

## PATHOGENICITY OF *Macrophomina* SPECIES COLLECTED FROM WEEDS IN COWPEA<sup>1</sup>

RUI SALES JÚNIOR<sup>2\*</sup>, ALFREDO NOGUEIRA DA SILVA NETO<sup>2</sup>, ANDRÉIA MITSA PAIVA NEGREIROS<sup>2</sup>, THOMAZ RAUAN RODRIGUES GOMES<sup>2</sup>, MÁRCIA MICHELLE DE QUEIROZ AMBRÓSIO<sup>2</sup>, JOSEP ARMENGOL<sup>3</sup>

**ABSTRACT** - Charcoal rot caused by *Macrophomina phaseolina* is a major cowpea disease causing substantial losses to growers. In the semi-arid region of Brazil, cowpea is one of the most widely used alternatives for crop rotation during the off-season of melon. This favors *Macrophomina* multiplication because both crops are hosts of this pathogen. The objective of this study was to verify the pathogenicity of *Macrophomina phaseolina* and *M. pseudophaseolina* on cowpea. The *Macrophomina* spp. isolates used were obtained from the roots of *Trianthema portulacastrum* and *Boerhavia diffusa*, weed species prevalent in melon production areas in North-east Brazilian. The experiment was carried out in a greenhouse. Cowpea plants cv. 'Paulistinha' were inoculated with 30 *M. phaseolina* isolates, 30 *M. pseudophaseolina* isolates and a reference isolate of *M. phaseolina* obtained from cowpea roots. All *Macrophomina* isolates were able to cause disease on cowpea and there were no statistical differences between both *Macrophomina* species regarding disease incidence and severity. Moreover, 65.2 and 100.0% of the *M. phaseolina* isolates, and 56.2 and 92.8% of the *M. pseudophaseolina* isolates, obtained from *T. portulacastrum* and *B. diffusa*, respectively, were as severe to cowpea as the *M. phaseolina* reference isolate from cowpea. These results emphasize the need to establish management practices aiming to control *T. portulacastrum* and *B. diffusa* from cowpea production areas, as they can act as potential sources of inoculum and survival for *Macrophomina* spp.

**Keywords:** *Boerhavia diffusa*. Inoculation. Soil-borne pathogen. *Trianthema portulacastrum*. *Vigna unguiculata*.

## PATOGENICIDADE DE ESPÉCIES DE *Macrophomina* COLETADAS DE PLANTAS DANINHAS EM FEIJÃO-CAUPI

**RESUMO** - A podridão de carvão causada por *Macrophomina phaseolina* é uma das principais doenças do feijão-caupi, causando perdas substanciais para os produtores. Na região semiárida do Brasil, o feijão-caupi é uma das alternativas utilizadas para rotação de culturas durante a entressafra do melão. Isso favorece a multiplicação de *Macrophomina*, uma vez que ambas as culturas são hospedeiras desse patógeno. O objetivo deste estudo foi verificar a patogenicidade em caupi de *Macrophomina phaseolina* e *M. pseudophaseolina* em caupi. Isolados de *Macrophomina* spp. obtidos das raízes de *Trianthema portulacastrum* e *Boerhavia diffusa*, espécies de plantas daninhas prevalentes em áreas de produção de melão no Nordeste brasileiro foram utilizadas neste estudo. O experimento foi realizado em casa de vegetação. Plantas de feijão-caupi 'Paulistinha' foram inoculadas com 30 isolados de *M. phaseolina*, 30 isolados de *M. pseudophaseolina* e um isolado de referência de *M. phaseolina* obtido de raízes de feijão-caupi. Todos os isolados de *Macrophomina* foram patogênicos ao feijão-caupi, não havendo diferenças estatísticas entre as duas espécies de *Macrophomina* em relação à incidência e severidade da doença. Além disso, 65,2 e 100,0% dos isolados de *M. phaseolina*, e 56,2 e 92,8% dos isolados de *M. pseudophaseolina*, obtidos de *T. portulacastrum* e *B. diffusa*, respectivamente, foram tão severos ao feijão-caupi quanto o isolado de referência. Esses resultados enfatizam a necessidade de estabelecer práticas de manejo visando o controle de *T. portulacastrum* e *B. diffusa* nas áreas de produção de feijão-caupi, pois podem atuar como fontes de inóculo e sobrevivência para *Macrophomina* spp.

**Palavras-chave:** *Boerhavia diffusa*. Inoculação. Patógeno habitante do solo. *Trianthema portulacastrum*. *Vigna unguiculata*.

\*Corresponding author

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<sup>2</sup>Department of Agronomic and Forest Sciences, Universidade Federal Rural do Semi-Árido, Mossoró, RN, Brazil; jrui@hotmail.com – ORCID: 0000-0001-9097-0649, feumsn@hotmail.com – ORCID: 0000-0002-4080-7162, deia-mitsa@hotmail.com – ORCID: 0000-0002-9544-2527, thomas-rauan@hotmail.com – ORCID: 0000-0003-3868-7904, marciamicelle@ufersa.edu.br – ORCID: 0000-0002-5033-9745.

<sup>3</sup>Mediterranean Agroforestry Institute, Universitat Politècnica de València, Valencia, Spain; jarmengo@eaf.upv.es – ORCID: 0000-0003-3815-8578.

## INTRODUCTION

Cowpea (*Vigna unguiculata* [L.] Walp.) is one of the main legumes cultivated in semi-arid regions of African countries, with 95.9% of world production (7.1 million tons) (FAOSTAT, 2019). However, world production is underestimated because countries such as Brazil and India do not have separate production data between cowpea and common bean (*Phaseolus vulgaris* L.) (DAMASCENO-SILVA; ROCHA; MENEZES-JÚNIOR, 2016).

In Brazil, cowpea production is concentrated mainly in the North-east and North Regions, with increasing progress in the Midwest region. This crop is considered one of the main protein sources of human food, and an important generator of employment and income in the growing regions (ROCHA et al., 2009; DAMASCENO-SILVA; ROCHA; MENEZES-JÚNIOR, 2016). The importance of cowpea is also due to the possibility of being cultivated by small farmers in family farms under irrigated and rainfed conditions, due to its easy management and low production costs, playing an important socioeconomic role (RAMOS et al., 2012; DAMASCENO-SILVA; ROCHA; MENEZES-JÚNIOR, 2016).

It is estimated that in the 2016/17 growing season, Brazilian cowpea production reached 789.8 thousand tons in 1.5 million hectares (CONAB, 2019).

Fungal diseases are among the most important limiting factors for cowpea production in Brazil, being responsible for high qualitative and quantitative losses. “Charcoal rot” caused by *Macrophomina phaseolina* (Tassi) Goid. (RIOS, 1988) is among the most relevant diseases of cowpea crops in Brazil. Due to the thermotolerant condition of this fungus, this disease becomes even more important in the North-east Brazilian Region, as climatic conditions such as high temperature and low humidity favor its development (ATHAYDE SOBRINHO, 2016; GOMES-SILVA et al., 2017; NEGREIROS et al., 2019b).

The primary source of inoculum for *M. phaseolina* are soil microsclerotia, infected seeds, and crop debris (KAUR et al., 2012; REIS; BOARETTO; DANELLI, 2014). Microsclerotia can survive for long periods, about 2 to 15 years, and can germinate throughout the growing season (GUPTA; SHARMA; RAMTEKE, 2012).

A study conducted in Senegal with *Macrophomina* isolates from *Abelmoschus esculentus* (L.) Moench., *Arachis hypogaea* L., *Hibiscus sabdariffa* L., and cowpea plants, identified a new species of *Macrophomina* named *M.*

*pseudophaseolina* Crous, Sarr & Ndiaye (SARR et al., 2014). In Brazil, recent studies have reported the occurrence of three species of *Macrophomina*: *M. phaseolina*, affecting numerous crops, among them cowpea; *M. pseudophaseolina* affecting *A. hypogaea*, *Gossypium hirsutum* L. and *Ricinus communis* L.; and *M. euphorbiicola* A.R. Machado, D.J. Soares & O.L. Pereira, sp. nov. a new species, affecting *Jatropha gossypifolia* L. and *R. communis* plants (MACHADO et al., 2019). Negreiros et al. (2019b) studying phytopathogenic fungi present in roots of prevalent weeds in melon fields in the states of Rio Grande do Norte (RN) and Ceará (CE) (North-east Brazilian Region) reported the occurrence of these three *Macrophomina* species in *Trianthema portulacastrum* L. and *Boerhavia diffusa* L. Very recently, Zhao et al. (2019) reported a novel *Macrophomina* species, *M. vaccinii* Y. Zhang ter & L. Zhao, sp. nov. affecting *Vaccinium* spp. in China.

This study aims to verify the pathogenicity of *M. phaseolina* and *M. pseudophaseolina* isolates obtained from roots of the weed species *T. portulacastrum* and *B. diffusa* on cowpea.

## MATERIALS AND METHODS

### *Macrophomina* spp. Isolates

The experiment was carried out in a greenhouse from December 2016 to March 2017 to evaluate the pathogenicity of 61 isolates of two *Macrophomina* species on cowpea. These isolates were obtained from asymptomatic *T. portulacastrum* and *B. diffusa* weed roots collected from commercial melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* [Thunb.] Matsum & Nakai) production areas in the municipalities of Icapuí (CE), Assú and Mossoró (RN). Crop rotations in these areas include cowpea.

In the experiment, 30 *M. phaseolina* isolates (23 from *T. portulacastrum* and 7 from *B. diffusa*), 30 *M. pseudophaseolina* isolates (16 from *T. portulacastrum* and 14 from *B. diffusa*) and one reference isolate from *M. phaseolina* obtained from cowpea roots (Strain number: MFE-01, collected in 2013, location: Brazil, Rio Grande do Norte, Mossoró, GenBank accession number: MN136201) were used (Table 1).

The identity of the fungal isolates used in this experiment was molecularly confirmed (NEGREIROS et al., 2019b), and a copy of each was deposited in the “Profa. Maria Menezes” (CMM) fungi collection, at the Universidade Federal Rural de Pernambuco (Pernambuco, Recife, Brazil) (Table 1).

**Table 1.** List of isolates used in the pathogenicity test of *Macrophomina* species.

<i>Macrophomina pseudophaseolina</i>			<i>Macrophomina phaseolina</i>		
Strain number	Host	Location	Strain number	Host	Location
CMM-4765	<i>Tp</i> <sup>1</sup>	Brazil - Icapuí	CMM-4749	<i>Tp</i>	Brazil - Mossoró
CMM-4766	<i>Tp</i>	Brazil - Icapuí	CMM-4750	<i>Tp</i>	Brazil - Mossoró
CMM-4767	<i>Tp</i>	Brazil - Icapuí	CMM-4751	<i>Tp</i>	Brazil - Mossoró
CMM-4768	<i>Tp</i>	Brazil - Icapuí	CMM-4752	<i>Tp</i>	Brazil - Mossoró
CMM-4770	<i>Tp</i>	Brazil - Assú	CMM-4753	<i>Tp</i>	Brazil - Mossoró
CMM-4772	<i>Tp</i>	Brazil - Assú	CMM-4754	<i>Tp</i>	Brazil - Mossoró
CMM-4773	<i>Tp</i>	Brazil - Assú	CMM-4755	<i>Tp</i>	Brazil - Mossoró
CMM-4774	<i>Tp</i>	Brazil - Assú	CMM-4756	<i>Tp</i>	Brazil - Mossoró
CMM-4775	<i>Tp</i>	Brazil - Assú	CMM-4757	<i>Tp</i>	Brazil - Mossoró
CMM-4777	<i>Tp</i>	Brazil - Assú	CMM-4733	<i>Tp</i>	Brazil - Icapuí
CMM-4778	<i>Tp</i>	Brazil - Assú	CMM-4734	<i>Tp</i>	Brazil - Icapuí
CMM-4779	<i>Tp</i>	Brazil - Mossoró	CMM-4735	<i>Tp</i>	Brazil - Icapuí
CMM-4780	<i>Tp</i>	Brazil - Mossoró	CMM-4736	<i>Tp</i>	Brazil - Icapuí
CMM-4784	<i>Tp</i>	Brazil - Mossoró	CMM-4737	<i>Tp</i>	Brazil - Icapuí
CMM-4787	<i>Tp</i>	Brazil - Mossoró	CMM-4738	<i>Tp</i>	Brazil - Icapuí
CMM-4788	<i>Tp</i>	Brazil - Mossoró	CMM-4739	<i>Tp</i>	Brazil - Icapuí
CMM-4789	<i>Bd</i> <sup>2</sup>	Brazil - Assú	CMM-4740	<i>Tp</i>	Brazil - Icapuí
CMM-4792	<i>Bd</i>	Brazil - Assú	CMM-4741	<i>Tp</i>	Brazil - Icapuí
CMM-4793	<i>Bd</i>	Brazil - Assú	CMM-4742	<i>Tp</i>	Brazil - Icapuí
CMM-4794	<i>Bd</i>	Brazil - Assú	CMM-4743	<i>Tp</i>	Brazil - Icapuí
CMM-4795	<i>Bd</i>	Brazil - Assú	CMM-4746	<i>Tp</i>	Brazil - Icapuí
CMM-4796	<i>Bd</i>	Brazil - Assú	CMM-4747	<i>Tp</i>	Brazil - Icapuí
CMM-4797	<i>Bd</i>	Brazil - Assú	CMM-4748	<i>Tp</i>	Brazil - Icapuí
CMM-4806	<i>Bd</i>	Brazil - Assú	CMM-4758	<i>Bd</i>	Brazil - Assú
CMM-4810	<i>Bd</i>	Brazil - Assú	CMM-4759	<i>Bd</i>	Brazil - Assú
CMM-4811	<i>Bd</i>	Brazil - Assú	CMM-4760	<i>Bd</i>	Brazil - Assú
CMM-4815	<i>Bd</i>	Brazil - Mossoró	CMM-4761	<i>Bd</i>	Brazil - Assú
CMM-4817	<i>Bd</i>	Brazil - Mossoró	CMM-4762	<i>Bd</i>	Brazil - Mossoró
CMM-4821	<i>Bd</i>	Brazil - Mossoró	CMM-4763	<i>Bd</i>	Brazil - Mossoró
CMM-4826	<i>Bd</i>	Brazil - Mossoró	CMM-4764	<i>Bd</i>	Brazil - Mossoró
-	-	-	MPH-FE	<i>Vu</i> <sup>3</sup>	Brazil - Mossoró

<sup>1</sup>*Trianthema portulacastrum*; <sup>2</sup>*Boerhavia diffusa*; <sup>3</sup>*Vigna unguiculata*.

### Inoculum preparation

All isolates were placed in potato-dextrose-agar (PDA) culture medium and incubated at  $28 \pm 2$  °C in the dark for seven days to be used for inoculum preparation.

Cowpea plants were inoculated following the methodology of the infested toothpick (AMBROSIO et al., 2015). Toothpick tips (1.5 cm) were vertically inserted, with the sharp portion of the toothpicks facing upwards, on a filter paper with the same internal diameter as the Petri plate. Subsequently, these plates, properly closed, were autoclaved at 121 °C for 30 min. PDA was then poured into these plates about 4 mm from the end of the toothpicks. After solidification of the culture medium, four 0.5 mm diameter discs with fungal structures (mycelium and sclerotia) were transferred to these plates, equidistantly distributed, and incubated for eight days in an incubator at  $30 \pm 2$  °C, for complete colonization of the toothpicks.

### Experimental design and evaluation

Three seeds of cowpea cultivar 'Paulistinha' were sown in plastic pots, with a capacity for 0.75 L,

containing commercial substrate Tropstrato HT<sup>®</sup>. Then, they were autoclaved twice at 121 °C for one hour, with a 24 h interval in between. Eight days after sowing, thinning was performed to have only one plant per pot. At this moment, toothpicks previously infested with each fungal isolate were inserted into the main stem of the plant at a height of 0.5 cm from ground level. For the uninoculated control, only autoclaved toothpicks were used.

The experimental design was completely randomized, with 61 treatments (representing the isolates) with five replications each, and one uninoculated control. The experimental unit consisted of a plastic pot with a plant. The pots were kept in a greenhouse at an average temperature of 32 °C for 30 days, under natural daylight conditions, and irrigation was manually performed. The experiment was repeated.

The incidence and severity of the *Macrophomina* isolates were evaluated 30 days after inoculation. Disease incidence was determined by counting plants with symptoms of charcoal rot, and the data were transformed as a percentage (%). To assess the severity of the disease, a scale proposed by Abawi e Pastor-Corrales (1990) was used, with modifications, where 1 = no symptoms; 3 = lesions

limited to cotyledonary leaves; 5 = progressing lesions from cotyledonary leaves up to 2 cm in the stem; 7 = extensive lesions, presence of chlorosis and necrosis in leaves and stem; and 9 = presence of pycnidia on stem and plant death.

After the evaluation, all plants were analyzed for fungal isolation to confirm Koch's Postulates. Fragments of the diseased areas were disinfested in a 1.5% sodium hypochlorite solution for 1 min and washed in sterile water. The disinfested fragments were placed in PDA-tetracycline (0.05 g L<sup>-1</sup>) medium and incubated for five days at 30 ± 2 °C.

For each experiment, the preliminary ANOVA was performed to determine whether there were significant differences between the two repetitions of the experiments and whether the data could be combined. Severity and incidence results by isolates of *M. phaseolina* and *M. pseudophaseolina* were analyzed with the non-parametric Kruskal-

Wallis test at the probability level of 5% ( $p \leq 0.05$ ) using the Assistat software, version 7.7 (SILVA; AZEVEDO, 2016). Differences in severity and incidence caused by *Macrophomina* species were determined using Mann-Whitney test at the 5% significance level using statistix v.9.0 (Analytical Software).

## RESULTS AND DISCUSSION

All isolates of both *Macrophomina* species (*M. phaseolina* and *M. pseudophaseolina*) isolated from *T. portulacastrum* and *B. diffusa* caused disease on cowpea plants (Table 2). The results of both experiments were combined because there were no statistical differences between the two experiments ( $p \leq 0.05$ ).

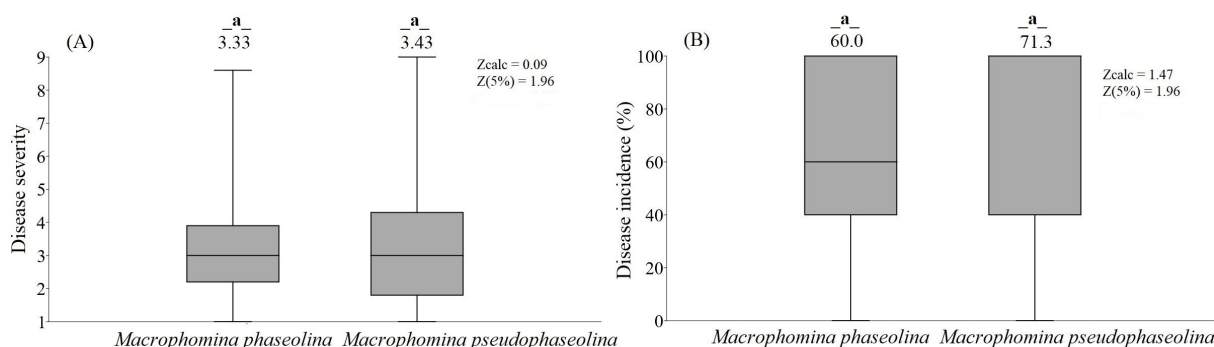
**Table 2.** Reaction of *Vigna unguiculata* cv. 'Paulistinha' to inoculation with isolates of *Macrophomina* spp.

Isolates	<i>Macrophomina phaseolina</i>				Isolates	<i>Macrophomina pseudophaseolina</i>			
	Disease Severity		Disease Incidence			Disease Severity		Disease Incidence	
	Rank	Mean <sup>c</sup>	Rank	Mean <sup>c</sup> (%)		Rank	Mean <sup>c</sup>	Rank	Mean <sup>c</sup> (%)
CMM-4733 <sup>a</sup>	44.2 ab	1.4	49.0 ab	20	CMM-4765 <sup>a</sup>	35.9 a	1.4	40.5 ab	20
CMM-4734 <sup>a</sup>	85.2 abc	3.8	81.0 ab	60	CMM-4766 <sup>a</sup>	90.4 abc	3.4	104.5 b	100
CMM-4735 <sup>a</sup>	102.2 abc	3.8	113.0 b	100	CMM-4767 <sup>a</sup>	81.5 abc	3.0	104.5 b	100
CMM-4736 <sup>a</sup>	67.4 abc	3.0	65.0 ab	40	CMM-4768 <sup>a</sup>	47.3 ab	1.8	56.5 ab	40
CMM-4737 <sup>a</sup>	66.6 abc	2.2	81.0 ab	60	CMM-4770 <sup>a</sup>	99.3 abc	3.8	104.5 b	100
CMM-4738 <sup>a</sup>	55.4 ab	1.8	65.0 ab	40	CMM-4772 <sup>a</sup>	47.3 ab	1.8	56.5 ab	40
CMM-4739 <sup>a</sup>	33.0 a	1.0	33.0 a	0	CMM-4773 <sup>a</sup>	35.9 a	1.4	40.5 ab	20
CMM-4740 <sup>a</sup>	89.8 abc	3.8	97.0 ab	80	CMM-4774 <sup>a</sup>	58.7 abc	2.2	72.5 ab	60
CMM-4741 <sup>a</sup>	95.6 abc	3.4	113.0 b	100	CMM-4775 <sup>a</sup>	47.3 ab	1.8	56.5 ab	40
CMM-4742 <sup>a</sup>	62.0 abc	2.2	65.0 ab	40	CMM-4777 <sup>a</sup>	70.1 abc	2.6	88.5 ab	80
CMM-4743 <sup>a</sup>	44.2 ab	1.4	49.0 ab	20	CMM-4778 <sup>a</sup>	95.2 abc	4.2	104.5 b	100
CMM-4746 <sup>a</sup>	56.2 ab	2.6	49.0 ab	20	CMM-4779 <sup>a</sup>	47.3 ab	1.8	56.5 ab	40
CMM-4747 <sup>a</sup>	106.7 abc	4.6	97.0 ab	80	CMM-4780 <sup>a</sup>	65.1 abc	2.6	56.5 ab	40
CMM-4748 <sup>a</sup>	91.0 abc	3.4	97.0 ab	80	CMM-4784 <sup>a</sup>	24.5 a	1.0	24.5 a	0
CMM-4749 <sup>a</sup>	77.8 abc	2.6	97.0 ab	80	CMM-4787 <sup>a</sup>	67.6 abc	3.0	56.5 ab	40
CMM-4750 <sup>a</sup>	90.6 abc	4.6	81.0 ab	60	CMM-4788 <sup>a</sup>	44.8 ab	1.8	40.5 ab	20
CMM-4751 <sup>a</sup>	62.0 abc	2.2	65.0 ab	40	CMM-4789 <sup>b</sup>	113.0 abc	5.0	104.5 b	100
CMM-4752 <sup>a</sup>	56.2 ab	2.6	49.0 ab	20	CMM-4792 <sup>b</sup>	104.1 abc	4.6	104.5 b	100
CMM-4753 <sup>a</sup>	84.8 abc	3.8	81.0 ab	60	CMM-4793 <sup>b</sup>	81.5 abc	3.0	104.5 b	100
CMM-4754 <sup>a</sup>	73.2 abc	2.6	81.0 ab	60	CMM-4794 <sup>b</sup>	81.5 abc	3.0	104.5 b	100
CMM-4755 <sup>a</sup>	56.2 ab	2.6	49.0 ab	20	CMM-4795 <sup>b</sup>	108.2 abc	4.2	104.5 b	100
CMM-4756 <sup>a</sup>	33.0 a	1.0	33.0 a	0	CMM-4796 <sup>b</sup>	58.7 abc	2.2	72.5 ab	60
CMM-4757 <sup>a</sup>	71.7 abc	3.0	65.0 ab	40	CMM-4797 <sup>b</sup>	117.8 bc	5.8	104.5 b	100
CMM-4758 <sup>b</sup>	101.0 abc	4.2	113.0 b	100	CMM-4806 <sup>b</sup>	135.6 bc	6.6	104.5 b	100
CMM-4759 <sup>b</sup>	95.6 abc	3.4	113.0 b	100	CMM-4810 <sup>b</sup>	133.3 bc	6.2	104.5 b	100
CMM-4760 <sup>b</sup>	77.8 abc	2.6	97.0 ab	80	CMM-4811 <sup>b</sup>	47.3 ab	1.8	56.5 ab	40
CMM-4761 <sup>b</sup>	135.3 bc	7.0	113.0 b	100	CMM-4815 <sup>b</sup>	150.0 c	9.0	104.5 b	100
CMM-4762 <sup>b</sup>	119.6 abc	5.8	113.0 b	100	CMM-4817 <sup>b</sup>	131.5 bc	7.0	104.5 b	100
CMM-4763 <sup>b</sup>	146.1 bc	8.6	113.0 b	100	CMM-4821 <sup>b</sup>	99.3 abc	3.8	104.5 b	100
CMM-4764 <sup>b</sup>	114.2 abc	5.0	113.0 b	100	CMM-4826 <sup>b</sup>	81.5 abc	3.0	104.5 b	100
MPH-FE	149.0 c	9.0	113.0 b	100	MPH-FE	150.0 c	9.0	104.5 b	100
Control	33.0 a	1.0	33.0 a	0	Control	24.5 a	1.0	24.5 a	0
H <sub>estatic</sub>	77.5		70.1		H <sub>estatic</sub>	107.4		88.9	

H<sub>estatic</sub> value significant at 5% by Kruskal-Wallis test. <sup>a</sup>Isolates obtained from *Trianthema portulacastrum*; <sup>b</sup>Isolates obtained from *Boerhavia diffusa*. <sup>c</sup>Mean of 10 inoculated plants.

For both analyzed variables, incidence and disease severity, there were no statistical differences

between the *Macrophomina* species for Mann-Whitney test ( $p \leq 0.05$ ) (Figure 1).



**Figure 1.** Boxplots showing (A) Disease Severity and (B) Incidence of the *Macrophomina* species in cowpea plants. The boxes show the first and third quartiles. Bold horizontal line represents median of group. Lower and upper whiskers extend from the boxes to the extreme values. Different lowercase letters indicate significant differences according to Mann-Whitney test ( $p \leq 0.05$ ).

Noronha; Damasceno-Silva and Silva (2012) analyzed the genetic resistance of 35 cowpea genotypes to *M. phaseolina* and concluded that none of the genotypes showed resistance to the pathogen. Regarding *M. pseudophaseolina*, studies conducted by Sarr et al. (2014) reported that this species was able to damage different crops, among them cowpea, in Senegal. Subsequent pathogenicity studies of *M. phaseolina* and *M. pseudophaseolina* isolates, conducted by Ndiaye et al. (2015) on three varieties of cowpea under two temperature regimes (24-34 and 26-36 °C) in a climatic chamber, showed that there were small differences in pathogenicity between *M. phaseolina* and *M. pseudophaseolina*. These authors indicated that the latter *Macrophomina* species seemed somewhat more aggressive in the susceptible cv. 'Mouride' (more microsclerotia per gram tissue and less biomass production) at the higher temperature tested (26-36 °C).

This fact is quite worrying, given that the production of this crop is prevalent in the Northeast and North regions of Brazil, where temperatures are quite high throughout the year. In fact, *M. phaseolina* is considered economically important in subtropical regions and tropical countries with a semiarid climate (ATHAYDE SOBRINHO, 2016; GOMES-SILVA et al., 2017; NEGREIROS et al., 2019b), but there is a lack of knowledge about the risks posed by *M. pseudophaseolina* on cowpea and other crops, because of its recent description (SARR et al., 2014).

In Brazil, *M. phaseolina* was first reported in 1935, infecting common bean plants in Campinas-SP (BITANCOURT, 1935). In cowpea, under favorable environmental conditions, this fungus can be highly virulent, attacking from the seed to the pod of the plant. Athayde Sobrinho, Viana and Santos (2005) detected the presence of *M. phaseolina* in 62% of cowpea seed samples analyzed in Brazil. According to Dhingra and Sinclair (1978), infected seeds, symptomatic or asymptomatic, represent the main

form of spread of this fungus over long distances.

It is also important to note that 65.2 and 100.0% of the *M. phaseolina* isolates, and 56.2 and 92.8% of the *M. pseudophaseolina* isolates, obtained from *T. portulacastrum* and *B. diffusa*, respectively, were as severe to cowpea as the *M. phaseolina* reference isolate from cowpea (Table 2).

Although both *Macrophomina* species isolated from *T. portulacastrum* and *B. diffusa* were pathogenic to cowpea, more than 90% of these isolates, derived from *B. diffusa*, showed severity in cowpea equal to the *M. phaseolina* reference isolate. However, the isolates obtained from *T. portulacastrum* were less severe to cowpea when compared to the isolate from *B. diffusa* (Table 2).

Weeds are important alternative hosts of plant pathogens (NEGREIROS et al., 2019a; b; SALES JÚNIOR et al., 2019). They can actively contribute to the survival of soil-borne phytopathogenic fungi, especially during the off-season (SALES JÚNIOR et al., 2012).

The coexistence of cowpea with weeds is one of the main factors compromising the development and productivity of this crop. Thus, weeds control is considered one of the main components of the production costs (FONTES; GONÇALVES; MORAIS, 2010). According to Freitas et al. (2009), when uncontrolled, weeds can reduce cowpea grain yield by up to 90%. Moreover, prior knowledge of weed participation as alternative hosts of soil-borne phytopathogenic fungi may be of great relevance in the management of root diseases, especially in the off-season, as they may play an important role in disease epidemiology (SALES JÚNIOR et al., 2012).

The weeds species *T. portulacastrum* and *B. diffusa* occur frequently in the main areas of melon (SALES JÚNIOR et al., 2019), watermelon (SILVA et al., 2013), and corn (*Zea mays* L.) (NASCIMENTO et al., 2011) cultivation in the state of RN, Brazil. In addition to *Macrophomina* spp., they have also been reported to host, *Monosporascus* spp. and *Rhizoctonia solani* Kühn, which are soil-

borne pathogens associated with root rot and vine decline on melon crops (SALES JÚNIOR et al., 2012; NEGREIROS et al., 2019a).

Reports estimate that there are over 700 species of *Macrophomina* host plants in the world (FARR; ROSSMAN, 2019). In Brazil, this genus has been reported in approximately 60 species of cultivated and weed plants, with the weeds being also responsible for the survival of the pathogen in the absence of host crops in the field. Some of the most important crops are: *Allium sativum* L., *Arachis hypogaea*, *Citrus* sp. L., *Cocos nucifera* L., *Coffea arabica* L., *Cucumis melo*, *Cucurbita* sp., *Daucus carota* L., *Glycine max* (L.) Merr., *Gossypium hirsutum*, *Helianthus annuus* L., *Jatropha gossypifolia*, *Phaseolus aureus* Roxb., *P. vulgaris*, *Ricinus communis*, *Solanum tuberosum* L., *Sorghum bicolor* (L.) Moench., *Triticum aestivum* L., *V. unguiculata*, and *Zea mays* (SALES JÚNIOR et al., 2012; MACHADO et al., 2019; NEGREIROS et al., 2019b; SALES JÚNIOR et al., 2019).

In Brazil, there are no chemicals registered for field control of this pathogen in any crop, and only products for seed treatment are available (AGROFIT, 2019). *Trianthema portulacastrum* and *B. diffusa*, even with no visible symptoms in the plants, when present in a cowpea production area can compete for water, nutrients and light, and also act as a source of inoculum for *M. phaseolina* and *M. pseudophaseolina*, which may cause charcoal rot in the plants and, consequently, a reduction in yield.

## CONCLUSIONS

Our results emphasize the need to establish management practices aiming to control *T. portulacastrum* and *B. diffusa* from cowpea production areas, as they can act as potential sources of inoculum and survival for *Macrophomina* spp.

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