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# Pathogenicity and aggressiveness of *Colletotrichum gloeosporioides* isolates in ornamental pepper<sup>1</sup>

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## ABSTRACT

Anthrachnose, a disease caused by fungi of the *Colletotrichum* genus, affects many crops, including ornamental plants. This study aimed at evaluating the pathogenicity and aggressiveness of the *Colletotrichum gloeosporioides* Penz. (Sacc.) isolates Cg<sub>1</sub> (papaya), Cg<sub>2</sub> (guava) and Cg<sub>3</sub> (mango) in the ornamental pepper varieties Stromboli, Etna and Pirâmide, during two different periods (July/August and November/December 2013). The fungi pathogenicity and the severity of plant symptoms were assessed using a grading scale. The three *C. gloeosporioides* isolates were pathogenic to the three ornamental pepper varieties studied, exhibiting different levels of aggressiveness among them. The environmental conditions influenced the isolates aggressiveness, with the most severe symptoms observed in the second assessment period.

KEY-WORDS: *Capsicum frutescens*; phytopathogenic fungi; anthracnose.

## RESUMO

Patogenicidade e agressividade de isolados de *Colletotrichum gloeosporioides* em pimenta ornamental

A antracnose é uma doença causada por fungos do gênero *Colletotrichum* que afeta inúmeras culturas, inclusive plantas ornamentais. Objetivou-se avaliar a patogenicidade e agressividade de isolados de *Colletotrichum gloeosporioides* Penz. (Sacc.) Cg<sub>1</sub> (mamoeiro), Cg<sub>2</sub> (goiabeira) e Cg<sub>3</sub> (mangueira), nas variedades de pimentas ornamentais Stromboli, Etna e Pirâmide, em duas épocas do ano (julho/agosto e novembro/dezembro de 2013). Avaliou-se a patogenicidade dos fungos e a severidade dos sintomas nas plantas utilizando-se uma escala de notas. Os três isolados de *C. gloeosporioides* foram patogênicos às três variedades de pimenta ornamental avaliadas, apresentando níveis diferenciados de agressividade entre as variedades. Houve influência das condições ambientais na agressividade dos isolados, sendo que a maior severidade foi obtida na segunda época de condução do experimento.

PALAVRAS-CHAVE: *Capsicum frutescens*; fungos fitopatogênicos; antracnose.

## INTRODUCTION

In Brazil, the ornamental plant and flower trade generates around BRL 5.7 billion per year. This supply chain has 8,000 registered producers and creates approximately 215,000 direct jobs, distributed among the production (39.5 %), distribution (3.1 %) and retail (49.7 %) sectors, as well as supporting functions (7.7 %) (Ibraflor 2016). Flower farming encompasses a wide range of products, such as ornamental plants, fresh or dried flowers, potted plants, fresh and dried foliage, grafted plants, cuttings, seedlings and large trees (Thomaz & Raquel 2007).

Chili peppers exhibit significant genetic variability and considerable potential for commercialization as potted ornamental plants (Stomel et al. 2006). Any species of chili pepper may be used as ornamental plants, but the most

recommended ones for potted planting are smaller species such as *Capsicum frutescens* (L.) and *Capsicum annuum* (L.), which can be kept indoors (Xavier et al. 2006, Neuhaus et al. 2010). Shape, size and color vary in leaves and fruits, with the latter displaying different levels of pungency. The most commonly observed color is red, but milky yellow, bright yellow, orange, salmon pink, purple and black also occur. Factors that contribute to their success as ornamental plants include easy seed germination, short germination time and heat tolerance. In landscapes, they may be used to create thick and bright colored hedges (Stommel & Bosland 2006).

Anthrachnose, caused by species of the *Colletotrichum* genus, is a common and highly destructive disease in a number of crops, including ornamental plants. It occurs worldwide and causes significant losses, particularly in tropical and

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subtropical regions (Agrios 2005). Plants can be infected during any stage of development and symptoms emerge after fungi colonization and are characterized by dark, sunken round or oval-shaped lesions on leaves, inflorescences or fruits. Under favorable conditions, conidial masses are formed (Menezes 2006).

The species *C. acutatum* (Sim.) and *C. gloeosporioides* Penz. (Sacc.) have been associated with anthracnose in chili peppers, bell peppers and scarlet eggplants, in four Brazilian States (Tozze Júnior et al. 2006). In the Rio Grande do Sul and São Paulo States, *Colletotrichum boninense* (Moriwaki) has also been reported, causing lesions on fruits and leaves of the bell pepper “Amanda” cultivar (Tozze Júnior et al. 2009). Costa et al. (2006) reported the occurrence of *Colletotrichum coccodes* (Wallr.) S. Hughes as another causal agent of anthracnose, in nightshades in the country.

Although anthracnose has been studied in several plant species, there is little information on its occurrence, aggressiveness of its isolates or resistance of ornamental plant species. Thus, this study aimed at determining the susceptibility of ornamental pepper varieties to isolates of *Colletotrichum gloeosporioides* obtained from fruit species.

## MATERIAL AND METHODS

The experiment was conducted in a greenhouse, at the Universidade Estadual do Piauí, in Picos (7°2'S and 41°29'W), where the average annual temperature is 30.2 °C (Brasil 2013). Assays were conducted during two periods, within the same year: July to August and November to December, 2013.

The experiment was carried out using a 3 x 3 + 4 (control treatments) factorial arrangement, in a completely randomized design, with nine replicates and one plant per plot. The treatments consisted of three *Capsicum frutescens* varieties inoculated with three *Colletotrichum gloeosporioides* isolates, in addition to a negative and a positive control. A negative control was used for each variety, in the form of plants sprayed only with sterile distilled water, totaling three negative control treatments. The aji pepper variety (*Capsicum baccatum* L.) was used as a positive control due to its high susceptibility to the pathogen.

Commercial seeds from the following *Capsicum frutescens* varieties were obtained for the experiment: Etna, Pirâmide and Stromboli. The seeds

were planted in plastic bags (17 cm high x 9 cm wide) with capacity for 600 g of substrate, which consisted of washed sand and hillside soil, in a proportion of 1:2. The plants were grown for 30 days, until they reached a height of approximately 20 cm and 6-8 leaves.

Three isolates of the *Colletotrichum gloeosporioides* (Cg) species were used. They were obtained from the Prof. Maria Menezes' collection (CMM), at the Universidade Federal Rural de Pernambuco, and named Cg<sub>1</sub> (CMM\_0797, papaya isolate), Cg<sub>2</sub> (CMM\_0847, guava isolate) and Cg<sub>3</sub> (CMM\_0978, mango isolate).

Conidial suspensions were prepared using 7-day-old cultures of the pathogen, grown on potato-dextrose-agar in Petri dishes, to which 30 mL of sterile distilled water were added. The conidia were removed using a soft bristled brush, with circular movements, and the suspension was filtered through a double layer of sterile gauze. Next, conidia were counted with a Neubauer chamber, adjusting the concentration to 10<sup>6</sup> conidia mL<sup>-1</sup>.

The 30-day-old plants were placed in a moist chamber for stomatal opening. After 24 h, they were inoculated by spraying the conidia suspension. *Capsicum* plants remained in the chamber for another 48 h, according to the methodology described by Pereira et al. (2011), adapted to local conditions.

Assessments were conducted at 3, 8, 13, 18 and 23 days after inoculation. Severity was evaluated using an adaptation of the grading scale proposed by Pereira et al. (2011): 1: plants with no symptoms; 2: 1-10 % of plants with symptoms; 3: 11-25 % of plants with symptoms; 4: 26-50 % of plants with symptoms; 5: 51-75 % of plants with symptoms; 6: more than 75 % of plants with symptoms and/or plant death.

At the end of the experiment, the infection rate was calculated using the McKinney (1923) index and the area below the disease progression curve (ABDPC), as it follows:  $ABDPC = \sum (y_i + y_{i+1}) / 2 \cdot d_i$ , where  $y_i$  and  $y_{i+1}$  are the severity values observed between two consecutive evaluations and  $d_i$  the interval between evaluations (Shaner & Finney 1977). The infection rate and ABDPC were analyzed using the Shapiro-Wilk test and, on confirmation of data normality, submitted to analysis of variance, via the Tukey test ( $p \leq 0.05$ ), using the Assistat software, version 7.6 beta (Silva & Azevedo 2002). Regression analysis was performed to obtain the disease progression curve, using the same statistical software.

## RESULTS AND DISCUSSION

The three *Colletotrichum gloeosporioides* isolates were pathogenic when inoculated in the three ornamental pepper varieties assessed. The isolates aggressiveness was influenced by the inoculation period (July/August or November/December) and the variety analyzed (Table 1).

In the first assessment period (July/August), the Cg<sub>2</sub> isolate showed the greatest aggressiveness in the Stromboli variety, differing statistically, in relation to Pirâmide and Etna (Table 1). There was no difference in the symptoms severity among the fungal isolates for each chili pepper variety, demonstrating that all three isolates may infect these three *Capsicum* varieties. All the positive control plants (*Capsicum baccatum*) died at 15 days after inoculation, demonstrating the plants vulnerability to the isolates tested. Initially, brown spots were observed on the leaves, progressing to necrosis. The seedlings also began to lean, after necrotic spots were seen on the stem. Thus, the pathogens were reisolated from the damaged tissue.

In assessments conducted during the second period (November/December), the Stromboli variety was the most susceptible to the Cg<sub>2</sub> isolate, which differed statistically from the other isolates tested (Table 1). The Etna variety exhibited the greatest susceptibility to Cg<sub>1</sub> and Pirâmide to Cg<sub>2</sub> and Cg<sub>3</sub>, demonstrating the difference in sensitivity among the varieties analyzed.

The Cg<sub>1</sub> isolate was the most aggressive to Etna, differing statistically from the other varieties. Cg<sub>2</sub> showed no significant difference in aggressiveness among the varieties and Cg<sub>3</sub> displayed greater aggressiveness to Pirâmide (Table 1). Freitas-Astúa et al. (2005) reported that humidity and high temperatures favor the development of anthracnose in ornamental plants. These favorable conditions occurred in the Picos region during the second assessment period (November/December), due to high

rainfall rates. This weather condition may explain the greater severity of symptoms observed in the pepper x *C. gloeosporioides* pathosystem studied here.

According to Soares et al. (2008), temperatures above 35 °C do not favor the development of anthracnose, since they may decrease the conidia germination and delay the disease development and symptoms emergence, regardless of relative humidity. Temperatures above 40 °C inhibit the formation of appressoria, limiting the disease development and the symptoms onset, while low temperatures may also restrict the pathogen development.

The first assessment period was characterized by high temperatures (above 35 °C) and low relative humidity, due to low rainfall levels. This is the most likely explanation for the differences occurred between the two assessment periods, as the high temperatures achieved in the region, from July to August, were unfavorable to the development of symptoms. Low aggressiveness may have contributed to maintain similar values of severity among isolates, in the first assessment period.

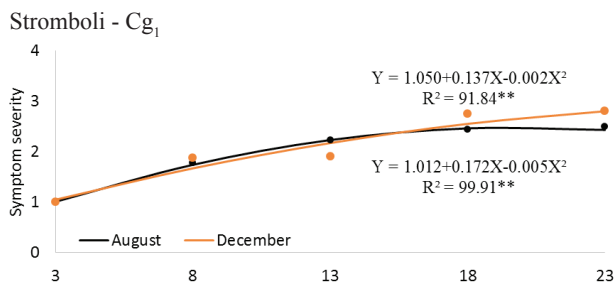
In a study with 63 chili pepper and bell pepper genotypes, four *Capsicum chinense* (Jacq.) genotypes were found to be resistant to *Colletotrichum gloeosporioides*, whereas *Capsicum annum* genotypes with yellow or orange fruits were more sensitive to the pathogen (Lobo Júnior et al. 2001). Sources of resistance to five isolates of *Colletotrichum acutatum* were investigated in seedlings and fruits of 129 varieties of chili pepper and bell pepper. The varieties analyzed ranged from extreme susceptible to resistant, and no significant correlation was observed between symptoms severity in fruits and inoculated plants (Pereira et al. 2011).

Tozze Júnior et al. (2006) studied 44 *Colletotrichum* isolates from nightshades, in different regions of Brazil. The authors found that the main anthracnose-causing species in this family were *C. gloeosporioides* and *C. acutatum*. Castilho & Reis (2007) investigated the causal agent of anthracnose

Table 1. Severity of anthracnose in three varieties of ornamental pepper inoculated with *Colletotrichum gloeosporioides* (Cg) isolates.

Pepper	<i>Colletotrichum gloeosporioides</i> isolates					
	Cg <sub>1</sub>	Cg <sub>2</sub>	Cg <sub>3</sub>	Cg <sub>1</sub>	Cg <sub>2</sub>	Cg <sub>3</sub>
	July/August 2013			November/December 2013		
Stromboli	38.88 aA*	59.26 aA	38.88 aA	44.44 bB*	66.66 aA	38.88 bB
Etna	50.00 aA	40.74 bA	27.77 aA	62.69 aA	55.55 aB	48.15 bB
Pirâmide	50.00 aA	33.33 bA	46.29 aA	46.29 bB	55.56 aA	66.66 aA
CV (%)	25.52			21.67		

\* Means followed by the same upper case letter, in the rows, and lower case letter, in the columns, do not differ according to the Tukey test ( $p < 0.05$ ). Estimates were obtained using the McKinney (1923) index.



in plants from this same family and observed that *C. gloeosporioides* was more widespread in Brazil than *C. acutatum*, with 31 of the 46 isolates studied belonging to this species.

After regression analysis of the severity within the period of 23 days after inoculation, a pattern of anthracnose symptoms development could be observed during the two assessment periods (Figure 1).

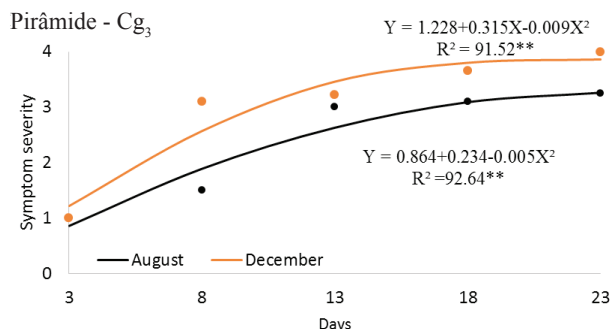
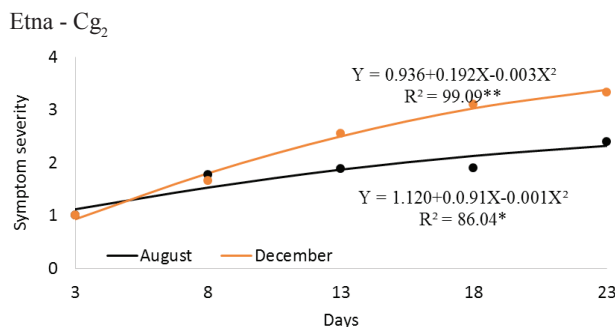
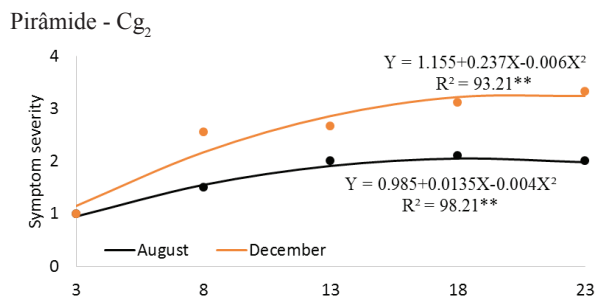
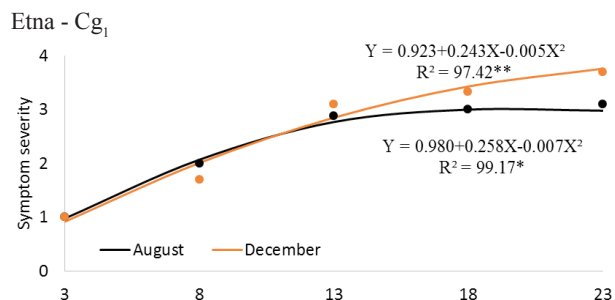
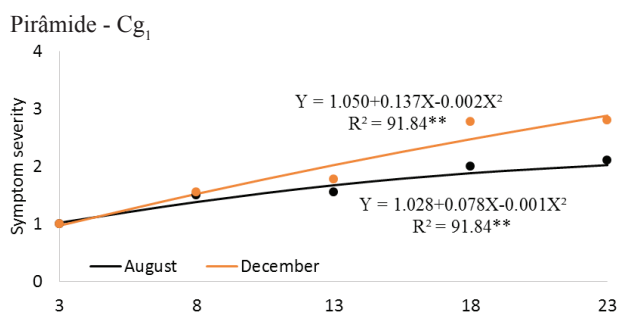
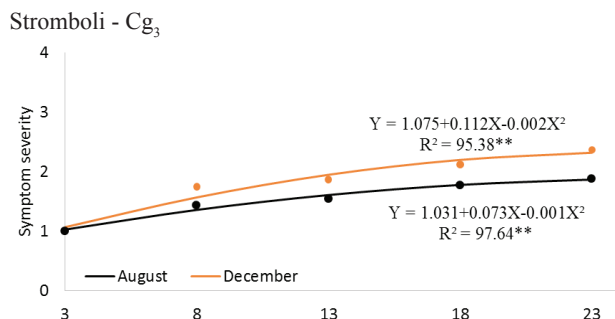
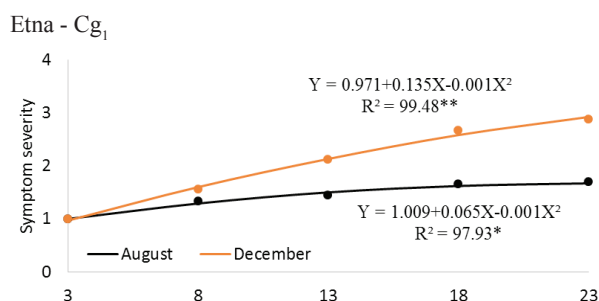
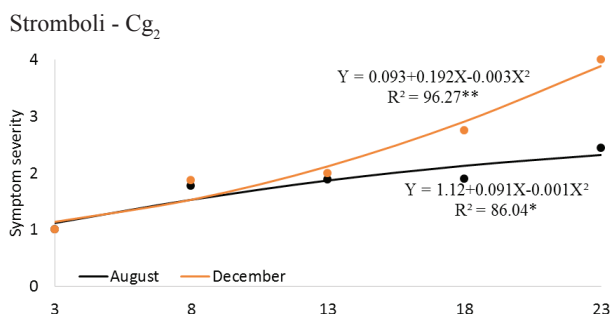


Figure 1. Development of anthracnose, caused by *Colletotrichum gloeosporioides* isolates (Cg<sub>1</sub>, Cg<sub>2</sub> and Cg<sub>3</sub>), in three varieties of ornamental pepper plants (Stromboli, Etna e Pirâmide), during two assessment periods. Model significance: \* 0.05; \*\* 0.01.



Symptoms severity of the isolates was greater in the second period (November/December) for all cultivars, with a significant increase in the disease development curve from the eighth day after inoculation.

Some studies on *Colletotrichum* spp. indicate that the temperature contributes to the colonization of tissues and symptoms onset, with the optimum temperature generally occurring between 22 °C and 28 °C (Dias et al. 2005, Poltronieri et al. 2013). These conditions were observed during the second assessment period of the experiment, favoring the disease development, which is also evident in the ABDPC assessment (Table 2). During this period, Cg<sub>1</sub> exhibited a significant difference in symptoms severity in the Etna pepper variety. Cg<sub>2</sub> was more aggressive in the second experiment for all the varieties studied, while Cg<sub>3</sub> showed an increase in severity only in the Etna and Pirâmide varieties, when compared to the first assessment period.

The aggressiveness of *C. gloeosporioides* isolates was evaluated in anthuriums, torch ginger and heliconias. Although some pathogen isolates exhibited specificity in relation to the host plant, most of them were capable of infecting all three plant species tested, suggesting a lack of specificity for the isolate analyzed (Barguil et al. 2011). This also indicates the pathogen ability to colonize different hosts, which hampers control measures such as crop rotation and contributes to maintaining the pathogen inoculum in the field.

Similarly, Lima Filho et al. (2003) studied the cross-pathogenicity of *Colletotrichum* spp., testing *C. gloeosporioides* obtained from cashew apple, passion fruit, mango, papaya and *C. musae* (Berk & Curt.) von Arx. isolated from banana, in these same fruits. They observed that only the passion fruit

isolate exhibited pathogenic specificity, while the others displayed cross-pathogenicity. This explains the fact that *C. gloeosporioides* isolates obtained from different fruit species were pathogenic to the ornamental pepper plant varieties. The data obtained in this study reinforce the need to implement practices that inhibit *C. gloeosporioides* infestation and infection in ornamental pepper plants.

## CONCLUSIONS

1. The ornamental pepper varieties Stromboli, Etna and Pirâmide are susceptible to the different *Colletotrichum gloeosporioides* isolates tested;
2. The isolates showed no pathogenic specificity for the varieties studied;
3. Anthracnose symptoms varied with season, indicating that environmental conditions influence the severity of this disease in ornamental pepper plants.

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Table 2. Area below the disease progression curve (ABDPC) for the three *Colletotrichum gloeosporioides* isolates inoculated in three ornamental pepper varieties, in two assessment periods (August and December 2013).

Peppers	<i>Colletotrichum gloeosporioides</i> isolates					
	Cg <sub>1</sub>		Cg <sub>2</sub>		Cg <sub>3</sub>	
	Aug	Dec	Aug	Dec	Aug	Dec
Stromboli	22.91 a	25.41 a	30.50 b	51.64 a	14.57 a	19.50 a
Etna	17.91 b	36.65 a	17.00 b	35.82 a	13.32 b	29.17 a
Pirâmide	15.50 a	23.57 a	21.50 b	39.29 a	14.50 b	51.24 a
CV (%)	32.84					

\* Means followed by the same letter, in the rows, for each isolate, do not differ according to the Tukey test ( $p \leq 0.05$ ).

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