

***Fusarium* and *Verticillium* Resistance Ranking Protocol for Cotton Varieties**

Purpose of this protocol

Through the Australian Cotton CRC, a committee of cotton pathologists and representatives from CSIRO, CSD, Deltapine, CRDC and ACGRA developed protocols for describing cotton variety resistance to *Fusarium* (F.rank) and *Verticillium* wilt (V.rank). The purpose of this system was to provide cotton growers and other industry members with a quantitative measure of the relative wilt resistance or susceptibility of new or existing cotton varieties. Recommended modifications arising from reviews in 2004 and 2006 have been incorporated in this 2008 version of the protocol.

1. *Fusarium* Resistance Ranking for cotton varieties – ‘F.rank’

1.1 A standard for describing cotton variety resistance to *Fusarium* wilt

- Sicot 189 is nominated as the standard variety for *Fusarium* wilt resistance ranking to Australian strains of *Fusarium* wilt.
- The resistance of all other varieties will be expressed relative to the resistance of the standard, which is given the value of 100
- All varieties will be ranked on a scale limited to between ‘0’ and ‘200’
- Varieties more resistant than the standard will be ranked between ‘100’ and ‘200’
- An F.rank of ‘200’ indicates completely resistant or immune (all plants survive unaffected)
- The F.rank for varieties that are more susceptible than the standard will be between ‘0’ and ‘100’
- An F.rank of ‘0’ indicates completely susceptible (all plants affected substantially)
- The number of comparisons (experiments/variety trials) must be indicated in brackets after the resistance ranking value.

1.2 Criteria for applying the *Fusarium* Resistance Ranking protocol

Field trials must

- be registered with the CRDC according to the ‘Trial Registration Procedures’ – see Section 3;
- have confirmed the strain of *Fov* present at the site by sending specimens for analysis;
- be laid out in a statistically valid design eg. appropriate number of replicated plots or repeated check design;
- have plots of no less than 10 meters in length;
- be planted to achieve a commercially acceptable stand;
- include the standard for *Fov* resistance ie. Sicot 189 or Sicot 289BR
- have a ‘Proportion of Plants Rating 0 & 1’ (ie. survival) of no more than 70% in the standard variety; *It may also be necessary to discard the results of trials where disease pressure is extreme and less than 5% of plants survive in the standard variety.*

Other considerations

- since trials may include both Bt-transformed and conventional varieties, 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 variety with that of a Bollgard II variety in an unsprayed trial;
- the use of terms like ‘slight tolerance’ and ‘some resistance’ to describe varieties is not recommended.
- For varieties with an F.rank of <50 use of the term ‘susceptible’ is acceptable as it may be difficult to include these varieties in sufficient F.rank trials to generate the required data.

1.3 Assessments and calculations to determine the 'F.rank'

The following values are calculated for each variety:

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).

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 1.4 below) when the stems of plants are cut at or near ground level at the end of the season.

C. Survival (Proportion of Plants Rating 0 and 1)

The survival is the number of plants rating '0' or '1' at harvest as a percentage of the initial plant stand. This value is calculated by dividing the value of B by the value of A and converting to a percentage.

D. 'F.rank'

The calculation of the F.rank differs depending on whether the survival in the test variety (T) is lower or higher than the survival in the standard variety (S).

The calculation is easily done in MS Excel using an 'IF' statement:

$$=IF(T<S, 100*T/S, 100+((T-S)/(100-S)*100))$$

This statement says that if survival in the test variety (T) is lower than survival in the standard variety (S) use the equation $100*T/S$, but if T is higher than S use the equation $100+((T-S)/(100-S)*100)$.

1.4 Vascular Browning Index for assessing Fusarium wilt infection

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 where:

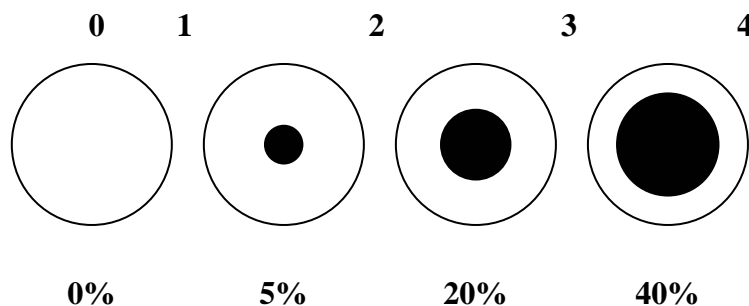
0 = no vascular discoloration

1 = discoloration restricted to small spots or 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



Note: the discoloured areas show the upper limit for each category

2. *Verticillium* Resistance Ranking for cotton varieties – ‘V.rank’

2.1 A standard for describing cotton variety resistance to *Verticillium* wilt

- Sicala V2 is nominated as the standard variety for *Verticillium* wilt resistance ranking.
- The resistance of all other varieties will be expressed relative to the resistance of the standard, which is given the value of 100
- All varieties will be ranked on a scale limited to between ‘0’ and ‘200’
- Varieties more resistant than the standard will be ranked between ‘100’ and ‘200’
- A V.rank of ‘200’ indicates completely resistant or immune (all plants survive unaffected)
- The V.rank for varieties that are more susceptible than the standard will be between ‘0’ and ‘100’
- A V.rank of ‘0’ indicates completely susceptible (all plants affected substantially)
- The number of comparisons (experiments/variety trials) must be indicated in brackets after the resistance ranking value.

2.2 Criteria for applying the *Verticillium* Resistance Ranking protocol

- Trials must be registered with the CRDC according to the ‘Trial Registration Procedures’ – see Section 3;
- *Verticillium* disease assessments should **not** be made before the final irrigation;
- Plots must be at least 10 metres in length and be planted to achieve a commercially acceptable stand;
- Trials are to be laid out in a statistically valid design eg. An appropriate number of replicated plots or repeated check design;
- For data to be used for this ranking system there should be no more than 90% disease-free plants in the recommended standard variety (see 2.3 below);

2.3 Assessments and calculations for determining the ‘V.rank’

- Assess disease incidence (%) based on the presence or absence of vascular discoloration in the main stem at, or just above, ground level - immediately following harvest.
- Determine survival (% healthy plants) by subtracting % incidence from 100
- Calculate the ‘V.rank’ using the same ‘IF’ statement as for the ‘F.rank’

3 Trial Registration Procedures

- *A ‘Cotton Disease Resistance Ranking Trial Registration Checklist’ must be completed and submitted to either the QDPI&F or NSW DPI cotton Pathologist for registration purposes.
- *After allocation of a number - one copy each for registrar, registrant and CRDC.
- *A trial plan with initial stand counts must be made available at registration for sighting only.
- *Either a results summary or a reason for abandoning the trial must be submitted to CRDC on completion of trial.

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Cotton Disease Resistance Ranking Trial Registration Checklist

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A trial plan with initial stand counts must be made available for sighting only.

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Ranking trial identification number	NSWDPI – N..... QDPI&F - Q.....
Disease	Fusarium/Verticillium
Organisation	
Contact	
Trial location (inspection may be undertaken)	
Trial plans and initial stand counts sighted	Yes / No
Trial design appropriate	Yes / No
Replications	
Plot size (minimum 1 row by 10metres)	
Initial stand count (commercially acceptable stand)	
Industry standard variety used	
Minimum disease pressure (maximum survival) for including trial results in F.rank or V.rank calculations	Fusarium – 70% survival Verticillium – 90% survival
FOV strain checked/ to be checked	01111 / 01112 / _____?_____
Insecticide Applications	Fully sprayed conventional Fully sprayed BollgardII Unsprayed BollgardII with hand sprayed standard as required
Signature	
Date	

NOTE – A results summary is to be lodged with the CRDC on completion of trial.

OR - CRDC must be advised of reason for abandoning trial