

# Ramularia areola sporulation potential in Brazilian cotton

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## ABSTRACT

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Ramularia blight, caused by *Ramularia areola*, is one of the most important diseases affecting cotton crop in Brazil. For its effective control, 5-9 fungicide applications on susceptible cultivars are necessary. The aim of the present study was to evaluate, *in vitro* and *in vivo*, the sporulation potential of *R. areola* isolates from different Brazilian regions at distinct temperatures. Spore production was assessed in the laboratory and under green house conditions by using leaves from plants of eight cotton cultivars. The *in vitro* results indicated that the potential of spore production was dependent on temperature. Maximum sporulation of the

fungus occurred at 17°C for isolates from São Paulo State and 23°C for isolates from Goiás and Mato Grosso States. In the *in vivo* study, there was a variation in spore production according to the cultivar and the isolate. Most isolates showed to be highly aggressive on cultivars FM966 LL and DELTAOPAL. The obtained results suggest a more rational use of fungicides and cultivars with decreased fungal sporulation and can form the basis for further studies of the pathogenic variability of this fungus in cotton crops in Brazil. This is the first report on the sporulation potential of Brazilian *R. areola* isolates.

**Additional keywords:** Ramularia blight, *Gossypium hirsutum*.

## RESUMO

Galbieri, R.; Cia, E.; Morello, C.L.; Fanan, S.; Andrade Junior, E.R.; Kobayast, L. Potencial de esporulação de *Ramularia areola* em algodoeiro no Brasil. *Summa Phytopathologica*, v.41, n.3, p.233-235, 2015.

A mancha de ramulária causada por *Ramularia areola* é uma das mais importantes doenças para a cultura do algodoeiro no Brasil. Para o controle efetivo da doença, são necessárias 5-9 aplicações de fungicidas em cultivares suscetíveis. O objetivo do presente trabalho foi avaliar, *in vitro* e *in vivo*, o potencial de esporulação de isolados de *R. areola*, originários de diferentes regiões do Brasil, sob diferentes regimes de temperaturas. A produção de esporos foi avaliada em laboratório e em folhas de plantas de oito cultivares de algodoeiro em condições de casa-de-vegetação. Os resultados obtidos *in vitro* indicaram que o potencial de produção de esporos foi dependente da temperatura.

A máxima esporulação do fungo ocorreu com 17°C para isolados originários do Estado de São Paulo e 23°C para isolados de Goiás e Mato Grosso. No estudo *in vivo*, houve variação na esporulação em função da cultivar e do isolado. A maioria dos isolados apresentou alta agressividade na cultivar FM 966 LL e DELTAOPAL. Com os resultados obtidos, é possível indicar uso mais racional de fungicidas, cultivares que apresentaram menor esporulação do fungo, além de formar base para novos estudos com relação à variabilidade desse fungo em algodoeiro no Brasil. Esse é o primeiro relato sobre o potencial de esporulação de isolados brasileiros de *R. areola*.

**Palavras-chave:** Mancha de ramulária, *Gossypium hirsutum*

Ramularia leaf blight in cotton, also known as false mildew, is caused by *Ramularia areola* (7) and is an economically important disease in Brazil, especially in the Center-Western Region of the country (1, 4). Highly favorable environmental conditions to the pathogen, as well as the use of susceptible cultivars, have made it potentially damaging in the absence of effective control measures (1, 2, 5).

The initial symptoms of this disease are characterized by small angular necrotic lesions of 3-4 cm. In the infection advanced stages, the lesions coalesce and become necrotic with severe chlorosis, causing the premature death of leaves and affecting the production (7, 5). In the Brazilian Center-Western Region, yield losses caused by this disease are estimated to be around 20-30% when control measures are not adopted (1), but losses can reach up to 60%, especially for highly

susceptible cultivars (2). In countries like India, yield losses of over 60% have been recorded (8).

At present, this disease has been partially controlled in Brazil by 5 to 9 fungicide applications, which has increased the production cost (1). Although some resistant cultivars are available for commercial cultivation, their resistance has not been stable, and the disease response is variable in different Brazilian regions (3). The epidemiology of this disease is directly influenced by the pathogen's sporulation and dissemination capacity (5), and such processes depend on the interaction between the fungal genetic factors and the cotton plant, as well as on environmental conditions such as temperature (7, 5). Thus, it is important to study the variability of this fungus under different aspects in order to better understand what happens to this path system

in Brazil.

The aim of the present investigation was to identify, *in vitro* and *in vivo*, the spore production potential of *R. areola* isolates originated from some endemic areas in Brazil.

First, ten *R. areola* monosporic isolates were collected from diseased cotton leaves at three different Brazilian States (São Paulo, Mato Grosso and Goiás), and the pathogen was cultivated on V8-juice agar. Then, to assess the fungal spore production, a trial was carried out on Petri plates at different temperatures and 12h photoperiod in growth chambers (12, 17, 21, 23, 26, 29 and 33 °C). Each treatment was replicated eight times at random, totaling 560 plates. Suspensions (10<sup>6</sup> conidia/mL) were prepared individually for each isolate by dispersing 1 mL in V8 culture medium (7) on each plate. The suspension was homogeneously spread throughout the plate surface by using a Drigalski spatula; the incubations were conducted according to the assigned treatment. Ten days later, 40 mL of distilled water were added to each plate and the surfaces were scraped with a no. 14 brush to release the conidia. A subsequent spore count was carried out by using a Neubauer chamber and an optical microscope. A drop of Tween 20 was added to each plate to facilitate the procedure.

A second trial was carried out under controlled conditions in the greenhouse, where the temperature was kept at about 25 ± 2 °C. The experimental design was in randomized blocks, 8 x 11 factorial design,

totaling eight cultivars and eleven *R. areola* isolates with four replicates. Experimental plots consisted of 12-L pots with four plants. Isolates were: No.308 from Luiz Eduardo Magalhães-BA; 309 from Barreiras-BA; 294 from Primavera do Leste-MT; 282 from Campo Verde-MT; 293 from Primavera do Leste-MT; 283 from Sorriso-MT; 281 from Lucas do Rio Verde-MT; 300 from Londrina-PR; 290 from Mococa-SP; 285 from Adamantina-SP; and 289 from Caiabu-SP. The inoculum was prepared as described for the first trial. Plants were inoculated at 60 days after planting by using a backpack sprayer until runoff. After twenty days, the spores on a third true leaf of each plant were counted. The spores on the upper and the lower leaf surfaces were scraped with a no. 16 brush and 80 mL distilled water. Spore counts were carried out in the resulting suspension by using a Neubauer chamber and an optical microscope. The average spore count of the four plants from each experimental plot represented the variable that was considered for analysis. Spore counts were subjected to analysis of variance and Tukey's test at 5% probability using the transformation  $\sqrt{x + 1}$ .

Figure 1 shows the *in vitro* effect of temperature on *R. areola* sporulation. Considering the extreme temperatures studied, fungal sporulation was minimal at 12 °C and there was not any sporulation at 33 °C. It can be assumed that the optimal temperature range for the event to occur is very close to the presented values, which corroborates the results obtained by Rathaiah (7). It is interesting to note that the

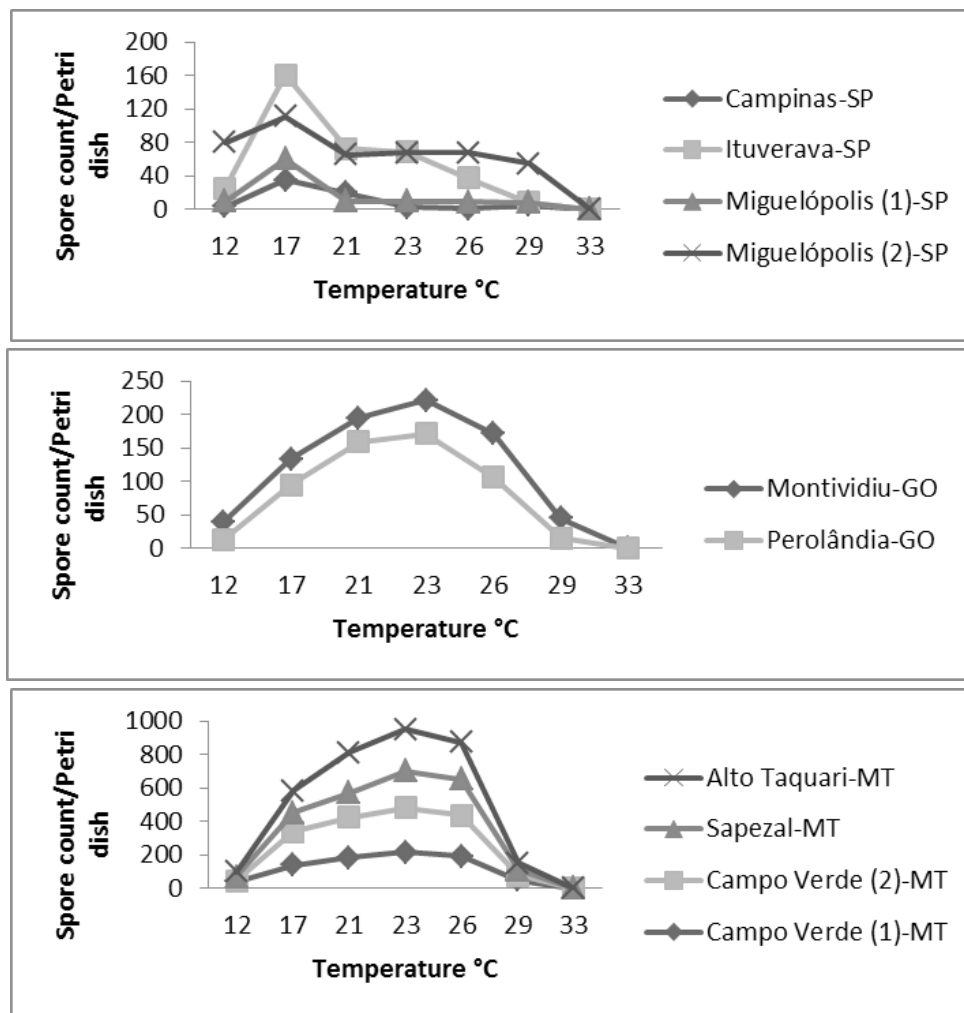


Figure 1. Mean spore count (x 100,000) per Petri dish (9 cm of diameter) for *Ramularia areola* isolates from different Brazilian States (SP, GO and MT) incubated at different temperatures.

**Table 1.** Mean spore count (x 100,000) per cotton leaf for eight genotypes inoculated with *Ramularia areola* isolates originated from different Brazilian States\*.

| <i>R. areola</i><br>Isolates | States* | Cultivars** |    |     |     |     |    |    |   |    |   |    |   |    |   |    |   |
|------------------------------|---------|-------------|----|-----|-----|-----|----|----|---|----|---|----|---|----|---|----|---|
|                              |         | 1           |    | 2   |     | 3   |    | 4  |   | 5  |   | 6  |   | 7  |   | 8  |   |
| 308                          | BA      | 3           | ab | 188 | ab  | 197 | a  | 1  | a | 5  | a | 17 | a | 0  | a | 46 | a |
| 309                          | BA      | 2           | b  | 29  | bcd | 3   | b  | 2  | a | 0  | a | 5  | a | 0  | a | 0  | a |
| 294                          | MT      | 1           | b  | 32  | bcd | 28  | ab | 4  | a | 2  | a | 10 | a | 12 | a | 0  | a |
| 282                          | MT      | 0           | b  | 167 | abc | 20  | b  | 0  | a | 3  | a | 24 | a | 2  | a | 2  | a |
| 293                          | MT      | 3           | ab | 173 | ab  | 21  | b  | 3  | a | 4  | a | 27 | a | 4  | a | 10 | a |
| 283                          | MT      | 6           | ab | 269 | a   | 172 | a  | 4  | a | 2  | a | 45 | a | 3  | a | 4  | a |
| 281                          | MT      | 2           | b  | 89  | abc | 18  | b  | 11 | a | 6  | a | 54 | a | 8  | a | 46 | a |
| 300                          | PR      | 7           | ab | 22  | cd  | 19  | b  | 15 | a | 3  | a | 4  | a | 11 | a | 2  | a |
| 290                          | SP      | 31          | ab | 9   | cd  | 19  | b  | 13 | a | 11 | a | 5  | a | 4  | a | 7  | a |
| 285                          | SP      | 97          | a  | 77  | a-d | 15  | b  | 19 | a | 9  | a | 32 | a | 9  | a | 11 | a |
| 289                          | SP      | 8           | ab | 18  | cd  | 5   | b  | 60 | a | 29 | a | 19 | a | 35 | a | 8  | a |

\*Brazilian States. \*\*1: CNPA-GO-06-158; 2: DELTAOPAL; 3: FM 966 LL; 4: FMT 705; 5: FMT 707; 6: IAC 25RMD; 7: IMA 03-1318; 8: IMACD 05-8276. Letters in the columns refer to the comparison of isolates within genotypes using Tukey's test at 5% probability. Coefficient of variation = 35.4 %.

temperature at which maximum sporulation occurred varied with the origin of the isolates. The optimal temperature was approximately 17°C for isolates from the state of São Paulo and approximately 23°C for isolates from the states of Goiás and Mato Grosso. The average temperature during the cotton cultivation period differs considerably among different regions. Temperature may have been responsible for the spore production potential and, at the end of the plant cycle, for the disease severity and for the survival mechanism of the regional isolates.

The results of spore count per leaf shown in Table 1 indicate variability in the aggressiveness of isolates originated from different geographic regions. Most of the isolates showed aggressiveness on two highly susceptible cultivars, DELTAOPAL and FM966LL, which produced the largest quantity of spores. The other isolates yielded relatively smaller number of spores on the remaining six cultivars. These data evidence the aggressiveness of such isolates on the two cultivars grown in Brazil. Nonetheless, further studies on this subject are needed to understand the distribution frequency of such isolates in each Brazilian State, since the spore production period of a single lesion was not studied. Spores are produced during a very short period of time, causing rapid senescence and premature death of the leaves; thus, studies on the sporulation period of a single lesion or a single leaf would still be desirable.

The results found in this study show the possibility of optimizing *Ramularia* spot control in Brazil due to the sporulation potential of this fungus. On the one hand, susceptible cultivars of high sporulation capacity should be avoided in order to keep the disease at low levels. On the other hand, fungicide applications to restrain fungal sporulation will be more efficient at temperatures around 17 °C for São Paulo State and 23 °C for Mato Grosso and Goiás States. Such a strategy is believed

to contribute to a more efficient disease control, as well as to a better and more rational fungicide use, since the 5-9 applications presently carried out for the pathogen control may be unsustainable.

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