



## The Development of New Strains of *Verticillium dahliae* in Australia

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### ABSTRACT

*Verticillium wilt was once the most widespread and significant disease of cotton in Australia. Several cultivars with some resistance to the disease have been released by CSIRO since 1989. These cultivars have been widely adopted and grown repeatedly. The results of annual disease surveys and field experiments have shown that repeated cultivation of a resistant cultivar leads to a declining incidence of Verticillium wilt. However, the incidence and severity of Verticillium wilt in a resistant cultivar has increased in several small patches at a site where the resistant cultivar has been grown each year for the last six years. Up to 100 per cent of plants were infected within these small patches.*

### Introduction

*Verticillium wilt of cotton is caused by Verticillium dahliae* Kleb.. Work by Schnathorst and Evans (1971) and Bell (1994) showed that strains of the pathogen isolated from cotton in Australia cause mild symptoms (vegetative compatibility group 4) when compared to those strains that occur overseas (vegetative compatibility groups 1 and 2). The severe defoliating strain of *V. dahliae* is not known to be present in Australia.

The repeated use of susceptible cultivars and the widespread adoption of permanent bed or reduced tillage systems contributed to a significant increase in the incidence of *Verticillium wilt of cotton* in NSW production areas (Allen and Lonergan, 1998). The results of disease surveys in commercial crops throughout NSW during the 1989/90 season showed that the mean incidence of the disease had reached 16.6%. In the same season the mean incidence of *Verticillium wilt of cotton* in the Namoi Valley of NSW reached 31.2%.

When cultivars with some resistance to *V. dahliae* were released by CSIRO in 1990 they were widely adopted. By 1996/97 the area sown to resistant cultivars had increased to 85 per cent of the area sown to cotton in NSW. The new cultivars are not immune to the *Verticillium wilt* pathogen and their resistance is indicated by reduced disease incidence and reduced disease severity. The introduction and quick adoption of these new cotton cultivars has resulted in a steady reduction in disease incidence (Allen and Lonergan, 1998).

The adoption and repeated use of cotton cultivars with resistance to *V. dahliae* in California resulted in the selection and increased prevalence of more virulent strains of the pathogen and consequently an increased incidence of disease in successive years (Ashworth *et*

*al.*, 1983). Their observations had confirmed the earlier work of Schnathorst and Mathre (1966) who had suggested that the 'demise' of *Verticillium wilt* tolerant cotton cultivars in California was caused by a build-up of more virulent strains of the pathogen in the soil as a result of host selection pressure.

The objective of the study reported here was to evaluate the durability of the resistance to *Verticillium wilt* in commercial Australian cotton cultivars. Commercial cotton fields where resistant cultivars of cotton have been grown for several consecutive seasons were inspected as part of annual disease surveys. A field experiment was established to investigate the effect of six consecutive cotton crops on the incidence of *Verticillium wilt*.

### Materials and methods

Between 60 and 100 commercial cotton crops were inspected in March of each year since 1985. The incidence of *Verticillium wilt* in each field was estimated in at least 20 samples of 10 plants selected with a step-point method and the results have been maintained in a database. The database was used to identify fields on the Auscott property at Narrabri where the incidence of *Verticillium wilt* exceeded 60 per cent prior to the introduction of resistant cultivars. The incidence of *Verticillium wilt* was again assessed in these fields in March 1998.

A *Verticillium wilt* 'nursery' was established in a field at the Australian Cotton Research Institute by incorporating gin trash collected during the processing of cotton harvested from fields where the incidence of the disease was known to be high. The development of disease symptoms in the 'nursery' was further encouraged by delaying the sowing date, increasing the irrigation frequency and using higher nitrogen fertilizer rates (El-Zik, 1985). These practices effectively delayed crop maturity and favoured the pathogen.

A susceptible cultivar, Siokra 1-4 was grown over the entire area prior to starting the long-term experiment. The trial area was subsequently divided into six plots with each plot being eight rows wide and 45m long. Three plots were planted to the cultivar Siokra 1-4 for the next six seasons and the remaining three plots were sown with the partially resistant cultivars Sicala V1 or Sicala V2. The cultivar Sicala V2 was a selection from the original Sicala V1 and was released commercially in 1994.

The incidence of *Verticillium* wilt was assessed on the basis of vascular discoloration at the end of each season after cutting the stem with secateurs at ground level. Prior to 1997 assessments were based on ten groups of ten plants selected along a zig-zag path from one end of the plot to the other using only the four centre rows (100 plants/plot). In March of 1997 and 1998 four groups of ten plants were assessed in each of the eight rows of each plot (320 plants/plot).

### Results and discussion

The repeated use of resistant cultivars along with limited crop rotation has resulted in dramatic reductions in the incidence of *Verticillium* wilt in commercial cotton crops (Table 1). This is consistent with the declining incidence of *Verticillium* wilt that has been observed across all cotton growing areas of NSW (Allen and Lonergan, 1998). As a result of the combination of good disease resistance and excellent agronomic qualities the resistant cultivars have been widely adopted and grown repeatedly.

The replicated field experiment in the *Verticillium* nursery at the Australian Cotton Research Institute was established to confirm the observations made in commercial cotton crops. The incidence of *Verticillium* wilt in the susceptible cultivar, Siokra 1-4, was 95 per cent in the first year and increased to over 99 per cent in each of the last three seasons (Figure 1). The incidence of the disease in the resistant cultivar, Sicala V2, declined significantly during the first three seasons. However, the decline ceased during the last three seasons (Figure 1). Closer examination of the plots revealed that there were areas within the plots where the incidence of *Verticillium* wilt had increased (Figure 2). Consequently the assessments of disease incidence in the Sicala V2 plots were more detailed in the 1996/97 and 1997/98 seasons.

In the 1996/97 season, when the mean incidence of *Verticillium* wilt across the three replicates of the resistant cultivar was 35 per cent, there were seven areas where the incidence was greater than, or equal to, 70 per cent. In the 1997/98 season, when the mean incidence of *Verticillium* wilt across the three replicates of the resistant cultivar was 37.5 per cent, there were thirteen areas where the incidence was greater than, or equal to, 70 per cent (Figure 2). There appeared to be no increase in the severity of the disease

symptoms in plants where the incidence of *Verticillium* wilt was high.

The pathogen has been isolated from plants growing within these areas of higher incidence and these isolates are being compared to isolates from elsewhere in the field.

### Conclusion

Schnathorst and Mathre (1966) and Ashworth *et al.* (1983) in California found that the repeated use of cotton cultivars with resistance to *V. dahliae* resulted in the selection of more virulent strains of the pathogen and a consequent breakdown in the level of cultivar resistance. Despite the widespread and repeated use of resistant cultivars in Australia there has been no evidence of the development of more virulent strains of the pathogen in commercial cotton fields to date.

However, the occurrence of small areas in the *Verticillium* wilt nursery experiment, where the incidence of disease is increasing, has indicated that the selection of more virulent strains of the pathogen may be possible under Australian conditions. It should be remembered that the environmental conditions provided in this experiment favoured the pathogen and there were no crop rotations included with either treatment.

Isolates of the pathogen collected from within these areas of higher incidence need to be characterized. It is essential that the incidence of *Verticillium* wilt in commercial cotton crops should continue to be monitored.

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### References

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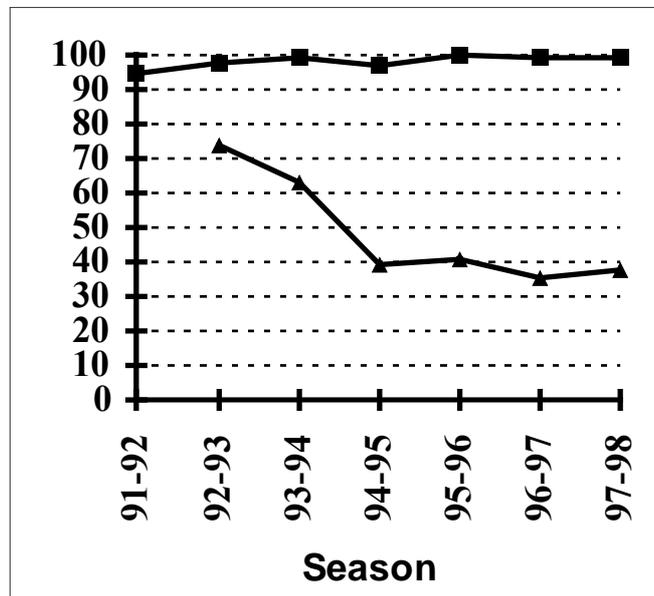
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**Table 1. The effect of the repeated use of resistant cotton cultivars on the incidence of *Verticillium* wilt in commercial crops on the Auscott property near Narrabri.**

Field	Incidence * (season)	Number of cotton crops	Incidence in 1997/98 season
12	70% (1988/89)	6 cotton crops in 9 seasons	4.5%
31	67% (1988/89)	5 cotton crops in 9 seasons	6.5%
33	74% (1990/91)	5 cotton crops in 7 seasons	7.0%
21	72% (1993/94)	3 cotton crops in 4 seasons	22.5%

\* - Incidence = percentage of plants with vascular symptoms of *Verticillium* wilt.

**Figure 1. The incidence of *Verticillium* wilt of cotton in a resistant (s) and a susceptible (n) cultivar when grown repeatedly in the same plots for six consecutive seasons.**



**Figure 2.** Trial plan showing areas within the trial plots where the incidence of *Verticillium* wilt has increased in the last two growing seasons. Plots were eight rows wide by 45 metres long and four groups of ten plants were assessed in each row at the end of the season. The number within each square represents the number of plants with vascular symptoms out of ten plants inspected at each position in the plot.

**1996/97 season (mean incidence – 35.0%)**

3	2	4	7	7	5	4	5	4	8	9	9	2	2	2	2	1	5	4	2	1	3	2	5
5	3	5	1	2	3	2	4	5	4	4	4	4	5	6	3	1	3	1	0	4	3	1	3
10	4	5	5	8	3	3	3	4	3	4	5	3	3	7	3	2	3	2	2	2	1	4	6
5	1	6	5	1	1	3	4	1	1	3	4	3	4	5	3	0	3	4	2	3	4	3	4
<b>Replicate 1</b>								<b>Replicate 2</b>								<b>Replicate 3</b>							

**1997/98 season (mean incidence – 37.5%)**

2	4	5	3	3	3	5	6	9	8	6	9	5	6	6	5	4	3	2	4	4	3	5	8
3	4	3	3	1	1	5	7	5	4	1	2	1	4	5	2	2	3	3	2	1	2	5	5
9	9	8	3	7	3	3	0	3	6	4	2	4	4	3	4	5	3	2	3	4	1	1	7
7	5	7	4	5	0	2	3	2	4	1	1	7	6	3	1	3	1	0	2	2	1	1	2
<b>Replicate 1</b>								<b>Replicate 2</b>								<b>Replicate 3</b>							