Scientific Electronic Archives
Issue ID: Sci. Elec. Arch. Vol. 13 (7)
July 2020
DOI: http://dx.doi.org/10.36560/13720201131
Article link
http://sea.ufr.edu.br/index.php?journal=SEA&page=article&op=view&path%5B%5D=1131&path%5B%5D=pdf
Included in DOAJ, AGRIS, Latindex, Journal TOCs, CORE, Discoursio Open Science, Science Gate, GFAR, CIARDRING, Academic Journals Database and NTHRYS Technologies, Portal de Periódicos CAPES, CrossRef

ISSN 2316-9281

Geographical distribution, diversity and pathogenicity of *Colletotrichum* associated with soybean anthracnose in Brazil

S. R. G. Moraes[1], S. M. Bonaldo[1] and A. R. R. Gaspareto[1]

[1] Universidade Federal do Mato Grosso

*** Author for correspondence:** sylmoraes@yahoo.com.br

Abstract. Eighty-five *Colletotrichum* isolates were obtained from soybean croplands in 34 municipalities of Mato Grosso state. Our objectives were characterize the isolates through mycelial growth, cultural and morphological analyses, as well as assessing pathogenicity. To evaluate cultural characteristics, the mycelial growth was daily measured, and the color of colonies was characterized after ten days incubation. The morphology, length, and width of 50 conidia per isolate were assessed. The pathogenicity of twenty-one isolates was evaluated on seed germination rate, incidence, and detection of extracellular enzymes. The cultural and morphology characteristics of *Colletotrichum* isolates were greatly variable. All isolates studied were able to reduce seed germination, produce sign and proteinase activity. These results suggest there are more than one *Colletotrichum* species associated with soybean anthracnose in Mato Grosso State.
Keywords: morphological and cultural traits, *Glycine max.*

Introduction

Soybean crop (*Glycine Max* (L.) Merr.) is one of the most important agricultural commodities, which 358.65 million metric tons produced in 2018/2019. Brazil and USA were the largest producers of soybeans (~35 million hectares), with 120 and 117 million metric tons produced in USA and Brazil, respectively. Brazil's 2019/20 soybean production and area are estimated at a record 126.0 million metric tons (mmt) and 36.9 million hectares (mha), respectively (USDA, 2020). The state of Mato Grosso is the largest producer, which is responsible for 28% of the Brazilian production (CONAB, 2019). However, disease is one of the most limiting factors to enhance crop yields, especially in the tropical region. Anthracnose is an important disease, which can cause severe damage under high temperature and moisture. Symptoms are dark, depressed, irregular lesions on pods, leaves, stems and petioles. Anthracnose may induce pods rotting and falling, immature pods opening and premature grain germination, resulting in a reduced pod number, leaf retention and green stem (Hartman et al., 2015).

Disease reduce stands, seed quality and yields by 16-26% or more in the United States, 30-50% in Thailand, and 100% in certain areas of Brazil and India (Hartman et al., 2015). Most recent studies, in the Brazilian Cerrado biome, indicate that for each 1% increment in the disease incidence (in the range of 9 to 17% incidence), a reduction of 90 kg.ha^{-1} of soybean grain may occur (Dias et al., 2016).

Pathogen-seed association is the most effective cause of an epidemic. *Colletotrichum* is able to establish latent infection without any visible symptom or cause cotyledon lesions, and pre-emergence and post-emergence damping-off at the V1 and V2 development phase. This infection resulting in seed physiological quality losses, as germination, viability and vigor (Begum et al., 2008).

The most common pathogen associated with soybean anthracnose is *Colletotrichum truncatum* (Schw.) Andrus & Moore (syn. C. *dematium* (Pers. exFr) Grove var. *truncata* (Schw.) Arx), but another species have been associated to soybean anthracnose. In Brasil, there are reports of C. *cliviae* in soybean crop in Mato Grosso (Barbieri et al., 2017) and Tocantins states (Dias et al., 2018). In addition, *C. incanum*, *C. chlorophyti, C. gloeosporioides*, *C. coccodes, C. destructivum, C. graminicola* were detected in USA (Yang et al., 2014; Yang et al., 2012; Riccioni et al., 1998), Malaysia (Mahmodia et al., 2013), Taiwan (Chen et al., 2006) and Argentina (Ramos et al., 2013).

Traditionally, the species identification of the genus *Colletotrichum* relied on the host infected species, morphological and cultural characteristics, even as size and shape of the conidia (Sutton,

1980). Vinnere (2004) reported that *C. acutatum* has slower growth than *C. gloeosporioides* in culture media, which is a reliable characteristic for identification among them.

Our objective were to identify *Colletotrichum* isolates currently affecting soybean crops in the state of Mato Grosso; compare their morphological and cultural characteristics, including the size and shape of the conidia; and describe their pathogenicity in soybean seeds. Moreover, preserve the isolates in fungal databases at the Federal University of Mato Grosso.

Methods

Colletotrichum isolates

Soybean plants with anthracnose symptoms on pods, stems and petioles were collected from thirty-four municipalities in the Mato Grosso state (Central Brazil) from 2011 to 2014 (Figure 1; table 1).

To obtain fungal isolates, disease pods, stems and petiole were cut into 0.5 x 0.5 cm pieces and surface-disinfested by sequential immersion in sterile water for 3 min, 70% ethanol for 1 min, 1% (w/w) sodium hypochlorite for 2 min, and rinsed three times in sterile distilled water for 2 min and dried on sterile filter paper. Subsequently, the pieces were placed on water agar and incubated at 25 °C in 12 h light/dark regimes. When a fungus grew, hyphen tips were transferred onto potato dextrose agar (PDA) and incubated at 25 °C in 12 h light/dark regimes for seven days. Then, pure cultures were obtained by monosporic culture, using the technique of successive dilution.

Eighty-five isolates were preserved in filter paper (Alfenas & Mafia, 2007) and in sterile distilled water (Castellani, 1939) and kept at 4 ºC in the Plant Pathology and Microbiology laboratory, Federal University of Mato Grosso.

Mycelial growth and cultural characterization

The mycelial growth of the eighty-five isolates was compared. Mycelial discs (diameter 6 mm) were dissected from previous colony margins and deposited in the center of Petri dishes (9 cm) containing PDA and then incubated at 25 °C, in dark conditions. After two days, two orthogonal diameters of the colonies were measured daily, during 10 days to evaluate the colony size.

Average mycelial growth rate (AMGR) were calculated according to the formula described by Oliveira (1991): AMGR = Σ(D-Da)/N. Where, D is current average diameter of colony; Da is the last day average diameter of colony; N is the number of days after inoculation.

After 7 days of incubation, cultural characterizations were identified according to 25 color (7.5YR) range determined by Munsell® Soil Color Charts (1971). In our study, the identified colors were: white (8/0), light gray (7/0), gray (6/0), dark gray (4/0), very dark gray (3/0), pinkish gray (6/2), pinkish gray (7/2), pink (7/4), pinkish white (8/2), and pink (8/4).

The experimental design was a completely randomized with five replicates (Petri dish) per isolate.

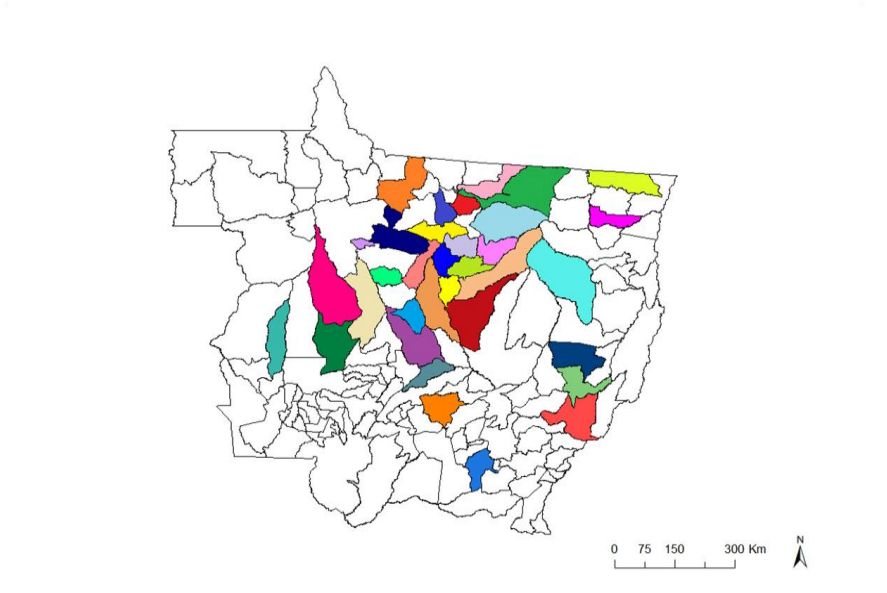

Figure 1. Municipalities of Mato Grosso state where soybean plants with anthracnose symptoms were taken

Table 1: Identification isolate code, municipalities and year of *Colletotrichum* isolates collection from soybean anthracnose symptoms in Mato Grosso state

Isolate code	Geographic origin	Year	Isolate code	Geographic origin	Year	Isolate code	Geographic origin	Year
AB-1	Água Boa	2013	LU-1	Lucas do Rio Verde	2012	RO-1	Rondonópolis	2013
AF-1	Alta Floresta	2014	LU-2	Lucas do Rio Verde	2013	SC-1	Santa Carmen	2012
BG-1	Barra do Garça	2012	LU-3	Lucas do Rio Verde	2014	SC-2	Santa Carmen	2013
BG-2	Barra do Garça	2012	LU-4	Lucas do Rio Verde	2014	SI-1	Sinop	2012
BR-1	Brasnorte	2012	LU-5	Lucas do Rio Verde	2013	SI-2	Sinop	2013
CH-1	Chapada dos Guimarães	2014	LU-6	Lucas do Rio Verde	2014	SI-3	Sinop	2013
CH-2	Chapada dos Guimarães	2014	MA-2	Marcelândia	2012	SI-4	Sinop	2012
CH-3	Chapada dos Guimarães	2014	MA-3	Marcelândia	2014	SI-5	Sinop	2013
CJ-1	Campos de Julho	2013	MA-4	Marcelândia	2014	SI-7	Sinop	2013
CL-1	Claúdia	2011	MT-1	Matupá	2014	SI-8	Sinop	2013
CL-3	Claúdia	2012	NA-1	America do Norte	2012	SI-9	Sinop	2014
CL-4	Claúdia	2012	NB-1	Nobres	2014	SO-3	Sorriso	2012
CL-5	Claúdia	2012	NB-2	Nobres	2014	SO-4	Sorriso	2013
CL-6	Claúdia	2013	NM-1	Nova Maringá	2012	SO-5	Sorriso	2013
CL-7	Claúdia	2013	NO-1	Nova Ubiratã	2013	SO-6	Sorriso	2013
CL-8	Claúdia	2013	NU-1	Nova Mutum	2012	SO-7	Sorriso	2013
CL-9	Claúdia	2013	NU-2	Nova Mutum	2012	SO-9	Sorriso	2014
CN-1	Campo Novo dos Parecis	2012	NU-3	Nova Mutum	2012	SO-10	Sorriso	2014
CN-2	Campo Novo dos Parecis	2013	NU-4	Nova Mutum	2014	SO-11	Sorriso	2104
CO-1	Colíder	2012	NU-5	Nova Mutum	2014	TB-1	Tabaporã	2013
FN-1	Feliz Natal	2012	NU-6	Nova Mutum	2014	TB-2	Tabaporã	2013
FN-2	Feliz Natal	2012	NX-1	Nova Xavantina	2014	TN-1	Terra Nova Norte	2014
IG-1	Itanhangá	2013	PE-1	Peixoto de Azevedo	2014	US-1	União do Sul	2014
IN-1	Ipiranga do Norte	2012	PE-2	Peixoto Azevedo	2014	VE-1	Vera	2013
IT-2	Itauba	2013	PE-3	Peixoto Azevedo	2014	VE-3	Vera	2013
IT-3	Itauba	2013	PE-4	Peixoto Azevedo	2014	VE-4	Vera	2013
IT-4	Itauba	2013	PN-1	Porto A. Norte	2014	VE-5	Vera	2013
IT-5	Itauba	2014	QU-1	Querência	2012	VR-1	Vila Rica	2014
IT-6	Itauba	2014	QU-2	Querência	2013			

Morphometric

Conidia were characterized by the sizes and shapes. *Colletotrichum* isolates were grown in PDA Petri dish and incubated at 25 °C, in dark conditions. After 7 days, conidia were observed in semi-permanent slides in lacto-glycerol, and 50 conidia were measured in a light microscope Zeiss, Axio Scope A1 (Gottingen, Germany) equipped with Zeiss Axion Cam Erc 5s. The shapes were classified in three groups: 1) falcate; 2) cylindrical, straight; or 3) fusiform, straight attenuated at each end (Sutton, 1980).

Colletotrichum pathogenicity to soybean seed

Twenty-one isolates of *Colletotrichum* (CH-1, CH-3, CJ-1, CN-1, CO-1, IT-3, IT-4, IT-6, LU-2, LU-3, MA-3 , NB-1, NB-2, NU-5, PE-4, QU-2, SI-9, SO-11, TN-1 and VR-1) were grown in PDA Petri dish with mannitol solute (Salisbury & Ross, 1991) at water potential of -0.6 MPa and incubated in 25 °C for 10 days.

Soybean seeds (cultivar TMG 132) were surface-disinfested in 1% (w/w) sodium hypochlorite for 1 min and air-dried for 24 h and then, put over the 10 day old colony for 24 h at 25 °C and 12 h photoperiod. After inoculation, 100 seeds per isolate were equidistantly distributed on a Petri dish with wet filter paper. They were incubated at 20 ºC with a 12 h photoperiod for 1 week. Un-inoculated seed (control, 20 seeds) were placed directly on PDA with mannitol. Inoculated and un-inoculated seeds were used for germination and incidence (acervuli and mucilage) rates.

The experiment was a completely randomized design, 5 replicates (Petri dish) with 20 seeds each.

Enzyme assays

Specific solid culture media on Petri dishes were used for detection of extracellular enzymes amylase and protease (Pereira, 2009). To determine amylase activity, *Colletotrichum* isolates were grown in solid culture media (6 g $NaNO_3$, 1.5 g KH_2PO_4, 0.5 g KCl, 0.5 g $MgSO_4.7H_2O$, 0.01 g $FeSO_4$, 0.01 g $ZnSO_4$, 10 g starch, 15 g agar and 1 L distilled water, at pH 6.8). The halo formation was measured after the 5-old-day colony had been in contact with Lugol's iodine solution (1 g iodine, 2 g KI, 300 mL distilled water) for 15 min. The starch is hydrolyzed by amylase when isolates forming a clear halo around the colony, whereas isolates that did not form a halo not produce an extracellular enzyme.

To evaluate protease activity identification, isolates were grown in solid culture media (5 g peptone, 3 g yeast extract, 1g NaCl, 15 g agar, 0.4 g gelatin and 1 L distilled water, at pH 6.0). The gelatin was autoclaved separately and mixed in the media before pouring it onto the Petri dish. Isolates were incubated at 25 °C for 48 h, in the dark.

The halo diameter measurements were done after 10 mL of 2% methyl red been applied on the colony. In addition, the ratio between the halo and the colony diameter (H/C) for each isolate was calculated.

The experiment design was a complete randomized, with 21 isolates and five replicates (Petri dish), totaling 55 experimental units.

Statistical analysis

The data were analyzed using the analysis of variance (ANOVA) of the statistical software SAMS – Agri (Canteri et al., 2001). The treatment means were estimated and comparison was performed using the Scott Knott test ($P < 0.05$).

Results and discussion

Colletotrichum isolates presented high difference in colony color and average mycelial growth rate (Table 2). No correlation was observed between the geographical origin of the isolates and morph-cultural characterization and aggressiveness.

Among 85 *Colletotrichum* isolates, 82% presented gray color colony, range from white to very dark gray, and the remaining isolates presented pinkish gray, pinkish white and pink color colony (Table 2). This pattern was also reported by Hartman et al. (2015). Usually, *Colletotrichum truncatum* produce whitish colonies that eventually turn smoky black and, for that reason, isolates of *C. truncatum* greatly vary the colony characteristics and pathogenicity (Torres-Calzada et al., 2017; Majonjo & Kapooria, 2003).

On the other hand, *C. truncatum* isolates obtained from the symptomatic soybean plants in several states of Brazil presented colonies dark grey or orange-colored colony (Rogerio et al., 2016). Cultural characters of *Colletotrichum truncatum* are dark olive, light olive and yellow brown colonies, and to *C. gloeosporioides* are dark olive and light olive colonies from soybean in Taiwan (Chen et al., 2006).

In papaya and apple fruits, a broad variation of *Colletotrichum gloeosporioides* and *C. truncatum* isolates was also observed. The authors have organized the isolates into several groups and report no correlation between the species and the colony color in BDA and peptone-glucose-agar (PGA) (Velho et al., 2015; Andrade et al., 2007).

Mycelial growth rate in vitro differed among isolates, which were classified into twelve groups (Table 2), however the growth rate ranged from 0.16 $cm.dia^{-1}$ (AB-1) to 1.24 $cm.dia^{-1}$ (VE-3). Rogerio et al. (2016), evaluating *C. truncatum* isolates, reported growth rate in PDA from 0.60 to 0.89 $cm.dia^{-1}$. In our study, only 28% of the isolates were identified in this range, 70% were above and 2% below it.

Table 2. Isolates of *Colletotrichum*, size and colony color, and morphological characteristics

Isolate code	Colony		Conidia		
				Size (µm)	
			Shape	Length	Width
	Munsell® Color	AMGR (cm.day^{-1})		Average (min-max)	Average (min-max)
AB-1	7.5YR 7/0	0.39 d	falcate	20.62 (16.49-22.84)	3.01 (3.53-2.52)
AF-1	7.5YR 8/0	1.00 k	*	*	*
BG-1	7.5YR 6/0	0.70 h	*	*	*
BG-2	7.5YR 4/0	0.77 i	*	*	*
BR-1	7.5YR 3/0	0.78 i	cylindrical	14.85 (12.27-18.51)	4.35 (2.97-3.53)
CH-1	7.5YR 7/0	0.43 e	falcate	22.05 (19.72-24.60)	3.66 (3.06-4.45)
CH-2	7.5YR 7/2	0.16 a	falcate	22.75 (17.35-26.88)	3.35 (2.56-4.04)
CH-3	7.5YR 7/2	0.45 e	fusiform	24.45 (18.49-28.69)	4.41 (3.37-5.23)
CJ-1	7.5YR 7/0	0.50 f	falcate	7.27 (5.95-8.29)	1.11 (0.84-1.51)
CL-1	7.5YR 4/0	0.81 j	cylindrical	14.08 (11.96-17.25)	4.55 (3.63-5.84)
CL-3	7.5YR 7/4	0.27 b	cylindrical	16.33 (13.70-19.71)	4.26 (2.90-5.31)
CL-4	7.5YR 8/2	0.33 d	cylindrical	28.43 (24.90-32.36)	3.23 (2.39-3.76)
CL-5	7.5YR 7/0	0.69 h	cylindrical	16.73 (14.25-19.90)	4.02 (2.97-5.09)
CL-6	7.5YR 7/0	0.69 h	fusiform	20.88 (16.00-26.67)	5.90 (4.05-7.28)
CL-7	7.5YR 8/0	0.69 h	cylindrical	18.38 (14.85-22.42)	4.31 (2.95-5.43)
CL-8	7.5YR 8/2	0.32 c	fusiform	20.17 (16.94-25.53)	3.74 (2.93-4.66)
CL-9	7.5YR 8/2	0.29 b	fusiform	19.55 (15.30-23.00)	3.78 (2.91-4.72)
CN-1	7.5YR 7/0	0.51 f	falcate	13.93 (11.57-16.50)	2.21 (1.54-2.71)
CN-2	7.5YR 6/0	0.53 f	falcate	24.80 (20.44-28.08)	4.18 (2.97-5.93)
CO-1	7.5YR 3/0	0.76 i	cylindrical	13.91 (11.25-16.65)	5.10 (4.26-6.43)
FN-1	7.5YR 3/0	0.83 j	cylindrical	14.22 (12.83-16.72)	4.65 (3.67-5.55)
FN-2	7.5YR 4/0	0.39 d	*	*	*
IG-1	7.5YR 8/0	0.44 e	falcate	23.17 (17.46-28.75)	4,15 (3.22-5.34)
IN-1	7.5YR 4/0	0.37 d	falcate	20.40 (15.41-23.87)	3.39 (2.42-4.13)
IT-2	7.5YR 8/0	0.41 e	falcate	23.34 (20.09-26.97)	3.09 (2.41-3.68)
IT-3	7.5YR 4/0	0.26 b	falcate	25.54 (22.16-29.74)	3.68 (2.94-4.42)
IT-4	7.5YR 8/2	0.30 c	falcate	20.44 (17.27-23.98)	3.77 (3.06-4.92)
IT-5	7.5YR 7/0	0.56 f	falcate	23.34 (17.24-27.80)	3.50 (2.70-4.08)
IT-6	7.5YR 7/0	0.27 b	falcate	21.46 (13.13-24.79)	3.76 (3.08-4.50)
LU-1	7.5YR 7/0	0.44 e	falcate	21.34 (14.59-26.48)	3.84 (2.70-5.58)
LU-2	7.5YR 7/0	0.37 d	falcate	18.03 (13.02-22.42)	3.58 (2.57-4.33)
LU-3	7.5YR 7/0	0.44 e	falcate	23.84 (20.14-27.94)	3.72 (2.57-4.96)
LU-4	7.5YR 7/0	0.42 e	falcate	22.80 (18.50-26.44)	3.62 (2.70-4.32)
LU-5	7.5YR 7/4	0.46 e	falcate	19.36 (13.35-23.56)	3.94 (3.30-4.72)
LU-6	7.5YR 4/0	0.32 c	falcate	21.73 (19.71-24.29)	3.07 (2.41-3.61)
MA-2	7.5YR 8/0	0.52 f	falcate	22.37 (17.27-25.83)	3.69 (2.85-4.53)
MA-3	7.5YR 7/0	0.42 e	falcate	24.26 (20.69-26.97)	4.06 (2.99-5.57)
MA-4	7.5YR 7/0	0.42 e	falcate	25.08 (21.76-28.38)	3.51 (2.87-4.32)
MT-1	7.5YR 7/0	0.37 d	falcate	18.89 (14.25-24.20)	3.67 (2.95-4.47)
NA-1	7.5YR 8/0	0.81 j	cylindrical	15.74 (11.44-18.92)	4.36 (3.55-5.01)
NB-1	7.5YR 7/0	0.45 e	cylindrical	15.49 (11.65-19.36)	4.66 (3.68-5.66)
NB-2	7.5YR 6/0	0.30 c	cylindrical	22.75 (19.25-27.11)	3.44 (2.79-4.08)
NM-1	7.5YR 7/0	0.84 j	cylindrical	15.82 (12.16-19.40)	4.83 (3.64-6.47)
NO-1	7.5YR 7/0	0.77 i	cylindrical	13.67 (9.04-16.61)	4.26 (3.01-5.46)
NU-1	7.5YR 3/0	0.85 j	cylindrical	14.47 (11.97-17.55)	4.67 (3.54-5.61)

to be continue...

NU-2	7.5YR 7/0	0.32 c	fusiform	18.94 (15.72-22.56)	3.35 (2.61-4.03)
NU-3	7.5YR 7/0	0.40 e	falcate	23.95 (19.11-28.73)	3.62 (2.92-4.49)
NU-4	7.5YR 6/0	0.55 f	falcate	24.65 (21.83-28.76)	3.70 (2.76-4.93)
NU-5	7.5YR 7/0	0.38 d	falcate	23.23 (18.24-25.30)	3.08 (2.28-3.84)
NU-6	7.5YR 7/0	0.54 f	falcate	24.76 (21.78-28.34)	3.22 (2.64-3.87)
NX-1	7.5YR 7/0	0.45 e	falcate	20.87 (15.02-23.34)	4.09 (3.26-4.78)
PE-1	7.5YR 7/0	0.64 g	falcate	20.37 (17.84-22.93)	3.21 (2.70-3.69)
PE-4	7.5YR 3/0	0.61 g	cylindrical	15.31 (12.45-18.92)	4.77 (3.85-5.58)
PN-1	7.5YR 4/0	0.37 d	falcate	23.66 (21.56-27.26)	3.56 (2.61-4.58)
QU-1	7.5YR 8/4	0.44 e	cylindrical	5.94 (4.18-6.64)	1.60 (1.24-1.99)
QU-2	7.5YR 6/2	0.26 b	fusiform	19.75 (15.48-23.10)	3.73 (3.06-4.37)
RO-1	7.5YR 7/0	0.63 g	cylindrical	17.17 (13.41-21.20)	4.62 (3.73-5.75)
SC-1	7.5YR 7/2	0.43 e	fusiform	21.92 (18.36-25.43)	3.49 (2.73-4.33)
SC-2	7.5YR 7/0	0.39 d	falcate	21.41 (16.89-25.71)	3.76 (3.14-4.78)
SI-1	7.5YR 7/0	0.86 j	cylindrical	15.66 (13.43-18.10)	4.40 (3.61-5.30)
SI-2	7.5YR 7/0	0.39 d	falcate	22.87 (20.33-26.33)	4.07 (3.26-5.08)
SI-3	7.5YR 8/0	0.62 g	cylindrical	12.74 (10.10-17.06)	4.11 (3.34-5.26)
SI-4	7.5YR 8/4	0.38 d	cylindrical	6.29 (4.35-7.96)	1.68 (1.25-2.25)
SI-5	7.5YR 7/0	0.44 e	falcate	23.59 (21.38-27.20)	3.33 (2.65-3.94)
SI-7	7.5YR 7/2	0.38 d	*	*	*
SI-8	7.5YR 8/0	0.37 d	falcate	15.40 (12.04-17.97)	2.12 (1.78-2.69)
SI-9	7.5YR 7/2	0.34 d	fusiform	21.02 (17.23-24.50)	3.37 (2.49-3.95)
SO-3	7.5YR 7/0	0.38 d	*	*	*
SO-4	7.5YR 7/0	0.37 d	cylindrical	15.23 (11.41-19.52)	4.91 (3.68-5.87)
SO-5	7.5YR 7/0	0.32 c	cylindrical	17.47 (13.96-20.14)	4.16 (3.30-5.23)
SO-6	7.5YR 7/0	0.64 g	falcate	22.25 (19.36-27.03)	3.55 (2.79-4.28)
SO-7	7.5YR 7/0	0.65 g	falcate	21.53 (17.02-24.88)	3.51 (2.83-4.25)
SO-9	7.5YR 7/0	0.36 d	cylindrical	15.34 (13.12-18.72)	4.49 (3.61-5.80)
SO-10	7.5YR 7/2	0.45 e	falcate	21.94 (17.98-25.53)	3.60 (2.97-4.86)
SO-11	7.5YR 4/0	0.67 h	falcate	24.58 (20.65-28.02)	3.91 (3.11-4.85)
TB-1	7.5YR 7/0	0.40 d	fusiform	19.67 (12.07-24.79)	3.73 (3.22-4.88)
TB-2	7.5YR 4/0	0.31 c	falcate	21.36 (16.26-26.66)	3.80 (3.20-4.40)
TN-1	7.5YR 7/0	0.69 h	cylindrical	*	*
US-1	7.5YR 4/0	0.43 e	falcate	23.63 (19.07-26.87)	2.99 (2.04-3.57)
VE-1	7.5YR 7/0	0.38 d	cylindrical	20.60 (16.42-25.50)	6.05 (4.22-7.92)
VE-3	7.5YR 7/0	1.24 l	cylindrical	17.54 (13.67-21.28)	4.67 (3.96-5.42)
VE-4	7.5YR 4/0	0.46 e	*	*	*
VE-5	7.5YR 6/0	0.82 j	falcate	21.73 (18.64-25.94)	3.62 (2.76-4.20)
VR-1	7.5YR 4/0	0.32 c	falcate	22.60 (18.47-26.79)	3.27 (2.62-4.23)

Means followed by the letter in the column did not differ ($P < 0.05$) by the Scott-Knott test.
* isolates did not sporulate under environmental conditions.

Comparing mycelial growth rate between two species of *Colletotrichum* Dias et al. (2018) reported slower growth rate in the *C. truncatum* than *C. cliviae*. Authors also observed, after 7 days of incubation at 25ºC, *C. cliviae* mycelial growth occupying all Petri dish (9 cm), while *C. truncatum* occupied less than 5 cm. In similar evaluation in the United States, *C. incanum* growth 0.75 cm.day^{-1}, differing from *C. truncatum* that growth 0.58 cm.day^{-1} (Yang et al., 2014).

The isolates conidia shape in our study were classified in three groups: falcate (58%), cylindrical (31%) and fusiform (11%). All conidia were hyaline, unicellular and aseptate, but with variable size (Table 2). Falcate conidia average size was from 18.9 to 25.5µm in length and 3.0 to 4.2µm in width (except isolate CJ-1, CN-1, SI-8 and CL-4). Cylindrical conidia average size varied from 12.8 to 18.4µm in length and from 4.0 to 5.1µm in length (except QU-1, SI-4 and VE-1). Fusiform conidia

average size was from 18.9 to 21.9µm x 3.3-3.8µm (except CH-3 and CL-6).

Average size measured for falcate conidia is similar to the values reported for *C. truncantum*, *C. chlorophyti* and *C. incanum* isolated from vegetative soybean around the world, except for CJ-1 isolate. The length x width range for *C. truncatum* conidia had been reported as 14-26 x 2-5µm (Chen et al., 2006); 20.54-25 x 2.95-3.4µm (Rogerio et al., 2016) and 18.81-25.5 x 3.56-4.63µm (Jagtap & Sontakke, 2009). On the other hand, *C. chlrophyti* presented conidia ranged from 15.5-21.3 µm × 2.5-4.3µm (Yang et al., 2012), and *C. incanum* produce shorter and narrower falcate conidia (16.9-21.9 x 2.3-3.7 µm) (Yang et al., 2014).

Average conidia length and width in isolates CJ-1, CN-1 and SI-8 were lower than average. According to Sutton (1980), falcate conidia with shorter width have wide host range and, usually is saprophytic. And the largest falcate conidia, as observed in the CL-4 isolate (28, 43 x 3.23µm) was reported on Gramineae.

Colletotrichum cliviae (Barbieri et al., 2017), *C. destructivum* (Damm et al., 2014) and *C. gloeosporioides* (Chen et al., 2006) have cylindrical conidia and size (length and width) similar to observed in our study. Except QU-1 and SI-4 isolates presents lower length and width conidia. And VE-1 isolate that had highest size conidia. *Colletotrichum sojae* is new specie that presents cylindrical conidia measuring from 14 to 17 µm in length and from 5 to 6 µm in width (Damm et al., 2019) however the width is greater than found in our work.

Colletotrichum coccodes obtained from the stem soybean presented straight and fusiforme conidia ranged from 15 to 23 µm long and 3 to 4 µm wide (Riccioni et al., 1998) as observed to fusiform conidia in our study. While CL-6 isolate had the highest width and CH-3 isolate the highest length and width.

The twenty-one of *Colletotrichum* spp. were pathogenic when inoculated on soybean seeds presenting acervuli and mucilage. However, there was difference in the isolate aggressiveness, which negatively affect seed germination. Seeds germination percentages were classified in five groups (Table 3). The control germination rate was greater than all other isolates. These low germination rate of the control is likely related to the decrease in the seed vigor due to the storage condition.

Table 3. Germination rate and incidence of acervuli and mucilage in soybean seeds (cultivar TMG132) inoculated with *Colletotrichum* isolates, and halo size

Isolate code	Germination	Acervuli (%)	Mucilage (%)	Protease (cm)
Control	64,5 a	0 c	0 d	-
CH-1	0,8 e	100 a	97,5 a	2,1 a
CH-3	3,3 e	100 a	49,1 b	0 c
CJ-1	0 e	100 a	97,5 a	3,2 a
CL-4	36,6 c	100 a	100 a	2,5 a
CN-1	0 e	100 a	97,5 a	2,7 a
CO-1	44,1 c	13,3 c	13,3 c	1,5 b
IT-3	0 e	100 a	95 a	1,8 b
IT-4	0 e	100 a	86,6 a	2,4 a
IT-6	0 e	100 a	97,5 a	1,6 b
LU-2	0 e	100 a	100 a	1,4 b
LU-3	0 e	100 a	100 a	2,7 a
MA-3	0 e	99,1 a	90 a	0,6 b
NB-1	32,5 c	77,5 b	92,5 a	2,0 a
NB-2	50,8 b	99,1 a	91,6 a	2,1 a
NU-5	3,3 e	99,1 a	95,8 a	1,7 b
PE-4	48,3 b	70,8 b	95 a	2,0 a
QU-2	4,1 e	100 a	100 a	1,3 b
SI-9	16,6 d	83,3 b	87,5 a	3,2 a
SO-11	10 d	98,3 a	100 a	2,6 a
TN-1	23,3 d	91,6 a	99,1 a	1,8 b
VR-1	3,3 e	100 a	100 a	1,9 a

Means followed by the letter in the column did not differ ($P < 0.05$) by the Scott-Knott test.

The seeds inoculated with NB-2 and PE-4 isolates presented the highest germination rate, followed by control. These isolates inhibited 21 and 25%, respectively, seed germination considering the control as a reference (100%). After these two, the lesser aggressive isolates were CO-1, NB-1 and CL-4 isolates, which inhibited 32%, 50% and 58%, respectively. The CH-1, CH-3, CJ-1, CN-1, IT-3, IT-4, IT-6, LU-2, LU-3, MA-3, VR-1 isolates greatly affect the germination rates, with values below 23%. Among them, CJ-1, CN-1, IT-3, IT-4, IT-6, LU-2, LU-3 and MA-3 isolates present no seed germination. There was no correlation between the isolate aggressiveness (low germination) and conidial morphology, since the more aggressive isolates present falcate and fusiforme conidia. Cylindrical conidia was identified in moderate or low aggressiveness isolates, although high acervuli and mucilage incidence was observed.

All isolates had acervuli and mucilage incidence on seeds, which was positively related with germination rate (81% of the isolates). In un-inoculate seeds, there were no acervuli and/or mucilage incidence from *Colletotrichum* spp.

Low seed quality was observed in previous studies evaluating soybean-*Colletotrichum* pathosystem. *C. truncatum* isolates reduced in vitro soybean seed germination and viability by 29.2% and 26.8%, respectively. Under greenhouse conditions, the germination rate was 46.4% less and an increased frequency of pre and post-emergence damping-off was observed compared with the control (Begum et al., 2008). Although, according to Galli et al. (2005), the contact period between soybean seeds and *C. dematium* var. *truncata* influences the germination rate and 40 hours of incubation was sufficient to warrant completely infected seeds.

Extracellular enzymes amylase was not detected in vitro, however halo formation would indicates degradation by amylase activity. Similar results were found for *Colletotrichum* spp. (Tozze et al., 2016). According to the authors, amylase production cannot be correlated with halo formation or insufficient amylase for halo formation, because amylase production by filamentous fungi varies according to genus and species.

The proteinase activity was identified in all *Colletotrichum* isolates, except CH-3. The *Colletotrichum* isolates were classified into two groups, according to the halo size. The first group (CH-1, CJ-1, CL-4, CN-1, IT-4, LU-3, NB-1, NB-2, PE-4, VR-1 isolates) present halo size larger than 1.98 cm. The second group was composed by CO-1, IT-3, IT-6, LU-2, NU-5, QU-2, TN-1 and MA-3 isolates. Broad variation of proteolytic activity from *Colletotrichum* spp. (Tozze et al., 2016) and *C. gloeosporioides* (Assis et al., 2010) isolates corroborate with the results of our study. However, *Colletotrichum* spp. from avocado fruits presented protease values up to eight times higher (Tozze et al., 2016) than registered in our study.

The pathogenicity of some fungi is related to the ability to produce enzymes that degrade cell wall (Ramos et al., 2010). Redman & Rodriguez (2002) reported proteinase is an essential enzyme to support pathogenicity and virulence of *C. coccodes* in tomato fruits. The enzyme activity was confirmed by mutants that did not produce the proteinase, which had nonpathogenic endophytic action.

Biochemical markers, such as amilolytic, cellulolytic, lipolytic and and physiological markers, such as mycelial growth, did not contribute to distinguish isolates into groups by aggressiveness. There were no correlation between in vitro amilolytica and proteolytic enzymatic activities and *Colletotrichum* isolates aggressiveness. The same result was also reported to *Colletotrichum gloeosporioides* (Almeida & Coelho, 2007). However, enzymatic activity was significantly different among five isolates of *C. gloeosporioides* in guava fruits (Wahid, 2010).

Conclusion

Our results indicate a broad morphological variation in the *Colletotrichum* isolates in the soybean cropland in Mato Grosso State, Brazil, and the great potential impact on the seed germination rate. It indicates that there are more than one *Colletotrichum* species able to cause anthracnose due to the identified population diversity. Our data indicates that future research is needed to molecular identify the species and, therefore, support the decision-make process in the agricultural practices (e.g., crop rotation, chemical control) and to driven plant breeding programs.

References

Alfenas, A.C., Mafia, R.G. Métodos em Fitopatologia. Viçosa. Ed. UFV, 2007. 382p.

Almeida, L.C.C., Coelho, R.S.B. Caracterização da agressividade de isolados de *Colletotrichum* de maracujá amarelo com marcadores bioquímico, fisiológico e molecular. Fitopatologia Brasileira, v.32, n.4, p.318-328, 2007. https://doi.org/10.1590/S0100-41582007000400006

Andrade, E.M., Uesugi, C.H., Ueno, B., Ferreira, M.A.S.V. Caracterização morfocultural e molecular de isolados de *Colletrotichum gloeosporioides* patogênicos ao mamoeiro. Fitopatologia Brasileira, v. 32, n.1, p.21-31, 2007. http://www.scielo.br/pdf/fb/v32n1/03.pdf

Assis, T.C., Menezes, M., Andrade, D.E.G.T., Coelho, R.S.B. Differentiation of *Colletotrichum gloeosporioides* isolates by using total proteins and esterase electrophoretic patterns and extracellular enzymes production. Summa Phytopatologica, v.36, n.2, 2010. https://doi.org/10.1590/S0100-54052010000200006

Barbieri, M.C.G, Ciampi-Guillardi, M., Moraes, S.R.G., Bonaldo, S.M., Rogerio, F., Linhares, R.R., Massola

Junior, N.S. First report of *Colletotrichum cliviae* causing anthracnose on soybean in Brazil. Plant Disease, v.101, n.9, p.1677, 2017. https://doi.org/10.1094/PDIS-07-16-0963-PDN

Begum, M.M., Sariah, M., Puteh, A.B., Zainal-Abidin, M.A. Pathogenicity of *Colletotrichum truncatum* and its influence on soybean seed quality. International Journal of Agriculture and Biology, v.10, n.4, p.393-398, 2008. ISSN Print: 1560–8530; ISSN Online: 1814–9596 07–443/MFA/2008/10–4–393–398 http://www.fspublishers.org

Canteri, M.G., Althaus, R.A., Virgens Filho, J.S., Giglioti, E.A., Godoy, C.V. SASM-Agri: Sistema para analise e separação de médias em experimentos agrícolas pelos métodos Scott Knott, Tukey e Duncan. Revista Brasileira de Agrocomputação, v.1, p.18-24, 2001.

Castellani, A. Viability of some pathogenic fungi in distilled water. The Journal of tropical medicine and hygiene, v.42, p.225–226, 1939.

Chen, L.S., Chu, C., Liu, C.D., Chen, R.S., Tsay, J.G. PCR-based detection and differentiation of anthracnose pathogens, *Colletotrichum gloeosporioides* and *C. truncatum*, from vegetable soybean in Taiwan. Journal of Phytopathology, v.154, p.654-662, 2006. https://doi.org/10.1111/j.1439-0434.2006.01163.x

CONAB - Companhia Nacional de Abastecimento, 2019.https://www.conab.gov.br/info-agro/safras/graos. 20 Fev. 2020.

Damm, U., O'Connell, R.J., Groenewald, J.Z., Crous, P.W. The *Colletotrichum destructivum* species complex - hemibiotrophic pathogens of forage and field crops. Studies in Mycology, v.79, p.49-84, 2014. https://doi.org/10.1016/j.simyco.2014.09.003

Damm, U., Sato, T., Alizadeh, A., Groenewald, J.Z., Crous, P.W. The *Colletotrichum dracaenophilum*, *C. magnum* and *C. orchidearum*. Studies in Mycology, v. 92, p.1-46, 2019. https://doi.org/10.1016/j.simyco.2018.04.001

Dias, M.D., Fonseca, M.E.N., Dias-Neto, J.J., Santos, M.D.M., Pandolfo, G.M., Boiteux, L.S., Cafe-Filho, A.C. Biology, pathogenicity, and haplotype analyses of *Colletotrichum cliviae*: a novel soybean anthracnose agent in warm tropical areas. Tropical Plant Pathology, v. 43, p.439-451, 2018. http://dx.doi.org/10.1007/s40858-018-0249-6

Dias, M.D., Pinheiro, V.F., Cafe-Filho, A.C. Impact of anthracnose on the yield of soybean subjected to chemical control in the north region of Brazil. Summa Phytopathologica, v.42, n.1, p.18-23, 2016. https://doi.org/10.1590/0100-5405/2114

Galli, J.A., Panizzi, R.C., Fessel, S.A., Simone, F., Ito, M.F. Efeito de *Colletotrichum dematium* var. *truncata* e *Cercospora kicuchii* na germinação de sementes de soja. Revista Brasileira de Sementes, v.27, n.2, p.182-187, 2005. https://doi.org/10.1590/S0101-31222005000200026

Hartman, G.L., Rupe, J.C., Sikora, E.J., Domier, L.L., Davis, J.A., Steffey, K.L. Compedium of soybean diseases and pets, 5th ed APS Press, St. Paul, Minnesota. 2015.

Jagtap, G.O., Sontakke, P.L. Taxonomy and morphology of *Colletotrichum truncatum* isolates pathogenic to soybean. African Journal of Agricultural Research, v.4, p.1483-1487, 2009.

Mahmodi, J.B., Kadir, M.Y., Wong, M.Y., Nasehi, A., Puteh, A., Soleimani, N. First Report of Anthracnose Caused by *Colletotrichum gloeosporioides* on Soybean (*Glycine max*) in Malaysia. Plant Disease, v.97, n.6, 2013. https://doi.org/10.1094/PDIS-10-12-0944-PDN

Majonjo, D.M., Kapooria, R.G. Ocorrence and variability of *Colletotrichum truncatum* on soybean in Zambia. EPPO Bulletin, v.33, p.339, 2003. https://doi.org/10.1046/j.1365-2338.2003.00657.x

Munsell® Soil Color Charts, Munsell Color Company, Inc. Baltimore, Maryland 21218, USA. 1971.

Oliveira, J.A. Efeito do tratamento fungicida em sementes no controle de tombamento de plântulas de pepino (*Cucumis sativas* L.) e pimentão (*Capsicum annanum* L.). Lavras: Escola de Agricultura de Lavras, 1991. 111p. Dissertação Mestrado.

Pereira, V.W. Caracterização e identificação molecular de espécies de *Colletotrichum* associadas à antracnose da goiaba no Estado de São Paulo. Piracicaba:Escola Superior de Agricultura "Luiz de Queiroz"/Universidade de Sao Paulo, 2009. 79p. Dissertação Mestrado.

Ramos, A.M., Gally, M., Garcia, M.C., Levin, L. Pectinolytic enzyme production by *Colletotrichum truncatum*, causal agent of soybean anthracnose. Revista Iberoamericana de Micología, v. 27, p.186-180, 2010. https://doi.org/10.1016/j.riam.2010.06.002

Ramos, A.M., Franco, T.L., Cinto, I., Carmona, M., Gally, M. Molecular characterization of *Colletotrichum* species causing soybean anthracnose in Argentina. Mycotaxon, v.123, p.457-465, 2013. https://doi.org/10.5248/123.457

Redman, R.S., Rodriguez, R.J. Characterization and isolation of an extracellular serine protease from the tomato pathogen *Colletotrichum coccodes*, and it's role in pathogenicity. Mycolical Research, v.106, n.12, p.1427-1434, 2002. https://doi.org/10.1017/S0953756202006883

Riccioni, L., Conca, G., Hartman, G.L. First report of *Colletotrichum coccodes* on soybean in the United States. Plant Disease, v.82, n.8, p.959, 1998. https://doi.org/10.1094/PDIS.1998.82.8.959C

Rogerio, F., Ciampi-Guillardi, M., Barbieri, M.C.G., Bragança, C.A.D., Seixas, C.D.S., Almeida, A.M.R., Massola Jr., N.S. Phylogeny and variability of *Colletotrichum truncatum* associated with soybean anthracnose in Brazil. Journal of Applied Microbiology, v.122, n.2, p.402-415, 2016. https://doi.org/10.1111/jam.13346

Salisbury, F.B., Ross, C.W Plant physiology. 4.ed. Belmont: Wadsworth, 1991. 682p.

Sutton, B.C. The Coelomycetes. Commonwealth Mycological Institute, Kew. 1980. 696p.

Torres-Calzada, C., Tapia-Tussell, R., Higuera-Ciapara, I., Huchin-Poot, E., Martin-Mex, R., Nexticapan-Garcez, A., Perez-Brito, D. Characterization of *Colletotrichum truncatum* from papaya, pepper and physic nut based on phylogeny, morphology and pathogenicity. Plant Pathology, v.64, p.821-830, 2017. https://doi.org/10.1111/ppa.12800

Tozze Jr, H.J., Firmino, A.C., Fischer, I.H., Furtado, E.L., Massola Jr, N.S. Caracterização da agressividade e atividade enzimática de isolados de *Colletotrichum* spp. associados à antracnose do abacate. Summa Phytopathologica, v.42, n.3, p.264-267, 2016. https://doi.org/10.1590/0100-5405/2107

USDA-United States Department of Agriculture, Foreign Agricultural Service Circular Series 2020 World Agricultural Production. Circular Series WAP 3-20 March 2020. https://apps.fas.usda.gov/psdonline/circulars/production.pdf 20 Fev. 2020

Velho, A.C., Alaniz, S., Casanova, L., Mondino, P., Stadnik, M.J. New insights into the characterization of *Colletotrichum* species associated with apple diseases in southern Brazil and Uruguay. Fungal Biology, v.119, n.4, p.229-244, 2015. https://doi.org/10.1016/j.funbio.2014.12.009

Vinnere, O. Approaches to Species Delineation in Anamorphic (mitosporic) Fungi: A Study on Two Extreme Cases. Uppsala: Acta Universitates Upsalienses, 2004. 72p. Doctor of Philosophy. http://uu.divaportal.org/smash/get/diva2:163839/FULLTEXT01.pdf

Yang, H-C, Haudenshield, J.S., Hartman, G.L. First report of *Colletotrichum chlorophyll* causing soybean anthracnose. Plant Disease, v.96, n.11, p.1699, 2012. https://doi.org/10.1094/PDIS-06-12-0531-PDN

Yang, H-C, Haudenshield, J.S., Hartman, G.L. *Colletotrichum incanum* sp. nov., a curved-conidial species causing soybean anthracnose in USA. Mycologia, v.106, p.32-42, 2014. https://doi.org/10.3852/13-013

Wahid, O.A.A. Occurrence of *Colletotrichum* anthracnose disease of guava fruit in Egypt. International Journal of Pest Management, v. 47, p.147-152, 2001. https://doi.org/10.1080/09670870151130679