

EFFECT OF TIME IN THE MOISTURE CHAMBER ON THE ANTHRACNOSIS SEVERITY IN *Annona squamosa* L. AND *A. muricata* L. LEAVES

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ABSTRACT: Edaphoclimatic conditions exert great influence on the development of pathogens; therefore, knowledge about pathogen, host and environment interaction for disease prevention systems and to define control actions is required. The aim of the present study was to evaluate the influence of time in moisture chamber on the anthracnose severity in sugar apple and soursop leaves. Two times of two and five days in moisture chamber at temperatures of 20, 25 and 30 °C were used. The experimental design was completely randomized in a triple factorial arrangement (2x5x3), represented by two times in moisture chamber, five *Colletotrichum* species (*C. fructicola*, *C. theobromicola*, *C. karstii*, *C. siamense* and *C. tropicale*) three temperatures, with four replicates / leaf. The diameter of lesions was determined by measuring each lesion in two perpendicular directions on the second and fifth days. It was observed that in the time of 2 days of inoculation, there were no anthracnose symptoms in soursop and sugar apple leaves at any of temperatures tested. After times of 5 days, there was appearance of dark brown to black spots. Temperature of 25 °C and time of 5 days favored the appearance of anthracnose symptoms in sugar apple (*Annona squamosa* L.) and soursop leaves (*Annona muricata* L.).

KEYWORDS: *Colletotrichum*, anonaceous, temperature.

INFLUÊNCIA DE PERÍODOS DE PERMANÊNCIA EM CÂMARA ÚMIDA SOBRE A SEVERIDADE DA ANTRACNOSE EM FOLHAS DE *Annona squamosa* L. E *A. muricata* L.

RESUMO: As condições edafoclimáticas exercem grande influência no desenvolvimento dos patógenos, devido a isso, se faz necessário conhecer sobre a interação patógeno, hospedeiro e ambiente para sistemas de prevenção de doenças e para definir ações de controle. O presente trabalho teve como objetivo avaliar a influência dos períodos de permanência em câmara úmida sobre a severidade da antracnose em folhas de pinheira e gravioleira. Foram utilizados dois períodos de permanência em câmara úmida de dois e cinco dias, nas temperaturas de 20, 25 e 30 °C. O delineamento experimental foi inteiramente casualizado em arranjo fatorial triplo (2x5x3), representado por dois períodos de permanência em câmara úmida, cinco espécies de *Colletotrichum* (*C. fructicola*, *C. theobromicola*, *C. karstii*, *C. siamense* e *C. tropicale*) três temperaturas, com quatro repetições/folha. O diâmetro das lesões foi determinado medindo-se cada lesão em dois sentidos perpendiculares, no segundo e quinto dia. Foi observada que no período de permanência de 2 dias de inoculação não houve sintomas de antracnose nas folhas de gravioleira e pinheira em nenhuma das temperaturas testadas. Após 5 dias de permanência, houve o aparecimento de manchas de coloração pardo-escura a preta. A temperatura de 25 °C e o período de permanência de 5 dias favoreceu o aparecimento de sintomas de antracnose em folhas de pinheira (*Annona squamosa* L.) e gravioleira (*Annona muricata* L.).

PALAVRAS CHAVE: *Colletotrichum*, Annonaceae, temperatura.

INTRODUCTION

Anthrachnose, caused by *Colletotrichum* species, is considered the most frequent fungal disease in Annonaceae, causing losses of up to 70% in periods of prolonged rains (Junqueira; Junqueira, 2014; Kamei et al., 2014; Álvarez et al. 2014; Costa et al., 2019).

Species of the genus *Colletotrichum* as including *Colletotrichum tropicale* E.I. Rojas, S.A. Rehner & Samuels, *C. karstii* L. Yang, Zuo Y. Liu, K.D. Hyde & L. Cai, *C. siamense* Prihastuti, L. Cai & K.D. Hyde, *C. theobromicola* Delacr., Bull, *C. gigasporum* E.F. Rakotoniriana & Munaut, *C. gloeosporioides* (Penz) Penz. & Sacc, *C. fructicola* Prihastuti, L. Cai & K.D. Hyde, *C. brevisporum* Phoulivong S., P. Noireung, L. Cai & K. D. Hyde have been reported as etiological agents of anthracnose on *Annona squamosa* L. and *A. muricata* L. leaves in Brazil (Costa et al., 2017, Costa et al., 2019).

Knowledge on the effects of the environment on the development of pathogens can aid in disease management strategies. Information on environmental factors is valuable in the development of disease prevention systems and can contribute to defining control actions (Maia et al., 2011).

Temperature exerts influence on the development of fungi of the genus *Colletotrichum* on the mycelial growth by the diameter of lesions developed on the host, on sporulation and germination of conidia and formation of appressoria that are favored by temperatures of 25°C and period of 12 to 18 hours of moisture (Poltronieri, 2012; Tavares, Souza, 2005; Lopez, 2005). These factors affect the progression of the disease in plants (Silveira et al., 2001) and are able to regulate the rate at which epidemics develop (Poltronieri, 2012); however, there are few studies about *Colletotrichum* species.

Pathogens differ in their preference for high or low temperature, since it affects spore germination and the number of spores formed (Agris, 2005).

Considering the importance of anonaceous species and the scarcity of studies in literature related to the effects of environmental conditions on the development of anthracnose, the aim of this study was to evaluate the influence of times in moisture chamber on the anthracnose severity in *A. squamosa* and *A. muricata* leaves.

MATERIAL AND METHODS

The work was conducted at the Laboratory of Molecular Phytopathology of the Department of Agricultural Sciences (CECA) of the Federal University of Alagoas (UFAL) located at km 85 of BR 101 Norte (9°27'54.71"S - 35°49'39.27" W), in the municipality of Rio Largo, state of Alagoas.

To evaluate the influence of times in moisture chamber, asymptomatic sugar apple (*A. squamosa*) and soursop leaves (*A. muricata*) were used, from 'Chacara das Anonáceas', located in the municipality of Maceió/AL and Department of Agricultural Sciences (CECA)/UFAL, respectively. Leaves were washed, disinfected and conditioned in plastic Gerbox box (11 x 11 x 3.5 cm) containing filter paper moistened with 5 mL of autoclaved distilled water. To avoid contact with the moist surface, sterile Petri dish covers or bottoms were used and petioles were wrapped with cotton swab moistened with autoclaved distilled water to maintain leaf vigor and favor the germination of pathogen reproduction structures.

The inoculum consisted of BDA plugs (5 mm) removed from the borders of monosporic culture with seven days of growth, containing pathogen structures deposited equidistant from one another on the surface of asymptomatic leaves. Each isolate was inoculated separately in leaves with wounds from their respective hosts. Only BDA plugs without the pathogen were used as controls.

The experiment was kept in a Biochemistry Oxygen Demand (BOD) with temperatures of 20, 25, 30 ° C ± 1 ° C in the dark between 2 and 5 days. The experimental design was completely randomized in a triple factorial arrangement (2x5x3), represented by two times in moisture chamber, five *Colletotrichum* species (*C. fructicola*, *C. theobromicola*, *C. karstii*, *C. siamense* and *C. tropicale*), three temperatures, with four replicates / leaf.

Colletotrichum isolates from sugar apple and soursop were obtained from the Collection of Phytopathogenic Fungi at the Universidade Federal de Alagoas (COUFAL).

After the times in moisture chamber, the diameter of lesions was determined by measuring the two perpendicular directions with the aid of a digital caliper. The mean values obtained were submitted to analysis of variance (ANOVA). The diameter of lesions

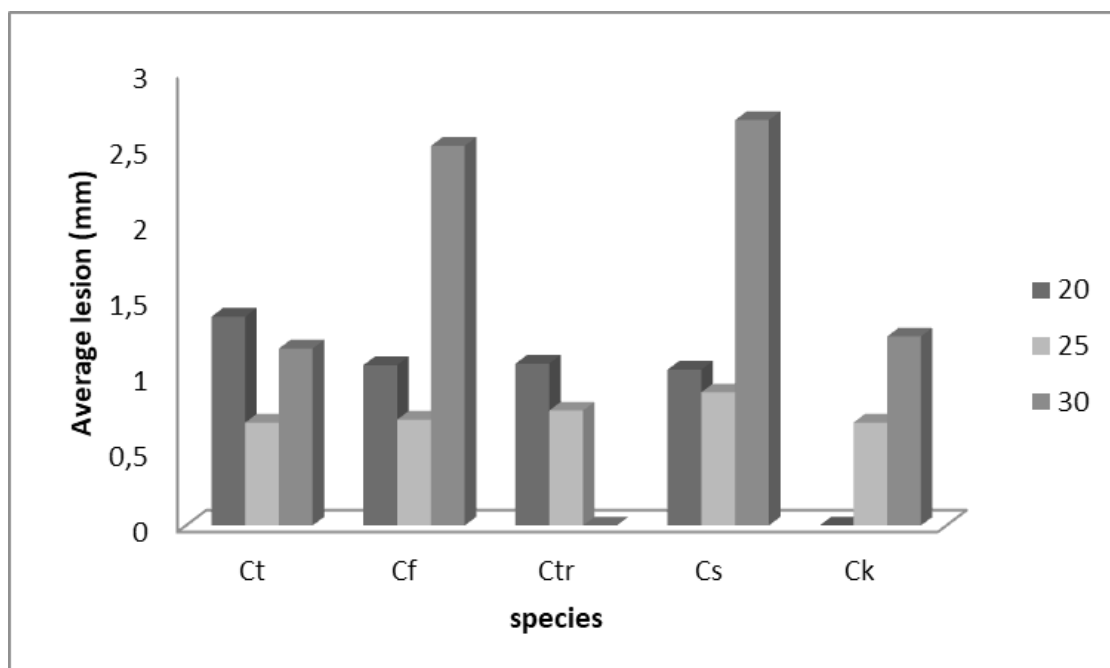
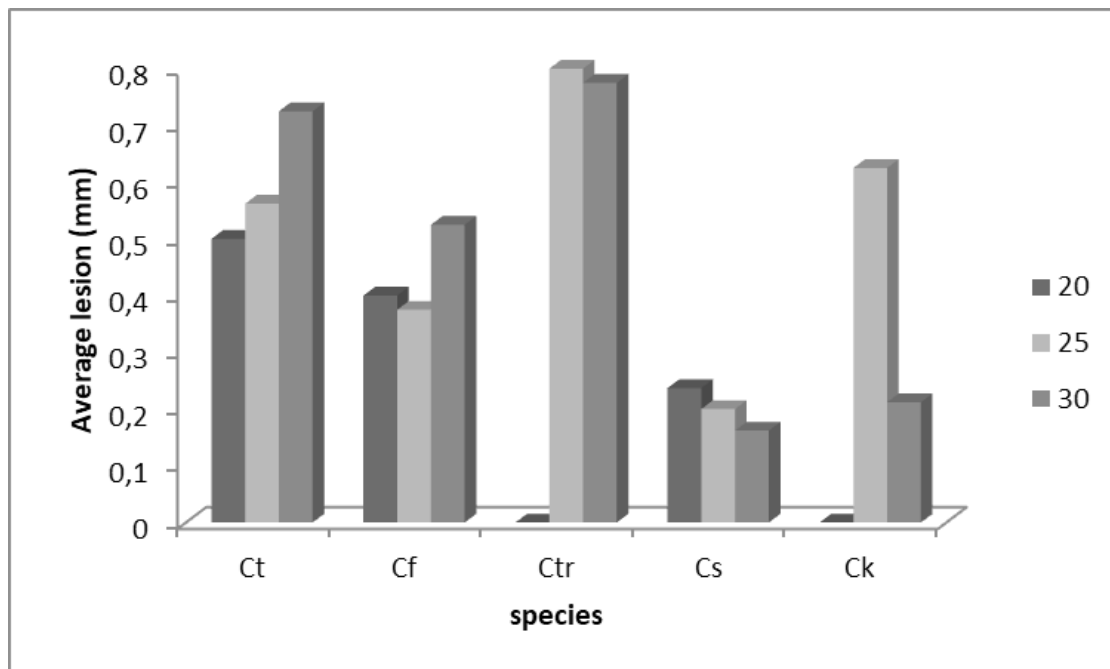
caused by the different *Colletotrichum* species was compared by the Tukey test at 1% probability, using ASSISTAT 7.7 beta software.

RESULTS AND DISCUSSION

Treatments performed to evaluate the effect of temperature on the severity of *Colletotrichum* species on sugar apple leaves after 5 days in moisture

chamber showed the appearance of dark brown spots with clearer center and irregular contour consistent with typical anthracnose symptoms at 25°C and 30°C and in soursop leaves, significant results at 1% probability level by the Tukey's test were observed (Figure 1 and 2). In the time of two days of inoculation, there were no anthracnose symptoms in sugar apple and soursop leaves at any of temperatures tested.

Figure 1. Evaluation of the five-day residence time in humid chamber of sweet apple (A) and soursop leaves (B) at different temperatures. Ct (*Colletotrichum theobromicola*), Cf (*C. fructicola*), Ctr (*C. tropicale*), Cs (*C. siamense*) and Ck (*C. karsti*). Means followed by the same letter do not differ from each other by the Tukey a test at 1% probability.



C. theobromicola, *C. fructicola* and *C. siamense* species caused symptoms at all temperatures tested. Anthracnose symptoms were not observed in sugar apple leaves inoculated with *C. tropicale* and *C. karstii* species when using temperature of 20 °C. All *Colletotrichum* species caused typical anthracnose symptoms at temperatures of 25 °C and 30 °C. However, temperature of 25 °C provided greater severity in sugar apple leaves.

In soursop leaves after 5 days in moisture chamber, the appearance of dark brown to black spots was observed. *C. theobromicola*, *C. tropicale*, *C. fructicolae* *C. siamense* species caused anthracnose symptoms when submitted to temperature of 20°C, except for *C. karstii* species. All species caused anthracnose symptoms in soursop leaves when submitted to temperature of 25 °C. At temperatures of 30 °C, *C. siamense*, *C.fructicola*, *C. theobromicolae* *C. karstii* species caused anthracnose symptoms; however, symptoms were not observed for *C. tropicale*.

Maia (2011) observed that *Colletotrichum* isolates obtained from mango showed better development when submitted to temperatures of 20 °C and 25 °C. The ideal temperature for vegetative growth in most *Colletotrichum* species is in the range from 25 °C to 30 °C (SUTTON, 1992).

Soares et al. (2008) reported that temperature influences the speed of conidia germination, appressoria formation, penetration and colonization of *C. acutatum* and *Colletotrichum gloeosporioidese*, causal agents of anthracnose in guava. In *C. gloeosporioides* isolates obtained from solanaceae, it was observed that the development of colonies occurred at temperatures from 25°C and 28 °C (TOZZE JUNIOR et al., 2006). Araújo et al., 2006, reported that the intensity of the disease in cotton seeds is not influenced by the time of exposure to the pathogen, with temperature being the determining factor. Low temperatures lead to the paralysis of the pathogen activities or even death, and very high temperatures can cause the desiccation of fungal structures present in the source of inoculum (BEDENDO; AMORIM, 2011).

The results of this work demonstrate the influence of temperature, time in the moisture chamber on the severity of *Colletotrichum* species, showing that the temperature of 25 °C and the times of five days in the moisture favored the appearance of anthracnose symptoms in sugar apple (*A. squamosa*) and soursop leaves (*A. muricata*).

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