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## Morphocultural and pathogenic characterization of *Colletotrichum gossypii* and *Colletotrichum gossypii* var. *cephalosporioides* under different temperatures<sup>1</sup>

Caracterização morfo cultural e patogênica de *Colletotrichum gossypii* e  
*Colletotrichum gossypii* var. *cephalosporioides* sob diferentes temperaturas

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### HIGHLIGHTS:

Temperatures influenced the mycelial growth and sporulation.

The highest mycelial growth was at 25 and 30 °C.

*Colletotrichum gossypii* and *Colletotrichum gossypii* var. *gloeosporioides* expressed symptoms in the BRS Cedro cotton cultivar.

**ABSTRACT:** Considering the importance of identifying *Colletotrichum* species associated with cotton plants, this study aimed to characterize the morphological and pathogenic isolates of *C. gossypii* var. *cephalosporioides* and *C. gossypii* under different temperatures. Five isolates of *C. gossypii* var. *cephalosporioides* and *C. gossypii* were incubated at 20, 25 and 30 °C. The cultural characteristics data were analyzed through ANOVA and the means compared by the Tukey test. There were differences between the isolates of the two species concerning mycelial growth and sporulation at different temperatures. Temperatures between 20 to 30 °C increased the length of conidia but did not influence the width, whereas between 25 and 30 °C, there was the highest mycelial growth. *Colletotrichum gossypii* expressed anthracnose symptoms, and ramulosis symptoms were observed only in plants inoculated with *C. gossypii* var. *cephalosporioides*.

**Key words:** *Gossypium* spp., anthracnose, ramulosis

**RESUMO:** Considerando a importância da identificação de espécies de *Colletotrichum* associadas à cultura do algodoeiro, objetivou-se realizar a caracterização morfológica e patogênica de isolados de *Colletotrichum gossypii* var. *cephalosporioides* e de *Colletotrichum gossypii* submetidos a diferentes temperaturas. Foram utilizados cinco isolados de *C. gossypii* var. *cephalosporioides* e cinco isolados de *C. gossypii*, os quais foram incubados a 20, 25 e 30 °C. Os dados de caracterização cultural foram submetidos à análise de variância e comparados pelo teste de Tukey. Houve diferenças entre os isolados em relação ao crescimento micelial e esporulação das duas espécies nas diferentes temperaturas. As temperaturas de 20 e 30 °C aumentaram o comprimento dos conídios, mas não influenciaram na largura, enquanto a 25 e 30 °C houve maior crescimento micelial. *Colletotrichum gossypii* expressou sintomas de antracnose, enquanto sintomas de ramulose foram observados apenas em plantas inoculadas com *C. gossypii* var. *cephalosporioides*.

**Palavras-chave:** *Gossypium* spp., antracnose, ramulose

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## INTRODUCTION

Cotton is a crop of great importance worldwide (Sharif et al., 2019; Silva et al., 2021). In Brazil, the crop contributes significantly to the national economy (IBGE, 2020). Among the primary diseases that affect the cotton crop in Brazil, ramulosis stands out, caused by the fungus *Colletotrichum gossypii* var. *cephalosporioides* (Moreno-Moran & Burbano-Figueroa, 2016), a physiological variant of the pathogen that causes anthracnose disease (*Colletotrichum gossypii*) of cotton. Considered a non-regulated quarantine pest (Almeida et al., 2020), the losses caused by the disease can be 70% or more when the incidence is severe.

The fungus infects the leaves, petioles, and the stem, causing dwarfism and over-sprouting of the branches, damaging the formation of bolls (Puia et al., 2020). In the management of ramulosis, it has been recommended to use pathogen-free seeds or seed treatment, crop rotation, use of resistant cultivars, and chemical control of the aerial part (Salustiano et al., 2014).

The causal agent of anthracnose of cotton, *C. gossypii*, is found only in Latin America. It is considered that ramulosis is caused by a distinct pathogen, *C. gossypii* var. *cephalosporioides* (Almeida et al., 2020). Ramulosis and anthracnose pathogens belong to the *C. gloesporioides* species complex. This complex comprises species that share common morphological traits (Nawaz et al., 2018).

The objective was to perform the morphological and pathogenic characterization of isolates of *C. gossypii* var. *cephalosporioides* and *C. gossypii* under different temperatures.

## MATERIAL AND METHODS

The experiment was conducted in the Embrapa Algodão (7°13'51"S, 35°52'54"W, 512 m altitude), in Campina Grande, Brazil. Ten isolates (already molecularly characterized) were used, five of *C. gossypii* (95, 109, 457-1, 457-2, CNPA 37) and five of *C. gossypii* var. *cephalosporioides* (CNPA 50, CNPA 67, CNPA 74, CNPA 86, CNPA 105), collected in cotton cultivations from Mato Grosso, Brazil. To ensure pathogenicity, the isolates were used for inoculation into cotton plants of the BRS Cedro cultivar, which is known to be susceptible to ramulosis. The completely randomized design was used with five replicates of four plants each, with two plants per pot. The treatments were composed of ten isolates: T1 - 95; T2 - 109; T3 - 457-1; T4 - 457-2; T5 - CNPA 37; T6 - CNPA 50; T7 - CNPA 67; T8 - CNPA 74; T9 - CNPA 86; T10 - CNPA 105.

Monospore cultures were obtained from each isolate by preparing a spore suspension containing between 1 and 10 spores per microscopic field when examined on a slide at low magnification (10x). An aliquot of 1 ml of the suspension was poured over the surface of solid water-agar in Petri dishes and spread evenly with the help of a Drigalski spatula until the medium surface was entirely covered. The plates with the spores were incubated at 25 °C for 24 hours. Small fragments of the medium containing a germinating spore were removed and transferred to potato dextrose agar (PDA), thus obtaining monospore colonies, later used for further testing.

For the morphological and cultural characterization of *C. gossypii* and *C. gossypii* var. *cephalosporioides* isolates, the mycelial growth and the morphological characteristic of the colony of each isolate were evaluated. Fungal colony discs (8 mm diameter) were extracted from the margins of the colonies, grown at 25 °C under constant light for seven days in Petri dishes. These discs were transferred to the center of new plates containing PDA. The plates containing the discs were incubated at 20, 25 and 30 °C ± 2 with a 12-hour photoperiod for 14 days.

During seven days, daily readings of two orthogonal diameters of the colonies were taken, and the averages obtained were used to calculate the colony diameter. Seven days after incubation, the morphological aspect of each colony was visually observed (Crous et al., 2009). Semi-permanent slides were made to observe the isolates for conidia shape and size under an optical microscope and compare with specialized literature (Seifert et al., 2011). The width and the average length of 25 conidia per isolate were characterized.

For pathogen characterization, cotton seeds of the BRS Cedro cultivar were sown in plastic pots with 5 L capacity, containing vegetable soil substrate, fertilized according to the recommendations for the crop (Borin et al., 2014). The pots were placed in a greenhouse with two plants per pot. The inoculation was performed 35 days after emergence (DAE) (Guerra et al., 2014).

In each Petri dish, 10 mL of distilled water was placed on preparing the suspensions containing the isolates of the two species. With the help of a Drigalski spatula, the conidia were detached from the mycelial mass. The suspensions were filtered into a Becker using two overlapping surgical gauzes. The inoculum was quantified under an optical microscope with the aid of a Neubauer chamber, adjusting the concentration to 10<sup>5</sup> conidia mL<sup>-1</sup>. The inoculation was performed by spraying the spore suspension on seedling leaves in two different situations: 1) inoculation only in the apical meristem where, for this, the mature leaves of the plants were isolated; 2) inoculation in leaves at the V4 stage (Marur & Ruano, 2001) and, in this case, the apical meristems were isolated. The pathogenicity was evaluated considering their ability to cause or not symptoms of ramulosis.

The growth data were submitted to analysis of variance considering the 10 x 3 factorial scheme (isolates x temperature). The means were compared by the Tukey test, using the SAS' software (SAS Institute).

## RESULTS AND DISCUSSION

There was a significant interaction between isolates and temperature for colony diameter and sporulation. It means that mycelial growth and sporulation occurred depending on the isolates and temperature to which the pathogen was exposed. There was no significant interaction between isolates and temperature for conidia length and width (Table 1).

In the highest temperatures, it was verified reduction in the length of the conidia. It was also found that there was no influence of temperatures in the width of the conidia (Table 2). Carvalho & Carvalho (2007), studying the effect of

**Table 1.** Summary of analysis of variance of colony diameter, sporulation, length, and width of conidia of *Colletotrichum gossypii* and *Colletotrichum gossypii* var. *cephalosporioides* grown *in vitro*

SV	DF	Mean squares			
		Colony diameter	Sporulation	Length	Width
Iso (I)	9	1.9334**	36239**	0.3479**	0.0295**
Temp (T)	2	22.677**	56566**	0.4363*	0.0113*
I × T	18	1.1996**	34651**	0.0715 <sup>ns</sup>	0.0068 <sup>ns</sup>
Error	72	0.1174	36971	0.1140	0.0073
CV (%)		7.5	49.6	28.2	27.9

SV - Sources of variation; Df - Degrees of freedom; Iso (I) - Isolate; Temp (T) - Temperature; ns, \*\* and \* - Not significant and significant (p ≤ 0.01) and (p ≤ 0.05) by F test, respectively

**Table 2.** Length and width of conidia of isolates of *C. gossypii* and *C. gossypii* var. *cephalosporioides* at three temperatures

Species	Factors	Length	Width
		(µm)	
<i>Colletotrichum gossypii</i>	Isolates		
	95	1.195 ab	0.310 ab
	109	1.209 ab	0.313 ab
	457-1	0.826 b	0.190 c
	457-2	1.180 ab	0.286 abc
	CNPA 37	1.308 a	0.377 a
<i>C. gossypii</i> var. <i>gloesporioides</i>	CNPA 50	1.140 ab	0.276 cb
	CNPA 67	1.305 a	0.306 ab
	CNPA 74	1.094 ab	0.260 cb
	CNPA 86	1.377 a	0.323 ab
	CNPA105	1.354 a	0.310 ab
	Temperatures		
20 °C	1.283 a	0.306 a	
25 °C	1.217 ab	0.302 a	
30 °C	1.096 b	0.278 a	

\* Means followed by the same letters in the columns do not differ by the Tukey test (p ≤ 0.05)

incubation temperature on vegetative growth, sporulation, and morphology of *C. gossypii* var. *cephalosporioides*, found that the conidia produced at extreme temperatures of 12 and 33 °C were slightly shorter than the others, corroborating the data obtained in this work.

The isolates showed similar development for conidia length, except isolate 457-1, which had the shortest length differing only from isolates CNPA 37, 67, 86 and 105. For the variable conidia width, the isolate CNPA 37 had higher width than the isolates 457-1, CNPA 50 and 74, not differing from the isolates 95, 109, 437-2, CNPA 67, 86 and 105 (Table 2).

A significant difference was observed in the growth of isolates between temperatures, with the highest temperatures, 25 and 30 °C, inducing higher mycelial growth. Concerning this variable, the isolates of *C. gossypii* presented the same behavior as the isolates of *C. gossypii* var. *cephalosporioides*. The isolate CNPA 37 of *C. gossypii* showed the lowest mycelial growth at 20, 25 and 30 °C, differing statistically from the other isolates, except for isolate CNPA 67, which showed the same behavior at 30 °C (Table 3).

Temperature plays an essential role in *Colletotrichum* species complex development, spore germination, and appressorium formation (Morkeliūne et al., 2021). In the literature, it is considered that the different species of anthracnose are pathogen adapted to warmer climate zone, with an optimal temperature of 26.7-32 °C. He et al. (2019) observed that the fastest colony growth occurred in the temperature range of 25-28 °C; however, in this study, the optimal temperature for *C. acutatum* growth was between 25-30 °C.

Regarding the sporulation, it was observed that the isolates of *C. gossypii* var. *cephalosporioides*, in general, sporulated less

**Table 3.** Effect of different temperatures on mycelial growth (mm) of isolates of *C. gossypii* and *C. gossypii* var. *cephalosporioides*

Isolate	Temperature (°C)		
	20	25	30
95	4.410 aB*	4.958 bdacA	5.132 cbA
109	3.938 abcB	4.843 bdacA	5.215 cbA
457-1	3.730 bcB	4.667 bdcA	4.612 cA
457-2	3.270 cC	4.482 dcB	6.630 aA
CNPA 37	2.604 dB	4.333 dA	4.326 dA
CNPA 50	4.284 baB	5.264 baA	4.819 cbA
CNPA 67	3.512 cB	4.755 bdacA	4.710 dA
CNPA 74	4.294 baB	5.206 baA	4.901 cbA
CNPA 86	3.807 bacB	5.173 bacA	4.902 cbA
CNPA 105	4.398 baB	5.414 aA	5.423 ba

\* Means followed by the same lowercase letters in the lines and uppercase letters in the columns do not differ by the Tukey test (p ≤ 0.05)

than the isolates of *C. gossypii*, with the highest sporulation observed in the isolates CNPA 37 and 105 at 20 °C. At 25 °C, higher sporulation was observed for isolate 95 than the other temperatures and isolates (Table 4). The temperature is also related to the transmissibility of *C. gossypii* var. *cephalosporioides* in cotton crops, as confirmed by Araújo et al. (2006), who obtained higher incidence and severity of the disease with increasing temperature (25 and 30 °C).

Regarding the effect of temperature on sporulation, studies consider that the disease caused by *Colletotrichum* spp. is harmful under milder temperature conditions (Gadaga et al., 2020; Morkeliūne et al., 2021). For fungi of the genus *Colletotrichum*, the optimal temperature for sporulation and conidia germination can vary greatly (Almeida et al., 2020). Gadaga et al. (2020) cited the range of 20-26 °C as optimal for *C. lindemuthianum* in common bean seeds. Considering the sporulation of each isolate at the different temperatures, the isolates CNPA 37 of *C. gossypii* and CNPA 105 of *C. gossypii* var. *cephalosporioides* behaved differently at the three evaluated

**Table 4.** Sporulation (n° conidia x 10<sup>5</sup>) of isolates of *C. gossypii* and *C. gossypii* var. *cephalosporioides* in three temperatures

Isolates	Temperatures (°C)		
	20	25	30
95	14503 aB*	18620 aA	12783 aB
109	6011.5 bA	2520.8 cdB	4493.3 cbAB
457-1	977.00 cA	726.00 dA	813.00 cdA
457-2	1206.6 cB	3996.6 cbdAB	5052.2 bA
CNPA 37	13000 aA	5267.2 cbB	1897.0 cbdC
CNPA 50	114.00 cA	535.00 dA	53.000 dA
CNPA 67	278.2 cA	127.40 dA	22.000 dA
CNPA 74	413.8 cA	80.260 dA	19.500 dA
CNPA 86	364.4 cA	394.80 dA	919.00 cdA
CNPA 105	13080 aA	7687.5 bB	3115.0 cbdC

\* Means followed by the same lowercase letters in the lines and uppercase letters in the columns do not differ by the Tukey test (p ≤ 0.05)

temperatures, decreasing the sporulation as the temperature increased.

Colony coloration was a characteristic that ranged little among the isolates studied according to temperature. Isolates 95, 109, 437-1 of *C. gossypii*, and CNPA 67, 74, 86 and 105 of *C. gossypii* var. *cephalosporioides* showed similar coloration at all temperatures (Table 5). The isolate of *C. gossypii*, CNPA 37, showed grey coloration with pinkish tones, and this coloration was different at 30 °C. The isolate CNPA 50 behaved differently at 25 °C. The colonies showed changes in shades within these isolates, primarily when replicating. As for colony topography, colony elevation and margin characteristics showed variation only at 30 °C (Table 5).

The colors of the colonies ranged within the color shades described for the genus *Colletotrichum*. The variations in coloration of isolates of *Colletotrichum* spp. have already been confirmed by Chung et al. (2020), ratifying those differences in isolates of *Colletotrichum* spp. coloration is a very variable characteristic.

In general, *Colletotrichum* species have been identified and separated according to their morphological characteristics (Puia et al., 2020). Among the main characteristics used by taxonomists are conidia morphology, considering size and shape and morphology of the appressoria; presence or absence of arrows; formation of sclerotia, acervuli, and teleomorph stage; coloration and texture of the colony; pigment

**Table 5.** Cultural characteristics of isolates of *C. gossypii* and *C. gossypii* var. *cephalosporioides*

Isolate	Colony coloration	Elevation of the colony	Colony margin
20 °C			
95	Pink	Flat	Whole
109	Grey	Flat	Whole
457-1	Light grey	Flat	Whole
457-2	Light grey	Flat	Whole
CNPA 37	Pink/Grey	Flat	Whole
CNPA 50	Grey/light edge	Raised	Whole
CNPA 67	Light grey	Raised	Whole
CNPA 74	Grey	Raised	Whole
CNPA 86	Light grey	Flat	Whole
CNPA 105	Light grey	Flat	Whole
25 °C			
95	Pink	Flat	Whole
109	Grey	Flat	Whole
457-1	Light grey	Flat	Whole
457-2	Pink/grey	Flat	Whole
CNPA 37	Pink/grey	Flat	Whole
CNPA 50	Grey	Raised	Whole
CNPA 67	Light grey	Raised	Whole
CNPA 74	Grey	Raised	Lobed
CNPA 86	Light grey	Raised	Whole
CNPA 105	Light grey	Raised	Whole
30 °C			
95	Pink/grey	Flat	Corrugated
109	Grey	Flat	Whole
457-1	Light grey	Flat	Whole
457-2	Pink/grey	Flat	Corrugated
CNPA 37	Light grey	Flat	Whole
CNPA 50	Grey/light edge	Raised	Lobed
CNPA 67	Light grey	Umbonate	Lobed
CNPA 74	Grey	Raised	Lobed
CNPA 86	Light grey	Flat	Lobed
CNPA 105	Light grey	Raised	Whole

production, and growth rate (Kamei et al., 2014). A factor that significantly influences the development of the *Colletotrichum* genus is temperature being one of the main climatic variables responsible for infection and colonization by this pathogen (Morkeliūne et al., 2021). In this work, isolates 86 and 105 of *C. gossypii* var. *cephalosporioides* presented flat colonies under the temperature of 20 °C, the isolates of *C. gossypii* presented this characteristic when submitted to growth at the three temperatures studied.

The plants of cotton BRS Cedro cultivar inoculated in the meristem region and on the leaves at the V4 stage showed different reactions to the inoculated species. When the plants were inoculated with *C. gossypii* suspension in the meristem region, they did not express leaf necrosis or overgrowth symptoms. On the other hand, anthracnose symptoms were identified on leaves at the V4 stage when the pathogen was inoculated on them (Table 6).

Ramulosis symptoms, especially leaf necrosis with a star spot symptom, typical of the early stages of the disease, and breakdown of apical dominance, were observed on plants inoculated in the meristem with a suspension using isolates considered to be *C. gossypii* var. *cephalosporioides*. In contrast, no disease symptoms were identified when the same suspension was inoculated on leaves at the V4 stage (Table 6).

Although there are controversies regarding the taxonomy of *C. gossypii*, it suggested the possibility that the symptoms of ramulosis are caused when the inoculum occasionally comes into contact with the plant meristem. In contrast, anthracnose symptoms are expressed when the inoculum, not reaching the meristem, comes into contact with leaves at the vegetative stage, ranging between V4 and V5 stages according to the scale of Marur & Ruano (2001). It was observed in this study that *C. gossypii* does not cause characteristic symptoms of ramulosis when in contact with the meristem.

The results obtained in this study agree with those already verified by Tanaka et al. (1996), who considered that ramulosis is caused by a variant of *C. gossypii* whose induced symptoms and growth characteristics are different from those observed in this species.

**Table 6.** Reaction of cotton plants, BRS Cedro cultivar, inoculated with *C. gossypii* and *C. gossypii* var. *cephalosporioides*

Isolates	Leaf symptoms	Meristem symptoms
	Necrotic spots	Overgrowth
95	Present	Absent
109	Present	Absent
457-1	Present	Absent
457-2	Present	Absent
CNPA 37	Present	Absent
CNPA 50	Absent	Present
CNPA 67	Absent	Present
CNPA 74	Absent	Present
CNPA 86	Absent	Present
CNPA105	Absent	Present

## CONCLUSIONS

1. Conidia had the largest size at 20 °C.
2. Temperatures of 25 and 30 °C induced the highest mycelial growth.
3. Colony coloration was slightly variable.

4. Characteristics of colony elevation and margin showed variation only at 30 °C.

5. The cotton BRS Cedro cultivar plants showed different reactions concerning the inoculated species under study. *C. gossypii* expressed anthracnose, and symptoms of ramulosis (*C. gossypii* var. *gloesporioides*) were observed.

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