Trop. plant pathol. (2017) 42:468–474
DOI 10.1007/s40858-017-0165-1

ORIGINAL ARTICLE

Cotton blue disease in central-west Brazil: Occurrence, vector (*Aphis gossypii*) control levels and cultivar reaction

Rafael Galbieri[1,2] · Alberto S. Boldt[1] · Leonardo B. Scoz[1] · Sandra M. Rodrigues[3] · Diego O. Rabel[4] · Jean L. Belot[1] · Maite Vaslin[5] · Tatiane da Franca Silva[5,6] · Leimi Kobayasti[7] · Luiz Gonzaga Chitarra[3]

Received: 10 January 2017 / Accepted: 30 May 2017 / Published online: 16 June 2017

Abstract Cotton blue disease (CBD) is the viral disease which poses the greatest threat to cotton in Brazil. One efficient way of controlling this disease is by using resistant cultivars. However, the recent emergence of an atypical form of CBD (ACBD), caused by a new virus genotype capable of overcoming these resistant cultivars, is causing concern. Thus, the aims of this study were to evaluate the distribution of ACBD in the states of Mato Grosso (MT) and Goiás (GO), to determine the relationship between vector infestation level, disease incidence and yield, and to check the reaction of cotton cultivars to two viral isolates. In both cotton production areas, 1128 plots were surveyed and 6.5% showed plants with the virus, 97.3% and 2.7% with ACBD and CBD, respectively. In cultivars susceptible to ACBD, a positive linear relationship between changes in the levels of aphid infestation and incidence of viral infection was indentified, and a negative linear relationship between infestation level and yield. The maximum acceptable level of aphids up to 80 days after sowing for susceptible cultivars was approximately 15%. Although 83% of the cultivars were shown to be resistant to CBD, only 19.2% were resistant to ACBD. There was also a number of cultivars with considerable resistance to both isolates.

Keywords Atypical cotton blue disease · Genotype resistance · CLRDV · Cotton leafroll dwarf virus · Vein mosaic virus

Section Editor: Jorge Rezende

✉ Luiz Gonzaga Chitarra
luiz.chitarra@embrapa.br

[1] Instituto Mato-grossense do Algodão, BR 070, Km 266, Primavera do Leste, MT 78850-000, Brazil

[2] Programa de Pós-Graduação em Agricultura Tropical, Universidade Federal de Mato Grosso, Cuiabá, MT 78060-900, Brazil

[3] Embrapa Algodão, Núcleo de P&D do Cerrado, Sinop, MT 78550-970, Brazil

[4] Setor de Ciências Agrárias, Universidade Federal do Paraná, Curitiba, PR 80035-050, Brazil

[5] Departamento de Virologia, Inst. de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 99415-047, Brazil

[6] Present address: Departamento de Biotecnologia, Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, SP 12602-810, Brazil

[7] Laboratório de Fitopatologia, Faculdade de Agronomia e Medicina Veterinária, Universidade Federal de Mato Grosso, Cuiabá, MT 78060-900, Brazil

Introduction

Cotton is the most important fiber crop in the world. In the 2014/15 crop season, global cotton production was 26.4 million tons of fiber. In the same period, the cotton growing area in Brazil was approximately one million hectares, producing 1.7 million tons of fiber, marking Brazil as one of the top five global producers of cotton. The states of Mato Grosso (MT) and Goiás (GO) produce 55% and 10% of all cotton in Brazil, respectively (Kist et al. 2015). Due to the rainfall regime, high temperatures and quality farming management, the cotton yield from the drylands in Brazil is the best in the world, with an average of 1545 kg per hectare (Conab 2015).

However, as in any other tropical region, environmental conditions are conductive to the development of insects and disease. These conditions increase the cost of production giving rise to the need to strengthen the methods of controlling pests and diseases (Suassuna and Coutinho 2015).

There are many pathogens that cause damage to cotton plants in the central-west region of Brazil and disease accounts for approximately 14–20% of all cotton yield losses (Cia and

Galbieri 2016). The major problems are areolate mildew (*Ramularia areola)*, nematodes and viruses (Chitarra and Galbieri 2015). Cotton blue disease (CBD) caused by *Cotton leafroll dwarf virus* (CLRDV; family *Luteoviridae*, genus *Polerovirus*) is the most important cotton virus in Brazil (Costa and Carvalho 1962; Corrêa et al. 2005; Distéfano et al. 2010). When cotton is sown in the savanna region, one of the main technical problems is the high incidence of CBD in cultivars from the United States of America and Australia, since they are very susceptible to this disease. Thus, research focuses on developing cultivars which are both resistant to CBD and adapted to conditions in the central-west (Freire et al. 2005; Freire 2011). Nowadays, almost all cotton cultivars sown in Brazil are resistant to CBD (Galbieri et al. 2010).

CLRDV is transmitted in a persistent-circulative manner by the cotton aphid *Aphis gossypii* Glover, a species which has a number of hosts including certain annual crops and weeds (Michelotto and Busoli 2003, 2007). CBD is capable of reducing the yield of susceptible cultivars by up to 80% if cotton aphids are not properly controlled during the early crop season (Silva et al. 2008) and losses up to 1500 kg/ha in infected cotton production attributable to CBD have been reported in Brazil (Freire 1998). It is important to understand the relationship between aphid control level and plant virus incidence if control of the disease is to be achieved. For CBD, Santos et al. (2004) showed the direct relationship between aphid infestation and plants with symptoms.

In 2006, a new disease, called atypical vein mosaic virus or atypical cotton blue disease (ACBD) was observed in fields planted with CBD-resistant cotton cultivars (Silva et al. 2008; Galbieri et al. 2010). Recently, Silva et al. (2015) have shown that ACBD is caused by a new resistance-breaking (RB) CLRDV genotype. These RB isolates have a high degree of nucleotide and amino acid sequence identity to those of isolates causing typical CBD. However, its P0 (movement protein) is only 86.1%, identical to the P0 of the typical CLRDV isolate, CLRDV-PV1.

Currently, more than 90% of all cotton cultivars sown in Brazil are susceptible to ACBD (Chitarra and Galbieri 2015). Cotton farmers have maintained aphids at lower levels during the crop season in an effort to control of ACBD efficiently. However, there are three consequences to such management: it increases production costs, it can be harmful to the environment, and because of the excessive rainfall in the early crop season period, it is very difficult to handle insecticide applications. Resistant cultivars are the best method for controlling cotton virus diseases (Cia et al. 2007; Santos et al. 2004). At the very least, the use of cultivars with an intermediate resistance level can maintain vector populations below economic injury level (Santos 2015).

This study evaluated the occurrence of CLRDV in MT and GO, determined the relationship between aphid control level and incidence of ACBD in the field, and studied the reaction of cotton cultivars to both CBD- and ACBD-associated virus isolates.

Material and methods

Occurrence of CBD and ACBD in the states of Mato Grosso and Goiás

In the 2013/14, 2014/15 and 2015/16 crop seasons, 1128 cotton plots were surveyed across a production area of approximately 225,600 ha in MT and GO. In each area the occurrence of plants exhibiting symptoms typically induced by viruses such as stunting, leaf rolling, intense green foliage or reddish and withered leaves (Fig. 1) was identified. Two symptomatic apex leaves were collected and stored in plastic bags containing 15 g of silica gel in order to maintain moisture at lower levels inside the bags. The materials collected were stored at a temperature of 10 °C and then analysed with specific primers to identify the type of virus present in the leaves.

The samples were processed in duplicate for the extraction and purification of RNA molecules using the Nucleospin RNA Plant kit (Marcherey-Nagel). Subsequently, CLRDV was detected by reverse transcription-polymerase chain reaction (RT-PCR) using the GoScript Reverse Transcription System (Promega). In order to differentiate CBD from ACBD, quantitative RT-PCR with specific primers/probes was performed. Reactions were performed with the QuantiTec Multiplex PCR kit (Qiagen) with primers CLRDV-F (5′-GTA AAT TGT CTA CCC TCT CCT CCA CTA-3′) and CLRDV-R (5′-TGA TGA

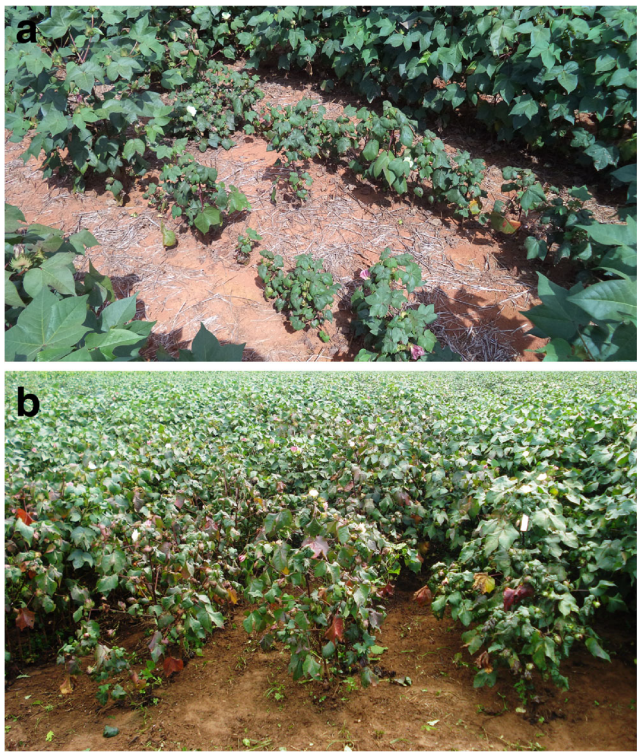

Fig. 1 Symptoms of atypical cotton blue disease (ACBD). **a** Infection occurring during the first stage of cotton development. **b** Infection at a later stage in development

AAG ACG CCG CAA ATT G-3′), and probes CLRDV1 (5′-FAM-CTT CCT CCC GTT CTT G-MGB-3′) for CBD and CLRDV2 (5′-VIC-TTC CTC CCA TTC TTG-MGB-3′) for ACBD. Reactions were generated and analyzed directly on a 7500 Real Time PCR System device (Applied Biosystems). Plants under greenhouse conditions completely free of both disease and vector were used as negative controls. Positive controls were obtained from leaves infected by two virus isolates under separate greenhouse conditions. The CBD isolate was obtained from cultivar FM 966 and the ACBD isolate (IMA2) from cultivar FMT 701. These two isolates have already been used and characterized in previous works by Galbieri et al. (2010) and Silva et al. (2015).

Relationship between vector (*Aphis gossypii*) control level and ACBD incidence

Three experiments were conducted under field conditions to evaluate the relationship between aphid control levels and the incidence of ACBD. The work was carried out in Primavera do Leste, MT (S15°31′34.1″, W54°12′20.1″) under natural infestation of the aphid/virus. The composition of the soil texture of the experimental area was 64% sand, 6.6% silt and 29.4% clay. Four fungicide sprays were applied to control *Ramularia areola* and specific insecticides were applied to control boll weevil, especially at 80 days after sowing.

Five threshold levels of aphid incidence, 5%, 20%, 40%, 60% and 80% were established. Monitoring of the aphid population was carried out in each plot by visual inspection every two days up to 80 days after crop sowing, and the percentage of plants attacked by colonies of 5–10 aphids was recorded. When the threshold levels were reached, foliar insecticide (thiamethoxam, 50 g of a.i./ha, and acetamiprid, 20 g a.i./ha) was applied using a precision backpack sprayer distributing CO_2 with a 2 m bar and 4 nozzles, extended range type (XR TeeJet; Green), spaced at 45 cm, at a constant pressure of 45 psi and spray solution consumption of 200 L/ha. Aphid control based on the threshold levels was carried out up to 80 days after sowing.

Each experiment was conducted in a randomized block design with five replications and the plots consisted of eight rows spaced at 0.90 m, each seven meters in length. The areas used for evaluation and harvest were the four center lines.

In experiment 1, the cotton cultivar FMT 701 was sown on Dec. 20, 2010 and harvested on Jun. 23, 2011. The threshold control levels were 5, 20, 40, 60 and 80%. In experiment 2, the cotton cultivars used were Delta Opal and CD 6468, sown on Dec. 21, 2010 and harvested on Jun. 26, 2011. In experiment 3, the cultivars used were Delta Opal, FM 993 and FMT 701, sown on Dec. 23, 2011 and harvested on Jun. 22, 2012. The threshold control levels in experiments 2 and 3 were 5, 20, 40 and 80%.

ACBD evaluations took place 80 days after sowing and were based on a disease severity scale of 1 to 5 (Cia et al. 2007). After harvesting, the data were converted to kg of fiber/ha according to the total weight of the useful four center lines. For fiber yield patterns, 30 cotton bolls were collected prior to harvest. Regression analysis between aphid control levels and disease incidence (score) as well as fiber yield per hectare was carried out.

Cultivar reaction to CBD and ACBD

Five plants from cultivar FMT 701 with viral symptoms were collected from the previously described experiment. These plants were transplanted into 12 L pots under greenhouse conditions. After 20 days, the plants were infested with aphids free of virus under controlled conditions. Seven days later, the aphids were transferred to new seedlings of cultivar FMT 701 in order to maintain the population of the isolated virus (IMA2), which was retained as the inoculum source for subsequent trials. This isolate had already been further characterized by Silva et al. (2015) and it was confirmed that it was ACBD. The CBD inoculum used for the characterization work was from the same isolate used in the work of Galbieri et al. (2010).

In the cultivar characterization tests for viruses, two separate greenhouses were used, one for CBD and one for ACBD. The methods used were the same described by Galbieri et al. (2010). The greenhouses were maintained at 27 ± 2 °C throughout the experiments. In total, four trials were conducted, two in 2014 and two in 2015, one for each virus, CBD and ACBD, with 16 and 26 cultivars for the first and second year, respectively.

The experiments were set up in a randomized block design with five replications, each consisting of 12 L pots with four plants. As substrate, a washed mixture of sand, soil and manure (5:13:4) was used. Fifteen viruliferous aphids were inoculated into each plant 10 days after sprouting, with a soft brush aid (#2). The virus acquisition period was about 10 days. Seven days after inoculation, the aphids were eliminated from the plants through insecticide application of carbosulfan (1.2 g i.a/L). Symptomatic plants were assessed 30 days after inoculation based on a disease severity scale of 1 to 5 (Galbieri

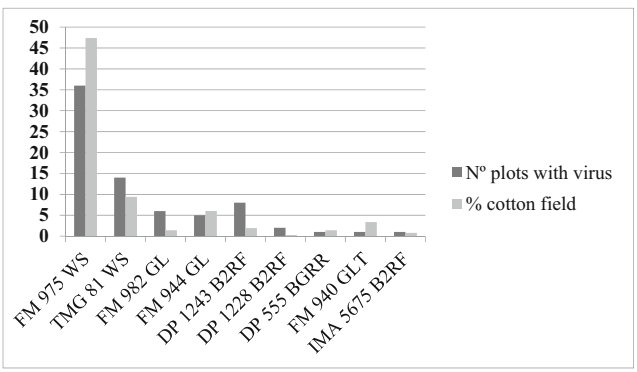

Fig. 2 Number of plots with presence of virus (CBD and ACBD) in different genotypes of cotton and percent distribution of cultivars in relation to the 1128 sampled plots

et al. 2010). The data were transformed into $\sqrt{x+1}$ and subjected to analysis of variance. Mean values were grouped by the Scott-Knott test at 5% significance.

Results

Occurrence of CBD and ACBD in the states of Mato Grosso and Goiás

Leaves from 1128 sampled plots with characteristic symptoms of CBD-like viruses, such as stunting due to internodal shortening, leaf rolling, intense green foliage and reddish and withered leaves were collected for molecular analysis (Fig. 1). The results showed that 6.5% of the plots had plants with symptoms of virus infection in the period prior to 80 days after sowing.

Virus symptoms were detected in 36 samples of the plots with cultivar FM 975WS (Fig. 2). Out of 74 samples of plants with symptoms of CBD-like disease, 97.3% were confirmed to be ACBD and only 2.7% in two plots had been affected by CBD (Fig. 3).

Relationship between vector (*Aphis gossypii*) control level and ACBD incidence

The ratio of aphid infestation level (up to 80 days after sowing) to disease incidence was positive and linear for both FM 993 ($p = 0.0015$) and FMT 701 ($p = 0.0122$), and assumed a quadratic function for CD 6468 ($p = 0.0017$) (Fig. 4, Table 1). In cultivar Delta Opal no plants showed virus symptoms, regardless of the aphid infestation level.

In terms of variation in fiber yield, the ratio of aphid infestation level was negative and linear with production of fiber per ha for CD 6468 ($p = 0.0282$), FM 993 ($p = 0.0515$) and FMT 701 ($p = 0.0124$). For Delta Opal, a significant model derived from the various levels of aphid infestation vs. production could not be determined.

Considering only the susceptible cultivars CD 6468, FM 993 and FMT 701, comparison of aphid incidence levels showed that the levels of 40% and 80% had similar responses. Thus, they were grouped according to the Scott Knott test results, which take the reaction to the virus into account (Table 2). Similarly, the levels of 5% and 20% were also grouped for both yield and reaction to the virus.

Cultivar reaction to CBD and ACBD

In the year 2014, the cultivars FM 966LL, FM 982GL and DP 555BGRR showed susceptibility to CBD. The other cultivars tested in this year were in the strongly resistant group with a score equal or close to one. The cultivars Delta Opal FM 951LL, BRS 368RF and TMG 42WS were in the top ACBD resistant group, while the others were in the lower susceptibility group (Table 3).

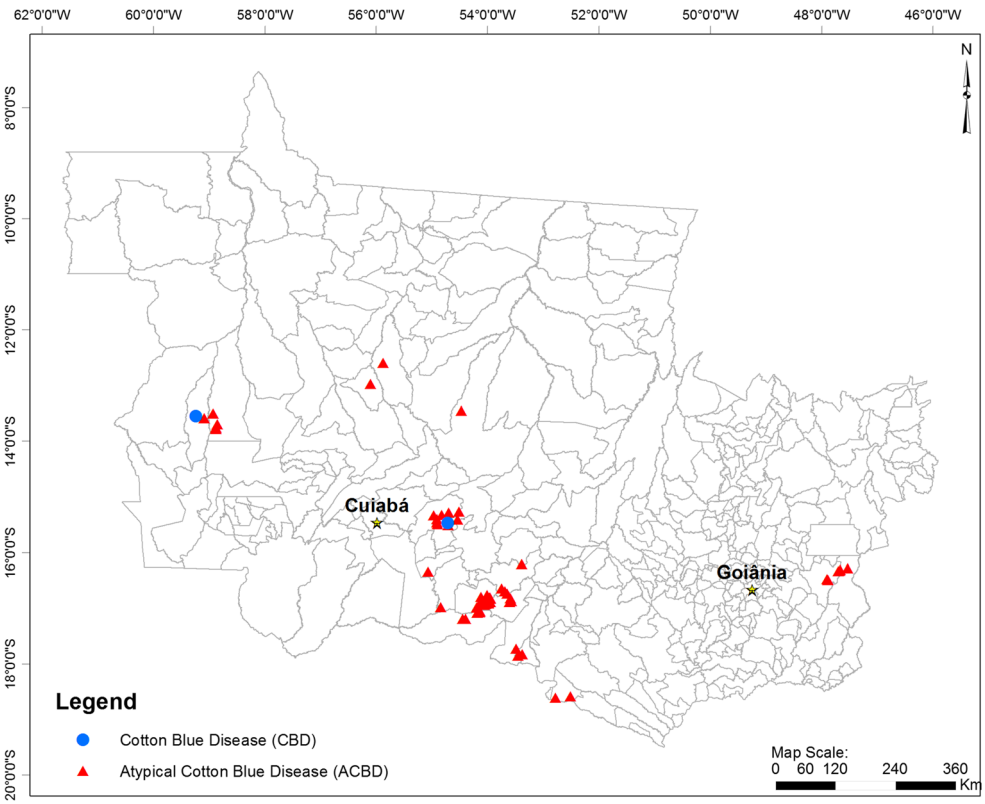

Fig. 3 Cotton areas in the states of Mato Grosso and Goiás with occurrence of CBD or ACBD. Total of 1128 sampled plots

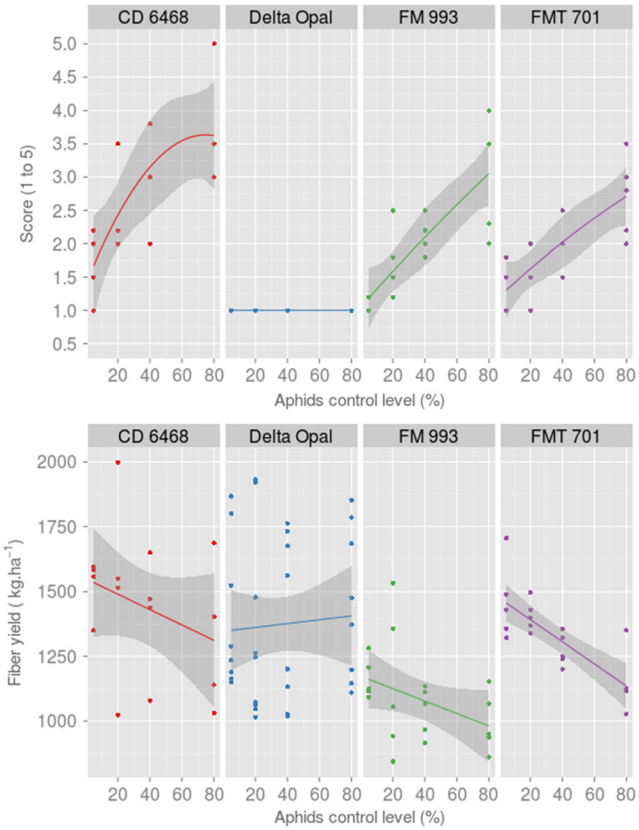

Fig. 4 Relationship of aphid control levels to incidence of ACBD and fiber yield in different cotton cultivars. Scores: 1, Plants without symptoms of the disease; 2, Up to 5% of plants with symptoms; 3, 6–25% of plants with symptoms; 4, 26–50% of plants with symptoms; 5, 51–100% of plants with symptoms

In the year 2015, cultivars FM 966LL, DP 1228B2RF and FM 982GL showed susceptibility to CBD, while the other cultivars were in the strongly resistant group with a score equal or close to one. With regard to ACBD, cultivars Delta Opal, BRS 368RF, FM 913GLT, TMG 42WS, and TMG 43WS remained in the top resistant group, while the others were in the lower susceptibility group (Table 4).

Comparing cultivars BRS 368RF, FM 944GL, FM 966LL, FM 975WS, FM 982GL, IAC 26RMD, IMA 5675B2RF, TMG 11WS, TMG 41WS, TMG 42WS, TMG 81WS, TMG 82WS, Delta Opal, and FMT 701, over two years of evaluation, it was observed that there was a high correlation of 0.96 and 0.71 ($p \leq 0.01$) for CBD and ACBD, respectively.

Discussion

For cotton blue disease, infection is most harmful up to 50 days after sowing, as shown by Santos et al. (2004), Santos (2015), and Michelotto and Busoli (2006). There was a high incidence of disease in cultivar FM 975WS, a fact observed in areas under cultivation in MT. In the area sampled in this study, approximately 48% was sown with FM 975WS. In surveys conducted by Tachinard (2015), this percentage was 61 and 40% of the sown area in MT during the crop season 2013/14 and 2014/15 respectively. Samples obtained between 60 and 90 days after sowing (late infection) were not considered in our work. If this kind of infection had been analyzed, the incidence probably would have been higher.

The results confirm the great predominance of ACBD over CBD in cotton in MT and GO. Since 2005, most of the cultivars sown in both states have been resistant to CBD (Galbieri et al. 2010), and the potential for this inoculum has been reduced throughout the cultivated area. However, according to the data presented, the virus survives, albeit at low impact level under the conditions found in the savanna. This information is important because, due to the unpredictable possibility of disease incidence, aphid levels are currently being maintained as low as possible. This may encourage breeding programs to re-launch cultivars susceptible to CBD, or at least not to take into account this feature in the selection process, as the vector is being effectively controlled. If this approach takes root, in combination with the presence of CBD, it can intensify the problem with the use of cultivars susceptible to both viruses. Thus, it is important to search for cultivars which are resistant to both CBD and ACBD if effective disease control is to be achieved.

Table 1 Regression models adjusted to represent the relationship between aphid control levels and the incidence of viral infection (score) and yield, in cultivars CD 6468, FM 993, FMT 701 and Delta Opal

Variable	Cultivar	Model[a]	*p*-value[b]	R^2
Score	CD 6468	$\hat{Y}$ =1.383 + 0.06026P – 0.0004P^2	0.0017**	0.99
	FM 993	$\hat{Y}$ = 1.0769 + 0.0248P	0.0015**	1.00
	FMT 701	$\hat{Y}$ = 1.2386 + 0.0186P	0.01224*	0.98
	Delta Opal	-	0.1026	-
Yield	CD 6468	$\hat{Y}$ = 1549.6223–2.9741P	0.0282*	0.94
	FM 993	$\hat{Y}$ = 1173.3500–2.4080P	0.0515	0.90
	FMT 701	$\hat{Y}$ = 1477.4714–4.2792P	0.0124*	0.98
	Delta Opal	-	0.2408	-

[a] P, aphid control level

[b] *$p \leq 0.05$, **$p \leq 0.01$

Table 2 Cluster test for aphid incidence levels considering only cultivars CD 6468, FM 993 and FMT 701

Control levels (%)	Score[a]	Yield (kg/ha)[a]
80	3.093a	1140.51a
40	2.386b	1228.86a
20	1.814c	1346.86b
5	1.378c	1372.14b
Mean	2.168	1272.09

[a] Means followed by the same letters in the column belong to the same group according to the Scott and Knott test, at 5% probability

Data from assays show that the severity of the disease was very high. Cultivars CD 6468, FM 993 and FMT 701 showed high susceptibility to ACBD.

According to the data shown on the prevalence of ACBD in MT and GO associated with the close relationship of the vector control level and the incidence of disease, it is necessary to maintain aphid infestation levels below 15% in susceptible cultivars. This index confirms the results reported by Santos (2015). However, it is difficult to maintain this low aphid level under the conditions prevalent in the savanna, mostly due to the high precipitation during the early crop development stage from December to February, especially in Primavera do Leste (aproximately 1000 mm) (Fietz et al. 2008). Under such conditions, it is difficult to carry out any field operations, in addition to the high fertility rate of the aphid in susceptible plants (Michelotto and Busoli 2009). In resistant cultivars such as Delta Opal, the aphid can be managed as an insect pest and not as a vector of the virus, since its level has no relationship with disease incidence. According to Santos (2015), CBD resistant cultivars can tolerate up to 50% of their plants being infested with aphids. In this study, Delta Opal showed resistance at an 80% threshold level of aphid infestation, with yield not being significantly affected.

Table 3 Cotton cultivar reactions to blue disease (CBD) and atypical cotton blue disease (ACBD) under greenhouse conditions in 2014

Cultivar	CBD[a]	ACBD	Cultivar	CBD	ACBD
BRS 368RF	1.0a	1.5a	IMA 5675BT2RF	1.0a	3.3b
DP 555BGRR	2.3b	2.0b	TMG 11WS	1.0a	3.3b
FM 944GL	1.0a	3.8b	TMG 41WS	1.0a	3.3b
FM 951LL	1.0a	1.3a	TMG 42WS	1.0a	1.8a
FM 966LL	5.0d	3.0b	TMG 81WS	1.0a	3.3b
FM 975WS	1.0a	2.5b	TMG 82WS	1.0a	2.8b
FM 982GL	3.8c	3.0b	Delta Opal	1.0a	1.0a
IAC 26RMD	1.3a	3.0b	FMT701	1.0a	4.5b
C.V. (%)	4.9	11			
r[b]	0.1n.s.				

[a] Scale from 1 to 5 with an increasing incidence of the disease. 1, plants without symptoms of the disease; 2, one plant per plot with symptoms of the disease; 3, two plants per plot with symptoms of the disease; 4, three plants with symptoms of the disease; 5, four (all) plants per plot with symptoms of the disease. Means followed by the same letter in the column within each virus do not differ significantly from each other according to the Scott and Knott test, at 5% probability

[b] Correlation between the results of CBD vs. ACBD

Table 4 Cotton cultivar reactions to blue disease (CBD) and atypical cotton blue disease (ACBD) under greenhouse conditions in 2015

Cultivar	CBD[a]	ACBD	Cultivar	CBD	ACBD
BRS 368RF	1.0a	1.0a	FM 975WS	1.0a	3.8d
BRS 369RF	1.0a	2.0b	FM 980GLT	1.0a	2.3b
BRS 371RF	2.0b	4.0d	FM 982GL	2.0b	4.0d
BRS 372	1.3a	3.0c	IAC 26RMD	1.3a	4.0d
IMA 2106GL	1.0a	3.5c	IMA 5675B2RF	1.0a	4.3d
DP 1227B2RF	1.3a	2.5b	TMG 11WS	1.0a	1.8b
DP 1228B2RF	2.8c	4.8d	TMG 41WS	1.0a	1.8b
DP 1240B2RF	1.0a	3.0c	TMG 42WS	1.0a	1.5a
IMA 8405GLT	1.0a	2.6b	TMG 43WS	1.0a	1.5a
FM 913GLT	1.0a	1.3a	TMG 81WS	1.0a	2.3b
FM 940GLT	1.0a	1.8b	TMG 82WS	1.0a	2.5b
FM 944GL	1.0a	4.5d	Delta Opal	1.0a	1.0a
FM 966LL	3.8d	3.3c	FMT 701	1.0a	4.5d
C.V. (%)	6.3	8.7			
r[b]	0.4n.s.				

[a] Scale from 1 to 5 with an increasing incidence of the disease. 1, plants without symptoms of the disease; 2, one plant per plot with symptoms of the disease; 3, two plants per plot with symptoms of the disease; 4, three plants with symptoms of the disease; 5, four (all) plants per plot with symptoms of the disease. Means followed by the same letter in the column within each virus do not differ significantly from each other according to the Scott and Knott test, at 5% probability

[b] Correlation between the results of CBD vs. ACBD

When fiber yield is analyzed, the difference between vector control at 5 and 80% threshold levels was 170, 205 and 314 kg/ha for cultivars FM 993, CD 6468 and FMT 701, respectively. These values represent a reduction in productivity in FM 993, CD 6468 and FMT 701 of approximately 14.6, 13.4 and 21.5%, respectively. These values are significant, and should be considered in crop management programs. However, according to the data reported earlier by Freire (1998), Santos et al. (2004) and Michelotto and Busoli (2006), CBD in susceptible cultivars seems to be more destructive than ACBD. This has also been highlighted by Galbieri et al. (2010) and Santos (2015).

In all experiments, cultivars Delta Opal and FMT 701 were used as controls. Delta Opal is resistant to both virus isolates and FMT 701 is susceptible to ACBD but resistant to CBD (Galbieri et al. 2010; Fang et al. 2010; Pupim Junior et al. 2008; Santos et al. 2004; Michelotto and Busoli 2003). In four experiments over a two-year period, the evaluation of disease severity in FMT 701 was 1.0 for CBD and 4.5 for ACBD. The symptomatology contrast between Delta Opal and FMT 701 can be seen in Fig. 5. Furthermore, the 14 cultivars that were used in the two years of evaluation were shown to be in the same classification group, attesting the replicability of the test.

Considering the two years of evaluation, 83% of the cultivars were resistant to CBD but only 19.2% to ACBD. These

Fig. 5 Contrast between a susceptible cultivar (FMT 701, left), with all plants showing symptoms of ACBD, and a resistant cultivar (Delta Opal, right), with all plants asymptomatic

data are similar to those presented by Galbieri et al. (2010) with regard to CBD. Similarly, the lack of a correlation between the incidence of the two viruses reinforces the fact that the isolates are distinct, as reported by Galbieri et al. (2010) and more recently by Silva et al. (2015).

In addition to Delta Opal, it was possible to find cultivars with strong resistance to both viruses. These data show an improvement in this feature compared to previous work performed by Galbieri et al. (2010). This is a result of the work that is being undertaken in breeding programs in Brazil, seeking to improve cultivar resistance to viruses. Further studies are necessary if an understanding is to be reached of the inherent resistance to ACBD and the development of a marker associated with resistance, as has already been reported in the case of CBD (Pupim Junior et al. 2008; Fang et al. 2010), to facilitate cultivar selection with higher resistance to disease.

Acknowledgements The present research was conducted with the financial support of IMA, AGOPA and IBA. Technical assistance was provided by Zeani C. Veloso and Antônio A. E. de Oliveira.

References

Chitarra LG, Galbieri R (2015) Controle de doenças no algodoeiro em Mato Grosso. In: Belot JL (ed) Manual de boas práticas de manejo do algodoeiro em Mato Grosso. Casa das Árvores, Cuiabá, pp 166–177

Cia E, Galbieri R (2016) Doenças do algodoeiro. In: Amorim L, Rezende JAM, Bergamin Filho A, Camargo LEA (eds) Manual de fitopatologia. Agronômica Ceres, São Paulo, pp 47–62

Cia E, Galbieri R, Fuzatto MG, Lüders RR, Kondo JI, Carvalho LH, Ruano O, Almeida WP, Ito MF, Oliveira AB, Cunha HF, Chiavevato EJ, Aguiar PU, Mehta YR, Martins ALM, Pettinelli Junior A, Bolonhezi D, Foltran DE, Kasai FS, Ito MA, Michelotto MD, Bortoletto N, Gallo PB, Reco PC, Souza PS, Rossetto R, Freitas RS, Furlani Junior E, Lebedenco A, Pedrosa MB, Lanza MA (2007) Comportamento de genótipos de algodoeiro na presença de patógenos e nematóides. Rev Bra Oleag Fib 1:99–109

CONAB (2015) Safras – grãos 2014/2015. Available at: www.conab.gov.br/conabweb/index.php?PAG=131. Accessed on 28 Sept 2015

Corrêa RL, Silva TF, Simões-Araújo JL, Barroso PAV, Vidal MS, Vaslin MFS (2005) Molecular characterization of a virus from the family *Luteoviridae* associated with cotton blue disease. Arch Virol 150: 1357–1367

Costa AS, Carvalho AMB (1962) Moléstia e vírus do algodoeiro. Bragantia 21:50–51

Distéfano AJ, Bonacic Kresic I, Hopp HE (2010) The complete genome sequence of a virus associeted with cotton blue disease, cotton leafroll dwarf virus, confirms that it is a new member of the genus *Polerovirus*. Arch Virol 155:1849–1854

Fang DD, Xiao J, Canci PC, Cantrell RG (2010) A new SNP haplotype associated with blue disease resistance gene in cotton (*Gossypium hirsutum* L.) Theor Appl Genet 120:943–953

Fietz CR, Comunello E, Cremon C, Dallacort R (2008) Estimativa da precipitação provável para o Estado de Mato Grosso. Série Documentos no. 97. Embrapa Agropecuária Oeste, Dourados.

Freire EC (1998) Doença azul tem solução. Cultivar 1:64–65

Freire EC (2011) História do algodão no cerrado. In: Freire EC (ed) Algodão no cerrado do Brasil. ABRAPA, Brasília, pp 23–60

Freire EC, Suinaga FA, Farias FJC, Morello CL, Silva Filho JL, Pedrosa MB, Chitarra LG, Vidal Neto FC, Andrade FP, Santos JW, Araújo GP, Lira AJS, Valmir LM (2005) BRS Araçá – cultivar precoce e com resistência a viroses para o cerrado do Mato Grosso. 5º Congresso Brasileiro de Algodão, Salvador, Proceedings... Embrapa Algodão, Campina Grande.

Galbieri R, Cia E, Fuzatto MG, Franzon RC, Belot JL, Dias JAC (2010) Transmissibilidade e reação de genótipos de algodoeiro a uma forma atípica do vírus do mosaico das nervuras. Trop Plant Pathol 35:88–95

Kist B, Santos C, Carvalho C, Reetz ER, Müller I, Beling RR (2015) Anuário brasileiro do algodão. Gazeta Santa Cruz, Santa Cruz do Sul

Michelotto MD, Busoli AC (2003) Aspectos biológicos de *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) em três cultivares de algodoeiro e em três espécies de plantas daninhas. Ciência Rural 33:999–1004

Michelotto MD, Busoli AC (2006) Efeito da época de inoculação do vírus do mosaico das nervuras por *Aphis gossypii* Glover (Hemiptera: Aphididae) no desenvolvimento e na produção do algodoeiro. Neotrop Entomol 35:251–256

Michelotto MD, Busoli AC (2007) Caracterização da transmissão do vírus do mosaico-das-nervuras do algodoeiro pelo pulgão *Aphis gossypii* com relação à persistência e ao tempo necessário para inoculação. Bragantia 66:441–447

Michelotto MD, Busoli AC (2009) Biologia de *Aphis gossypii* em plantas infectadas pelo vírus do mosaico das nervuras do algodoeiro. Bragantia 68:1017–1024

Pupim Junior O, Schuster I, Pinto RB, Pires E, Belot JL, Silvie P, Chitarra LG, Hoffmann LV, Barroso P (2008) Inherance of resistance to cotton blue disease. Braz Agric Res 43:661–665

Santos WJ (2015) Manejo das pragas do algodão com destaque para o cerrado brasileiro. In: Freire EC (ed) Algodão no cerrado do Brasil. ABRAPA, Brasília, pp 267–364

Santos KB, Neves PMJ, Santos WJ (2004) Resistência de cultivares de algodoeiro ao vírus do Mosaico das Nervuras transmitido pelo pulgão *Aphis gossypii* (Glover) (Hemiptera: Aphididae). Neotrop Entomol 33:481–486

Silva TF, Corrêa RL, Castilho Y, Silvie P, Belot JL, Vaslin MFS (2008) Widespread distribution and a new recombinant species of Brazilian virus associated with cotton blue disease. Virol J 5:1–13

Silva AKF, Romanel E, Silva TF, Castilhos Y, Schrago CG, Galbieri R, Belot JL, Vaslin MFS (2015) Complete genome sequences of two new virus isolates associated with cotton blue disease resistance breaking in Brazil. Arch Virol 160:1371–1374

Suassuna ND, Coutinho WM (2015) Manejo das principais doenças do algodoeiro no cerrado brasileiro. In: Freire EC (ed) Algodão no cerrado do Brasil. ABRAPA, Brasília, pp 365–408

Tachinard R (2015) O cultivo algodoeiro em Mato Grosso. In: Belot JL (ed) Manual de boas práticas de manejo do algodoeiro em Mato Grosso. Casa das Árvores, Cuiabá, pp 12–17