An Acad Bras Cienc (2020) 92(Suppl. 1): e20181205 DOI 10.1590/0001-3765202020181205
Anais da Academia Brasileira de Ciências | *Annals of the Brazilian Academy of Sciences*
Printed ISSN 0001-3765 I Online ISSN 1678-2690
www.scielo.br/aabc | www.fb.com/aabcjournal

AGRARIAN SCIENCES

Integrated control of target spot and yield of cotton in the Brazilian *cerrado* biome

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Abstract: Target spot is a disease that has caused serious damage to cotton crops. This study was carried out to examine if different cotton genotypes, plant heights and fungicide treatments can be used as tools of an integrated control methods of target spot. The experiment was carried out in the 2014/15 crop season, in *cerrado* biome of Chapadão do Sul - MS, Brazil. It was used a randomized block design in a 2 × 3 × 3 factorial arrangement with four replicates. The factors were two plant heights (1 and 1.5 m), three cultivars (FMT 701, FM 975, and FM 944), and three fungicide treatments (control, FT1, and FT2). Fungicide treatments consisted of sequential applications of different fungicides of the triazole, strobilurin, and carboxamide groups. Cultivar FM 944 showed lower susceptibility to target spot. The shorter plants exhibited lower disease severity. The fungicides pyraclostrobin + fluxapyroxad and trifloxystrobin + prothioconazole reduced the severity of target spot. Cultivar FM 975 had the highest yield. A higher yield was obtained in the upper stratum than in the lower stratum of the plant.

Key words: *Corynespora cassiicola*, Sustainability, Control, Management.

INTRODUCTION

Target spot, a disease caused by the fungus *Corynespora cassiicola*, was reported in the cotton plant for the first time in 1959, in the USA (Jones 1961). After this occurrence, it has frequently appeared in cotton crops in the United States (Conner et al. 2013, Fulmer et al. 2012), China (Wei et al. 2014), and Brazil (Dias et al. 2016, Galbieri et al. 2014).

The initial symptoms of target spot in cotton are characterized by small spots on the leaves located in the lower stratum of the plant (Conner et al. 2013). As the disease progresses, these spots acquire a rounded or irregular shape, with dark brown borders and a white brown center (Conner et al. 2013). Lesions may present as concentric rings (Fulmer et al. 2012) and, in cases of great severity, the leaves acquire a yellowish color and easily detach from branches, resulting in defoliation of the plant (Conner et al. 2013).

A study led by Conner et al. (2013) in the United States revealed that target spot may lead to a drop of over 336 kg ha^{-1} in the yield of cotton, in susceptible cultivars. However, the quantification of losses in the cotton crop is not often described in the Brazilian literature.

There is few information about the control of target spot in cotton, and the existing data are often from other crops such as soybean. In developing countries, an effective and sustainable plant disease control can be achieved by integrated disease management which combines some control strategies (El Khoury & Makkouk 2010).

Chemical control can be introduced as an alternative for the management of target spot in cotton. According to Price et al. (2015), the use of the fungicides pyraclostrobin and tetraconazole provided a reduction in the disease severity. However, there is a concern with the relationship and effectiveness of fungicides in the control of this disease (Woodward et al. 2016), because some studies have shown a reduction or loss of sensitivity of the pathogenic agent to fungicides in isolates from the soy crop (Teramoto et al. 2011, Avozani et al. 2014).

Manipulating the plant size is a crop management strategy that can help in the control of target spot, since it can change the microclimate, creating unfavorable conditions to the development of the pathogen (Ando et al. 2007). As stated by Pangga et al. (2011), taller canopies allow for accumulation of moisture and a reduction in wind flow and in the penetration of sun rays into the lower part, thereby promoting a favorable environment to the development of the pathogen.

The use of cultivars resistant to target spot is a highly desirable alternative for the control of this disease. Nevertheless, to this date, there are no reports of a single cultivar showing resistance to this disease (Galbieri et al. 2014). Thus, those authors suggested that research should be carried out to identify resistant genotypes in order to generate information to guide cotton producers in the choice of the best cultivar.

In view of the dearth of information on the effect of integrating different methods for the control of target spot in the cotton plant, the present study was undertaken to examine if plant height, cotton cultivars, and the use of fungicides influence on the control of target spot as well as on crop yield.

MATERIALS AND METHODS

The experiment was carried out under field conditions from December 2014 to August 2015 in Chapadão do Sul - MS, Brazil (18°41’33” S and 52°40’45” W, 810 m altitude).

According to the classification proposed by Santos et al. (2013), the soil in the area was classified as a dystrophic Red Latosol (Oxysol). Prior to the establishment of the experiment, samples were collected from the superficial layer of the soil, at the 0-0.20 m depth, and the following chemical properties were found: pH ($CaCl_2$) 5; Ca 5.15 cmolc dm^3; Mg 0.80 cmolc dm^3; Al 0.04 cmolc dm^3; K (Mehlich) 87 mg dm^3; P (resin) 46.55 mg dm^3; OM 43.20 g dm^3; CEC 10 cmolc dm^3; and SB 57.10%. Later, based on the results of the chemical analysis, the soil was fertilized according to the recommendations for the crop proposed by Souza & Lobato (2004). Seeding took place on December 22, 2014, in a no-tillage system, in an area previously cultivated with corn. The space between rows was 0.90 m and the population per cultivar was 90,000 plants ha^{-1}.

The experiment was set up as a randomized-block experimental design with four replicates in a 2 × 3 × 3 factorial arrangement consisting of two plant heights (1 m and 1.5 m), three cotton cultivars (FMT 701, FM 975, and FM 944), and three fungicide treatments. Unsprayed plants were considered as control treatment (Table I).

The plots were composed of four 12-m plant rows, with the two center rows considered the usable area of the plot, disregarding four meters from each extremity. Plant height was regulated by using mepiquat chloride, applying 150 g of the active ingredient (a.i.) for cultivars FMT 701 and FM 975 and 100 g of the a.i. for cultivar FM 944.

Fungicides and the growth regulator were applied using a backpack sprayer with a constant CO_2 pressure, with a 3-m bar and six spraying nozzles (XR 11002) spaced 0.50 m apart. A working pressure of 300,000 Pa was adopted, and the spray volume was set as 150 L ha^{-1}. The

Table I. Description of fungicide treatments used at work. Chapadão do Sul, MS. Crop season: 2014/15.

Treatments	Active ingredients	Phenological stage (+days) at spray	Dose of CP* L ha^{-1}	Dose of ai** Kg L^{-1}
Control	-	-	-	-
FT1	[1]Pyraclostrobin + Fluxapyroxad	B1	0,3	0,333+0,167
	[2]Trifloxystrobin + Prothioconazole	B1+ 15	0,4	0,150+0,175
	[1]Pyraclostrobin + Fluxapyroxad	B1+30	0,3	0,333+0,167
	[1]Pyraclostrobin + Fluxapyroxad	B1+45	0,3	0,333+0,167
	[1]Pyraclostrobin + Fluxapyroxad	B1+60	0,3	0,333+0,167
	[2]Trifloxystrobin + Prothioconazole	B1+75	0,4	0,150+0,175
FT2	Azoxistrobin+ Difenoconazole	B1	0,3	0,200+0,125
	Difenoconazole	B1+ 15	0,3	0,250
	Azoxistrobin+ Difenoconazole	B1+30	0,3	0,200+0,125
	Azoxistrobin+ Difenoconazole	B1+45	0,3	0,200+0,125
	Azoxistrobin+ Difenoconazole	B1+60	0,3	0,200+0,125
	Difenoconazole	B1+75	0,3	0,250

[1]Addition of mineral oil Assist® (500 ml.ha^{-1}); [2]Addition of mineral oil Áureo® (0,25% of spray volume); *Commercial product; **Active ingredient.

sprays started at B1 stage (Marur & Ruano 2001) with interval of 15 days until 75 days.

Severity was assessed by the method suggested by Campbell et al. (2012). Seven evaluations were undertaken using the Florida scale to quantify the disease severity (Chiteka et al. 1988). The Florida scale consists of the following grades: 1 - No spot on the leaves; 2 - Few lesions on the leaves, none in the upper canopy; 3 - Few lesions on the leaves in the upper canopy; 4 - Some lesions in the upper part of the plant and over 5% defoliation; 5 - Evident lesions, even in the upper canopy, and 20% defoliation; 6 - Visible number of lesions even in the upper canopy and 50% defoliation; 7 - Numerous lesions in the upper canopy and 75% defoliation; 8 - Upper canopy covered in lesions and 90% defoliation; 9 - Few leaves remaining, and those remaining covered with lesions, and 98% defoliation; and 10 - Plants completely defoliated and dead due to leaf spot. Subsequently, the area under the disease progress curve (AUDPC) was determined as proposed by Campbell & Madden (1990), using the following equation:

$$AACPMA=\sum_{n=1}^{n-1}\left(\left[\frac{yi+yi+1}{2}\right]*\left(ti+1-ti\right)\right)$$

where *n* is the number of evaluations; *yi* is the degree of severity of *Corynespora* leaf spot in the i-th evaluation; and *ti* + 1 – *ti* is the interval between evaluations (days).

Bolls were harvested manually on August 24, 2015. On the occasion, the bolls from the lower and upper parts of the plants were collected separately to stratify the plant's yield. A complementary evaluation was carried out to compare the yield of the lower with the upper part of the cotton plant, in the different cultivars. The data of the studied variables were subjected to analysis of variance and means were compared by the Scott Knott test at the 5% probability level.

RESULTS AND DISCUSSION

Target spot symptoms were observed at 119 days after plant emergence, when the plants were in development stage F14. The symptoms appeared initially in the lower part of the plant, as also reported by Conner et al. (2013).

The AUDPC was significantly affected by the plant height × cultivar and fungicide treatment × cultivar interactions (Table II).

There was a difference in the severity of target spot caused by the use of different fungicides in the cotton crops. The use of FT1 provided a better control of the disease in cultivars FMT 701 and FM 975 than FT2 (Table III). Cultivar FM 944 did not exhibit significant differences in the AUDPC values when subjected to the different fungicide treatments. According to Tormen et al. (2013), the different disease severities seen with the use of fungicides in the cultivars may be due to characteristics inherent to the diseases, resistance mechanisms that act upon the host, and mode of action of the applied fungicides.

In the analysis of performance of the cotton cultivars in each fungicide treatment, FMT 701 obtained the highest AUDPC values, followed by cultivars FM 975 and FM 944, respectively (Table III). These findings contrast with the results obtained by Galbieri et al. (2014), who evaluated different cotton genotypes in a greenhouse and observed that cultivars FM 975 and FM 944 were similar in terms of severity of target spot. The difference between the obtained results is possibly related to the environment in which the plants were grown and the aggressiveness variability of the pathogen (Terramoto et al. 2011).

According to Mukew & Mayee (2002), the different reactions of cotton cultivars to diseases may be related to histochemical, morphological, and anatomical factors of each cultivar. In this way, the cotton cultivars may show higher or lower disease severity due to those factors.

The influence of plant height on the AUDPC was observed only in cultivars FMT 701 and FM 975, since no differences were detected for plant height due to the lesser severity of target spot on cultivar FM 944. Taller plants may have higher AUDPC values in cultivars FMT 701 and FM 975 (Table III).

Table II. Analysis of variance of the area under the target spot progress curve (AUTSPC) in three cotton cultivars, two plant height and fungicide treatments. Chapadão do Sul, MS. Crop season: 2014/15.

Variation factor	F values
	AUTSPC
Height (H)	63,0729 *
Cultivar (C)	95,8543 *
Fungicide treatment (FT)	20,4846 *
H x C	5,2904 *
H x FT	1,9662 ns
FT x C	3,1004 *
H x C x FT	0,3467 ns
CV (%)	17,18

*** significant at 5% probability; ns: not significant.**

Table III. **Area under the target spot progress curve (AUTSPC) of three cotton cultivars submitted to fungicide treatments (FT) and two plant heights. Chapadão do Sul, MS. Crop season: 2014/15.**

Fungicide treatments*	AUTSPC		
	FMT 701	**FM 975**	**FM 944**
Control	173,92 aA	119,60 aB	82,27 aC
FT1	131,33 bA	99,88 bB	75,57 aC
FT2	195,15 aA	139,15 aB	88,78 aC
Height (m)			
1,0	141,73 bA	93,68 bB	73,89 aC
1,5	191,87 aA	145,40 aB	90,53 aC
CV %	----------------------------17,18---------------------------		

Averages followed by the same letter, lowercase letter in the columns and capital letter in the lines, do not differ among themselves by Scott-Knott's test at 5% of probability. *Control; FT1- Active ingredients and sprays starting at B1 stage with interval of 15 days until 75 days (Piraclostrobin + Fluxapyroxad (B1), Trifloxystrobin + Prothioconazole (B1+15), Piraclostrobin + Fluxapyroxad (B1+30), Piraclostrobin + Fluxapyroxad (B1+45), Piraclostrobin + Fluxapyroxad (B1+60), Trifloxystrobin + Prothioconazole (B1+75)); FT 2- Active ingredients and sprays starting at B1 stage with interval of 15 days until 75 days (Azoxistrobin + Difenoconazole (B1), Difenoconazole (B1+15), Azoxistrobin + Difenoconazole (B1+30), Azoxistrobin + Difenoconazole (B1+45), Azoxistrobin + Difenoconazole (B1+60), Difenoconazole (B1+75)).

As declared by Ando et al. (2007), alterations in the form of cultivation of plants, whether in population density, spacing, or plant height, may directly influence the development of diseases, possibly reducing or aggravating its severity. This fact was observed in the present study, in which plant height influenced the severity of target spot.

Some authors have reported the influence of environmental changes from crop treatments on the development of diseases in various plant species. Lima et al. (2012) evaluated the cross-sowing system in comparison with the conventional system in the soy crop and observed a higher severity of Asian rust using the former system. Those authors suggested that an increase the severity was due to the favorable microclimatic conditions provided by that plant cultivation method. Silva et al. (2012) reported that cultivating corn with a smaller spacing between plants reduces the severity of Cercospora leaf spot. According to those authors, the lower severity of Cercospora spot seen in the crops with a lesser spacing between plants resulted from the lower circulation of wind within the plant canopy, which hinders the spread of the pathogen, leading to decreased severity. Those studies demonstrate the influence of the growing system on disease development.

The higher AUDPC values observed in the taller plants (1.5 m) might have been due to the microclimatic conditions favorable to the development of the fungus *Corynespora cassiicola* provided by the taller plants. Marois et al. (2004) stated that a greater plant height allows for an increase in the relative humidity of the air and a reduction of the temperature within the cotton plants. These conditions allow for increased germination of the spores, leading to greater progression of the disease.

The cultivars behaved similarly at both plant heights, with FMT 701 showing the highest AUDPC, followed by FM 975 and then by FM 944. These results indicate that cultivar FM 944 was less susceptible to the disease than the other cultivars.

Yield in the upper portion and in the whole plant was influenced by the interaction between plant height and fungicide treatment. As shown in Table IV, in the lower portion, the seed cotton yield was influenced by the plant height × cultivar and fungicide × cultivar interactions.

The total yield of the shorter plants was higher when fungicide treatments FT1 and FT2 were used. Price et al. (2015) observed that fungicide use may provide a reduction in the severity of target spot, resulting in gains in yield.

In the taller plants, there were no differences in total yield across the different fungicides used (Table V). According to Reddy et al. (1990), taller plants reduced the fungicide application efficiency due to the larger amount of leaves (Sobrinho et al. 2007), which prevented the distribution of the fungicides within the plant canopy, possibly compromising its yield.

Control and FT2 treatments were influenced by plant height in the total yield. However, in FT1, the shorter plants achieved a higher total yield.

Fungicide treatments FT1 and FT2 in the shorter plants resulted in 15.54 and 14.62% higher cotton seed yields, respectively, than the taller plants, in the upper portion (Table V). These findings suggest that the shorter plants allowed for a better distribution of the fungicide within the plant canopy, favoring disease control, which might have culminated in increased yield.

There was a difference in yield between the evaluated cotton cultivars (Table VI). The yield of the cotton cultivars in both the upper part and in the whole plant followed the same trend. Cultivar FM 975 was the most productive in the upper portion and in the whole plant. Cultivars FMT 701 and FM 944 exhibited no differences in yield.

Anselmo et al. (2015) reported that cultivar FM 975 showed good adaptability in the region of Chapadão do Sul - MS, Brazil, yielding up to 6000 kg ha^{-1}. Freire et al. (2015) investigated the performance of cultivars in different locations of Brazil and observed that, in the overall mean, cultivar FM 975 had the largest yield, also surpassing FM 944.

The table VII presents the yield stratified by plant region. The main goal of this type of evaluation is to make it possible to investigate the influence of the disease on yield in the different parts of a plant. According to Ascari et al. (2016), the microclimatic condition in each plant region is different, which might influence disease development and thus compromise productivity.

Table IV. Analysis of variance of cotton yield in the upper, lower portions and whole plant (total yield). Chapadão do Sul, MS. Crop season: 2014/15.

Variation factor	F values for yield		
	Upper portion	Lower portion	Total yield
Height (H)	9,8297 *	0,7422 ns	3,7615 ns
Cultivar (C)	8,0612 *	8,9931 *	10,5746 *
Fungicide treatment (FT)	2,4732 ns	0,6381 ns	0,6318 ns
H x C	1,1215 ns	4,2390 *	1,3931 ns
H x FT	4,2373 *	0,2194 ns	3,5976 *
FT x C	2,0666 ns	5,4522 *	1,2636 ns
H x C x FT	0,5229ns	0,7867 ns	0,9222 ns
CV (%)	11,67	11,57	8,28

*** significant at 5% probability; ns: not significant.**

Table V. Cotton seed yield (kg ha^{-1}) in the upper portion of the plant and in the whole plant (total yield), submitted to two plant heights and three fungicide treatments in cotton. Chapadão do Sul, MS. Crop season: 2014/15.

Height (m)	**Upper portion yield (kg ha^{-1})**		
	Control	**FT1**	**FT2**
1,0	2767,2 aB	3238,0 aA	3077,2 aA
1,5	2844,7 aA	2802,4 bA	2684,6 bA
CV (%)	-----------------------------------11,68--------------------------------		
Height (m)	Total yield (kg ha^{-1})		
	Control	FT1	FT2
1,0	5047,7 aB	5483,8 aA	5332,2 aA
1,5	5232,6 aA	5055,1 bA	4986,6 aA
CV (%)	------------------------------------8,51-----------------------------------		

Averages followed by the same letter, lowercase letter in the columns and capital letter in the lines, do not differ among themselves by Scott-Knott's test at 5% of probability. *Control; FT1- Active ingredients and sprays starting at B1 stage with interval of 15 days until 75 days (Piraclostrobin + Fluxapyroxad (B1), Trifloxystrobin + Prothioconazole (B1+15), Piraclostrobin + Fluxapyroxad (B1+30), Piraclostrobin + Fluxapyroxad (B1+45), Piraclostrobin + Fluxapyroxad (B1+60), Trifloxystrobin + Prothioconazole (B1+75)); FT 2- Active ingredients and sprays starting at B1 stage with interval of 15 days until 75 days (Azoxistrobin + Difenoconazole (B1), Difenoconazole (B1+15), Azoxistrobin + Difenoconazole (B1+30), Azoxistrobin + Difenoconazole (B1+45), Azoxistrobin + Difenoconazole (B1+60), Difenoconazole (B1+75)).

Based on the cotton seed yields found in the different plant parts, the upper stratum produced a larger amount of cotton in all cultivars. The yield in the upper portion of cultivars FMT 701, FM 975, and FM 944 corresponded to 57.83%, 56.31%, and 53.62% of their respective total yields. These results show that the upper stratum had a greater impact on total yield.

The lower plant stratum is the part most severely affected by target spot, because, as stated by Corner et al. (2013), this disease appears initially in that region. Rosolem (2001) asserted that the greater severity of diseases in the lower portion of the cotton plant impairs its yield. Thus, the plant attempts to compensate for the decreasing yield by increasing the number of bolls in its upper portion, which possibly led to the increased yield in the upper canopy.

The rotting of the bolls is a possible factor that might have contributed to the lower yield in the lower stratum of the plant compared with the upper portion. According to Marois et al. (2004), the relative humidity of the air is higher in the lower part of plants, which favors the penetration of pathogens [*Corynespora cassiicola* (Lakshmanan et al. 1990), *Colletotrichum* spp., *Fusarium* spp., *Botryodiplodia* sp., *Aspergillus* sp., *Myrothecium*, and *Alternaria* (Zancan et al. 2011)] into the bolls, which may cause them to rot, reducing the yield in the lower stratum.

Results for yield in the lower part of the plant (Table VIII) revealed that the cotton cultivars responded differently in that region. These divergences between the cotton cultivars regarding yield in that part of the plant may stem from the form of production of each cultivar or even the action of genetic factors of the plant that ensure greater hardiness or even tolerance to diseases occurring in that region. Different plant architectures of the evaluated cultivars may also explain the results observed in the lower part of the plants (Hanan & Hearn 2003).

Table VI. Cotton seed yield (kg ha^{-1}) in the upper portion and whole plant (total yield) of three cultivars. Chapadão do Sul, MS. Crop season 2014/15.

Cultivars	Yield (kg ha^{-1})	
	Upper portion	Total yield
FMT 701	2884,9 b	4988,4 b
FM 975	3106,7 a	5516,2 a
FM 944	2715,9 b	5064,3 b
CV (%)	11,68	8,51

Averages followed by the same letter do not differ among themselves by Scott-Knott's test at 5% of probability.

Table VII. Cotton seed yield (kg ha^{-1}) in the upper and lower portion of the plants of three cultivars. Chapadão do Sul, MS. Crop season 2014/15.

Cultivars	Plant stratum	
	Upper	Lower
FMT 701	2884,9 bA	2103,5 bB
FM 975	3106,7 aA	2409,5 aB
FM 944	2715,9 bA	2348,8 aB
CV (%)	----------------------------14,66--------------------------	

Averages followed by the same letter, lowercase letter in the columns and capital letter in the lines, do not differ among themselves by Scott-Knott's test at 5% of probability.

Table VIII. Cotton seed yield (kg ha^{-1}) in the lower portion of the plant of three cultivars, submitted to two plant heights and three fungicide treatments in cotton. Chapadão do Sul, MS. Crop season: 2014/15.

Fungicide treatment*	Yield (Kg ha^{-1})		
		Cultivars	
	FMT 701	FM 975	FM 944
Control	2097,0 aB	2709,0 aA	2196,4 bB
FT1	2175,2 aA	2225,9 bA	2317,2 bA
FT2	2038,0 aB	2264,2 bB	2532,9 aA
Height (m)			
1,0	2185,0 aA	2268,7 bA	2327,4 aA
1,5	2021,9 aB	2550,2 aA	2370,4 aA
CV %	----------------------------11,57--------------------------------		

Averages followed by the same letter, lowercase letter in the columns and capital letter in the lines, do not differ among themselves by Scott-Knott's test at 5% of probability. *Control; FT1- Active ingredients and sprays starting at B1 stage with interval of 15 days until 75 days (Piraclostrobin + Fluxapyroxad (B1), Trifloxystrobin + Prothioconazole (B1+15), Piraclostrobin + Fluxapyroxad (B1+30), Piraclostrobin + Fluxapyroxad (B1+45), Piraclostrobin + Fluxapyroxad (B1+60), Trifloxystrobin + Prothioconazole (B1+75)); FT 2- Active ingredients and sprays starting at B1 stage with interval of 15 days until 75 days (Azoxistrobin + Difenoconazole (B1), Difenoconazole (B1+15), Azoxistrobin + Difenoconazole (B1+30), Azoxistrobin + Difenoconazole (B1+45), Azoxistrobin + Difenoconazole (B1+60), Difenoconazole (B1+75)).

Dias & Theodoro (2017) evaluated different cotton cultivars and fungicide treatments for the control of *Ramularia* spot and also observed different responses in terms of yield between the cultivars in that region of the plant.

No differences were found between the cultivars for cotton yield in the lower stratum in the shorter plants. This response was not observed in the taller plants, however, for which cultivar FMT 701 presented the lowest yield. The lowest yield observed in this cultivar among the taller plants may be due to the greater disease severity detected in this cultivar.

The results presented in this study showed that the disease control strategies based on the use of different cotton cultivars, plant heights, and fungicide treatments made it possible to generate valuable information to guide cotton producers in a sustainable management of target spot in the crop.

CONCLUSIONS

The cultivar FM 944 showed less susceptibility to target spot and it was observed greater disease progression in taller cotton plants (1.5 m). The fungicides pyraclostrobin + fluxapyroxad and trifloxystrobin + prothioconazole sprays in the taller plants provided increases in whole-plant yield and reduced the severity of target spot in cultivars FM 975 and FMT 701. Cultivar FM 978 obtained a higher yield in the whole plant and the yield of cotton seed was higher in the upper part of the plants of all evaluated cultivars.

Acknowledgments

This study was funded in part by Fundação Universidade Federal de Mato Grosso do Sul – UFMS/MEC – Brazil and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001. The authors thank Fundação de Apoio à Pesquisa Agropecuária de Chapadão for the donation of inputs for the realization of this work.

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How to cite

SOUZA HM, THEODORO GF, DIAS AR, SOUZA CRW & MAGALHÃES FF. 2020. Integrated control of target spot and yield of cotton in the Brazilian *cerrado* biome. An Acad Bras Cienc 92: e20181205. DOI 10.1590/0001-3765202020181205.

Manuscript received on November 29, 2018;
accepted for publication on February 28, 2019

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Author contributions

The authors contributed to this study as follow: Hugo Manoel de Souza performed the experiment, collected and analyzed the data, discussed the results, did the bibliographic review and the writing of the paper. Gustavo de Faria Theodoro conceived and designed the experiment, analyzed data, discussed the results, revised, corrected and translated the paper for english language. Alfredo Ricieri Dias performed the experiment; collected data. Christian Rones Wruck Souza performed the experiment; collected data. Fernando Fagner Magalhães performed the experiment; collected data.

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