

## Management strategies for Fusarium wilt of cotton

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### Introduction

Prior to the 1992-1993 cotton season, Australia was considered to be free from the fungal disease of cotton known as Fusarium wilt. The disease 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; Bentley *et al.* these proceedings). These strains, described as Vegetative Compatibility Groups (VCGs) 01111 and 01112, have caused severe losses to cotton production in Australia, particularly in susceptible varieties.

*Fov* is now considered by many growers, ginners, consultants and other industry personnel as the most important constraint to sustainable cotton production in Australia to have developed in recent years.

On the Darling Downs in Queensland, Fusarium wilt has spread over a large proportion of the current production area with the worst affected areas being the Central and Southern Downs. The first records of this disease in dryland cotton crops were confirmed this season. It is estimated to have caused losses of AUS\$57 million on the Darling Downs this season. On some severely affected properties the levels of disease have risen such that cotton production has not been possible after three seasons, even with the most resistant varieties that are available.

The discovery this season of *Fov* in many cotton districts previously thought to be free from the disease has sent shock waves throughout the industry. Districts where this disease has been confirmed now include: The Darling Downs, Goondiwindi, Talwood, Theodore, Baralaba, St George and Dirrinbandi in Queensland, and Boggabilla, Mungindi, Moree, Bourke, Boggabri, Carroll (upper Namoi), Warren and Narromine in New South Wales. The disease has not been found in the production areas of Emerald in Queensland, Tandou and Hillston in New South Wales, Western Australia or the Northern Territory.

Current research into strategies to manage this disease and lower disease incidence on affected farms will be described in this paper.

## **What has caused this disease to occur so widely this year?**

### ***Presence of the strains of *Fov* that can infect and cause wilt in cotton***

New occurrences of the disease this year are unlikely to be the result of spread this year. There is evidence that low levels (fungal spore numbers) of *Fov* in the soil will not cause wilt symptoms to appear in even the most susceptible varieties but high levels of *Fov* in the soil will kill even the most resistant varieties that are currently available.

### ***Seasonal conditions***

*Fov* is a stress related pathogen meaning that cotton plants are often able to resist attack until they become stressed by other factors, then enabling the fungus to colonise.

The seasonal conditions during the 1999-2000 growing season have been ideal for the development of *Fov*. Extreme temperatures experienced this past growing season have contributed to plants suffering severe stress. For example, November 1999 on the Darling Downs was the coldest on record, and was the reason that long periods of unusual cold shock conditions were experienced early in the growing period. The severe heat wave conditions experienced in January (immediately after periods of mild temperatures) contributed additional plant stress. Widespread plant deaths as a result of *Fov* infection were reported after both these events from many areas.

### ***Susceptible varieties***

Many of the specimens received from newly identified areas have been from very susceptible varieties. Unfortunately, many of the varieties that produce superior yields in many areas, and as a result are widely grown, are very susceptible to this disease. In other cases, growers had decided to change to transgenic lines for insecticide spray sensitive areas, or to reduce insecticide applications. Trial and field observations in recent seasons indicate that some varieties that have been transformed with the *Bt* gene appear more susceptible to *Fov* than their conventional counterparts. Research work is continuing to assess transformed varieties in an effort to understand this apparent loss of resistance and to better understand the mechanisms and heritability of resistance to *Fov* in cotton plants.

## **Can we live with this disease?**

Evidence from California suggests that once spores of *Fov* are established in an area they can survive in the soil for at least ten years, even in the absence of cotton crops (Smith *et al.*, 2000). There are no commercially viable means to eradicate this fungus from soil. If highly susceptible cotton varieties continue to be grown in infested fields, the *Fov* population will increase in the soil to the point where production with even the most resistant varieties of cotton will not be possible. Therefore, strategies to manage this disease are being investigated and continue to be developed. It is envisaged that implementation of these strategies will minimise disease levels and allow sustainable cotton production to continue. We have no choice but to find ways of living with *Fov*!

## **Strategies to manage Fusarium wilt**

### ***Maintenance of disease free areas***

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 smaller, shorter lived spores (conidia). Estimates suggest that a gram of infested soil may contain up to 5000 spores of *Fov* (Smith *et al.*, 1981). The first line of defence to maintain 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.

Details of vehicle cleaning procedures and use of disinfectant agents such as Castrol *Farmcleanse*, have been extensively detailed elsewhere (Kochman, 1995; Kochman, 1998; Salmond, *et al.*, 1998; O'Neill, 1999; Moore and O'Neill, 2000; Salmond *et al.*, 2000; and the ACCRC Information Sheet "Farm Hygiene for disease and weed control", 2000). Such measures are effective in preventing transfer of the disease from affected to clean areas particularly if a district adopts and adheres to measures on a district-wide basis.

### ***Field inspection for early detection***

Experience in Australia has shown that early detection and treatment of small outbreaks of *Fov* can be very effective in containing the spread of this disease. Any wilted, stunted or otherwise suspicious plants should be sent for analysis to the authors at the DPI laboratories at either Indooroopilly or Toowoomba (refer to the end of this paper for instructions). There is no charge for this analysis. It is vitally important to confirm if the problem is *Fusarium* wilt but also to detect any new strains of the disease as soon as possible should they appear in the future. External and internal disease symptoms appear the same regardless of which of the two strains currently known to occur in Australia [Vegetative Compatibility Groups (VCGs) 01111 and 01112] is causing the disease. Analysis is the only way to differentiate which strain is involved. 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, therefore it is important to know which strain occurs on an affected farm.

### ***Containment of small outbreaks***

The steps for containing a small outbreak on a farm may vary depending on the particular circumstances and location of the affected area, but they may include some or all of the following measures:

- Mark the affected area and send specimens for analysis
- Restrict access of vehicles and workers through the affected spot to zero, including a buffer zone of apparently healthy plants/soil around the suspected plants. Minimise traffic in adjacent areas and restrict to dry weather only where possible.
- Restrict or cease irrigation flows through the affected area. This is not always possible but is worth the effort involved as *Fov* spores can travel in irrigation water. Some farms have pushed up earth around affected sites to form a bund to minimise water (eg. rain) flow through, and from, the area.
- Kill the affected cotton plants and the 'healthy' plants in the buffer zone around them by spraying with herbicide and leave them to die in place. Do not remove the affected plants from this spot. Pulling and burning the plants on the site is an option, but do not carry plants from the site as the risk of spreading the disease further by accidental dropping of infected plant debris and attached soil is very high.

- After plants are treated, the area can be solarised to kill *Fov* spores in the soil. This is achieved by covering the area with a layer of thick, clear plastic and leaving this in place for 6 weeks or more. The effect of the sunlight on the affected soil under the plastic is enhanced if there is some moisture in the soil beforehand.
- If your district has protocols in place for collecting and processing cotton modules from Fusarium wilt affected areas, notify your local gin, contractors and consultants about which block has the outbreak. If you or your neighbours are involved in clean seed production, you must notify the relevant seed company as *Fov* has been shown to be transmitted in seed.
- If there are not already facilities for cleaning vehicles on your farm, consider where these would be best placed and how to minimise spreading infested soil from the affected block to other areas on the farm eg. by working that block last and cleaning soil and plant debris off machinery before it leaves that block. Each case will vary but small changes in how and when affected blocks are worked can make big reductions in risk of spreading this fungus.

The aim of these measures is to contain the spread of the fungus across the field and to other production areas and minimise unnecessary risks. In relation to efforts to contain outbreaks of this disease, sceptical comments are sometimes made about the possible spread from the affected areas before they have been detected. Whilst the opportunities for spread may have occurred prior to detection of the first symptomatic plants, the extent of subsequent spread is unknown. There is much to be gained by keeping the level of spores in the soil as low as possible. Until varieties with higher levels of resistance to this disease become available, early detection and containment efforts are the first and most important options to lower the spore levels and 'buy some time'. If nothing is done, the fungus will continue to build-up in the affected spot, which will remain a source of infection and spread for the rest of the farm and for neighbouring farms.

On farms where the disease appears first in a large area, there are fewer options available for containment. Bearing in mind that any soil that moves from an infested field may carry thousands of spores of *Fov*, it is advantageous to work affected fields after disease free-fields and to follow basic farm hygiene protocols between fields and between farms where possible. If cotton production is intended for that area, resistant varieties must be adopted.

### **Resistant varieties**

As with Fusarium wilts of other crops, host plant resistance is the primary strategy for long-term management of this disease. Whilst both cotton breeding programs in Australia (CSIRO and Deltapine Australia) 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. If highly susceptible varieties (eg. Siokra 1-4, DeltaJEWEL) are grown in affected areas, disease levels in the crop and in the soil will rapidly increase (Hillocks, 1992).

Unexpected problems with resistance to *Fov* have been encountered in recent seasons with the release of some INGARD® varieties. Trial and field observations in recent seasons



indicate that some *Bt* transformed varieties appear more susceptible to *Fov* than their conventional, untransformed counterparts.

The issue of integrity of disease resistance in all new varieties, including transgenic breeding lines, is of major concern to the industry. Varieties such as those transformed with the *Bt* gene are understandably desirable in requiring less pesticide sprays for control of *Helicoverpa* species but the impact of the introduction of this gene on other qualities in the plant, such as resistance to diseases, is a complex issue that requires more understanding. The mechanisms and heritability of resistance to *Fov* in cotton plants are not well understood. Research projects that have just commenced aim to better understand this issue by studying the segregation for resistance and susceptibility in transformed and untransformed breeding lines through various generations.

An improved rating scheme that relates the disease reaction of new varieties and breeding lines to both of the industry benchmark varieties has been recommended by the Cotton Fusarium Wilt Working Group. These guidelines recommend that in future the disease resistance performance of new varieties be expressed as a percentage of the performance of both the nominated industry benchmark varieties. Other recommendations to improve Fusarium wilt assessment protocols are: the inclusion of a moderately susceptible variety into each field trial, as an indicator of disease 'pressure' at the site; an assessment of seedling survival/plant stand in the early phase of the crop and; the rating for internal disease symptoms (vascular discoloration) at the end of the season. Yield ratings should also be included. Discussions to implement these recommendations are in progress.

Until, and even after, varieties with higher levels of resistance to *Fov* become available and widely adopted, a number of other important issues must be taken into account for the Australian cotton industry to manage this disease to maintain cotton producing capability.

### ***Effect of farming practices on survival of Fov*** ***Stubble management***

Spores of *Fov* as well as other cotton pathogens such as *Verticillium*, can survive in infected plant material. Trials to test the effect of three different stubble management regimes on the incidence of Fusarium wilt have been under way for three seasons at an *Fov* infested trial site on the Darling Downs where "back-to-back" cotton is grown. For experimental purposes varieties with moderate levels of resistance have been used in these trials. The treatments being investigated are 1) mulch cotton stubble and leave on the surface for up to 6 weeks before incorporation, 2) mulch cotton stubble and incorporate as soon as possible and 3) pull and rake stubble to the end of the rows and burn. Seedling survival counts are being made and soil samples have been taken throughout each treatment for use in glasshouse bioassays. Preliminary results indicate that there were up to 30% fewer seedling deaths in treatment 1. In addition disease levels in the bioassays have begun to drop in the soils from treatment 1 (pull, mulch and leave on the surface). This is likely 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) are not yet dropping significantly. Similar results have been obtained by Dr S. Allen in trials near Goondiwindi.

It is essential to take data from these trials for several more consecutive seasons to verify initial trends and to account for seasonal variations that may affect spore levels in the soil. The development of DNA detection systems for the Australian strains of *Fov* will significantly assist the soil assay aspects of these and other studies into disease management strategies (refer Bentley *et al.*, these proceedings).

### **Irrigation**

*Fov* spores can be carried in water, especially attached to soil particles in colloidal solution. If irrigation through fields affected by the disease can be separated from that used on the rest of the farm, this will minimise the potential for spores to be spread across the farm. With the type of flood irrigation practices used across much of the cotton industry in Australia, this is not always possible. Research into possible chemical or filtration treatments to kill *Fov* spores in irrigation water has commenced but at this stage there appear to be no cost effective methods to treat the large volumes of water used on irrigated cotton farms throughout each season.

To minimise the numbers of spores that can be transported in irrigation water on-farm, some simple measures can be considered. Since spores and the soil particles to which they are attached will settle once in storage, it is advisable to allow recycled water from tail drains to settle in storage for as long as possible before use. The practice of direct irrigation on to fields with water from rivers or overland flows directly downstream from affected farms is not recommended. The use of flocculants such as alum, to settle particles in storage water may be applicable in some cases but research has not yet verified the efficacy of such treatments on *Fov*.

The related issue of managing irrigation for least stress to the cotton crop should be mentioned at this point. Fusarium wilt is a stress-related disease and commonly appears in a crop after periods of stress, such as waterlogging. It is no coincidence that the first cases of Fusarium wilt of cotton often appear in the tail drain ends of fields where irrigation water can pool causing plants to become waterlogged for several days at a time. The association of wilt disease with waterlogging has been found in other crops. For example, the Cavendish variety of banana is normally highly resistant to the strain of the banana Fusarium wilt pathogen known as 'race 1'. However, after periods of heavy waterlogging, Cavendish plantations in Western Australia became diseased with race 1 Fusarium wilt due to the colonisation of the waterlogged plants by the pathogen (Pegg *et al.*, 1995). Studies by Aguilar *et al.* (2000) on tissue differentiation in waterlogged banana roots showed that root functioning in normally resistant varieties was severely affected after periods of sustained waterlogging and hypoxia (4% O<sub>2</sub>) and anoxia (0% O<sub>2</sub>) in the root stele. Furthermore, the survival and colonisation of the banana Fusarium pathogen was favoured in the root cells (aerenchyma) of waterlogged plants during hypoxia and after re-aeration.

Short term waterlogging stress and its negative effects on cotton plants must not be confused with the long-term anaerobic effects of flooding *Fov* infested fields throughout the summer in the absence of cotton plants (S. Allen, pers. comm.). Managing the crop for minimum stress and optimum growing conditions is a recommendation for managing Fusarium wilt disease in many crops.

It is significant to note that until this (1999-2000) season, *Fov* had not been confirmed from any dryland cotton farms in Australia. In general, they remain free from the disease despite significant increases in the number of irrigated farms that have become severely

affected over the past five seasons. It is likely that, if early containment of initial outbreaks in non-irrigated fields is achieved and resistant varieties are adopted, the disease will have far less opportunity for subsequent spread around the dryland farm. Monitoring outbreaks in dryland farms over coming seasons will enable this assumption to be tested.

### **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 nutsedge (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. Future research will continue to sample weeds from cotton farms for the presence of *Fov* to confirm additional weed hosts.

### **Rotation crops**

Crop rotation studies are yet to be completed. Dr Stephen Allen (CSD, Narrabri) has trials underway in northern NSW (eg. wheat, barley and bare fallow). In small plot trials, Wang *et al.* (1999) found that *Fov* populations under sorghum and maize did not decrease significantly. The effects of treatments on non-host crop residues will also be investigated. Crop rotation trials are to be expanded on the Darling Downs. Initial research trends must be verified over several seasons, and expanded trials are being planned for the next season.

### **Biofumigant crops**

Crops grown for 'green manure' can have multiple benefits if the plant varieties used also release compounds that are toxic to soil-borne pathogens as the crop residues break down. Green manure crops can also deliver organic matter to the soil and in some cases a nitrogen bonus to the following crop. Kirkegaard *et al.* (1999) have described beneficial effects from varieties of the Brassicaceae (Brassica) family (eg. Indian mustard, canola) which produce compounds known as glucosinolates (GSLs) as the plant tissue breaks down after mulching and incorporation into the soil. These compounds in turn release isothiocyanates (ITCs) which have been shown to have a deleterious effect on the survival of a range of soil-borne pathogens (Sarwar *et al.*, 1998). Little data is available yet on the effect of such crops on Fusarium wilt of cotton. If such crops were incorporated into the cotton production regime, they may affect essential mycorrhizal development in cotton plants. However, in the short-term, if particular varieties are found to be effective against *Fov*, they may offer a good broad-scale method for decreasing pathogen levels on affected farms until cotton varieties with higher levels of resistance become available.

Allen and Nehl (1999) have discussed the deleterious effect of Indian mustard on the soil-borne cotton pathogen *Theilaviopsis basicola*, the causal agent of Black Root Rot in cotton, and also suggested the potential of 'Hairy' vetch (*Vicia* spp.) for use as a

biofumigant in cotton rotation systems. When vetch tissues breakdown in the soil they release ammonium compounds which are toxic to some microorganisms. Vetch is also not thought to have deleterious effects on mycorrhizal fungi. Rochester and Peoples (1998) have shown that vetch provided a significant nitrogen bonus (average 171 kg/ha N fixed and returned to the soil) for the following cotton crop when used as a winter rotation.

Several cotton farms affected by *Fusarium* wilt will be experimenting with soft-seeded varieties of vetch as a green manure biofumigant crop this season. Soil samples have been taken from some of these farms prior to planting to enable glasshouse soil bioassays to monitor any changes in the levels of *Fov* in these soils after the vetch residues have been incorporated and broken down. Protocols such as timing and depth of incorporation as well as the suitability of different varieties of biofumigant crops to different cotton growing regions will need to be fine-tuned as experience is gained from field trials.

### **Biocontrol**

Research into bacterial agents for biocontrol, including field evaluation of such agents for their ability to decrease the incidence of *Fusarium* wilt of cotton, is reported elsewhere in these proceedings and will not be covered here (please refer to paper by Dr S. Putcha in these proceedings).

### **Seed treatments**

The *Fusarium* wilt fungus can also be seed-borne. Both seed companies took this fact into account as soon as the disease was identified and precautions were 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.

Recent investigations indicate that the fungus is not present in seed from plants not showing symptoms of infection. Fifty-six percent infection was found in 'fuzzy' seed from plants showing wilt symptoms but acid de-linting treatment of the seed resulted in the fungus being isolated from only 11% of the seed. A number of seed treatments, including the current treatment of Quintozene/Apron, have been found to prevent all transmission of the fungus in germinating seed under laboratory conditions. These investigations are continuing.

### **Conclusion**

Cotton varieties with high levels of resistance to *Fusarium* wilt are the cornerstone for management of this disease. Varieties with higher levels of resistance than the current top benchmarks Sicot 189 and DeltaEMERALD may be several seasons away from commercial release and it may be even longer until resistance can be incorporated into varieties with a range of other characteristics for application to different cotton growing regions in Australia. Until such varieties are widely available, production will only be maintained by vigilant efforts to minimise the spread of this disease, particularly into disease free areas, and to engage in 'best bet' practices to lower disease levels on affected farms. This includes not growing highly wilt susceptible varieties and incorporating a range of risk minimisation strategies into farming practices on affected farms. Research into the ecology and survival of *Fov* is under way, but many of the trials to test different treatments (eg. crop rotations, biofumigant crops etc.) must be carried out over several seasons to gather data from several consecutive years to verify initial trends.



### **Sending specimens for analysis**

Six to eight sections of stem (approx 20-30 cm long) from plants which show symptoms of wilting or have internal discoloration, should be wrapped in dry paper and sent in paper (not plastic) packaging to either:

- Dr Natalie Moore or Mr Wayne O'Neill, Plant Pathology Bldg, DPI, 80 Meiers Rd, Indooroopilly, Q 4068 or,
- Dr Joe Kochman, DPI, 203 Tor St, Toowoomba, Q 4350.

Please do not send suspected *Fusarium* wilt specimens to centres in NSW (eg. ACRI, Narrabri). It is not necessary to include leaf material with the stems unless unusual symptoms are observed. Please include details of the variety and location.

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