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Reaction of non-cucurbitacea to *Monosporascus* spp.[1]

Reação de não-cucurbitáceas à *Monosporascus* spp.

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ABSTRACT - With the recent discovery of five new species of the *Monosporascus* genus, a fungal genus involved in "*Monosporascus* root rot and vine decline," pathogenicity studies have become important to understand the impact of these new species on cultivated plants, mainly on plant species used as an alternative for crop rotation. This study aimed to evaluate the pathogenicity of *Monosporascus* spp. in non-cucurbitaceous cultures. The tests were carried out in duplicate in a greenhouse in a completely randomized design, with the treatments: One isolate of each species of *Monosporascus* (*M. brasiliensis*, *M. caatinguensis*, *M. cannonballus*, *M. mossoroensis*, *M. nordestinus*, and *M. semiaridus*) and the absolute control, and five cultures (cowpea, jack bean, corn, sorghum, and bell pepper). Seeds of these cultures were sown in pots containing soil + Tropstrate HT® (ratio 2:1, v / v), previously inoculated with wheat seeds colonized with the species of *Monosporascus*. After 50 days of sowing, plants were evaluated for incidence, disease severity, length of shoot and roots, fresh and dry weight of shoots and roots. All inoculated *Monosporascus* species were able to induce damage in cowpea and bell pepper cultures, with *M. cannonballus* being the most aggressive. Corn, jack beans, and sorghum presented few or no symptoms of the disease and may be indicated as alternative cultures to be used in crop rotation with cucurbits. Studies with a greater number of isolates and cultivars for each culture tested in this study should be carried out to strengthen the data obtained.

Key words: Crop rotation. Host range. Pathogenicity test. Soilborne pathogen. Virulence.

RESUMO - Com a descoberta recente de cinco novas espécies do gênero *Monosporascus*, gênero fúngico envolvido na "podridão das raízes por *Monosporascus* e declínio das ramas", estudos de patogenicidade tem se tornado importante para conhecer o impacto dessas novas espécies em plantas cultivadas, principalmente sobre espécies vegetais utilizadas como alternativa de rotação de culturas. Este estudo teve como objetivo avaliar a patogenicidade de *Monosporascus* spp. em culturas não-cucurbitáceas. Os ensaios foram realizados em duplicata, em casa-de-vegetação, utilizando o delineamento inteiramente casualizado, tendo como tratamentos: um isolado de cada espécie de *Monosporascus* (*M. brasiliensis*, *M. caatinguensis*, *M. cannonballus*, *M. mossoroensis*, *M. nordestinus* e *M. semiaridus*) e a testemunha absoluta, e cinco culturas (feijão-caupi, feijão-de-porco, milho sorgo e pimentão). Sementes destas culturas foram semeadas em vasos contendo solo + Tropstrato HT® (proporção 2:1, v / v), previamente inoculados com sementes de trigo colonizadas com as espécies de *Monosporascus*. Após 50 dias da semeadura, as plantas foram avaliadas quanto a incidência, severidade da doença, comprimento da parte aérea e das raízes, peso fresco e seco da parte aérea e raízes. Todas as espécies de *Monosporascus* induziram danos em feijão-caupi e pimentão, sendo *M. cannonballus* a espécie mais agressiva. O milho, feijão-de-porco e sorgo apresentaram poucos ou nenhum sintoma da doença, podendo ser indicadas como culturas alternativas a serem usadas em rotação de cultivo com cucurbitáceas. Estudos com um maior número de isolados e cultivares para cada cultura testada neste estudo deverão ser realizados para fortalecer os dados obtidos.

Palavras-chave: Rotação de cultivos. Hospedeiras. Teste de patogenicidade. Patógeno habitante do solo. Virulência.

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INTRODUCTION

Monosporascus Pollack & Uecker (Diatrypaceae, Ascomycota) is one of the fungal genera that has species of phytopathological importance in different cucurbits, including melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* [Thunb.] Matsum. & Nakai) (BEN SALEM *et al*., 2013; COHEN *et al*., 2012; MARTYN; MILLER, 1996; SALES JÚNIOR *et al*., 2019). For a long time, the existence of five species in this genus was considered: *M. eutypoides* (Petrak) von Arx, *M. monosporus* (Malloch & Cain) D. Hawksw. & Ciccar, *M. cannonballus*, *M. adenantherae* (S. D. Patil & C. Ramesh) A. Pande and *M. ibericus* Collado, Ant. González, Stchigel, Guarro & Peláez (CASTRO *et al*., 2020). However, recent phylogenetic studies have identified five new species in this genus: *M. brasiliensis* A. Negreiros, M. León, J. Armengol & R. Sales Júnior, *M. caatinguensis* A. Negreiros, M. León, J. Armengol & R. Sales Júnior, *M. mossoroensis* A. Negreiros, M. León, J. Armengol & R. Sales Júnior, *M. nordestinus* A. Negreiros, M. León, J. Armengol & R. Sales Junior and *M. semiaridus* A. Negreiros, M. León, J. Armengol & R. Sales Júnior (NEGREIROS *et al.*, 2019).

Among the *Monosporascus* species reported so far, *M. cannonballus* stands out as one of the main root pathogens associated with “root rot and vine decline” (RRVD) in melon and watermelon plants worldwide, reported in 22 countries, including Brazil (AL-MAWAALI *et al*., 2013; COHEN *et al*., 2012; MARKAKIS *et al*., 2018; YAN *et al*., 2016).

Experiments performed by Cavalcante *et al.* (2020) studied the components of adaptability (pH and salinity), sensitivity to fungicides, and pathogenicity of these new species to different cucurbits. All *Monosporascus* species tested were tolerant to pH and salinity variations in *in vitro* mycelial growth, with average pH values ranging from 5.72 (*M. caatinguensis*) to 8.05 (*M. cannonballus*), and salinity ranging from 903.69 mM (*M. cannonballus*) to 975.49 mM (*M. nordestinus*). The actives fludioxonil and fluazinam were the fungicides that presented the best results in the *in vitro* control of the *Monosporascus* species tested. All isolates tested were pathogenic to melon and watermelon, causing damage to roots.

In Brazil, to date, there is no fungicide product registered for the control of *M. cannonballus*, and crop rotation and management of crop residues are the best alternatives for the management of this pathosystem (COHEN *et al*., 2012; SALES JUNIOR *et al*., 2017). Crop rotation is considered promising in terms of reducing diseases associated with different crops. The effectiveness of this technique is related to different mechanisms of action in relation to pathogens such as: Release of toxic substances to phytopathogenic organisms, increase in biodiversity, and establishment of antagonism relationships between the soil biological community and pathogens (DE GRAAFF *et al*., 2010; SU *et al*., 2015; TRIVEDI *et al*., 2015). This technique, from a phytopathological point of view, requires the use of species not susceptible to pathogens commonly associated with the main crop, that is, the tissues of the crops used in crop rotation should not be susceptible to colonization by the pathogen and, consequently, this tends to decrease their survival and inoculum production (SU *et al*., 2015).

Therefore, the purpose of this study was to evaluate the behavior of different non-cucurbits cultures when inoculated with *Monosporascus* spp.

MATERIAL AND METHODS

The experiments were carried out in a greenhouse and laboratory in Mossoró – RN, Brazil, located at coordinates 5º11’15” S and 37º20’39” W, with an altitude of 16 meters. The climate of the region, according to the Köppen’s classification, is type B*Sh* (hot semi-arid) (ALVARES *et al*., 2013).

For the experiments, six isolates of *Monosporascus* spp. (Table 1), and an absolute control (negative control) were used. These isolates were identified through a phylogenetic study (NEGREIROS *et al*., 2019), and are deposited in the collection of phytopathogenic fungi “Profª. Maria Menezes” (CMM), at Universidade Federal Rural de Pernambuco (Pernambuco, Recife, Brazil).

Inoculum production and pathogen inoculation

Isolates of *Monosporascus* species, preserved in “Falcon” tubes by the “Castellani” method, were transferred to Petri dishes with potato dextrose agar - PDA culture medium, and incubated in BOD in the dark at 28 ± 2 °C for seven days to grow. Previously, wheat grains were placed in distilled water for 12 h and placed in erlenmeyer flasks, then they were autoclaved three times for 30 min at 120 °C, with a 24-hour interval between each process. Five fungal disks from the colonies of *Monosporascus* spp., with 8.0 mm in diameter, were obtained from each Petri dish/species, and transferred to erlenmeyer flasks containing the autoclaved wheat grains, according to the methodology suggested by Ben Salem, Armengol and Boughalleb-M ‘hamdi (2015), for colonies of *Monosporascus* species to develop and colonize the grains.

The recipients containing the fungi were kept in BOD at 28 ºC, being daily shaken for four weeks, so that the colonization of the wheat grains by the respective fungi was standardized. Then, pots with 1.5 L capacity were filled with an autoclaved mixture of soil + Tropstrato® HT Vegetables substrate, at 2:1 v/v ratio. Soil infestation

Table 1 - Isolates of the *Monosporascus* spp. used in this study

Monosporascus Species	Code (CMM)[1]	Host	Location[2]	GenBank ITS Region[3]
M. brasiliensis	4839	*Trianthema portulacastrum*	Brazil, RN	MG735234
M. caatinguensis	4833	*Boerhavia diffusa*	Brazil, CE	MG735228
M. cannonballus	2429	*Cucumis melo*	Brazil, RN	JQ762366
M. mossoroensis	4857	*Trianthema portulacastrum*	Brazil, RN	MG735252
M. nordestinus	4846	*Trianthema portulacastrum*	Brazil, RN	MG735241
M. semiaridus	4830	*Trianthema portulacastrum*	Brazil, CE	MG735222

[1] CMM = Culture Collection of Phytopathogenic Fungi “Prof. Maria Menezes” of the Universidade Federal Rural de Pernambuco (Recife, PE, Brazil). [2] CE = Ceará state and RN = Rio Grande do Norte state. [3] Sequence of the Internal Transcribed Spacer Region (ITS) of the isolates deposited at GenBank

was performed by adding 12 g of colonized wheat per kilo of the mixture in each pot, with the different isolates of *Monosporascus* spp. Then, the pots were placed to rest for a week, so that the soil was properly colonized by the fungal species, before transplanting and/or direct seeding of the tested plant species.

Pathogenicity experiment assembly

Five experiments were simultaneously set up to evaluate the incidence and severity of *Monosporascus* spp. for the following crops: corn (*Zea mays* L.) ‘BR 5037’, sorghum (*Sorghum bicolor* [L.] Moench) - ‘BRS Ponta Negra’, cowpea (*Vigna unguiculata* [L.] Walp) ‘Rizo do Ano’, jack bean (*Canavalia ensiformis* L.) - native seed and sweet pepper (*Capsicum annuum* L.) ‘Block Yellow’.

A completely randomized design (DIC) was used, with seven treatments (*Monosporascus* species + control) and six replications for each plant species. The experimental plot consisted of a pot containing one plant each. These experiments were performed in duplicate.

Assessments of disease incidence and severity

The evaluations happened 50 days after sowing, and the plants were carefully removed from the containers to minimize damage and loss of the root system. Subsequently, the roots were washed in running water to eliminate soil particles adhered to the roots.

Disease incidence was determined through the number of infected plants for each *Monosporascus* species and is expressed as a percentage (%).

To assess the severity of the disease, a diagrammatic scale of notes was used (AEGERTER; GORDON; DAVIS, 2000), with modifications. The values of the applied grades ranged from 0 to 4, where: 0 (symptomless); 1 (less than 10% of the roots with weak discoloration or lesions); 2 (moderate discoloration or rot, with lesions reaching 25 to 35% of the roots); 3 (lesions converging to 50% of the roots and death of secondary roots); and 4 (generalized necrosis of the roots or dead plant).

The average reaction for each hybrid or cultivar (tested culture) was calculated by the sum of the scores obtained from each treatment, divided by the total number of evaluated plants. This value was used to discriminate cultures into five reaction classes:0= immune-like (SI); 0.1-1.0= highly resistant (AR); 1.1-2.0= moderately resistant (MR); 2.1-3.0= susceptible (SU); 3.1-4.0= highly susceptible (AS) (ARMENGOL *et al*., 1998; SALES JÚNIOR *et al*., 2018).

Reisolation of each fungal species was performed in Petri dishes containing BDA culture medium supplemented with streptomycin (500 ppm) (BDAS). In an aseptic environment, fragments of symptomatic roots, seven per plate, previously disinfected in a 2% sodium hypochlorite solution for one minute, and washed in distilled and sterilized water three times, were seeded in Petri dishes with BDAS. Subsequently, the plates were kept in a BOD-type oven at 28 ± 2 ºC, in the dark, for 5-7 days. After the colonies growth, they were counted to obtain the percentage of positive growth points for *Monosporascus* spp. All the roots of the inoculated plants were analyzed to confirm the ‘Koch’ Postulates.

The following biometric variables were measured: root (RL) and shoot length (SL), fresh root weight (FRW) and fresh shoot weight (FSW), root (DRW) and shoot (DSW) dry weight. RL and SL were measured after washing the roots, with the aid of a ruler graduated in cm. FRW and FSW were obtained using a digital analytical scale. To obtain DRW and DSW, the plants were added to paper bags and placed in an oven with forced air circulation at 65 ºC until constant weight was obtained. Subsequently, the samples were weighed to obtain the dry mass.

Data analysis

The data on the incidence and severity of the disease did not present statistical normality, being analyzed using non-parametric tests of Kruskal-Wallis and Mann-Whitney at the 5% probability level, through the Assistat 7.7 software (SILVA; AZEVEDO, 2016). For the variables RL, SL, FRW, FSW, DRW, and DSW, the data was submitted to the F-test, and later to the Tukey test at the 5% probability level, when there was significance of the F values for each variable.

RESULTS AND DISCUSSION

The results obtained for the two replications of the experiment were statistically similar for all variables (ANOVA, $p > 0.05$), and the data from both experiments was analyzed together.

Corn

The statistical analysis of the data obtained for the variables incidence and severity of the disease in the corn culture showed a significant difference for the treatments tested. In terms of disease severity and incidence, the species *M. semiaridus* differed from the other species tested, but did not differ from *M. caatinguensis*, according to the non-parametric Kruskal-Wallis test ($p < 0.05$). The disease severity and incidence values ranged from 0.0 to 0.2 and from 0.0 to 25.0%, respectively (Table 2). According to the reaction class suggested by Armengol *et al.* (1998) and Sales Junior *et al.* (2018), the corn cultivar 'BR5037' used in these experiments was highly resistant to the species *M. caatinguensis* (0.1) and *M. semiaridus* (0.2). For the other inoculated *Monosporascus* species, 'BR5037' was immune (Table 2).

For the variables FRW, DRW, SL, FSW, and DSW there was no significant difference between the treatments (Table 3), however, a statistical difference was observed between the treatments for the variable RL, where the control stood out in relation to the other treatments, with exception of *M. cannonballus* (34.2 cm) which statistically differed from *M. brasiliensis* (33 cm) (Table 3). All inoculated pathogenic isolates/species were reisolated.

Table 2 - Pathogenicity of *Monosporascus* spp. in different cultures

Treatments	Corn				Reaction Class[2]
	Disease Incidence		Disease Severity		
	Rank[1]	Mean (%)	Rank[1]	Mean (%)	
M. brasiliensis	40.5 a	0.0	40.5 a	0.0	SI
M. caatinguensis	44.0 ab	8.3	44.0 ab	0.1	AR
M. cannonballus	40.5 a	0.0	40.5 a	0.0	SI
M. mossoroensis	40.5 a	0.0	40.5 a	0.0	SI
M. nordestinus	40.5 a	0.0	40.5 a	0.0	SI
M. semiaridus	51.0 b	25.0	51.0 b	0.2	AR
Testemunha	40.5 a	0.0	40.5 a	0.0	-
$\chi 2$	14.0		14.0		
			Sorghum		
M. brasiliensis	42.5 a	0.0	42.5 a	0.0	SI
M. caatinguensis	42.5 a	0.0	42.5 a	0.0	SI
M. cannonballus	42.5 a	0.0	42.5 a	0.0	SI
M. mossoroensis	42.5 a	0.0	42.5 a	0.0	SI
M. nordestinus	42.5 a	0.0	42.5 a	0.0	SI
M. semiaridus	42.5 a	0.0	42.5 a	0.0	SI
Testemunha	42.5 a	0.0	42.5 a	0.0	-
$\chi 2$	0.0		0.0		
			Cowpea		
M. brasiliensis	42.0 abc	50.0	40.3 ab	0.5	AR
M. caatinguensis	56.0 bc	83.0	54.6 bc	0.9	AR
M. cannonballus	63.0 c	100	73.4 c	2.3	SU
M. mossoroensis	42.0 abc	50.0	40.3 ab	0.5	AR
M. nordestinus	38.5 abc	42.0	35.6 ab	0.4	AR
M. semiaridus	35.0 ab	33.0	32.5 ab	0.3	AR
Testemunha	21.0 a	0.0	21.0 a	0.0	-
$\chi 2$	30.6		41.9		

Continuation table 2

Jack bean					
M. brasiliensis	42.5 a	0.0	42.5 a	0.0	SI
M. caatinguensis	42.5 a	0.0	42.5 a	0.0	SI
M. cannonballus	42.5 a	0.0	42.5 a	0.0	SI
M. mossoroensis	42.5 a	0.0	42.5 a	0.0	SI
M. nordestinus	42.5 a	0.0	42.5 a	0.0	SI
M. semiaridus	42.5 a	0.0	42.5 a	0.0	SI
Testemunha	42.5 a	0.0	42.5 a	0.0	-
χ2	0.0		0.0		
Bell pepper					
M. brasiliensis	32.5 b	100	27.9 ab	2.0	MR
M. caatinguensis	32.5 b	100	37.8 b	2.8	SU
M. cannonballus	32.5 b	100	49.0 b	3.8	AS
M. mossoroensis	32.5 b	100	29.5 b	2.1	SU
M. nordestinus	32.5 b	100	25.3 ab	1.8	MR
M. semiaridus	32.5 b	100	25.5 ab	1.8	MR
Testemunha	4.5 a	0.0	4.5 a	0.0	-
χ2	55.0		34.7		

χ2 = significant chi-square values; values followed by the same letter in the columns show no statistical difference between them by the nonparametric Kruskal-Wallis test ($p < 0.05$). 1 Classification average from all observations in each sample. Data represents mean values from two experiments, each with six replications (pots) per treatment and one plant per replication. 2 Reaction class: 0= immune-like (SI); 0.1-1.0= highly resistant (AR); 1.1-2.0 = moderately resistant (MR); 2.1-3.0=susceptible (SU); 3.1-4.0= highly susceptible (AS), according to Armengol et al. (1998) and Sales Junior et al. (2018)

Table 3 - Effect of inoculation with *Monosporascus* spp. on biometric variables in different cultures

Treatments	Corn					
	RL[1] (cm)	FRW[2] (g)	DRW[3] (g)	SL[4] (cm)	FSW[5] (g)	DSW[6] (g)
M. brasiliensis	33.0 c	106.0 a	14.2 a	79.2 a	101.7 a	17.9 a
M. caatinguensis	34.2 bc	138.9 a	13.2 a	78.7 a	106.1 a	20.1 a
M. cannonballus	44.3 ab	123.0 a	14.0 a	77.0 a	90.6 a	18.6 a
M. mossoroensis	34.7 bc	141.4 a	14.3 a	80.2 a	104.1 a	18.9 a
M. nordestinus	42.1 bc	125.3 a	16.2 a	77.0 a	94.2 a	20.0 a
M. semiaridus	41.6 bc	131.2 a	16.2 a	72.2 a	89.7 a	20.0 a
Testemunha	56.2 a	157.1 a	20.2 a	85.4 a	108.7 a	22.6 a
CV (%)	21.0	25.5	23.3	21.4	22.5	28.3
F	8.52 **	0.6^{ns}	1.5^{ns}	0.68^{ns}	0.7^{ns}	0.9^{ns}
	Sorghum					
M. brasiliensis	48.6 a	107.8 a	15.6 a	78.9 a	67.9 a	16.8 a
M. caatinguensis	58.2 a	100.0 a	17.3 a	82.7 a	74.9 a	17.5 a
M. cannonballus	57.6 a	105.5 a	17.0 a	70.6 a	70.2 a	15.9 a
M. mossoroensis	58.9 a	106.8 a	17.4 a	75.4 a	75.2 a	17.7 a
M. nordestinus	57.6 a	106.6 a	17.6 a	80.9 a	71.6 a	17.7 a
M. semiaridus	61.5 a	93.6 a	16.8 a	74.8 a	67.6 a	15.9 a
Testemunha	63.6 a	112.1 a	19.2 a	84.9 a	76.4 a	18.3 a
CV (%)	21.5	23.5	18.4	28.3	22.8	27.43
F	0.5^{ns}	0.1*	0.3^{ns}	0.6^{ns}	0.3^{ns}	0.5^{ns}

Continuation table 3

			Cowpea			
M. brasiliensis	23.0 a	90.2 a	5.7 a	31.8 a	32.5 ab	3.8 a
M. caatinguensis	21.7 a	77.6 ab	5.3 a	33.0 a	41.2 a	4.3 a
M. cannonballus	23.9 a	47.6 b	2.2 b	21.0 a	18.3 b	2.2 a
M. mossoroensis	20.3 a	85.1 ab	5.5 a	31.3 a	38.8 ab	4.3 a
M. nordestinus	20.4 a	87.6 ab	6.0 a	29.3 a	40.9 a	3.5 a
M. semiaridus	21.5 a	87.7 ab	5.0 ab	27.8 a	30.1 ab	3.3 a
Testemunha	26.2 a	92.4 a	6.8 a	34.0 a	43.8 a	4.7 a
CV (%)	24.4	22.2	27.4	22.8	20.41	27.4
F	1.8^{ns}	2.5*	4.8**	1.4^{ns}	3.0*	1.9^{ns}
			Jack bean			
M. brasiliensis	29.3 a	38.7 a	3.9 a	72.4 a	48.3 a	9.4 a
M. caatinguensis	28.8 a	37.8 a	3.8 a	73.6 a	48.8 a	10.5 a
M. cannonballus	31.4 a	37.8 a	3.3 a	69.0 a	39.9 a	7.9 a
M. mossoroensis	29.8 a	35.6 a	3.6 a	71.6 a	46.2 a	8.4 a
M. nordestinus	29.5 a	36.1 a	3.2 a	67.0 a	38.6 a	8.9 a
M. semiaridus	30.5 a	40.7 a	3.9 a	66.1 a	43.4 a	8.4 a
Testemunha	32.0 a	42.8 a	4.3 a	82.0 a	49.8 a	10.7 a
CV (%)	22.9	20.16	28.9	23.7	28.15	25.2
F	0.3^{ns}	6.2^{ns}	0.3^{ns}	0.6^{ns}	1.4^{ns}	1.4^{ns}
			Bell pepper			
M. brasiliensis	13.6 bc	5.2 bc	0.5 bc	15.6 ab	5.2 bc	0.6 b
M. caatinguensis	15.6 ab	5.9 bc	0.5 bc	17.3 a	5.2 bc	0.6 b
M. cannonballus	8.1 c	0.5 c	0.05 c	8.6 b	2.3 c	0.3 b
M. mossoroensis	14.5 abc	4.7 bc	0.4 bc	15.7 ab	4.5 bc	0.6 b
M. nordestinus	12.3 bc	4.5 bc	0.4 bc	15.1 ab	7.0 b	0.8 ab
M. semiaridus	14.8 abc	7.2 b	0.6 b	17.1 a	5.5 bc	0.6 b
Testemunha	21.8 a	13.9 a	1.4 a	19.1 a	11.5 a	1.2 a
CV (%)	22.9	25.3	22.7	20.6	27.35	26.73
F	5.7**	8.3**	10.5**	3.9**	8.4**	4.5**

CV (%) = significant coefficient of variation; values followed by the same letter in the columns show no statistical difference between them by the Tukey's test ($p < 0.05$). Data represents mean values from two experiments, each with six replicates (pots) per treatment and one plant per replication. [1] Root length. [2] Fresh root weight. [3] Dry root weight. [4] Shoot length. [5] Fresh shoot weight. [6] Dry shoot weight. F = test comparing variances between means. ns = not significant. * = $p < 0.05$. ** = $p < 0.01$.

The corn culture did not behave as a good host for *Monosporascus* spp. although *M. caatinguensis* and *M. semiaridus* produced a low level of damage to plant roots, and were, at low frequency, reisolated from inoculated plants. Previous works differ regarding the pathogenicity of *M. cannonballus* in corn, Mertely *et al*. (1993) inoculated *M. cannonballus* against 'Asgrow 405W' corn, and obtained a damage index of 0.7, and a percentage of 33% of the plants with positive re-isolation of the fungus. On the other hand, Sales Junior *et al*. (2018), tested 'BRS 205' and 'AG 7098' corn seeds against two *M. cannonballus* isolates (CMM2390 and CMM3646), and the results indicated that these two corn hybrids were susceptible to *M. cannonballus*, with an average percentage of positive re-insulation points of approximately 50%. This divergence is probably due to the difference between the corn hybrids used, and/or *M. cannonballus* isolates. Genetic studies of population structure performed by Bezerra *et al*. (2013) indicated that the maximum percentage of genotypic diversity of *M. cannonballus* isolates of Brazilian origin used in the study was only 6.9%. However, Correia *et al*. (2014) studied the adaptability components of 57 *M. cannonballus* isolates, obtained from melon producing areas in the states of Rio Grande do Norte and Ceará, and the results presented indicated the separation, by means of multivariate analysis, into 18 similarity groups, demonstrating that adaptability traits greatly differ within populations.

To date, there is no data regarding the reaction of corn to the inoculation of the five new species of *Monosporascus*.

Sorghum

In sorghum culture, there was no statistical difference for disease severity and incidence according to the non-parametric Kruskal-Wallis test (Table 2). There was no root damage produced by any of the inoculated fungal species, nor fungal reisolation from the roots. There was also no statistical difference for the analyzed biometric variables, according to the Tukey test at 5% probability (Table 3). The sorghum ‘Ponta Negra’ was not a host plant for any of the *Monosporascus* species tested, being considered by the reaction class proposed by Armengol *et al*. (1998) and Sales Junior *et al*. (2018) as similar to immune for all inoculated *Monosporascus* species (Table 2).

Similar to what was previously observed for corn, sorghum was not a host to the *Monosporascus* species used in these experiments. This data corroborates the results found by Mertely *et al*. (1993), who, when inoculating *M. cannonballus* against ‘Pioneer 8358’ sorghum, obtained a damage index of 0.3 and a percentage of 40% of the plants with positive reisolation of the fungus, and by Sales Júnior *et al*. (2018), who tested ‘BRS Ponta Negra’ and ‘BRS Santa Elisa’ sorghum seeds against two *M. cannonballus* isolates (CMM2390 and CMM3646), reported that these two sorghum hybrids were resistant to *M. cannonballus*, with a positive reisolation point percentage of approximately 16%.

To date, there is no data regarding the reaction of sorghum to the inoculation of the five new species of *Monosporascus*.

Cowpea

The statistical analysis of data on the variables incidence and severity of the disease in the cowpea culture showed a significant difference for the treatments tested. For severity, a significant difference was observed in relation to the species *M. caatinguensis* (0.9) and *M. cannonballus* (2.3) in relation to the control (0.0), with the treatments presenting average values of 0.3 (*M. semiaridus*) to 2.3 (*M. cannonballus*). Regarding the incidence of the disease, *M. caatinguensis* (83%) and *M. cannonballus* (100%) were the *Monosporascus* species that presented the highest values for this variable (Table 2). The cowpea cultivar ‘Rizo do Ano’, according to the class of reaction to inoculation with the different species of *Monosporascus*, behaved as highly resistant to the different species of *Monosporascus* inoculated, with exception of *M. cannonballus* (2.3), where this cultivar was susceptible (Table 2).

In the biometric variables RL, SL, and DSW, the tested treatments did not statistically differ from each other (Table 3). On the other hand, for the variable PRW, *M. cannonballus* showed a significant difference (47.6 g) when compared to the absolute control (92.4 g) and *M. brasiliensis* (90.2 g). Regarding the variable DRW, *M. cannonballus* (2.2 g) statistically differed from all other treatments, with exception of *M. semiaridus* (5.0 g). For the FSW variable, *M. cannonballus* (18.3 g) presented a statistically different value from the control (43.8 g), *M. caatinguensis* (41.2 g), and *M. nordestinus* (40.9 g) (Table 3). This culture was a host for all *Monosporascus* species tested. All inoculated pathogenic isolates/species were reisolated.

Although it belongs to the same botanical family as cowpea (Fabaceae), common bean (*Phaseolus vulgaris* L.) ‘Improved Commodore’ was used by Mertely *et al*. (1993) in an assay of host reaction to inoculation with *M. cannonballus*. The results obtained indicated that this culture did not behave as susceptible to the attack of the pathogen in artificial inoculation, presenting a value of damage in roots of 0.2. However, *M. cannonballus* was reisolated from 70% of the inoculated plants, which makes it a possible alternative host of this fungus in the field. Subsequently, Sales Júnior *et al*. (2018) inoculated *M. cannonballus* isolates [CMM2390 (0.0) and CMM3646 (0.0)] in cowpea ‘BRS Itaim’ and ‘BRS Cauamé’ in greenhouse experiments. These cultivars were classified as highly resistant to the inoculated pathogens, with no damage to roots, and/or positive points of isolation in culture medium. On the contrary, the results obtained in this study are totally different from those obtained in previous studies, considering that when *M. cannonballus* (CMM2429) was inoculated into ‘Rizo do Ano’ cowpea, the root damage was 2.3. The variation in the severity of the disease in the evaluation of the different studies may have occurred due to the pathogenicity of the different isolates, or the cultivars used in the respective studies with cowpea. The statistical difference evidenced in the analysis of the biometric variables performed in this study pointed to *M. cannonballus* as a pathogenic agent for this culture.

The results of the biometric evaluations indicated that only *M. cannonballus* statistically differed from the control for the variables FRW, DRW, and FSW. To date, there is no study in the consulted literature that can compare the reaction of *Monosporascus* spp. on the biometric indices analyzed for cowpea. However, during the experiment there was a delay in germination of three to four days for the treatments inoculated with *M. cannonballus* and with *M. semiaridus*, and *M. cannonballus* also delayed the vegetative development of the plants, where they presented smaller size when compared to the other treatments.

Jack bean

In the jack bean culture, there was no statistical difference for disease severity and incidence, according to the non-parametric Kruskal-Wallis test (Table 2), nor for any of the biometric variables analyzed, according to the Tukey test (Table 3). Jack bean was not a host to any of the *Monosporascus* species tested. The native variety of jack bean was considered immune, and no damage was observed to the roots of plants inoculated with the different species of *Monosporascus* (Table 2).

Jack bean is a species of the Fabaceae family that is already widely used in cultural management as green manure and has been reported as a strategy to control soil-dwelling phytopathogens that cause root diseases (CRUZ *et al*., 2013; MORAES *et al*., 2006; PORTO *et al*., 2016; SALES JÚNIOR *et al*., 2017).

According to Silva López (2012), jack bean presents, in its tissue composition, glycoproteins, polypeptides, enzymes, and compounds from the metabolism of amino acids, which when released to the soil after the incorporation and decomposition of the vegetable mass, may be able to reduce the population density of the pathogen. According to Mazuchowski and Derpsch (1984), it is recommended that botanical species to be used as green manure or as cover crops should not be susceptible to diseases and pests, in order to minimize the risk to the main crop, minimizing thus, the rate of reproduction and multiplication of the pathogen by the direct action of volatile and non-volatile toxic compounds released during the decomposition of green manures in the soil. These, in turn, may be exerting a suppressive activity against this pathogen, causing less disease severity, as they may be preventing the use of the plant tissues of the jack bean as a source of energy and nutrients (SALES JÚNIOR *et al*., 2017).

Bell pepper

All *Monosporascus* species used in these experiments proved to be pathogenic to the ‘Block Yellow’ bell pepper cultivar used in these experiments, causing disease severity damages that ranged from 1.8 (*M. nordestinus* and *M. semiaridus*) to 3.8 (*M. cannonballus*). A statistical difference was verified at the 5% of probability level, according to the non-parametric Kruskal-Wallis test, for the species *M. caatinguensis*, *M. cannonballus,* and *M. mossoroensis* in relation to the absolute control (Table 2). In relation to the variable incidence, it is worth noting that all *Monosporascus* species caused 100% of infected and symptomatic plants (Table 2). According to reaction class, ‘Block Yellow’ bell pepper was highly susceptible to *M. cannonballus* (3.8), and susceptible to *M. caatinguensis* (2.8) and *M. mossoroensis* (2.1). The bell pepper was moderately resistant to the other *Monosporascus* species tested in these experiments (Table 2).

For the variable RL, the species *M. brasiliensis* (13.6 cm), *M. cannonballus* (8.1 cm), and *M. nordestinus* (12.3 cm) statistically differed from the control (21.8 cm), according to the Tukey test (Table 3). All *Monosporascus* species differed from the control for the variables FRW and DRW, with difference between the species *M. cannonballus* (0.5 and 0.05 g) and *M. semiaridus* (7.2 g and 0.6 g) being observed for these same variables, respectively. The analysis of the variable SL identified a statistical difference between the species *M. cannonballus* (8.6 cm) and the control (19.1 cm). In addition, *M. cannonballus* also statistically differed from *M. caatinguensis* (17.3 cm) and *M. semiaridus* (17.1 cm) species. Statistical difference was detected between the species *M. cannonballus* (2.3 g) and *M. nordestinus* (7.0 g), and control (11.5 g). Most *Monosporascus* species, with exception of *M. nordestinus* (0.8 g), differed from the control for the DSW variable. All inoculated pathogenic isolates/species were reisolated.

The bell pepper culture was the most affected among the non-cucurbitaceous species tested in this study. We observed, during the experimental conduction, that all bell pepper plants (except those from the *M. cannonballus* treatment) flowered and reached reproductive maturity. However, only the control treatment managed to develop fruits and maintain these until the end of the experiment, possibly due to the stress caused by the attack of these pathogens. To date, this is the first study in which the reaction of *Monosporascus* spp. in bell pepper culture. It is worth noting that Mertely *et al*. (1993), when inoculating *M. cannonballus* on ‘Rutgers’ tomato, a species of the Solanaceae family, the same family as bell pepper, detected damage to roots and reduction in PSR, with values that differed statistically at a 5% probability level. However, *M. cannonballus* did not produce perithecia in the roots, nor was it reisolated from the plant’s root system.

CONCLUSIONS

The present study allowed us to conclude that the different species of *Monosporascus* caused damage in the tested cowpea cultivars ‘Rizo do Ano’ and bell pepper ‘Block Yellow,’ which presented different disease severity in relation to each inoculated pathogen species. Corn cultivars ‘BR 5037’, jack bean (native seed), and sorghum ‘BRS Ponta Negra’ had few or no symptoms of the disease and may be indicated as alternative cultures to be used in crop rotation with cucurbits. It is important to highlight the importance of new studies with a greater number of isolates and cultivars for each tested culture. This is the first report of reaction from non-cucurbit cultures to the five new species of *Monosporascus* reported in the world.

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