DISEASES – NATIONAL UPDATE

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Abstract

The results of annual disease surveys continue to indicate the importance of soil-borne plant pathogens. Seedling diseases are controlled by the use of fungicide seed treatments. Current and potential seed treatments are being evaluated in annual field experiments. Verticillium wilt has been effectively controlled by the repeated use of resistant cultivars. Black root rot and Fusarium wilt are widespread and particularly significant. Black root rot is found in all cotton production areas but is more important in the cooler areas. The pathogen has been dispersed by the activities of cotton growers. There is no evidence of host plant resistance to black root rot of cotton and current control options are limited to biofumigation with a green manure crop of vetch, summer flooding and delaying the planting time to avoid cool conditions early in the season. The distribution of Fusarium wilt is continuing to increase. The pathogen appears to have originated from native populations of *Fusarium oxysporum* and resistance to the pathogen has been found in some native *Gossypium* spp.. Slow but significant progress has been made in breeding cotton cultivars with better resistance to the pathogen. Progress has also been made in understanding some of the mechanisms of resistance. Several control options have been, or are being, evaluated and an integrated disease management strategy for Fusarium wilt is being developed. The strategy currently includes delayed incorporation of residues from the previous crop, bare fallow rotation, delayed planting into moisture, use of a cultivar with a high F.rank and use of rolling cultivators to minimise damage to the roots.

Disease Surveys

Staff of NSW Agriculture, Cotton Seed Distributors Ltd. and the Queensland Department of Primary Industries have annually surveyed commercial cotton crops in all production areas of Eastern Australia. The results of the 2003-2004 survey are presented in Table 1 and Table 2. Significant features of the survey results were as follows:-

* Seedling mortality in crops in the Lachlan and Murrumbidgee was approximately double the incidence of seedling mortality in other production areas. Seedling mortality is derived from the difference between the number of seeds planted/metre and the number of plants established/metre.

* Black root rot was most severe in the Namoi and Macquarie valleys where it was present in almost all fields inspected and where the mean incidence of the disease was 57%.

* Although the mean incidence of Verticillium wilt in crops in the Namoi valley was only 8% the disease was present in 79% of the fields inspected.
* Fusarium wilt was most common on the Darling Downs where it was observed in 75% of crops surveyed. The mean incidence of the disease was 10% with 45% of plants infected in one field.
* Boll rots caused significant losses in the Emerald area in the early maturing crops that were subjected to wet weather in January and early February.

Table 1. Distribution of the significant soil-borne cotton diseases as indicated by the percentage of fields inspected where the disease was found to be present during the 2003-2004 disease surveys.

Production region	Black root rot % of fields inspected	Verticillium wilt % of fields inspected	Fusarium wilt % of fields inspected
Emerald	20.0	0.0	0.0
Theodore	0.0	12.5	0.0
St George	50.0	25.0	0.0
Downs	75.0	12.5	75.0
Macintyre	81.8	45.5	36.4
Gwydir	27.3	30.0	20.0
Namoi	100.0	79.2	0.0
Macquarie	88.9	0.0	0.0
Lachlan	33.3	0.0	0.0
Murrumbidgee	25.0	25.0	0.0

Table 2. The incidence of the significant cotton diseases found to be present during the 2003-2004 disease surveys of commercial crops in Eastern Australian production regions.

	Seedling mortality (%)	Black root rot incidence (%)	Verticillium wilt incidence (%)	Fusarium wilt incidence (%)	Boll rots incidence (%)
Emerald	23.8	0.1	0.0	0.0	6.9
Theodore	24.8	0.1	0.2	0.1	1.9
St George	22.1	11.8	0.4	0.1	1.7
Downs	27.4	0.1	0.6	10.6	0.3
Macintyre	18.4	21.9	1.4	0.5	1.1
Gwydir	21.5	0.6	1.3	0.2	1.1
Namoi	25.0	57.5	8.4	0.1	2.1
Macquarie	22.9	57.3	0.1	0.1	0.4
Lachlan	48.2	12.3	0.0	0.0	0.0
M'bidgee	45.4	2.3	0.9	0.0	0.0

Apart from those diseases indicated in Tables 1 and 2 there were several other minor diseases noted during the 2003-2004 season. Alternaria leaf spot was common in most crops in most areas at trace levels. Bunchy top was observed in only three fields with an estimated 1.3% of plants affected in one field near Theodore. Phytophthora boll rot was the most common type of boll rot in NSW production areas. Reniform nematode was found associated with the roots of stunted cotton seedlings in one field near Emerald and this represents a new record for cotton in Australia. Reniform nematode is a major constraint to cotton production in parts of the USA. (Nehl, Allen, Kochman)

Significant Epidemics

Annual disease surveys are important because the results can provide information on the relationships between disease incidence and location, trash carry-over, planting time, cultivar planted or ground preparation. However, the most important aspect of the inter-relationship between farming practices and disease is whether or not the incidence is increasing or decreasing. Increasing disease distribution, incidence or severity indicates declining economic returns to farmers and the necessity to adjust the farming system to make it less favourable for the pathogen. Disease survey results indicate that we are experiencing major epidemics of both black root rot and Fusarium wilt (Figure 1).

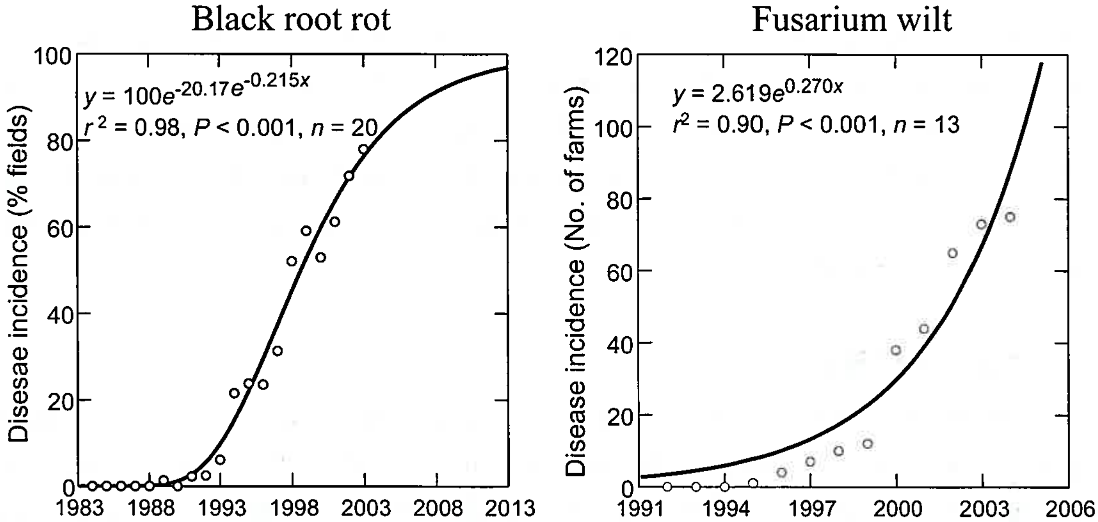

Figure 1. Epidemic progress curves for black root rot and Fusarium wilt show the increasing distribution and incidence of these cotton diseases in NSW production areas. (Courtesy DB Nehl, NSW Agriculture)

Black root rot and Fusarium wilt therefore present a real challenge to cotton pathologists. Current farming systems allow for efficient dispersal of the pathogens and provide ideal conditions for infection and survival. Black root rot was first observed on cotton in Australia in 1988 and is now found in all cotton production regions with disease incidence increasing steadily. Fusarium wilt was first observed in Queensland in the 1992-1993 season and in NSW in the 1993-1994 season. It has been estimated that, at current rates of increase, the disease will be present on 90% of farms in NSW by 2010. (Nehl, Allen, Kochman)

Soil-borne Pathogens

It is interesting to note that all of the significant diseases of cotton in Australia are caused by soil-borne pathogens and this has prompted some to suggest that this is an indication that cotton growing has ruined the soil and impaired soil health. However, the development of plant pest and pathogen populations indicates a 'healthy' ecosystem resistance to an imposed cotton monoculture. The presence of plant pests and pathogens indicates an ecosystem attempt to restore plant biodiversity.

Seedling Diseases

All cotton seed for planting in Australia is treated with fungicides for the control of *Pythium* sp. and *Rhizoctonia* sp.. Current and potential fungicide seed treatments are evaluated by NSW Agriculture in annual field experiments at multiple sites. Both Cotton Seed Distributors Ltd. and Deltapine Australia Pty Ltd. collaborate in these annual experiments. (Nehl)

Verticillium Wilt

The release and adoption of cultivars with resistance to Verticillium wilt was accompanied by a decline in the incidence and importance of the disease. Survey results show that a return to the use of the more susceptible cultivars was reflected in an increase in mean disease incidence. It is therefore important that growers use the Verticillium resistance ranking (V.rank) information to select the more resistant cultivars for fields where the disease is present. (Nehl)

Black Root Rot

The pathogen that causes black root rot has a large host range. However, a study of the pathogenicity and diversity of the pathogen in Australia has shown that the strains that cause disease in cotton seedlings are genetically different to those which cause disease on other hosts and also different from those strains isolated from disturbed soils in non-cultivated land. The pathogen was not isolated from undisturbed soils. This implies that the widespread distribution of black root rot is a result of poor farm hygiene allowing spread of the pathogen from cotton farm to cotton farm. The cotton strains are also able to infect and colonise legumes including soybeans (Harvey, Nehl, Mondal)

Control Strategies

There have been several unsuccessful attempts to identify host plant resistance to the black root rot pathogen. All cotton cultivars, from both local and overseas sources, appear to be equally susceptible. Resistance to black root rot has been identified in other species of *Gossypium*. Accessing such resistance in a cotton breeding program presents considerable difficulties. (Allen, Nehl, Stiller)

Symptoms of black root rot are most severe when early-season weather conditions are cool and symptoms dissipate when weather conditions warm up. Consequently the most commonly recommended control strategy has been to delay planting. Flooding a field for 30-60 days during summer has been promoted in parts of California as a method of black root rot control. A field near Merah North was flooded for 57 days in February and March, 2000 and the number of spores/gram of soil was reduced by 97% from 422 to 14. (Nehl)

The use of either a green manure vetch crop or a brassica crop to provide biofumigation results in a significant reduction in the concentration of inoculum in the soil. Incorporation and breakdown of hairy vetch residues produces ammonia in the soil and the ammonia is toxic to the spores of the black root rot pathogen. Brassica crops (especially Indian mustard) release glucosinulates into the soil resulting in the production of isothiocyanate (ITC) compounds that are also toxic to spores of the black root rot pathogen. (Nehl)

Several fungicide seed treatments and in-furrow treatments have been evaluated for black root rot control. These treatments have produced either inadequate or inconsistent control. A seed treatment that induces a systemic, natural resistance in cotton seedlings is being evaluated with encouraging results (Table 3). (Nehl, Allen, Mondal)

Table 3. Reduced severity of black root rot of cotton in the field following seed treatment with acibenzolar-S-methyl (ASM)

	Control	ASM	Difference	Probability
Narrabri (seed soaking)				
Disease severity (0-10)	6.9	4.6	**-33%**	*p*=0.021
Wee Waa (seed treatment)				
Disease severity (0-10)	6.53	5.35	**-18%**	*p*<0.001
Moree (in-furrow spray)				
Disease severity (0-10)	9.6	7.4	**-23%**	*p*<0.001
Lateral roots (no./plant)	1.5	6.8	**+350%**	*p*<0.001
Fruiting (bolls/metre)	21	27	**+29%**	*P*=0.004

Since black root rot severity is influenced significantly by temperature then strategies that increase early season soil temperatures should provide some control of the disease. Preliminary field experiments have evaluated modifications to bedshape, plastic mulches and the retention of standing cereal stubble as a shelterbelt. (Jhorar)

Host-pathogen interactions are being further investigated in an attempt to identify a weakness in the host pathogen relationship that can be exploited in a control strategy. (Pereg-Gerk)

Fusarium Wilt

Seventeen pathologists, molecular biologists and cotton breeders are currently directing either all, or at least part, of their research effort to the Fusarium wilt problem. Significant progress has been made. Fusarium wilt results from the interaction of a virulent pathogen with a susceptible host under favourable environmental conditions. Pathogen, host and environment represent the three sides of the 'disease triangle' and the development of an appropriate control strategy requires an understanding of these three aspects.

The Pathogen

Fusarium oxysporum is a common component of Australian soils. A comprehensive study of these indigenous *Fusarium oxysporum* showed that they could be divided into five distinct lineages and that some could infect susceptible cottons. Lineage A isolates were more closely related to the cotton field pathogens than any other lineage. This suggests that the pathogenic strains causing Fusarium wilt in cotton in Australia have originated in Australia and have not been introduced from elsewhere. (Brubaker, Wang)

An industry funded diagnostic service has been provided by the QDPI so that growers could confirm the presence of the pathogen on their farm. This service has processed 765 samples in the last five years and 352 (46%) have been found to be positive. A complete database and Fusarium reference collection have been established as part of this diagnostic service. Almost all records have been the 'Downs' strain of the pathogen (VCG 01111) with the 'Boggabilla' strain (VCG 01112) only occurring on some farms in the Boggabilla area plus in one field at St George and in one field in the Gwydir valley. Where both strains have been found on the same farm the 'Downs' strain appears to become predominant. (Smith, O'Niell)

It is very difficult to distinguish between pathogenic and non-pathogenic isolates of *Fusarium oxysporum* from the soil. Consequently it is very difficult to detect and quantify the amount of pathogen inoculum in soil. It is also very time consuming to determine the particular strain of the pathogen present in a sample. These three constraints have been overcome by the development of a molecular diagnostic technique utilizing real-time PCR. Using this diagnostic tool should enable detection of the pathogen in soil before plant symptoms become evident and quantification of the effects of imposing various control strategies. Standard diagnosis to confirm Fusarium wilt from infected material should also be much quicker. (Gulino, Bentley)

The Fusarium wilt pathogen can be dispersed within seed. Up to 11% of acid-delinted, graded seed that had been hand-picked from infected plants of a susceptible cultivar was found to carry the pathogen. Preliminary results suggest that the level of seed borne infection in the more resistant cultivars is much lower than that in susceptible cultivars. Seed borne infection declines with time in storage and is no longer evident after about 6 months. The standard fungicide seed treatments currently in use eliminate seed borne infection. (Kochman, Allen)

The Fusarium wilt pathogen can also be dispersed in water and crop residues. A new project based at Narrabri is investigating these modes of transmission and their significance. (Anderson)

It is important to understand where the pathogen inoculum is located in the soil profile. Intact soil cores were collected from six fields and divided into 0-8cm, 8-16cm and 16-24cm fractions. Mortality in seedlings of Siokra 1-4 clearly showed that most of the inoculum was in the top 16cm (Figure 2). The results further suggest that most of the inoculum is in the 0-8cm fraction following a summer crop and in the 8-16cm fraction following a cereal-summer fallow. This may indicate a partial sterilization of the 0-8cm fraction resulting from the exposure of bare soil to summer sun and the consequent high temperatures. (Allen, Swan)

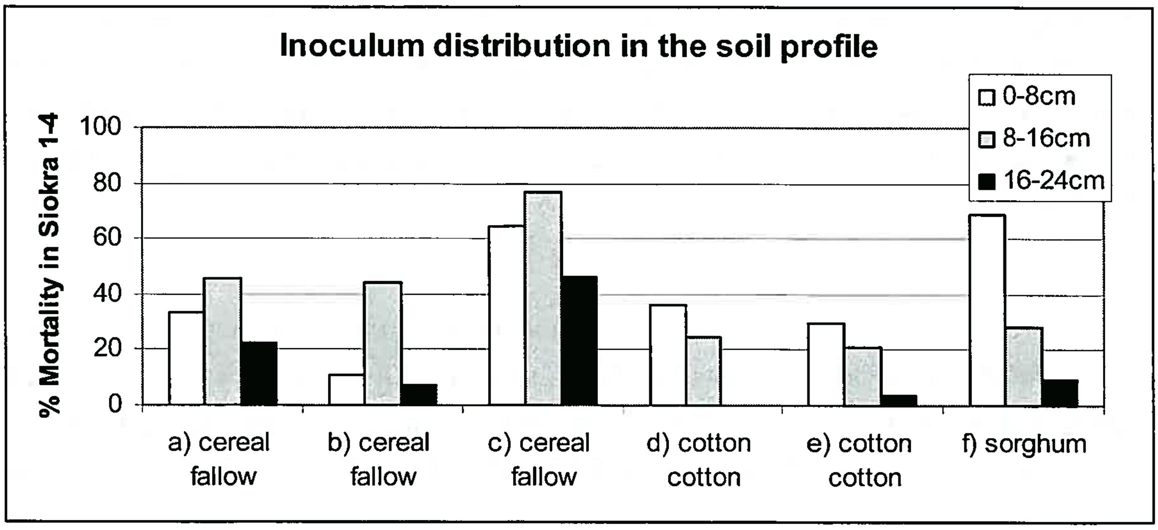

Figure 2. The distribution of inoculum in the soil profile at six sites in the Macintyre valley (b,c) and on the Darling Downs (a,d,e,f). Three of the sites (a,b,d) were sampled in November 2002 and three of the sites (c,e,f) were sampled in November 2003.

Host Plant Resistance

Initial evaluations of the level of resistance or susceptibility in the commercial cotton cultivars that were available in Australia showed that Sicot 189 was more resistant than most other cultivars. However, when inoculum levels are very high and the environment is particularly favourable then survival in Sicot 189 may be as low as 1%. A protocol for ranking cultivars on their resistance to Fusarium wilt has been developed and used by the industry for the last three seasons. Sicot 189 is the standard with a rank of 100 and is included in all cultivar comparisons. A cultivar with a rank (F.rank) less than 100 is more susceptible than Sicot 189 while a cultivar with a F.rank higher than 100 is more resistant than Sicot 189. If a cultivar has an F.rank of 200 then the proportion of plants surviving to the end of the season is double that of the standard Sicot 189. The number of field comparisons averaged to produce an F.rank are indicated in brackets following the F.rank. (FUSCOM)

Australian cotton breeders have made considerable progress in increasing the level of resistance to Fusarium in commercial cultivars. Examples include Sicot F-1 with an F.rank of 208(15), Sicala 45 with an F.rank of 154(15) and Sicot 14B with an F.rank of 145(15). It has been estimated that the average F.rank of the commercially available CSIRO cultivars has doubled from 60 to approximately 120 in the last 8 years. (Constable, Reid, Stiller, Leske)

These increases in resistance have been derived from several sources. Considerable advances have come from reselection from current commercial cultivars. Several exotic sources of resistance have been identified and utilized in breeding programs. A *Gossypium barbadense* cultivar with good resistance has been identified. These discoveries raise several important questions. Is the mechanism of resistance the same for all of these sources of resistance? If there are different mechanisms then is it possible to combine them to produce even higher levels of resistance to the Fusarium wilt pathogen? (Constable, Reid, Stiller)

The search for possible sources of resistance identified some of the native *Gossypium* spp as potential candidates. Attempts are being made to utilise the resistance expressed by some accessions of *Gossypium sturtianum.* This resistance is expressed in hybrids with cotton but further work is required to make it usable in a cotton breeding program. (Davis, Becerra)

The possibility of developing a transgenic solution to the Fusarium wilt problem is also being investigated. Cotton has been genetically modified with genes from tobacco that produce antifungal proteins known as defensins. New gene constructs have enabled these defensins to be produced within the vascular tissue of cotton. (Heath)

The infection process in susceptible and resistant cultivars has been studied in an attempt to understand the mechanisms of host plant resistance. Cultivar differences are apparent before the pathogen even enters the plant. The pathogen not only germinates and reproduces more readily in root diffusate from the most susceptible cultivar, but hyphal growth is also increased. Examination of the diffusate components is currently underway to pinpoint which might be responsible for this pathogen growth promotion. The pathogen forms a dense sheath around the root surface before easily entering via both inter- and intracellular penetration. Once the pathogen enters the cotton roots, its vertical expansion up the stem is restricted in the more resistant cultivar. This is possibly due to the plant accumulating toxic compounds around the infected cells to kill the fungus. Identification and quantification of this response is yet to be completed. (Hall)

The role of fusaric acid in the host-pathogen interaction is also being investigated. Australian isolates vary considerably in their capacity to produce fusaric acid. The levels of production do not seem to correlate with field assessments of virulence. (Smith)

Other Hosts

Several weeds have been identified as alternative hosts of the pathogen. These include bladder ketmia, sesbania pea, dwarf amaranth, paddy melon and bellvine. (Davis, Kent)

The Fusarium wilt pathogen has also been isolated from the stem of mung bean growing under field conditions, from the roots of symptomless sorghum, maize and sunflower when grown in pots in infested soil under glasshouse conditions and from the stems and roots of symptomless mung bean, vetch, field pea, chickpea, pigeon pea and lucerne when grown in pots in infested soil under glasshouse conditions. The pathogen has not been isolated from the seed of any of these crops. (Swan)

The Environment

The environment is an important component of the disease triangle. If the environment is unfavourable then symptoms of Fusarium wilt will not develop. For example - glasshouse temperatures need to be kept below 23C in order to have good symptom expression in pot experiments. At higher temperatures symptoms of Fusarium wilt will not develop. (Swan, Kochman)

The impact of seasonal conditions on the development of the external visible symptoms and the internal vascular symptoms of Fusarium wilt is being investigated. (Anderson)

Several field experiments have investigated the impact of delaying the time of planting to avoid the less favourable environmental conditions early in the season. In seven out of eight studies over three seasons the total survival to the end of the season was increased by delaying the planting by between 14 and 35 days. Survival was generally increased by 15-40%. However, in one of these studies total survival was increased by 80.5% ($P<0.001$) by delaying the planting from mid-October to the end of October. In the eighth study there was no significant difference in survival between earlier and later plantings. (Allen, Anderson, Nehl)

Symptoms of Fusarium wilt appear to be more severe when the spring environment is cool and wet. The relationship between rainfall in October and November and the total survival of Sicot 189 to the end of the season has been investigated over the last four seasons at the Pampas trial site (Figure 3). A clear and significant relationship appears to exist. Symptoms of the disease were most severe in the 2001-2002 season when 220mm rain was recorded in the October and November and least severe in the 2002-2003 season when only 60mm of rain was recorded in the October and November. (Allen)

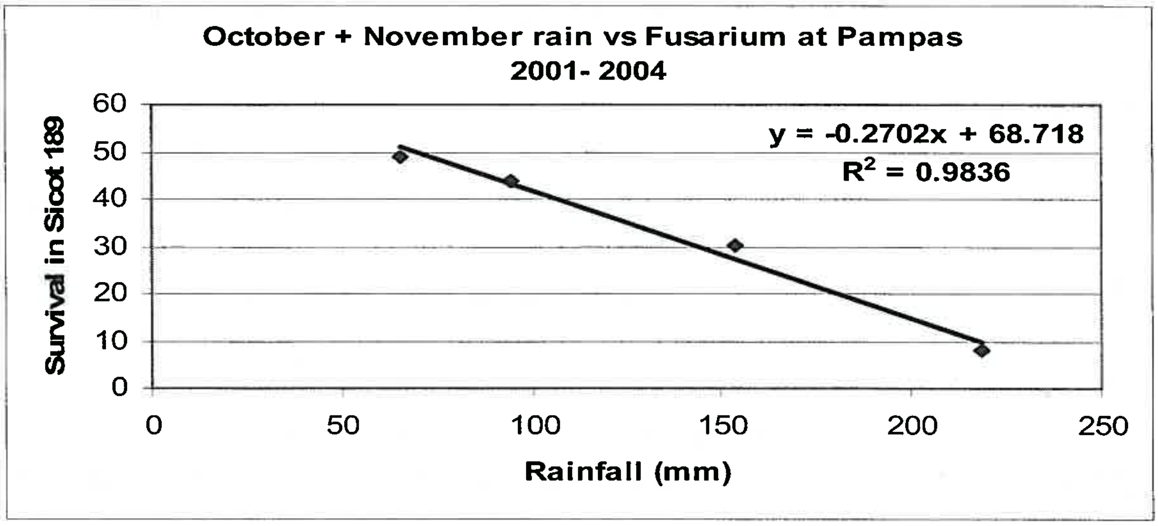

Figure 3. A clear relationship between spring rainfall and survival of Sicot 189 to the end of the season at the Pampas site on the Darling Downs over the last four seasons.

Why do factors like planting date and spring rainfall appear to have an impact on plant survival to the end of the season as assessed approximately six months later? A possible explanation is that a wet spring encourages shallow root development in that part of the soil profile that has the most inoculum. In order to part test this hypothesis an experiment was established using a hessian and plastic mulch to keep the beds moist and encourage shallow root development. Survival to the end of the season was reduced by 71% ($P<0.001$) in the mulched plots compared to survival in the control plots. Further investigations are required. (Allen)

Other Control Strategies

Using the most resistant cultivars available and delaying the planting date have been mentioned as important strategies for managing Fusarium wilt. Apart from these and obvious strategies such as maintaining good farm hygiene practices and minimising tailwater to reduce dispersal of the pathogen on farm, there are still other management strategies that have been investigated.

The most common crop included in rotations with cotton is wheat. The Fusarium wilt pathogen is able to colonise incorporated cereal residues. Cotton seedling death from Fusarium was 40% higher following either wheat or barley with the residues incorporated than in bare fallow plots. Current field experiments are comparing the effects of i) incorporating cereal residues, ii) burning cereal residues and iii) 'weathering' cereal residues before incorporation, on the incidence of Fusarium wilt in the subsequent cotton crop. (Allen)

A range of possible summer rotation crop alternatives to cotton have also been evaluated in glasshouse and field experiments. These alternatives have included bare fallow, cotton, maize, sorghum, soybean and mung bean. The incidence of Fusarium wilt was greatest following soybean and mung bean and lowest following a bare fallow. (Swan)

Crop residues are an important source of pathogen inoculum. In two out of three field experiments there has been a 30% reduction in disease incidence when residues have been pulled and mulched and retained on the surface and exposed to wet weather for 6 – 8 weeks instead of incorporating them straight away. There was no significant difference between the two treatments in the third experiment where weather conditions remained dry during exposure of the residues. (Kochman, Allen)

Five field experiments have evaluated the effects of cultivation on the incidence of Fusarium wilt. Disease incidence has not been increased by using rolling cultivators. Cultivation with knives significantly increased (by 15% and by 27%) disease incidence in only two of the experiments. Mixed results were also obtained in field experiments to study the impact of herbicides on disease incidence. In the 2001-2002 season survival was reduced by 41% in the Treflan-Cotogard treated plots while in the 2002-2003 season the Dual-Cotogard treatment had no effect at all on plant survival. It is suggested that these mixed results from cultivation and herbicide treatments may reflect seasonal conditions. When spring conditions are dry there is no shallow root development and consequently cultivation and herbicide treatments have no impact. (Allen)

Summer flooding has been recommended as an effective control strategy for soil borne diseases of cotton in California. Summer flooding has been applied to at least five cotton fields in the Gwydir and Macintyre valleys in recent years. While disease incidence is drastically reduced the pathogen is not eliminated. A field near Boomi was flooded during the summer of 2000-2001. Only 0.8% of plants in the 2001-2002 season and 1.9% of plants in the 2002-2003 season were found to be infected in an area where previously nearly all plants were killed. (Allen)

The cotton plant has natural defense mechanisms that can be activated by the application of acibenzolar-S-methyl (ASM) to the seed at, or prior to, planting. The use of ASM as a seed treatment has consistently reduced the incidence of Fusarium wilt of cotton throughout the season. While not providing complete control, systemic induced resistance is seen as a potentially important component of an integrated disease management strategy for Fusarium wilt of cotton. (Allen, Nehl)

Several novel control strategies are being investigated. The literature provides considerable support for the use of non-pathogenic strains of *Fusarium oxysporum* as biological control agents of Fusarium wilts. A collection of isolates is being assembled for testing of their biological control potential. The use of soluble silicon has been shown to significantly decrease fungal disease susceptibility in various crops. In banana, Fusarium wilt was significantly reduced in soil grown plants and initial studies have shown that the defense gene endochitinase was upregulated 6-15 fold following application of soluble silicon. Glasshouse studies have commenced to investigate the effect of silicon on Fusarium wilt of cotton. (Smith)

Numerous other products, fertilizers and 'potions' have been tested and tried without producing any disease control benefits. These treatments include fertilizers such as gypsum, sulphate of potash, urea, Perlka, Organic Life, feedlot and poultry manure, humates, micronutrients, BioActive, Brotomax, GeoSeedGard, herbal treatments, ti-tree oil, biologicals including four *Trichodermas* and Kodiak, mustard meal, Voom, phosphoric acid, etc. etc. Although fumigation with Metham Sodium produced some encouraging results there were difficulties with application and problems with re-colonisation. (Allen)

An Integrated Disease Management Strategy for Fusarium Wilt

Based on our current understanding of the pathogen – host – environment interaction the following integrated disease management strategy can be recommended:

- maintain good farm hygiene practices
- minimise tailwater to reduce dispersal of the pathogen around the farm
- use the most resistant cultivars available
- use seed treatments to induce resistance when they become available
- delay planting by 2 to 4 weeks – (until the end of October?)
- use rolling cultivators in preference to knives
- avoid legumes in the rotation, bare fallow is best
- after harvest pull and mulch residues and retain on surface for 6 to 8 weeks before incorporation
- flood affected fields for 4 to 8 weeks in summer if opportunity presents

Conclusion

The 'Integrated Disease Management Guidelines' have been developed as a resource for growers and consultants. They provide assistance in identifying, monitoring and managing the significant diseases of cotton.

A considerable and continuing effort is being made to provide solutions to the Fusarium wilt problem. A similar effort is required to address the black root rot problem. Our farming systems have nurtured these 'monsters'. Plant diseases are a man made problem.

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