# FUSARIUM RESISTANCE RANKING PROTOCOL FOR COTTON CULTIVARS IN AUSTRALIA Stephen Allen Cotton Seed Distributors Ltd Australian Cotton Cooperative Research Centre Narrabri, NSW, Australia

#### Abstract

Commercial cotton cultivars in Australia vary in their resistance/susceptibility to Fusarium wilt. Cotton growers, agronomists and consultants requested the development of a system that would provide a quantitative measure of the relative resistance or susceptibility of new or existing cotton cultivars to Fusarium wilt. A Fusarium Resistance Ranking protocol was developed and growers are using the ranking when selecting cultivars to grow in areas where the disease is present. The protocol is described along with assessment methods and the methods for calculating the ranks. The proportion of plants surviving throughout the season with little or no disease is used as a measure of the resistance of a cultivar and is expressed relative to an industry standard cultivar that is assigned a value of 100. The number of field comparisons contributing to the ranking is indicated in brackets. For example, if a cultivar has a Resistance Rank of 129(5) it indicates that the averaged results of five field experiments have shown that the cultivar is 29% better (more resistant) than the standard. This Resistance Ranking protocol provides a means of measuring the progress being made by plant breeders in their attempts to produce cultivars with higher levels of resistance.

#### **Introduction**

The increasing prevalence of Fusarium wilt of cotton in Australian production areas and the lack of effective control strategies caused considerable concern among growers. Field experiments indicated that all cultivars were susceptible to the disease and that some cultivars were much more susceptible than others. Seed companies began describing commercial cultivars as being 'susceptible' or as having 'some', 'fair, 'good', 'very good' or 'excellent' tolerance or resistance. Under high disease pressure the level of resistance in the best of these cultivars was insufficient to prevent complete crop failure and they could more appropriately be described as 'less susceptible'.

Cotton growers, agronomists and consultants requested the development of a system that would provide a quantitative measure of the relative resistance or susceptibility of new or existing cotton cultivars to Fusarium wilt. Through the Australian Cotton Cooperative Research Centre, a committee of cotton pathologists and representatives from Australian cotton industry organizations, cotton breeding teams and seed production companies have developed the following protocol for describing cotton cultivar resistance to Fusarium wilt. A similar protocol has been developed for describing resistance or susceptibility to Verticillium wilt. These protocols provide a means of measuring the progress being made by plant breeders in their attempts to produce cultivars with higher levels of resistance.

# **The Fusarium Resistance Ranking Protocol**

## 1 The Standard for Describing Cotton Cultivar Resistance to Fusarium Wilt

- Based on previous trial data, Sicot 189 has been selected as the current standard cultivar for ranking Fusarium wilt resistance to Australian strains of the Fusarium wilt pathogen. Sicot 189 must be included in all cultivar comparisons where the incidence of Fusarium wilt is to be assessed.
- The resistance of a cultivar will be expressed relative to the resistance of the standard, which is given the value of 100.
- The number of comparisons (field experiments/cultivar trials) will also be specified in the resistance ranking description. The more comparisons/trials contributing to a Resistance Rank, the more confidence a grower can have in the validity of that ranking.

*Example 1* - The Fusarium Resistance Ranking of new cultivar A = 129(5). This means that the averaged results of five comparisons have indicated that cultivar A is 29% better (more resistant) than the standard.

*Example 2* - The Fusarium Resistance Ranking of cultivar B = 87(7). This means that the averaged results of seven comparisons have indicated that cultivar B is 13% less resistant (more susceptible) than the standard.

## 2 Criteria for Applying the Fusarium Resistance Ranking Protocol

Field trials / Cultivar comparisons :

- must include the standard cultivar Sicot 189;
- must be located on sites where the distribution of the pathogen is as even as possible;

- must be laid out in a statistically valid design with an appropriate number of replications or a repeated check design;
- must have plots of no less than 10 meters in length and be planted to achieve approximately 10 plants per meter;
- must have no more than 70% of plants surviving through the season in the recommended standard cultivar;
- a plant specimen should be submitted to a laboratory to confirm which strain of the pathogen is present at the trial site;
- replication of trials at more than one site, and over several seasons is encouraged.

Other considerations:

- Since trials may include both Bt-transformed and conventional cultivars, it is important that they be sprayed to control insect pests eg. *Helicoverpa*. It would be unfair to compare the level of disease in a conventional cultivar with that of a Bollgard cultivar in an unsprayed trial;
- Yield data is not required for calculation of the Fusarium Resistance Ranking of a cultivar. Other data (eg. yield, fibre quality etc.) can be presented in addition to the Fusarium Resistance Ranking at the discretion of the seed company/breeding program;
- Qualitative terms such as 'slight tolerance' and 'some resistance' should not be used to describe resistance.

## 3 Example of Calculations to Determine the Fusarium Resistance Ranking

The following values are calculated for each cultivar:

- A. *Initial plant stand* This is the total number of seedlings in the row or plot (a minimum of 10m) assessed as soon as possible after emergence (and no later than 3 weeks). Eg. for cultivar X the initial stand count in the 10m plot is 110 seedlings.
- *B* Number of plants rating "0" and "1" at harvest This value describes the number of plants in a plot that have a Vascular Browning Index rating of 0 or 1 (see 4 below) when the stems of plants with 4 bolls or more are cut at ground level, immediately after harvest, at the end of the season. Eg. after stem cutting plants of cultivar X, a total of 46 plants had VBI scores of 0 or 1.
- *C.* Proportion of plants rating "0" or "1" ie the proportion of plants surviving with little or no disease throughout the season. This value is calculated by dividing the value of B by the value of A and converting to a percentage. Eg. for cultivar X, B/A = 46/110 x 100 = 41.8%

<u>The Fusarium Resistance Ranking</u>. To determine the Fusarium Resistance Ranking value for a cultivar the "Proportion of plants rating 0 or 1" (C) is expressed in relation to the industry standard, which is given the value of 100 for the purpose of this ranking system.

Eg. if the value of C above for the standard cultivar Sicot 189 in a trial is 30.5 (Number of Plants Rating 0 or 1 at Harvest =32/ Initial Plant Stand =105) and the value for cultivar X in the same trial is 41.8, then the Fusarium Resistance Ranking for cultivar X is expressed as:

 $41.8/30.5 \times 100 = 137(1)$  - The figure in brackets gives the number of trials used to determine the figure, in this case, one.

## **<u>4 Vascular Browning Index for Assessing Fusarium Wilt Infection</u>**

This rating system is based on the severity of vascular discoloration (browning) visible in a cross section of the main plant stem cut as close as practicable to ground level immediately after harvest where:

- 0 = no vascular discoloration
- 1 = discoloration restricted to an area less than 5% of the stem cross section
- 2 = discoloration of between 5% and 20% of the stem cross section
- 3 = discoloration of between 20% and 40% of the stem cross section
- 4 = greater than 40% vascular discoloration of the stem cross section

A rating of "0" indicates no visible evidence of the disease in the stem while a rating of "1" indicates that vascular discoloration has been restricted, usually to the center of the base of the stem.

## **<u>5 Presentation of Results</u>**

It is acknowledged that the presentation of a Fusarium Resistance Rank that is an average of the Resistance Ranks from several field trials can be misleading and hide significant variability. For this reason it is suggested that individual trial results should be made available in table or graph form and a Standard Error should be calculated to indicate the variability (Tables 1 and 2). An alternative approach is to plot survival in the test cultivar against survival in the standard cultivar for each of the field experiments where both cultivars are present. The slope of the relationship is an estimate of the Resistance Ranking and the 'r-squared' value is a measure of the variability.

#### **Discussion**

The Fusarium Resistance Ranking protocol is still undergoing some development and refinement. It has been well accepted by the Australian cotton industry and is being used by growers when selecting cultivars for areas where the disease is known to be present. There is some concern that growers are placing too much weight on position in the rankings rather than on statistically valid differences in rankings and some effort is being applied to the development of appropriate statistical methods to address this issue.

The standard cultivar was originally chosen because it was widely grown and considered to be one of the least susceptible cultivars available to growers. Cotton breeding programs have made some progress and there are now several commercial cultivars that appear to be as good as, or slightly better than, the standard cultivar. Some experimental breeding lines with preliminary Fusarium Resistance Ranks in excess of 150 have been identified in the last two seasons.

			VCG	Survival*	Resistance
Season	Location	Trial type	(strain)	in Std (%)	Rank
00/01	Boggabilla	CSD box trial	11	32.2	110
00/01	Pampas	CSD box trial	11	44.0	117
00/01	Bongeen	CSD box trial	11	65.0	103
00/01	Brookstead	CSD large plot	11	27.5	172
01/02	Boggabilla	CSD box trial	11	20.6	96
01/02	Brookstead	CSD small plot	11	28.2	113
01/02	Brookstead	CSD box trial	11	29.2	109
01/02	Pampas	CSD small plot	11	7.9	126
01/02	Norwin	QDPI small plot	11	37.6	97
01/02	Norwin	QDPI/CSIRO	11	26.9	106
01/02	Norwin	QDPI/CSIRO	11	33.2	115
01/02	Norwin	QDPI/CSIRO	11	27.4	109
01/02	Norwin	QDPI/CSIRO	11	39.9	89

Table 1. The results of 13 individual comparisons between the cultivar Sicot 80 and the standard - contributing to the average Fusarium Resistance Rank for Sicot  $80 = 112(13)^{\#}$ .

 $^{\#}$  - Standard Error = 20.37

\*- the proportion of plants surviving with little or no disease - indicates disease pressure.

Table 2. The results of 16 individual comparisons between the cultivar Sicala 40 and the standard - contributing to the average Fusarium Resistance Rank for Sicala  $40 = 78(16)^{\#}$ .

			VCG	Survival*	Resistance
Season	Location	Trial type	(strain)	in Std (%)	Rank
00/01	Boggabilla	CSD box trial	11	57.5	92
00/01	Boggabilla	CSD box trial	11	32.2	71
00/01	Bongeen	CSD box trial	11	65.0	98
00/01	Norwin	CSIRO small plot	11	15.4	83
00/01	Norwin	CSIRO small plot	11	8.1	89
00/01	Norwin	CSIRO small plot	11	21.8	52
01/02	Brookstead	CSD small plot	11	28.2	56
01/02	Pampas	CSD small plot	11	7.9	81
01/02	Norwin	QDPI small plot	11	37.6	90
01/02	Norwin	QDPI/CSIRO	11	26.9	74
01/02	Norwin	QDPI/CSIRO	11	33.2	72
01/02	Norwin	QDPI/CSIRO	11	27.4	68
01/02	Norwin	QDPI/CSIRO	11	39.9	79
01/02	Norwin	QDPI/CSIRO	11	24.3	75
01/02	Norwin	QDPI/CSIRO	11	37.4	80
01/02	Norwin	QDPI/CSIRO	11	36.6	87

<sup>#</sup> - Standard Error = 12.52

\*- the proportion of plants surviving with little or no disease - indicates disease pressure.