EFFECT OF DIFFERENT DISCS OF HOSTS' LEAVES ON THE SPORULATION OF *MACROPHOMINA PHASEOLINA* (TASSI) GOID.*

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ABSTRACT

This work aimed to study the effect of leaf discs of cowpea plant [*Vigna unguiculata* (L. Walp.], wheat plant (*Triticum aestivum* L.), and corn plant (*Zea mays* L.) on the sporulation of the fungi *Macrophomina phaseolina* (Tassi) Goid. The trials were assembled in a 2 x 5 factorial arrangement (temperature x sporulation substrate), with four replicates. The experimental plot was a Petri dish with PDA growth medium over which six leaf discs from the species mentioned (Ø 0.7 cm) were laid. The incubation temperature was 25°C and 30°C under a continuous light regime. A disc made of filter paper and PDA growth medium was used as a control. A 0.4-cm-diameter PDA disc colonized with fungal mycelium was placed at the central part of each Petri dish. The evaluation was performed at the 9th day of incubation by estimating the number of conidia.mL^{-1}.disc^{-1}.Petri dish^{-1}. The data were tested by ANOVA, followed by a multiple comparation test of averages (Tukey Test) α 0.01. The best sporulation was obtained from the discs contained wheat incubated at 25°C (42.6 x 10^3 conidia.mL^{-1}.disc^{-1}.Petri dish^{-1}), followed by the same substrate at 30°C (39.1 x 10^3 conidia.mL^{-1}.disc^{-1}. Petri dish^{-1}).

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INTRODUCTION

Macrophomina phaseolina (Tassi) Goid. is a mithosporic fungal species, being the only representative of the genus *Macrophomina* [27, 17]. It is a severe pathogen which attacks many types of cultivated plants, mainly corn (*Zea mays* L.), soybean [*Glycine Max* (L.) Merril], sesame (*Sesamum indicum* L.), peanut (*Arachis hypogea* L.), beans (*Phaseolus vulgaris* L.), cowpea [*Vigna unguiculata* (L.) Walp.], among others [15, 18].

The fungus causes a disease called charcoal rot on cowpea plants and it can occur along different growth phases, according to Rodrigues *et al*. [23]. Under the Brazilian Northeastern conditions, Ponte [21] and Athayde Sobrinho *et al*. [2] highlight that the disease initially occurs on the stem base, reaching, afterwards, the root system, the upper trunk, and the primary branches. Some gray and diffused lesions with a humid aspect, which evolve to an intense tissue rot, are observed on the branches. Depending on the severity, a total or a partial split on the parenchyma and on the vascular system can occur, resulting in a general yellowish, withering, drying, and death of the plants. Some dark spots of reproduction (pycnidia), which are in contrast with the gray background around the whole lesion, are found on the surface of the lesions [16, 12]. Because the production of these structures has not been obtained frequently in artificial cultivation, some isolated ones could be obtained [1, 7].

Researches related to the reproductive behavior of these fungi have demonstrated that many species passed down to seeds frequently loose the ability to sporulate when isolated and artificially cultivated. [26]. The use of extracts and part of host plants (fragments of leaves, trunks, roots, etc.) added to the growth medium have been thoroughly used in order to solve the problem [19, 8; 26]. This initiative has been successful in many cases; however, in some situations, it is inefficient at all [7]. Chidambaram and Mathur [4], using *M. phseolina* isolates from different hosts, tested growth media with leaves of certain crops obtaining promising results such as the production of fertile pycnidia. In this same research line, Machado [15] induced, in some isolates, higher fertile pycnidia production rates by overlaying filter paper on the agar growth medium.

Growth medium, photoperiod, and light quality were factors, highlighted by Chidambaram and Mathur [4], as interfering in the reproductive process of *M. phaseolina*. The authors obtained higher rates of pycnidia production when the fungal colonies were put in agar growth medium with wheat leaves and at near ultraviolet light (NUV). They yet observed that such treatments stimulated the reproduction of the majority of the isolates; however, some of them did not respond positively.

Dhar *et al*. [6] corroborated with the importance of the light factor over the sporulation. These authors verified that the highest rates were obtained under a daily 12-hour-light/dark-exposition period during the whole incubation period. It was also verified that a slight increase in the dark period was enough to inhibit

the sporulation process. Machado [15] demonstrated that this process was equally stimulated either under continuous light conditions or under light-alternating period with a 12-hour photophase. The author also highlighted that some isolated can only sporulate when under continuous light conditions.

The importance of fertile pycnidia production under experimental conditions is undoubtfull [14] when dealing with studies related to the pathogenicity of the fungi [28] as well as identifying and developing sources of resistance to disease caused by the own fungi. Due to the absence of conidia production in artificial cultivation, Athayde Sobrinho *et al*. [3] highlighted the difficulty in inoculating the fungi in cowpea plants either for testing the pathogenicity of the isolated organisms or for identifying the sources of resistance to the pathogen observed in *V. unguiculata*. The fungal mycelium and/or sclerotia did not demonstrate effectiveness when used in suspension.

The present work aimed studying the effect of temperature and overlaying of leaves of cowpea (*V. unguiculata*), wheat (*T. aestivum*), and corn (*Z. mays*) plants on PDA growth medium over the sporulation of *M. phaseolina*, for later studies related to pathogenicity, identification of source of resistance, and control of charcoal rot.

MATERIAL AND METHODS

The strain (MP002), apparently resistant to sporulation in artificial media, was obtained from cowpea cv. Fradinho seeds from Piauí state, Brazil and utilized in this study.

The assay was assembled in a 2 x 5 factorial arrangement (temperature x sporulation substrate) with four replicates. The experimental plot consisted of a Petri dish with PDA growth medium on which six 0.7-cm-discs of leaves of cowpea (*V. unguiculata*), wheat (*T. aestivum*), and corn (*Z. mays*) plants at 25º C and 30º C at continuous light were placed. The treatments were incubated on a BOD-type growth chamber Tecnal, model EP – II.

The controls plots were delineated with Paper filter discs in PDA growth medium and PDA growth medium only, both without adding of any kind of substance. A 0.4-cm-disc of PDA growth medium colonized with fungal mycelium was placed at the central part of each Petri dish. The PDA growth medium colonized

with fungal mycelium was obtained from a 4-day-old-pure culture of an strain of *M. phaseolina*, which was cultivated on PDA at alternating light regime (12 hours dark and 12 hours light).

The evaluation was performed at the 9^{th}-incubation day by removing the discs from each treatment. Each disc was ground in 2.5 mL of distilled water in order to free the conidia and pre-estimate their number. After filtering the collected material, the data were obtained through the average estimative of the conidia number by the Neubauer chamber.

The data related to the sporulation were transformed into log (x+1) in order to perform a statistical analysis. The averages were compared using the multiple comparation averages test (Tukey Test) at 1% of probability, according to Pimentel Gomes[20]. The referred analysis was performed by using SAS PROC ANOVA procedure [25].

RESULTS AND DISCUSSION

The variance analysis revealed a highly significative effect ($p \leq 0.01$) of temperature, discs of leaves as well as the interaction between them and the fungi sporulation (Table 1).

The values reveal that the temperature, independently from the disc, significantly affected the fungi sporulation (Table 2). The highest sporulation rates were obtained at 25ºC ($15{,}9 \times 10^3$ .conidia.mL^{-1}.$disc^{-1}$.Petri $dish^{-1}$), which was significatively superior to those obtained at 30ºC (7.9×10^3 .conidia.mL^{-1}.$disc^{-1}$.Petri $dish^{-1}$).

The data obtained in the present work are partially corrected by the existence of a common thought about the fact that the fungi sporulate better on mild temperature ranges. This fact was demonstrated in works developed by Dhar *et al*. [6], where the production of fertile pycnidia from four *M. phaseolina*

TABLE 1: Analysis of variance for *Macrophomina phaseolina* sporulation under different substrates (discs) and at different temperatures.

Source	Degree of freedom	Sum of square	Mean square	F Value	Pr >F
Temperature	1	26.03	26.03	43.28	0.0001
Disc	4	99.11	24.78	41.20	0.0001
Temp. x disc	4	26.98	6.74	11.21	0.0001
Residue	30	8.04	0.60		
Total	39	160.16			

TABLE 2: Influence of temperature and of superposition of leaf discs to PDA growth medium, on the sporulation of *Macrophomina phaseolina*

Treatment	Number of conidia (x 10^3 conidia.mL^{-1}. $Disc^{-1}$.Petri $dish^{-1}$) (1)		Average
	Temperature (º C)		
	25	30	
Wheat Disc	42.6 Aa	39.1 Ba	40.85 a
Corn Disc	34.8 Ab	0.0 Bb	17.40 b
Cowpea Disc	1.95 Abc	0.0 Ab	0.97 bc
Without Disc	0.39 Acd	0.37Ab	0.38 cd
Paper Disc	0.0 Ad	0.0Ab	0.00 d
Average	15.9 A	7.9 B	

[1] Average of four repetitions/treatment. Averages followed by the same letter (small letter in the vertical position and capital ones in the horizontal position) do not differ among them by Tukey Test at 1% of probability.

Note: Data were not transformed.

of leaves of cowpea (*V. unguiculata*), wheat (*T. aestivum*), and corn (*Z. mays*) plants at 25º C and 30º C at continuous light were placed. The treatments were incubated on a BOD-type growth chamber Tecnal, model EP – II.

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The data obtained in the present work are partially corrected by the existence of a common thought about the fact that the fungi sporulate better on mild temperature ranges. This fact was demonstrated in works developed by Dhar *et al*. [6], where the production of fertile pycnidia from four *M. phaseolina* explained by Hawker [10, 11] when referring to a differential behavior shown by fungi from the Order Sphaeoropsidales exposed to fluctuations on the nourishing quality of the growth medium. The author assures that the transference of the fungi from a rich medium to poorer one is effective in stimulating the sporulation, due to

the slight nourishing ‘shock’. This can explain the phenomenon verified in this work. The fungi when is grown on PDA medium, which is nourishing rich and with a high availability of nutrients, was stimulated to develop vegetatively, without needing reproducing themselves. When the fungi reach the discs through regular growth, they start nourish from this substrate, where the nutrients are probably less available; then the referred transition occurs and, in this case, the fungi is stimulated to start their reproductive process. Hawker [10, 11] still emphasizes that the abrupt changes cannot be effective for the reproduction. This can explain the absence of sporulation observed on the filter paper, corn, and cowpea mentioned above. This behavior seems to be supported on the Klebs Principles [9], which states, among other aspects, that fungi reproductive process is dependent from the nutrients’ exhaustion.

By analyzing the data from the point of view of the interactions of discs versus temperature, it is observed that the higher sporulation levels were verified when discs of wheat leaves were added to the PDA medium. The results revealed values such as 42.6 and 39.1 x 10^3 conidia.mL^{-1}.$disc^{-1}$.Petri $dish^{-1}$ at 25ºC and 30ºC, respectively, which are significantly superior to the other treatments. These results are in accordance with Chidambaram and Mathur [4] ones, where the higher sporulation rates were obtained when fragments of leaves of wheat, oat, and barley were added to the medium. A special emphasis was given to the leaves of wheat.

The results showed that leaves of corn could also stimulate the sporulation of the fungi at 25º C, similarly to those obtained using wheat leaves. The data displayed on Table 2 show that 34.80 x 10^3 conidia.mL^{-1}.$disc^{-1}$.Petri $dish^{-1}$ was similar to those obtained using cowpea discs (1.95x10^3 conidia.mL^{-1}.$disc^{-1}$.Petri $dish^{-1}$) and that it was significatively superior to those obtained from the treatment using filter paand PDA. Such results demonstrate that the interaction between leaves of corn and temperature (30º C) is not favorable to sporulation. Hawker [11] statement can explain this phenomenon.

Figure 1 shows details on the formation of pycnidia and scletoria on different substrates tested in this experiment. In discs of wheat leaves it was observed that the pycnidia showed to have bigger dimensions and were more individualized. Little or no formation of scletoria, indicating that the conditions were favorable to sporulation. Abundant quantities of scletoria and insignificant amounts of pycnidia, which presence justifies

the appearance of some conidia (0,39 and 0,37x 10^3 conidia. mL^{-1}. $disc^{-1}$.Petri $dish^{-1}$), were observed on PDA growth media and are shown on Table 2.

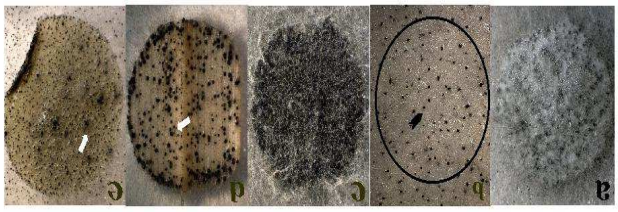

Figure 1: Details on the pycnidia formation (light arrow) and sclerotia (dark arrow) on different substrates: a- Paper; b- PDA; c – Corn; d- Wheat; e- Cowpea.

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