating that some of the herbicide resistant varieties being developed have about 75% of the resistance of their conventional counterparts, so further breeding and selection is required. The introduction of Bollgard II® varieties will also require very significant screening and selection of these varieties in *Fov* infected sites to ensure they maintain at least the same level of resistance as their conventional counterparts.

In response to requests from growers and consultants, a standard rating scheme to assess disease reaction has been developed, and continues to evolve, during the course of the field trials. The Cotton Fusarium Wilt Working Committee (FUSCOM) has used this scheme to develop a Fusarium wilt ranking for cultivars. The Fusarium wilt ranking will express the disease resistance performance of new varieties as a percentage of the performance of nominated industry benchmark varieties. There has been significant progress with the introduction of this ranking system but it will need to be used cautiously. Several factors, such as disease pressure at a location, environmental conditions and seed quality, can all affect a rating. Results from multiple sites, and several seasons will need to be collected to improve confidence in the Fusarium wilt ranking. This ranking has been designed to allow cumulative results from trials to be incorporated into the Fusarium wilt rank value.

Seed treatments

Fov can be seed-borne and both seed companies took this fact into account as soon as the disease was identified, with precautions being taken to ensure seed production only occurred in disease free areas. Hence, it is essential that seed companies be informed of any new affected areas. To date the fungus has not been isolated from seed collected from plants not showing symptoms of infection.

Seed harvested from infected plants during the1999/2000 and 2000/2001 seasons was plated at one month intervals in laboratory tests conducted at Toowoomba. In fuzzy seed, the initial infection levels were 56% in 1999/2000 and around 50% in 2000/2001. The proportion of *Fov* in seed was reduced with storage time. No *Fov* was found in either fuzzy or acid delinted seed, six months after harvest from plants infected with *Fov*, and storage at room temperature. Other species of *Fusarium* survived for longer periods but their numbers were also reduced in storage, particularly in acid delinted seed where the percentage of infested seed fell from 47% to 26%. These results augur well for preventing the dispersal of *Fov* in seed lines, by relatively short-term storage.

During 2001 the United States of America Plant Quarantine system deemed the two Australian strains of *Fov* to be prohibited pathogens, threatening the export of some 300,000 tones of fuzzy cottonseed to California, USA. This export trade had been built up over a number of years and is currently worth about $100 million annually. As a result exporters have to prove that the fuzzy seed is free of *Fov*. All export lots have had to be treated with methyl bromide for insect control so two major seed plating studies were undertaken at Indooroopilly to investigate the efficacy of methyl bromide in eliminating *Fov* in fuzzy seed. These studies were conducted in conjunction with GRAINCO.

Fumigation was found to eliminate all *Fusarium oxysporum* when methyl bromide was used at the appropriate concentration for the appropriate length of time.

Effect of farming practices on survival of *Fov*

Exclusion of the pathogen and farm hygiene practices

The *Fov* pathogen survives effectively in soil, even in the absence of cotton plants, for many years by producing thick-walled resting spores (chlamydospores) and also thin walled, shorter-lived spores (conidia). Estimates suggest that a gram of infested soil may contain up to 5000 spores of *Fov*. The first line of defence for disease free farms and production areas is to avoid the introduction of the *Fov* into the soil.

The disease can spread from field to field, farm to farm and even region to region. Spores of the fungus are effectively spread over long distances in infested soil attached to boots, vehicles, farm machinery and equipment and also in water (irrigation and over-land flows). It can also be transferred in infected plant material.

There has been a major extension effort by researchers, extension officers, consultants and grower bodies, to provide information about *Fov* to the industry. The "COME CLEAN GO CLEAN" campaign has been waged in every cotton growing area in Australia. Details of vehicle cleaning agents and procedures have been extensively detailed in industry publications and grower information meetings (Kochman *et al.*, 1998; O'Neill, 1999; Moore and O'Neill, 2000, Salmond, *et al.*, 1998; Salmond, 2000). Such measures are effective in preventing transfer of the disease from affected to clean areas particularly if a district adopts and adheres to protocols on a district-wide basis. When adopted as a regular practice, these measures will also retard the spread of other soil-borne diseases and weeds.

Even with these measures and practices in place the disease continues to be identified in new areas and fields within farms. This often occurs after flooding events but this season we have received several new samples from dryland crops where flooding has not occurred. **In almost every case of a new disease record a susceptible variety has been involved.** Unfortunately, many of our high yielding varieties, which are very attractive to growers, are very susceptible to *Fov*. The two Australian genotypes are genetically distinct from all overseas strains of *Fov* and the other species of *Fusarium* examined. Therefore, it is possible that the continued cultivation of highly susceptible varieties has been responsible for increasing the proportion of the pathogenic strains in the soil, which finally results in the expression of disease symptoms in crops. If highly susceptible varieties continue to be grown in affected areas, disease levels in the crop and *Fov* levels in the soil will rapidly increase (Hillocks, 1992).

Stubble management

Trials to test the effect of three different stubble management regimes on the incidence of Fusarium wilt were investigated for three seasons on the Darling Downs. The treatments being investigated were 1) pull, mulch cotton stubble and leave on the surface for up to 6 weeks before incorporation, 2) pull, mulch cotton stubble and incorporate immediately, and 3) pull, rake stubble and burn. Soil samples were taken throughout each treatment for use in glasshouse bioassays. In the first season results indicated that there were up to 30% fewer seedling deaths in treatment 1. In addition disease levels in the bioassays were lower in the soils from treatment 1 (pull, mulch and leave on the surface). This may have been due to the action of sunlight (UV radiation) killing the fungal spores in the mulched, infected stubble. Disease levels in the bioassays from soils in treatments 2 (mulch and incorporate) and 3 (rake and burn) were similar to one another.

Unfortunately, the differences in seedling disease incidence were not observed in following seasons and the trial was concluded in the 2000/2001 season.

Rotations

Growers and consultants frequently ask what effect various crops, that might be grown in rotation with cotton, have on *Fov* survival and development in the soil. A number of trials have been conducted providing some preliminary results but other trials had to be curtailed because of dry seasonal conditions during the last two years. In small plot trials, Wang *et al.*, 1999, found that *Fov* populations under sorghum and maize did not decrease significantly. Other trials are continuing. Dr Stephen Allen (CSD, Narrabri) has trials underway in northern NSW (e.g. wheat, barley and bare fallow) and we have about four hectares of rotation trials at "Cowan" involving five summer crops and a fallow treatment. In addition, trials involving 19 different rotations have also been established in the glasshouse at Toowoomba.

These trials are ongoing but some preliminary data from the rotation trial gives cause for concern. Isolation from an apparently clean (showing no disease symptoms) mung bean stem and sorghum roots have yielded *Fov*. Seed from chickpeas, mung bean, sorghum and soybean, produced on the Downs and some from known fields infected with *Fov* are currently being checked for the pathogen.

Biofumigant crops have also been used in rotations. Allen and Nehl (1999) discussed the effect of Indian mustard on Black Root Rot in cotton and suggested that "Hairy" vetch (*Vicia* spp.) might also be useful in cotton rotation systems. However, observations since then indicate that Fusarium wilt is more severe after "Hairy" vetch (D Nehl, pers. comm.), so it would appear that there can be no uniform recommendation for the control of pathogens by biofumigant crops.

Weed management

Early treatment to avoid the build-up of weeds in cotton fields is a major consideration in the management of Fusarium wilt. Weed species such as nut sedge (nut grass) have been reported in association with increased Fusarium wilt incidence in affected fields in California (Garber *et al.*, 1996). Australian strains of the fungus have been recovered from the roots of symptomless plants of several common weeds species by R. Davis (unpublished data), including Bladder Ketmia, Dwarf Amaranth and Sesbania pea. The authors are also aware of several reports from growers that severe disease incidence often coincides with an area of severe weed infestation in an affected field.

Treating weeds whilst small may have added efficacy. Experience in Australia with Fusarium diseases of other crops such as crown rot of wheat, have shown that the fungus can survive well in large patches of dead, herbicide treated weeds (L. Burgess, pers. comm.) suggesting that management to prevent large areas of weeds is a key strategy. Further research into the role of weeds as alternative hosts for *Fov* is being continued by Richard Kent as part of his Ph D studies at the University of New England.

Biocontrol agents

Research into bacterial agents for biocontrol, including field evaluation of such agents for their ability to decrease the incidence of Fusarium wilt of cotton, were conducted by Dr S. Putcha (NSW Ag) until June 2001.

During the 2001/2002 season, QDPI staff have conducted large-scale field trials with one species of bacteria selected in Dr Putcha's program. There were four large-scale trials and two smaller plot trials. There were no significant differences in yield obtained from all treatments in each of the large-scale trials even though there were some differences between treatments in percentage seedling survival and survival to maturity. In three of the four trials, stem-cut data showed that the percentage of plants showing a zero or one (0 or 1) rating were significantly higher in the bacterium plus benzoic acid treatments than untreated control treatments (differences ranged from 14 to 28%). In the long term, these differences might have some effect on survival of the pathogen in the soil if used in combination with more resistant varieties.

Conclusions

The research and extension effort to understand and manage Fusarium wilt has been akin to waging a war, with battles on several fronts occurring at the same time. There has been good collaboration between the many sectors of the cotton industry in Australia. This has resulted in considerable progress being made in advancing knowledge about *Fov* to develop and underpin strategies for Fusarium wilt management.

We know that the cornerstone of any management is based on resistant varieties and there has been considerable progress in the selection and development of these. There have been some reversals, particularly when some of the first Ingard® varieties released were found to be more susceptible to *Fov* than their conventional counterparts. This should not occur with future releases of new genetically modified varieties, now that the reasons for the increased susceptibility to *Fov* in some Ingard® varieties is understood. Another problem is that susceptible varieties continue to be produced and marketed, and growers continue to grow them.

Diagnostic tools to distinguish *Fov* from non-pathogenic *Fusarium oxysporum* and other species of *Fusarium* have been developed and are in the validation phase. These will be invaluable in rotation studies and to resolve possible quarantine issues.

Results from biofumigation trials and observations indicated that some biofumigant crops increase the incidence of *Fov* in subsequent cotton crops. Although these results were not as expected they are important in developing management strategies for *Fov*.

So to answer the question 'The Fusarium wilt threat – are we making the progress?' Definitely yes, but we have not won the war yet and need to continue our efforts.

Acknowledgments

The authors would like to acknowledge the financial support of research projects by the Farming Systems Institute of the Agency for Food and Fibre Sciences in the QDPI, the CRDC, the Australian Cotton CRC, the CRC for Tropical Plant Protection.

The assistance of many cotton growers, consultants and other industry representatives has been very valuable in setting and reviewing research directions and assisting with the conduct of field trials. In particular we would like to acknowledge our collaboration with the breeding programs of CSIRO (Greg Constable and team) and Deltapine Australia (Richard Leske and team), Plant Pathologists from NSW Ag and CSD, as well as the very long-term support of Graham and Wendy Clapham of "Cowan".

References

Allen, S.J. and Nehl, D.B. (1999) The dilemma between sustainable soil management and controlling soilborne diseases of cotton. In: Proceedings of the First Australian Soilborne Disease Symposium, Gold Coast. Ed. R.C. Magarey, Bureau of Sugar Experiment Stations. 212 pp.

Bentley, S., Kochman J.K., Moore, N.Y., Pattermore, J.A., Gulino, L and O'Neill, W.T. (2000) DNA diagnostics for Fusarium wilt of cotton. In: Proceedings of the 10th Australian Cotton Conference, Brisbane, Queensland. Australian Cotton Growers Association, Wee Waa, Australia. 722 pp.

Davis, R.D., Moore, N.Y. and Kochman, J.K. (1996) Characterisation of a population of , *Fusarium oxysporum* f.sp. *vasinfectum* causing wilt of cotton in Australia. *Australian Journal of Agricultural Research* **47:** 1143-1156.

Garber, R.H., Devay, J.E., Goodell, P.B. and Roberts, P.A. (1996) Cotton diseases and nematodes. In: Cotton Production Manual. Eds. S.J. Hale, T.A. Kerby and K.D. Hake. University of California, Oakland, Ca., USA. 417 pp.

Hillocks, R.J. (1992) Fusarium wilt. In: Cotton Diseases. Ed. R.J. Hillocks. CABI, Wallingford, UK. pp 127-160.

Kochman, J.K. (1995) Fusarium wilt on cotton – a new record in Australia. *Australasian Plant Pathology* **24:** p74.

Kochman, J.K., Obst, N.R., Moore, N.Y., O'Neill, W.T. and Bentley, S. (1998) Fusarium wilt of cotton in Australia. In: Proceedings of the 9th Australian Cotton Conference, Broadbeach, Queensland. Australian Cotton Growers Association, Wee Waa, Australia. 777 pp.

Moore, N.Y. and O'Neill, W.T. (2000) Detergent based degreaser for disinfecting machinery to reduce the spread of Fusarium wilt of cotton. Australian Cotton CRC Information Sheet. March 2000. Available through the Australian Cotton CRC webpage on the internet at: http://www.cotton.pi.csiro.au

O'Neill, W.T. (1999) New disinfectant gives extra insurance against Fusarium wilt. *The Australian Cottongrower.* March-April 1999, **Vol 20:** no. 2, p8.

Salmond, G., MacPherson, I. And Beetson, M. (1998) Industry approach to disease control. (1998) Fusarium wilt of cotton in Australia. In: Proceedings of the 9th Australian Cotton Conference, Broadbeach, Queensland. Australian Cotton Growers Association, Wee Waa, Australia. 777 pp.

Salmond, G. (2000) Fusarium wilt update. Australian Cotton CRC Information Sheet. March 2000. Available through the Australian Cotton CRC webpage on the internet at: http://www.cotton.pi.csiro.au

Wang, B., Dale, M.L., Kochman, J.K. and Obst, N.R. (1999) Effects of plant residue, soil characteristics, cotton cultivars and other crops on fusarium wilt of cotton in Australia. *Australian Journal of Experimental Agriculture* **39:** 203-209.