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Appendix 1

Variety Resistance Ranking

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 have developed the following protocols for describing cotton variety resistance to Fusarium and Verticillium wilt. The purpose of this resistance ranking system is 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.

1. FUSARIUM RESISTANCE RANKING FOR COTTON VARIETIES

1.1A STANDARD FOR DESCRIBING COTTON VARIETY RESISTANCE TO FUSARIUM WILT

Based on previous trial data DeltaEMERALD and Sicot 189 are nominated as the current standard varieties for Fusarium wilt resistance ranking to Australian strains of Fusarium wilt. It is crucial that at least one, and where possible both, of these varieties be included in all ranking assessments for Fusarium wilt. QDPI and NSW Agriculture will include DeltaEMERALD and Sicot 189 in all variety comparisons

- The resistance of a variety will be expressed relative to the resistance of the standard, which is given the value of 100
- The number of comparisons (experiments/variety trials) will also be specified in the resistance ranking description

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

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

1.2 CRITERIA FOR APPLYING THE FUSARIUM RESISTANCE RANKING PROTOCOL

Field trials must

- be located on sites where the level and distribution of disease in the previous season is known (involve the local IDO in this decision where possible);
- 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 with all plants of 4 bolls or more within this to be assessed at the end of the season ;
- be planted to achieve 10 plants per metre;
- include one or both standards for Fov resistance ie. DeltaEMERALD &/or Sicot 189;
- have a 'Proportion of Plants Rating 0 & 1' (ie. value C below) of no more than 70% in the recommended standard variety;
- replication of trials at more than one site is encouraged.

Other considerations

- trials should be inspected early in the season (eg. by the local IDO and in communication with CRDC) to have the trial requirements verified and a trial number assigned;
- 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 an Ingard variety in an unsprayed trial;
- yield data is not required for calculation of the Fusarium Resistance Ranking of a variety. 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 programme;
- the use of terms like 'slight tolerance' and 'some resistance' to describe new varieties is discouraged in preference to descriptions of resistance in percentage terms in relation to the standard varieties.

1.3 EXAMPLE OF CALCULATIONS TO DETERMINE THE FUSARIUM RESISTANCE RANKING

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). Eg. for variety XYZ the initial stand count in the 10m plot is 80 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 1.4 below) when the stems of plants with 4 bolls or more are cut at or near ground level at the end of the season. Eg. after stem cutting plants of variety XYZ, a total of 34 plants had VBI scores of 0 or 1.

C. Proportion of Plants Rating 0 and 1

This value is calculated by dividing the value of B by the value of A and converting to a percentage. Eg. for variety XYZ, $B/A = 34/80 \times 100 = 42.5\%$

D. Fusarium Resistance Ranking

To determine the Fusarium Resistance Ranking value of a variety the "Proportion of Plants Rating 0 and 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 Sicot 189 in a trial is 22.4 (Number of Plants Rating 0 and 1 at Harvest 32/ Initial Plant Stand 70) and the value for variety XYZ in the same trial is 42.5, then the Fusarium Resistance Ranking for variety XYZ is expressed as:

$42.5/22.4 \times 100 = 190(1)$ - The figure in brackets gives the number of trials used to determine the figure, in this case only one.

Note

Cotton breeders and seed companies may find it useful to divide the 'Proportion of plants rating 0 and 1'(C) into its two components that describe seedling and adult plant reaction to the disease. Seedling survival can be calculated by comparing the number of plants stem-cut at the end of the season with the 'Initial Plant Stand' (A) while adult plant reaction is described by expressing the number of plants with a 0 or 1 stem-cut rating (B) as a proportion of the number of plants that were stem-cut.

1.4 VASCULAR BROWNING INDEX FOR ASSESSING FUSARIUM WILT INFECTION

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

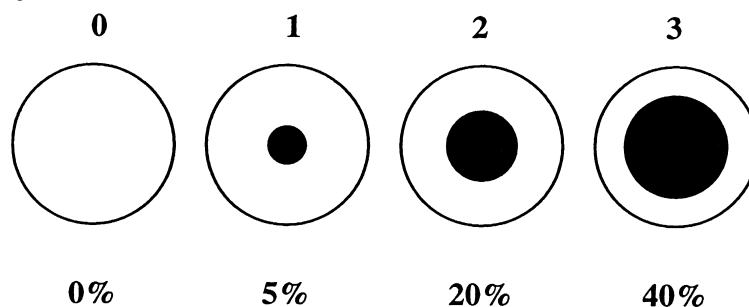
0 = no vascular discolouration

1 = discolouration restricted to small spots or an area less than 5% of the stem cross section

2 = discolouration of between 5% and 20% of the stem cross section

3 = discolouration of between 20% and 40% of the stem cross section

4 = greater than 40% vascular discolouration of the stem cross section



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



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2. VERTICILLIUM RESISTANCE RANKING FOR COTTON VARIETIES

2.1 A STANDARD FOR DESCRIBING COTTON VARIETY RESISTANCE TO VERTICILLIUM WILT

- Based on previous trial data Sicala V2 is nominated as the national standard variety for Verticillium wilt resistance ranking. It is strongly encouraged that this variety be included in all future ranking for Verticillium wilt
- QDPI and NSW Agriculture will include Sicala V2 in all variety comparisons
- The resistance of a new variety will be expressed relative to the resistance of the standard, which is given the value of 100
- The number of comparisons (experiments/variety trials) will also be specified in the resistance ranking description

Example 1 - The Verticillium Resistance Ranking of new variety XYZ = 121(5). This means that the averaged results of five comparisons have indicated that variety XYZ is 21% better (more resistant) than Sicala V2.

Example 2 - The Verticillium Resistance Ranking of variety ABC = 85(7). This means that the averaged results of seven comparisons have indicated that variety ABC is 15% less resistant than Sicala V2.

2.2 CRITERIA FOR APPLYING THE VERTICILLIUM RESISTANCE RANKING PROTOCOL

- Trials to be located on sites where the level of disease is known (involve the local IDO in this decision where possible);
- Verticillium disease assessments should not be made before the final irrigation
- at least 100 plants per plot (or given section of a plot) should be used in Verticillium resistance evaluation
- Trials are to be laid out in a statistically valid design eg. 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)
- Replication of trials at more than one site is encouraged

2.3 CALCULATIONS USED IN DETERMINING VERTICILLIUM RESISTANCE RANKING

- Assess disease incidence (%) at the end of the season (presence or absence of vascular discolouration)
- Determine % healthy plants by subtracting % incidence from 100
- Express relative to the standard, which is given the value of 100

Eg. if the disease incidence for Sicala V2 in a trial is 25% (ie. percentage healthy = 75%) and the disease incidence for variety XYZ in the same trial is 19% (ie. percentage healthy = 81%), then the Verticillium Resistance Ranking for variety XYZ is expressed as:

$81/75 \times 100 = 108(1)$ The figure in brackets gives the number of trials used to determine the figure, in this case only one.