



## Instability of ramulosis reaction of cotton cultivars with respect to protection and registration procedures

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**ABSTRACT** - The reaction to ramulosis is a descriptor in the protection and registration of cotton cultivars. This descriptor is however not as homogeneous, distinguishable and stable as desired, since it depends on the isolates and the edaphoclimatic conditions. To evaluate the stability of this descriptor for cotton cultivars the Specific combining ability (SCA), general combining ability (GCA) and the general aggressiveness (GA) were estimated for the cultivars EPAMIG 5 - Precoce 1, IAC-22, EPAMIG 4 - Redenção, CNPA – ITA 96 and CNPA – ITA 90, which were inoculated with isolates of *Colletotrichum gossypii* var. *cephalosporioides*, causal agents of ramulosis. The symptoms were evaluated 10 and 40 days after inoculation. The significance of the variables SCA, GCA and GA indicated variability of horizontal resistance and isolate aggressiveness. This performance suggests specific responses of the cultivars to the contact with different pathogen isolates and consequently, instability of this descriptor.

**Key-words:** *Colletotrichum gossypii* var. *cephalosporioides*, disease resistance, horizontal resistance.

### INTRODUCTION

One of the main fungal diseases of cotton (*Gossypium hirsutum* L.) is ramulosis, whose etiologic agent is *Colletotrichum gossypii* var. *cephalosporioides* A. S. Costa (Costa 1941). The great challenge of this disease is the possibility of transmission of the etiologic agent via seed, posing the risk of bringing the pathogen to yet uninfested areas (Lima et al. 1985).

The reaction to ramulosis has been used as one of the descriptors for the protection and registration of cotton cultivars (Brazil 1997). But ramulosis reaction, as well as the other descriptors, gives rise to doubts. A reliable descriptor must meet standards of homogeneity, stability and distinctness for a successful use, while these factors vary considerably under different conditions of cultivation,

soil fertility and climate. However, stability seems to be an important factor linked to the resistance of cultivated varieties, and to the genetic control involved in this resistance.

Cia and co-authors (1999) mentioned the instability of most cotton genotypes to diverse diseases indicating the need of genetic improvement for multiple disease resistance. Another aspect is that information on the distinct cultivar performance and the degree of aggressiveness of the agent causal or degree of favorable environmental conditions would be important, as they would allow an evaluation of the horizontal and vertical resistance for this descriptor.

However, there are few studies on the genetic control of the reaction to ramulosis (Carvalho et al. 1994) and

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few evaluations on the stability of this trait when tested across the diversity of pathogen races or genotypes found in nature.

This stability can be evaluated in studies of the pathogen-host interaction, as suggested by Melo and Santos (1999), where the general combining ability (GCA) of the host represents the horizontal resistance, and depends on the average performance of the host in the inoculations with different isolates of the pathogen. Likewise, the general aggressiveness (GA) of the pathogen is given by the mean pathogenicity of each isolate when inoculated into the different host genotypes. The interaction between pathogenicity and horizontal resistance corresponds to the Specific combining ability (SCA), which represents the vertical resistance. These estimates allow conclusions on the pathogen virulence, as well as estimations of the vertical resistance of the host genotypes.

This study aimed to estimate the SCA host-pathogen, the GCA of the host, and the GA of the pathogen in pathogenicity tests of plants inoculated with *C. gossypii* var. *cephalosporioides*. The data allowed to draw conclusions on the type of resistance that involves a genotype-host relationship, aiming to evaluate the stability of the variable “reaction to ramulosis” used in procedures of protection and registration of cotton cultivars.

## MATERIAL AND METHODS

The experiments were carried out in a greenhouse of the Department of Agriculture of the Universidade Federal de Lavras. In the first experiment four different isolates of *C. gossypii* var. *cephalosporioides* (CA-12, CA-1, CGC1, CGC 5) were used and eight isolates (10166, 11327, 11086, 11326, CR5, 6239, 6727, 3564) in the second (Table 1). Pathogenicity tests were carried out involving the cotton cultivars EPAMIG 5 - Precoce 1, IAC-22, EPAMIG 4 - Redenção, considered moderately ramulosis-resistant, and CNPA ITA 96 and CNPA ITA 90, considered fully ramulosis-resistant.

Seeds of the cultivars were sown in pots filled with about 3 kg soil and thinned to two plants per pot. Macro and micronutrients were complemented according to crop-specific fertilization recommendations.

The isolates were cultivated in PDA (Potato-Dextrose-Agar) medium. Fungal conidia suspended in sterilized water at a concentration of  $10^6$  conidia/mL were applied to 30-day-old plants, which were maintained in a moist chamber for 72 hours.

The symptoms were evaluated 10 and 40 days after inoculation (mean temperature 30 °C, relative humidity 80%). The disease severity was evaluated by the slightly modified score scale proposed by Cia (1977) (Table 2).

The resistance was estimated based on a methodology proposed by Melo and Santos (1999) that uses the model of Griffing IV (1954), with a partial diallel scheme, by Generaldi and Miranda Filho (1988).

A completely randomized design was used with four replications. The data were analyzed using the statistical software package SAS® (1993). The mathematical model Y proposed for the analysis of the pathogen-host interaction for the partial diallel was:

$$Y_{ij} = \mu + r_i + a_j + s_{ij}$$

$Y_{ij}$  = disease severity expressed by host i when inoculated with isolate j;

$r_i$  = effect of horizontal resistance of host i;

$a_j$  = effect of aggressiveness of race j;

$s_{ij}$  = effect of interaction of host i when inoculated with isolate j, compared to the virulence effect of race j with the vertical resistance of host i.

## RESULTS AND DISCUSSION

The results of the diallel analysis (Table 3) of the first experiment (1999) showed that GCA, GA and SCA were significant (1% probability) for the disease data 40 days after inoculation. This evidences the existence of variability for horizontal resistance and isolate virulence, as well as the existence of vertical resistance. In the second experiment (2000) SCA and GA were significant (1% probability) (Table 3). The significance of SCA implies the existence of variability in vertical resistance, that is, specific cultivar performances when inoculated with different isolates. This performance has been described in pathogenicity tests as symptom variations, probably due to variability of isolate aggressiveness (Tanaka and Lyn 1991).

In this study, the horizontal cultivar resistance differed in the first year of experiment. The cultivars EPAMIG 4 - Redenção and CNPA - ITA 96 were ramulosis-susceptible (Table 4). Ten days after inoculation, these cultivars did not differ from each other in the symptomology. After 40 days however, the horizontal resistance of cultivar ITA 96 was lower.

Independently of the evaluation date, the cultivar with the highest degree of horizontal ramulosis

**Table 1.** Provenance of isolates of *Colletotrichum gossypii* var. *cephalosporioides* used in the trials with cotton (*Gossypium hirsutum*)

| Isolate | Provenance    | Host                                       |
|---------|---------------|--|
| 10166   | Piracicaba-SP | <i>G. hirsutum</i> – stem                  |
| 11327   | Piracicaba-SP | <i>G. hirsutum</i> – stem                  |
| 11086   | Piracicaba-SP | <i>Euphorbia heterofilla</i> L.- plant tip |
| 11326   | Piracicaba-SP | <i>G. hirsutum</i> -stems                  |
| CR5     | Ituverava-SP  | <i>G. hirsutum</i> -stems                  |
| 6239    | Piracicaba-SP | <i>G. hirsutum</i> -stems                  |
| 6727    | Piracicaba-SP | <i>Bidens pilosa</i> -stems                |
| 3564    | Piracicaba-SP | <i>G. hirsutum</i> -stems                  |
| CA12    | Piracicaba-SP | <i>G. hirsutum</i> -IAC 21                 |
| CA1     | Piracicaba-SP | <i>G. hirsutum</i>                         |
| CGC5    | Andirá-PR     | <i>G. hirsutum</i> -seeds-IAPAR 71-PR3     |
| CGC1    | Piracicaba-SP | <i>G. hirsutum</i> -stems                  |

**Table 2.** Criteria to evaluate ramulosis symptoms in cotton plants (*Gossypium hirsutum*), inoculated with isolates of *Colletotrichum gossypii* var. *cephalosporioides*

| SYMPTOMS   | SCORE |
|--|-------|
| Plants without symptom (absence of lesions) .....  | 1     |
| Plants with yellowish spots on shoot leaves.....   | 2     |
| Plants with less than 40% reduction of the internodes, compared with the control, and with round and star-shaped leaf spots..... | 3     |
| Plants with excessive sprouting and growth reduction by 40 to 60%, compared with the control .....                               | 4     |
| Plant with excessive sprouting and affected development; growth reduced by 60% or more .....                                     | 5     |

**Table 3.** Summary of the analysis of variance of the diallel scheme for ramulosis severity 40 days after inoculation of cotton cultivars (*Gossypium hirsutum*) with different isolates of *Colletotrichum gossypii* var. *cephalosporioides*

| 1999                        |    |             |
|-----------------------------|----|-------------|
| Source of Variation         | df | Mean square |
| GCA (Horizontal resistance) | 4  | 4.106*      |
| GA (Aggressiveness)         | 3  | 20.466*     |
| SCA (Vertical resistance)   | 12 | 3.039*      |
| Error                       | 60 | 0.025       |
| 2000                        |    |             |
| GCA (Horizontal resistance) | 4  | 1.020 n.s.  |
| GA (Aggressiveness)         | 7  | 2.571*      |
| SCA (Vertical resistance)   | 28 | 2.111**     |
| Error                       | 40 | 0.8999      |

\* significant at 1%

\*\* significant at 5%

resistance was EPAMIG 5 - Precoce 1 (Table 4), followed by IAC-22 and CNPA- ITA 90, which performed similarly.

**Table 4.** Estimate of Resistance Capacity (horizontal resistance) to ramulosis severity 10 and 40 days after inoculation, of the cotton cultivars (*Gossypium hirsutum*) inoculated with different isolates of *Colletotrichum gossypii* var. *cephalosporioides* (1999)

| Cotton cultivar                       | GCA (severity) |         |
|---------------------------------------|----------------|---------|
|                                       | 10 days        | 40 days |
| EPAMIG 5 – Precoce 1                  | -0.587         | -0.637  |
| IAC-22                                | -0.150         | -0.200  |
| EPAMIG 4 - Redenção                   | 0.474          | 0.487   |
| CNPA ITA-96                           | 0.412          | 0.549   |
| CNPA ITA-90                           | -0.150         | -0.200  |
| DMS (G <sub>i</sub> )                 | 0.050          | 0.035   |
| DMS (G <sub>i</sub> -G <sub>j</sub> ) | 0.079          | 0.055   |

Regarding GA we observed that isolate CGC1 was the most aggressive 10 as well as 40 days after inoculation, with characteristic symptoms on the leaves, shortened internodes and height reduction between 40 and 60% (Table 5).

**Table 5.** Estimates of the General Aggressiveness Ability for ramulosis severity, 10 days after inoculation of cotton cultivars (*Gossypium hirsutum*) with different *Colletotrichum gloesporioides* isolates

| Isolates                              | 1999           |         |
|---------------------------------------|----------------|---------|
|                                       | GAA (severity) |         |
|                                       | 10 days        | 40 days |
| CA-12                                 | -0.850         | -0.850  |
| CA-1                                  | -0.950         | -0.850  |
| CGC1                                  | 1.199          | 1.149   |
| CGC5                                  | 0.599          | 0.549   |
| DMS (G <sub>i</sub> )                 | 0.043          | 0.030   |
| DMS (G <sub>i</sub> -G <sub>j</sub> ) | 0.070          | 0.050   |
| Year 2000                             |                |         |
| 10166                                 | 0.337          | 0.400   |
| 11327                                 | -0.462         | -0.600  |
| 11086                                 | -0.962         | -0.200  |
| 11326                                 | 0.237          | 0.300   |
| CR5                                   | 0.737          | 0.700   |
| 6239                                  | 0.137          | -0.500  |
| 6727                                  | -0.562         | -0.500  |
| 3564                                  | 0.537          | 0.400   |
| DMS (G <sub>i</sub> )                 | 0.270          | 0.280   |
| DMS (G <sub>i</sub> -G <sub>j</sub> ) | 0.409          | 0.424   |

The second most aggressive was CGC5, which presented ramulosis symptoms after 10 as well as after 40 days (Table 5).

The aggressiveness of these isolates was heterogeneous.

In the second experiment (2000), 10 days after inoculation the most aggressive isolates were CR5, 3564, 10166 and 11326 (Table 5).

The symptomology after 40 days indicated CR5, 3564, 10166, 11326 as the most aggressive isolates (Table 5). This allows conclusions on the difference of performance of the cultivars towards the different isolates, which must be taken into consideration when resistance is evaluated; here it was expressed in the SCA estimates.

The data of the AFLP marker analysis indicated values of genetic similarity of 69.47% for CGC1 and CGC5, of 97.21% for CR5, 3564, 10166 and 11326, and mean similarity values of over 95% for CR5, 3564, 10166, and 11326 (Silva-Mann et al. 2005).

In spite of the close relationship revealed by the AFLP marker analysis, the microorganisms presented different responses in association with different cotton

cultivars, which contributed to infer on the instability of the physiologic descriptor “reaction to ramulosis”.

The SCA estimates indicate the vertical resistance level of cultivars in relation to isolate aggressiveness (Tables 6 and 7). It was verified that the cultivars EPAMIG 5 –Precoce 1 and IAC-22, described as intermediate ramulosis-resistant in the literature presented vertical resistance to isolate CGC5 only, whereas cultivar CNPA-ITA 90, described as ramulosis-resistant (Beltrão 1999), presented vertical resistance to the isolates CA-12 and 10166 only. On the other hand, the cultivars IAC-22 and EPAMIG 4 - Redenção, described as intermediately ramulosis-resistant (Beltrão 1999), presented vertical resistance to isolate CA-12 in the evaluation after 10 days (Table 5). Likewise, the cultivars IAC-22, CNPA – ITA 96 and EPAMIG 4 - Redenção presented this type of resistance to this isolate when evaluated 40 days after inoculation (Table 7).

In the second experiment, cultivar EPAMIG 5 - Precoce 1 presented vertical resistance to isolate 6727, obtained from *Bidens pilosa* L. plants in the cotton field, which induced ramulosis symptoms when inoculated in cotton plants in the present study. The same was observed for cultivar IAC-22 when inoculated with isolate 11086 (Table 7). In studies with AFLP markers these isolates presented a similarity of 97.50% (Silva-Mann et al. 2005). In spite of the proximity, the isolates performed differently for pathogenicity in cotton plants.

Ramulosis-resistance has been used as physiologic marker in the procedure of protection and certification of cotton cultivars. However, as shown here, the way the

**Table 6.** Estimates of the Specific Combining Ability (SCA) resulting of the interaction between cotton cultivars and isolates of the *Colletotrichum gloesporioides* complex, regarding disease severity, evaluated 10 days after inoculation (1999)

| Varieties                                 | Isolates |        |        |        |
|---|----------|--------|--------|--------|
|   | CA-12    | CA-1   | CGC1   | CGC5   |
| EPAMIG 5-Precoce 1                        | 0.037    | 0.387  | 0.987  | -1.412 |
| IAC-22                                    | -0.399   | 0.700  | 0.550  | -0.849 |
| EPAMIG 5 Redenção                         | -0.274   | -0.174 | -0.074 | 0.525  |
| CNPAITA-96                                | 0.037    | -0.612 | -0.012 | 0.587  |
| CNPAITA-90                                | 0.600    | -0.299 | -1.449 | 1.150  |
| DMS (S <sub>ij</sub> )                    | 0.086    |        |        |        |
| DMS (S <sub>ij</sub> - S <sub>ik</sub> )  | 0.141    |        |        |        |
| DMS (S <sub>ijm</sub> - S <sub>kj</sub> ) | 0.136    |        |        |        |
| DMS (S <sub>ij</sub> - S <sub>kl</sub> )  | 0.117    |        |        |        |

**Table 7.** Estimates of the Specific Combining Ability (SCA) resulting of the interaction between cotton cultivars and isolates of the *Colletotrichum gloeosporioides* complex, with respect to disease severity, evaluated 40 days after inoculation

| 1999                      |        |        |        |         |        |        |         |        |
|---------------------------|--------|--------|--------|---------|--------|--------|---------|--------|
| Cultivar                  | CA12   | CA1    | 10166  | CGC5    |        |        |         |        |
| EPAMIG 5 Precoce 1        | 0.037  | 0.287  | 1.037  | -1.362  |        |        |         |        |
| IAC-22                    | -0.399 | 0.600  | 0.600  | -0.799  |        |        |         |        |
| EPAMIG 4 -Redenção        | -0.087 | -0.337 | -0.087 | 0.512   |        |        |         |        |
| CNPA ITA-96               | -0.149 | -0.149 | -0.149 | 0.450   |        |        |         |        |
| CNPA ITA-90               | 0.600  | -0.399 | -1.399 | 1.200   |        |        |         |        |
| DMS ( $S_{ij}$ )          | 0.061  |        |        |         |        |        |         |        |
| DMS ( $S_{ij} - S_{ik}$ ) | 0.100  |        |        |         |        |        |         |        |
| DMS ( $S_{ij} - S_{kj}$ ) | 0.096  |        |        |         |        |        |         |        |
| DMS ( $S_{ij} - S_{kl}$ ) | 0.082  |        |        |         |        |        |         |        |
| 2000                      |        |        |        |         |        |        |         |        |
|                           | 10166  | 11327  | 11086  | 11326   | CR5    | 6239   | 6727    | 3564   |
| EPAMIG 5 Precoce 1        | -0.212 | 1.287  | -0.112 | 1.387   | -0.013 | 0.688  | -1.812* | -1.212 |
| IAC-22                    | 0.288  | -1.212 | 0.888  | -2.112* | 0.487  | -0.312 | -0.688  | 1.288  |
| EPAMIG 4 -Redenção        | -0.275 | -0.275 | -1.175 | -0.675  | 0.075  | 1.125  | 1.125   | 0.225  |
| CNPA ITA-96               | -0.275 | 0.725  | -0.175 | 1.325   | 0.425  | -0.875 | -0.875  | -0.275 |
| CNPA ITA-90               | 0.475  | -0.525 | 0.575  | 0.075   | -0.825 | -0.625 | 0.875   | -0.025 |
| DMS ( $S_{ij}$ )          | 0.561  |        |        |         |        |        |         |        |
| DMS ( $S_{ij} - S_{ik}$ ) | 0.848  |        |        |         |        |        |         |        |
| DMS ( $S_{ij} - S_{kl}$ ) | 0.779  |        |        |         |        |        |         |        |
| DMS ( $S_{ij} - S_{kl}$ ) | 0.744  |        |        |         |        |        |         |        |

trait has been used can lead to a mischaracterization of a given cultivar when evaluated by this descriptor. This is a concern for the work of breeders and phytopathologists, since in the literature ramulosis-resistance is only classified as: highly resistant, moderately resistant, moderately susceptible, susceptible and highly susceptible. However, the cultivars EPAMIG 5 - Precoce 1 and CNPA – ITA 90, described in the literature as intermediately resistant and tolerant, varied in performance according to the isolate, and cultivar IAC-22 presented vertical resistance to isolate CGC5, but not to isolate 10166. In other words, in one field, where isolate CGC5 prevails, the cultivar will be ramulosis resistant, but ramulosis-susceptible in another, where isolate 10166 prevails. The same is true for cultivar IAC-22, considered moderately ramulosis-resistant. Our study demonstrates the need of more in-depth and accurate evaluations of ramulosis

resistance for the cultivars recommended for planting, especially covering a greater diversity of isolates, which differ in virulence and aggressiveness, according to the region and climate conditions.

Since this study was carried out under controlled greenhouse conditions, studies under field conditions are necessary to confirm the cultivar reactions to ramulosis, considering that which descriptors are also based on field observations, which can partially explain the conflicting results.

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## Instabilidade da reação a ramulose de cultivares de algodoeiro e sua relação com o processo de proteção e registro

**RESUMO** - Em algodoeiro a reação a ramulose é um descritor na proteção e registro de cultivares. No entanto, este descritor nem sempre apresenta homogeneidade, distinguibilidade e estabilidade, a depender dos isolados e condições edafoclimáticas. Visando avaliar a estabilidade deste descritor para cultivares de algodoeiro é que se estimou a Capacidade Específica de Interação (CEI), Capacidade Geral de Reação (CGR) e a Capacidade Geral de Agressividade (CGA) para as cultivares EPAMIG 5 - Precoco 1, IAC-22, EPAMIG 4 - Redenção, CNPA - ITA 96 e CNPA - ITA 90, inoculadas com isolados de *Colletotrichum gossypii* var. *cephalosporioides* causadores da ramulose. Os sintomas foram avaliados aos 10 e 40 dias após a inoculação. Observou-se significância para as variáveis CGA, CGR e CEI indicando a existência de variabilidade para a resistência horizontal e agressividade dos isolados. Este comportamento sugere respostas específicas das cultivares quando em associação com diferentes isolados do patógeno, e, portanto, instabilidade deste descritor.

**Palavras-chaves:** *Colletotrichum gossypii* var. *cephalosporioides*, resistência a doenças, resistência horizontal.

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