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## **Comparison of Bemisia tabaci Infestation, Virus Infection, and Yield in Conventional and Transgenic Bean Golden Mosaic Virus-Resistant Common Bean Elite Lines**

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# Comparison of *Bemisia tabaci* infestation, virus infection, and yield in conventional and transgenic Bean golden mosaic virus-resistant common bean elite lines

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## Abstract

In Brazil, the Cowpea mild mottle virus vectored by *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), is an emerging threat for common bean (*Phaseolus vulgaris* L.; Fabaceae), including genetically modified elite bean lines developed for resistance to the Bean golden mosaic virus. To investigate this interaction, we quantified the susceptibility of 10 Bean golden mosaic virus-resistant lines to Cowpea mild mottle virus infection in 2 regions in Brazil with natural incidence of viruliferous *B. tabaci*. In 2 field trials, *B. tabaci* established on all varieties, but showed preference for the conventional cultivars 'Pérola' and 'BRS Pontal' when compared with elite lines (averaging > 400 nymphs per leaflet, and > 97% infection rate in 1 study). However, whereas elite lines were resistant to Bean golden mosaic virus, all became infected with Cowpea mild mottle virus. Highest infection rates (19 to 99% infected plants) occurred in genetically modified elite lines derived from BRS Pontal versus Pérola (12 to 16%). When comparing seed yield, most elite lines outperformed their conventional recurrent parent. On average, elite lines achieved relative yield increases between 199 and 1,045%, and the varieties CNFCT 16205 and CNFCT 16210 were the most productive in our field trials. Our results showed that the use of common bean cultivars resistant to golden mosaic is an important tool within integrated management strategies for whiteflies and viruses. However, further studies are needed to elucidate the threat of Cowpea mild mottle virus and the nature of whitefly interactions between these different viruses.

Key Words: Aleyrodidae; isogenic lines; carioca; virus-whitefly interactions; yield

## Resumo

No Brasil, o Cowpea mild mottle virus transmitido pela *Bemisia tabaci* (Genn.) (Hemiptera: Aleyrodidae), é uma ameaça emergente ao feijão comum (*Phaseolus vulgaris* L.; Fabaceae), incluindo as linhas elites de feijão geneticamente modificadas desenvolvidas para resistência ao Bean golden mosaic virus. Para investigar essa interação, quantificamos a suscetibilidade de dez linhas geneticamente modificadas resistentes ao Bean golden mosaic virus à infecção pelo Cowpea mild mottle virus em duas regiões do Brasil com incidência natural de *B. tabaci* virulíferas. Em dois ensaios de campo, *B. tabaci* estabeleceu em todas as variedades, mas mostrou preferência pelas cultivares convencionais 'Pérola' e 'BRS Pontal' quando comparadas com as linhas elite geneticamente modificadas (média > 400 ninfas por folíolo e > 97% taxa de infecção por vírus em um dos estudos). No entanto, embora as linhas elite foram resistentes ao Bean golden mosaic virus, todas foram infectadas pelo Cowpea mild mottle virus. As maiores taxas de infecção (19 a 99% de plantas infectadas) ocorreram nas linhas elite geneticamente modificadas derivadas da BRS Pontal versus Pérola (12 a 16%). Ao comparar a produtividade, a maioria das linhas elite superou seu pai convencional recorrente. Os aumentos relativos de rendimento foi de 199 a 1.045% sendo as variedades CNFCT 16205 e CNFCT 16210 as mais produtivas. Nossos resultados mostraram que o uso de cultivares de feijão resistentes ao mosaico dourado é uma ferramenta importante dentro das estratégias integradas de manejo para moscas-brancas e vírus. No entanto, mais estudos são necessários para elucidar a ameaça do Cowpea mild mottle virus e a natureza das interações da mosca-branca com estes diferentes vírus.

Palavras Chave: Aleyrodidae; linhas isogênicas; carioca; interações vírus-mosca-branca; rendimento

Common bean (*Phaseolus vulgaris* L.; Fabaceae) is among the most widely consumed legume, with the commercial value exceeding all other legume crops combined (FAO 2020). Brazil is the largest producer and consumer, producing *P. vulgaris* on 1.7 million ha which yielded 2.6 million metric tons in 2017 (CONAB 2020). In addition to providing a nutritional source of protein, folic acid, dietary fiber, and complex

carbohydrates (Siloch et al. 2016), these beans are a key source of iron, with a single serving providing 23 to 30% of daily recommended levels (Carvalho et al. 2013a).

The silverleaf whitefly, *Bemisia tabaci* biotype B (Genn.) (Hemiptera: Aleyrodidae) is a key pest of *P. vulgaris* in Brazil (Faria et al. 2016). In addition to direct damage, in tropical and semi-tropical regions, *B.*

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*tabaci* vectors diseases including the Bean golden mosaic begomovirus (Morales 2010; Inoue-Nagata et al. 2016). Bean golden mosaic begomovirus can cause up to 100% yield losses in major bean-growing areas of Brazil (Faria et al. 2016), and at least 200,000 ha of common bean crop has been abandoned due to Bean golden mosaic begomovirus (Aragão & Faria 2009). Other begomoviruses vectored by *B. tabaci*, such as Macropodium yellow spot virus (Ramos-Sobrinho et al. 2014) and Sida micrantha mosaic virus (Fernandes-Acioli et al. 2011), also infect common bean in Brazil.

In Brazil, insecticides are used commonly to control whiteflies that migrate between host crops, including common bean, soybean, cotton, potato, and tomato (Quintela et al. 2016; Dempsey et al. 2017). However, intensive insecticide use has caused resistance to multiple insecticide classes worldwide (Basit 2019). Moreover, while insecticides may effectively reduce *B. tabaci* populations, generally they are less effective at reducing the incidence of viral diseases (Legg et al. 2014). Therefore, additional tools to supplement chemical controls are needed for effective whitefly management.

Host plant resistance provides a strategy to reduce impacts of insect-vectored plant virus (Nicaise 2014). In Brazil, conventional breeding programs during the past 40 yr identified bean lines with low level resistance or tolerance to golden mosaic diseases such as Bean golden mosaic begomovirus (Faria et al. 2014). The resistance to Bean golden mosaic begomovirus was achieved subsequently through genetic engineering. The Brazilian Agricultural Research Corporation (Embrapa) successfully developed 'Event Embrapa 5.1,' a transgenic common bean resistant to golden mosaic, using RNA interference (RNAi) technology and plant transformation through the biolistic methodology (Bonfim et al. 2007; Aragão & Faria 2009; Aragão et al. 2013). Homozygous plants showed complete resistance to golden mosaic disease under field conditions where non-transgenic plants showed severe disease symptoms or died (Bonfim et al. 2007; Aragão & Faria 2009; Souza et al. 2018). Field evaluations since 2007 suggest that Bean golden mosaic begomovirus resistance is stable (Faria et al. 2016; Souza et al. 2018).

However, during initial field trials to evaluate the agronomic characteristics of transgenic beans, it was noted that both transgenic and conventional lines were susceptible to another disease, the Cowpea mild mottle virus (Souza et al. 2018). Cowpea mild mottle virus is a Carlavirus that is transmitted in a non-persistent manner by *B. tabaci* (Muniyappa & Reddy 1983).

In Brazil, Cowpea mild mottle virus was recorded first in the states of São Paulo and Paraná in 1979, showing symptoms of angular yellow mosaic in common bean leaves (Costa et al. 1983), but yield losses were not considered significant (Gaspar et al. 1985). Since 2013 Cowpea mild mottle virus has been increasingly observed in Bean golden mosaic begomovirus-resistant common bean breeding lines developed by Embrapa (Faria et al. 2016).

Studies of the interactions between Event Embrapa 5.1 and *B. tabaci* and Cowpea mild mottle virus is fundamental to devise Cowpea mild mottle virus management strategies. The development of near isogenic lines from 2 carioca commercial cultivars ('Pérola' and 'BRS Pontal') provided an opportunity to evaluate Cowpea mild mottle virus transmission by *B. tabaci* under natural field conditions, because these transgenic isolines are immune to Bean golden mosaic begomovirus. Thus, the present study compared the susceptibility of 10 elite transgenic (Bean golden mosaic begomovirus-resistant) and 5 conventional (susceptible) bean line varieties to different whitefly densities as well as their yield.

## Materials and Methods

### TRANSGENIC EVENT AND GENETICALLY MODIFIED BEAN GOLDEN MOSAIC BEGOMOVIRUS-RESISTANT LINES

The event Embrapa 5.1 is the source of the transgene for resistance to Bean golden mosaic begomovirus (Bonfim et al. 2007). This event resulted from the introduction of a chimeric gene containing 411 base pairs of the rep gene of Bean golden mosaic begomovirus (Accession GenBank NC 004042, position 1836-2247), positioned in sense and antisense, to express a double strand RNA (dsRNA) that will form a hairpin structure. This dsRNA triggers the process known as RNA interference (RNAi) which finally resulted in resistance to Bean golden mosaic begomovirus. This event has been designated as RMD technology (RMD stands for resistance to golden mosaic) and became the source of the transgene for resistance to Bean golden mosaic begomovirus. Commercial lines were derived by crossing and backcrossing Embrapa 5.1 to commercial carioca cultivars. Ten genetically modified elite lines out of 26 homozygous lines and 5 conventional control cultivars were tested (Table 1). BRS Pontal and Pérola are Brazilian cultivars from the

**Table 1.** Genetically modified Bean golden mosaic virus-resistant common bean elite lines and conventional control cultivars evaluated in 2 field trials carried out in Brazil in the dry growing season of 2014.

Common bean line	Type	Commercial class	Recurrent parent
CNFCT 16201	Genetically modified	Carioca	Pérola
CNFCT 16202 <sup>1</sup>	Genetically modified	Carioca	Pérola
CNFCT 16203	Genetically modified	Carioca	Pérola
CNFCT 16204	Genetically modified	Carioca	Pérola
CNFCT 16205	Genetically modified	Carioca	BRS Pontal
CNFCT 16206	Genetically modified	Carioca	BRS Pontal
CNFCT 16207	Genetically modified	Carioca	BRS Pontal
CNFCT 16208	Genetically modified	Carioca	BRS Pontal
CNFCT 16209	Genetically modified	Carioca	BRS Pontal
CNFCT 16210	Genetically modified	Carioca	BRS Pontal
Pérola	Conventional	Carioca	NA <sup>2</sup>
BRS Pontal	Conventional	Carioca	NA
IPR Eldorado	Conventional	Carioca	NA
BRB 169	Conventional	Black	NA
CNFP 15882	Conventional	Black	NA

<sup>1</sup>This line was not tested in field Trial #1.

<sup>2</sup>NA, not applicable – conventional control cultivars.

carioca market class which were used as recurrent parents in backcrosses to develop the genetically modified elite lines CNFCT 16201 to CNFCT 16210 (Del Peloso et al. 2004; Faria et al. 2014; Souza et al. 2018).

The conventional controls included the cultivars Pérola and BRS Pontal, the cultivars 'IPR Eldorado' (carioca seed), 'BRB169,' and 'CNFP 15882' (both black seeded). The last 3 cultivars were included based on claims of partial resistance or tolerance to golden mosaic by their developers (Bianchini 2010).

## FIELD SITES AND EXPERIMENTAL DESIGN

Two field trials were carried out in the dry growing season of 2014 at the Embrapa Rice and Beans Experimental Station, Santo Antonio de Goiás, Goiás, Brazil. Field Trial #1 was conducted from 27 Feb 2014 through 22 May 2014 at Goianira, Goiás, Brazil (16.435888°S, 49.401611°W). Field Trial #2 was performed from 24 Mar 2014 through 19 Jun 2014 at Santo Antônio de Goiás, Goiás, Brazil (16.506825°S, 49.2851472°W). In both cases, genetically modified and conventional cultivars were arranged in a randomized block design with 3 replicate plots consisting of 4 rows (spacing 4 × 0.5 m). Treatments were seeded at 15 seeds per m<sup>2</sup> with the soil classified as a Typic Acric Oxisol in both locations. Plants were grown using recommended crop cultivation methods (Supplement Table 1).

Whiteflies were abundant at both field locations. The population of nymphs, adults, and virus incidence was higher in Field Trial #1 (Goianira), which was adjacent to a soybean field and a likely source of immigrating whiteflies and their viruses. Adults and nymphs in both locations were identified as *B. tabaci* biotype B, using molecular techniques based on the analysis of mitochondrial cytochrome oxidase I gene (mtCOI) (Quintela et al. 2016). No insecticides were applied for whitefly, although carbaryl at 72 g i.a. ha<sup>-1</sup> (Carbaryl Fersol® 480 SC, Fersol Corporation, Haili Guixi Chemical Pesticide Co., Jiangxi, China) and acephate at 375 g i.a. ha<sup>-1</sup> (Acefato Nortox®, Nortox Corporation, Arapongas, Paraná, Brazil) were sprayed for chrysomelid leaf beetle control (in Trials #1 and #2, respectively). Both insecticides were considered to have low efficacy for *B. tabaci* biotype B due to acquired insect resistance (E. D. Quintela, unpublished data). Phytophagous mites were controlled with abamectin at 7.2 g i.a. ha<sup>-1</sup> (Vertimec® 18 EC, Syngenta Corporation, North China Pharmaceutical Group Aino Co., Hebei, China) in both trials when whitefly samples were not taken.

## Whitefly Assessments

Assessments were conducted on 4 linear m in the 2 middle rows of each plot to minimize edge effects. Adult whiteflies were counted on the last fully expanded leaf (30 leaflets per plot). Sampling was performed in the morning and leaves were handled carefully to minimize dispersal. Nymphs were counted on 10 leaflets per plot. Leaflets (10 per plot) were held in a Gerbox-type box (11 × 11 × 3.5 cm) in a Styrofoam box, and samples were brought to the laboratory for processing using a stereoscope microscope (Leica EZ4, Wetzlar, Germany) at 40× magnification. Assessments were conducted approximately weekly over 35 and 31 d after plant emergence for the first and second trial, respectively. Plants were manually harvested 79 and 82 d after emergence for Trials #1 and #2, respectively, and the seeds were used for yield assessments.

## Virus Assessments

The incidence of Bean golden mosaic begomovirus and Cowpea mild mottle virus was evaluated visually in 2 m of the 2 central plot rows at 41 and 39 d after emergence for Trials #1 and #2, respectively.

Symptoms characteristic of Bean golden mosaic begomovirus were yellow-green mosaic, leaf curling, and stunted growth (Fig. 1A). Transgenic plants infected by Cowpea mild mottle virus showed crinkled leaves, often associated with vein enations visible as necrotic veins on abaxial leaf surfaces (Fig. 1B). In young inoculated plants, most common symptoms are a mild chlorosis along or between the veins, which may or may not be accompanied by a green banding of the veins (Fig. 1C). In addition, 10 leaves were selected randomly from each plot and taken to the laboratory for confirmation of the presence of Cowpea mild mottle virus via ELISA. An antiserum specific for Cowpea mild mottle virus was graciously supplied by Carvalho as well as the specific ELISA methodology (Carvalho et al. 2013b). The initial identification of the presence of Cowpea mild mottle virus was performed by electron microscopy (JEM 1011, JEOL USA, Inc., Peabody, Massachusetts, USA) and by serology (indirect ELISA) using antiserum produced by an isolate of Cowpea mild mottle virus infecting soybean plants (Faria et al. 2016). For conventional cultivars, the incidence of Bean golden mosaic begomovirus and Cowpea mild mottle virus was grouped because the single occurrence of each disease could not be distinguished visually (Fig. 1D).

## Statistical Analyses

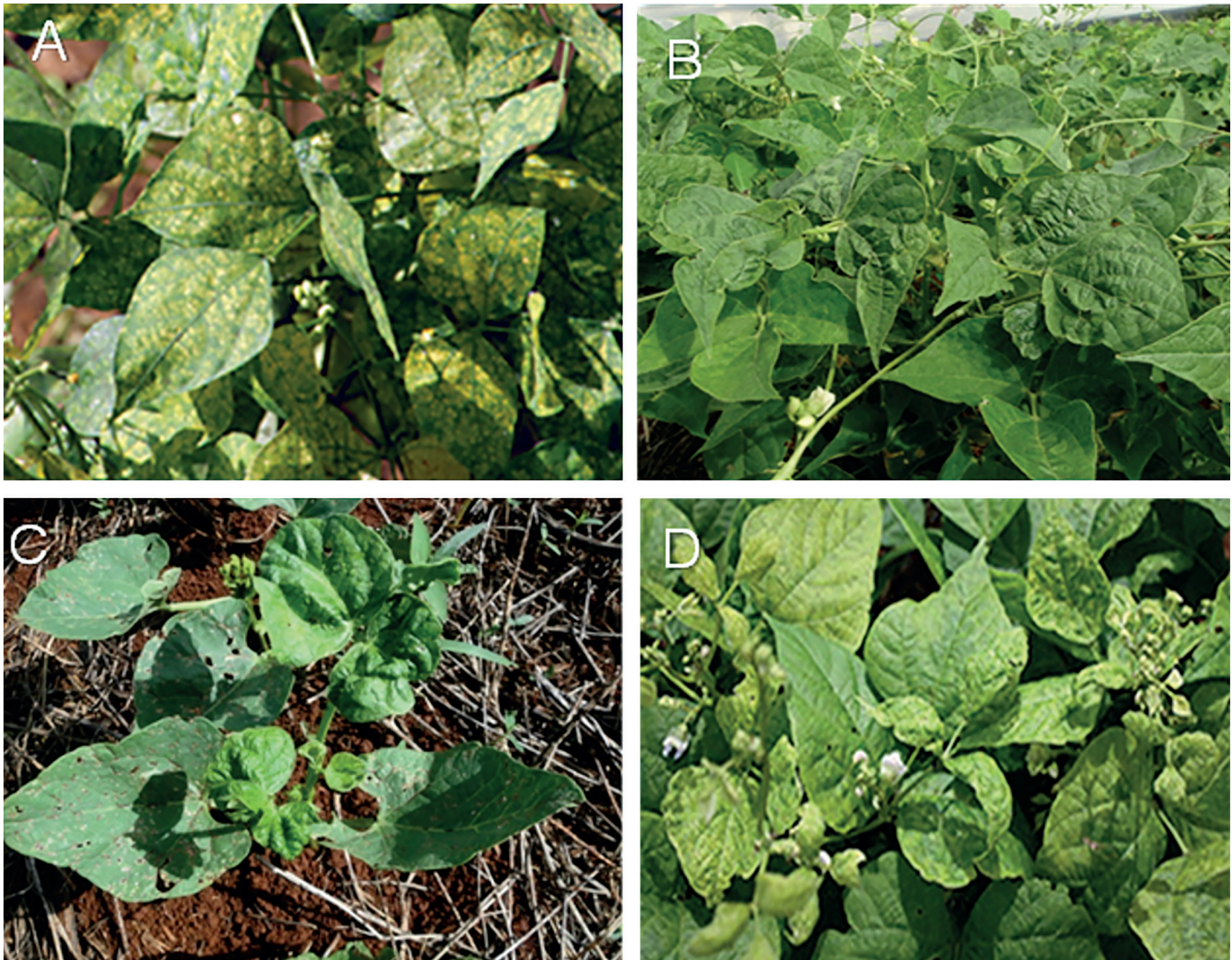
Data were checked for normality and homoscedasticity with Kolmogorov-Smirnov and Levene methods, respectively. Data for virus infected plants and whitefly numbers (grouped by sample date) were transformed into  $(x + 0.5)^{1/2}$  to meet the assumptions of analysis of variance (ANOVA). All data were submitted to ANOVA and means were grouped by the Scott-Knott method ( $P \leq 0.05$ ). The correlation between variables was determined by the Pearson test. The analysis was conducted with R Statistical Software, Version 3.1.2 (R Development Core Team 2018).

## Results

### Field Trial #1: Goianira, Goiânia, Brazil

Whitefly populations varied over the study, with adults decreasing over 35 d and nymphs (F1 generation) increasing with the plant growth cycle in the middle of the study (Table 2). The lower mean number of whitefly nymphs (mean 17.4 per leaflet) and higher number of adults (9.9 per leaflet) at 14 d after emergence, indicates that whiteflies were initiating the colonization of the plants at this date. Although found on all tested varieties, when averaged over sample dates, whitefly adults were significantly lower in 5 of the genetically modified elite lines (Table 2). The conventional commercial cultivars IPR Eldorado, BRB169, and CNFP15882 also had lower number of adults compared with the standard cultivars. The overall reduction observed for whitefly nymphs in genetically modified lines was more pronounced, with significantly fewer individuals on 7 out of 9 genetically modified lines tested, i.e., all except 16204 and 16209. The conventional cultivars IPR Eldorado, BRB169, and CNFP15882 also had lower numbers of nymphs. A positive correlation between whitefly adult and nymph populations was observed in both cases ( $r^2 = 0.64$ ;  $P < 0.054$  for genetically modified lines, and  $r^2 = 0.95$ ;  $P < 0.01$  for conventional cultivars).

No Bean golden mosaic virus symptoms were observed among genetically modified elite lines at 41 d after emergence. However, the presence of the Cowpea mild mottle virus was confirmed in all lines via ELISA test and by symptomatic plants. The incidence of Cowpea mild mottle virus was lower in genetically modified elite lines derived from Pérola compared to BRS Pontal, except for CNFCT 16208 (Table 3). Genetically modified lines derived from Pérola had a mean of 11.7 Cow-



**Fig. 1.** Symptoms of virus infection on common bean plants under field situation: (A) Bean golden mosaic virus; (B) Cowpea mild mottle virus on old transgenic plants and (C) young transgenic plant; (D) symptoms of mixed infection caused by Bean golden mosaic virus and Cowpea mild mottle virus.

pea mild mottle virus incidence compared with 67.9 mean incidence in genetically modified lines derived from BRS Pontal. The correlations between whitefly adults and nymphs with Cowpea mild mottle virus for genetically modified elite lines were not significant ( $r^2 = 0.16$ ;  $P < 0.6884$ , and  $r^2 = 0.32$ ;  $P < 0.3044$ , respectively).

Among conventional varieties, almost all plants ( $\geq 97.4$ ) in the cultivars Pérola and BRS Pontal were infected with Bean golden mosaic begomovirus or Cowpea mild mottle virus (Table 3). Virus-incidence was lower statistically in IPR Eldorado. The percentage virus incidence was lower among both black seeded varieties (BRB 169 and CNFP 15882) compared with all carioca varieties. The reductions probably were due to the lower number of adults and nymphs observed in IPR Eldorado and the black seeded cultivars (Table 2). There were positive correlations between the number of adult and nymphs with symptomatic plants (Bean golden mosaic begomovirus + Cowpea mild mottle virus) ( $r^2 = 0.82$ ;  $P < 0.01$ , and  $r^2 = 0.88$ ;  $P < 0.049$ , respectively).

Among genetically modified lines, there was no difference in seed yields for BRS Pontal and Pérola derived lines, except CNFCT 16204. Among conventional cultivars, seed yield was higher in black seeded varieties (BRB 169 and CNFP 15882) compared with carioca cultivars, with Pérola having the lowest yield.

A positive correlation ( $r^2 = 0.74$ ;  $P < 0.02$ ) was observed between Cowpea mild mottle virus-infected genetically modified and bean seed yield. Seed yield was higher in conventional cultivars BRB 169 and CNFP 15882 with less incidence of Cowpea mild mottle virus + Bean golden mosaic begomovirus compared with Pérola, BRS Pontal, and IPR Eldorado (Tables 3 & 4). Therefore, a negative correlation ( $r^2 = -0.95$ ;  $P < 0.0001$ ) was observed between seed yield and Cowpea mild mottle virus + Bean golden mosaic begomovirus incidence.

Seed weight expressed per 100-seed weight was significantly higher for genetically modified elite lines (ranging from 20.9 to 25.1 g, except for CNFCT16208 and CNFCT16209 at 18.0 and 19.9 g, respectively). By contrast, conventional cultivars had mean values from 16.3 to 18.8 g (Table 4).

#### Field Trial #2: Santo Antônio De Goiás, Goiânia, Brazil

Whiteflies and virus incidence was lower overall in the second trial (Table 5). However, like the first trial, adult whiteflies gradually declined ( $\leq 2.6$  per leaflet), while F1 nymphs increased over 31 d (reaching  $> 100$  per leaflet). Averaged over sample dates, adult whiteflies were lower in 5 of the genetically modified elite lines

**Table 2.** *Bemisia tabaci* biotype B (mean whiteflies per leaflet) on transgenic (Bean golden mosaic virus-resistant CNFCT lines 16201 to 16210) and conventional common bean lines at 4 sampling dates in Trial #1 (Feb to Mar 2014, Goianira, Goiás, Brazil).

Bean lines	Adult whitefly per leaflet <sup>1</sup>				Nymphs per leaflet <sup>1</sup>				Adults pooled <sup>1</sup>	Nymphs pooled <sup>1</sup>
	D after plant emergence									
	14	21	28	35	14	21	28	35		
16201	7.2 b	2.4 b	1.0	0.53	14.0 b	278.1 c	373.3 c	58.2 c	2.8 b	180.9 b
16203	8.4 b	2.6 b	0.7	0.73	20.9 a	372.8 b	542.2 b	38.1 c	3.1 b	243.5 b
16204	13.2 a	4.4 a	0.8	1.27	26.2 a	422.2 b	682.4 a	94.0 b	4.9 a	306.2 a
16205	7.1 b	3.2 b	1.1	0.70	13.4 b	411.4 b	692.7 a	60.3 c	3.0 b	294.5 b
16206	9.9 a	2.6 b	0.8	0.60	10.6 c	391.8 b	355.8 c	60.6 c	3.5 b	204.7 b
16207	10.1 a	3.0 b	1.1	0.63	13.5 b	448.3 b	470.3 b	46.1	3.7 b	244.5 b
16208	11.2 a	4.7 a	0.6	0.70	21.7 a	421.6 b	487.6 b	81.9 c	4.3 a	253.2 b
16209	11.8 a	5.4 a	0.7	0.83	21.6 a	463.5 b	745.7 a	68.9 c	4.7 a	324.9 a
16210	11.8 a	4.3 a	1.0	0.77	19.8 a	565.3 a	318.7 c	102.3 b	4.5 a	251.5 b
Pérola	12.3 a	3.8 a	1.0	1.30	14.5 b	643.0 a	814.6 a	140.4 a	4.6 a	403.1 a
BRS Pontal	10.3 a	4.1 a	1.0	0.87	15.5 b	609.7 a	849.1 a	190.1 a	4.1 a	416.1 a
IPR Eldorado	8.1 b	3.1 b	0.6	0.87	17.9 a	423.5 b	581.2 a	54.0 c	3.2 b	269.2 b
BRB 169	9.20 b	3.1 b	0.7	0.53	17.3 a	378.5 b	603.0 a	88.0 c	3.4 b	271.7 b
CNFP 15882	6.80 b	2.1 b	0.6	0.93	9.7 c	278.2 c	428.3 c	52.4 c	2.6 b	192.2 b
CV (%)	23.4	33.3	36.8	38.9	27.9	19.9	33.4	49.0	56.5	68.3
F	5.80	4.40	1.02	1.45	9.05	1.62	6.52	5.61	2.52	4.03
P	0.0001	0.0001	0.431	0.132	0.0001	0.0001	0.0001	0.0001	0.001	0.0001

<sup>1</sup>Means followed by the same letter in the table columns are not significantly different by the Scott-Knott method ( $P < 0.05$ ).

and 2 conventional cultivars (IPR Eldorado and CNFP15882) (Table 5). Lower numbers of nymphs, overall, was observed for 7 out of 9 of the genetically modified elite cultivars. There was no significant correlation between adult and immature whitefly numbers among all cultivars ( $r^2 = 0.57$ ;  $P < 0.09$ ).

While no genetically modified elite lines showed Bean golden mosaic begomovirus symptoms, Cowpea mild mottle virus symptoms were observed to varying degrees (Table 3). Similar to the first trial, Cowpea mild mottle virus incidence was lower in Pérola derived lines (CNFCT 16201 to CNFCT 16204) when compared to BRS Pontal lines. Positive

**Table 3.** Mean infected plants (%) by incidence Cowpea mild mottle virus in transgenic Bean golden mosaic virus-resistant common bean elite lines (CNFCT 16201 to 16210) or Bean golden mosaic virus- + Cowpea mild mottle virus in conventional lines.

Genetically modified bean lines (CNFCT)	Trial #1 – Goianira 41 d after emergence <sup>1</sup>	Trial #2 – Santo Antonio de Goiás 39 d after emergence <sup>1</sup>
16201	7.6 d	2.0 d
16202	– <sup>2</sup>	1.0 d
16203	15.5 c	12.8 c
16204	12.1 c	8.7 c
16205	76.4 a	41.9 a
16206	49.2 b	29.3 b
16207	99.2 a	52.7 a
16208	19.0 c	19.3 b
16209	82.1 a	27.8 b
16210	81.9 a	20.7 b
CV	22.4	26.41
F	34.0	9.51
P	0.0001	0.0001
Cowpea mild mottle virus + Bean golden mosaic virus		
Pérola	100.0 a	76.1 a
BRS Pontal	97.4 a	78.2 a
IPR Eldorado	65.5 b	60.5 b
BRB 169	18.0 c	9.6 d
CNFP 15882	27.4 c	17.2 c
CV	17.31	10.40
F	25.70	95.34
P	0.0001	0.0001

<sup>1</sup>Means followed by the same letter in columns are not significantly different (Scott-Knott method,  $P < 0.05$ ).

<sup>2</sup>Not tested.

**Table 4.** Seed (yield and weight) for transgenic Bean golden mosaic virus-resistant common bean elite lines (CNFCT 16201 to 16210) and conventional common bean lines (Pérola, BRS Pontal, IPR Eldorado, BRB 169, CNFP 15882) in 2 field trials carried out in Brazil in the dry growing season of 2014.

Bean line (CNFCT)	Trial #1 – Goianira		Trial #2 – Santo Antonio de Goiás	
	Seed yield <sup>1,2</sup> (Kg per ha)	W100 <sup>1,2</sup> (g)	Seed yield <sup>1,2</sup> (Kg per ha)	W100 <sup>1,2</sup> (g)
16201	913 a	23.1 a	1,897 b	23.5 a
16202	ND <sup>3</sup>	ND <sup>3</sup>	2,186 b	22.5 b
16203	771 a	20.9 a	1,955 b	21.9 b
16204	476 b	22.3 a	1,546 c	24.2 a
16205	1261 a	25.1 a	2,460 a	21.5 b
16206	947 a	23.0 a	2,170 b	24.1 a
16207	991 a	23.9 a	2,086 b	19.6 c
16208	793 a	18.0 b	1,804 b	20.5 b
16209	1090 a	19.9 b	2,105 b	21.4 b
16210	1056 a	24.7 a	2,495 a	23.7 a
Pérola	69 c	ND	510.0 e	17.1 d
BRS Pontal	315 b	18.8 b	1,100 d	17.6 d
IPR Eldorado	396 b	17.4 b	1,306 d	17.3 d
BRB 169	789 a	17.2 b	2,074 b	17.6 d
CNFP 15882	613 a	16.3 b	1,533 c	17.5 d
CV (%)	16.2	7.7	11.5	4.4
F	6.44	10.97	19.6	25.9
P	0.0001	0.0001	0.0001	0.0001

<sup>1</sup>Means followed by the same letter in the table columns are not significantly different by the Scott-Knott method ( $P < 0.05$ ).

<sup>2</sup>Seed yield and 100-seed weight (W100) corrected to 13% moisture content.

<sup>3</sup>Not determined.

correlations were observed between nymphs and adults with Cowpea mild mottle virus-infected genetically modified ( $r^2 = 0.65$ ;  $P < 0.0408$ , and  $r^2 = 0.64$ ;  $P < 0.0455$ , respectively). Also similar to the first trial, among conventional varieties, black class seeds (BRB 169 and CNFP15882) had significantly lower virus incidence compared with the carioca commercial class. The proportion of virus-infected plants was again lower in IPR Eldorado compared to Pérola and BRS Pontal. No significant correlations were observed between adults with Bean golden mosaic begomovirus + Cowpea mild mottle virus-infected plants ( $r^2 = 0.15$ ;  $P < 0.8149$ ).

Seed yields followed broadly similar patterns to Trial #1. Highest yields (reaching about 2,500 Kg per ha) occurred from elite lines 16205 and 16210 when compared to other genetically modified lines and conventional cultivars (Table 4). Lowest yields were observed among CNFCT 16204, with no significant yield differences among other genetically modified elite lines. No correlation was observed between Cowpea mild mottle virus-infected genetically modified and seed yield ( $r^2 = 0.42$ ;  $P < 0.224$ ).

Among conventional cultivars, seed yield again was highest from black seeded BRB 169 and lowest for Pérola. Seed yield for BRS Pontal and IPR Eldorado was similar. No significant correlation was observed between Cowpea mild mottle virus-infected genetically modified and seed yield ( $r^2 = 0.42$ ;  $P < 0.224$ ). Seed yield of conventional cultivars was negatively correlated with Bean golden mosaic begomovirus + Cowpea mild mottle virus incidence ( $r^2 = -0.87$ ;  $P < 0.001$ ).

Seed weight was significantly higher for genetically modified elite lines compared with conventional cultivars (Table 4). Among the genetically modified lines, CNFCT 16201, 16204, 16206, and 16210 exhibited the highest seed weight (from 23.5 to 24.2 g) and CNFCT 16207 the lowest (19.6 g). Among the 5 conventional control cultivars, the mean values of 100-seed weight ranged from 17.1 to 17.6 g.

#### Comparisons between Transgenic Genetically Modified Elite Lines and Conventional Cultivars

When comparing seed yield, Bean golden mosaic begomovirus-resistant genetically modified lines outperformed their conventional

recurrent parent lines (BRS Pontal and Pérola) in both field trials, i.e., relative yield increase between 199 and 1,045% (Table 6). In some cases, this reduction was probably related to high virus titer affecting plant development. For example, in Trial #1 with higher whitefly pressure, 100% of conventional Pérola-cultivars were infected (versus 11.7% in genetically modified line), yielding > 10% of the genetically modified line seed yield. Seed yield in Trial #2 was relatively higher in Trial #2, possibly due to the lower virus and whitefly incidence in the second trial (Table 4).

In both trials, the average incidence of Cowpea mild mottle virus was significantly higher in genetically modified elite lines derived from BRS Pontal when compared to Pérola (Table 6). However, seed yield was higher for genetically modified elite lines derived from BRS Pontal.

While no overall numerical difference in abundance of whitefly adults was noted between genetically modified lines and recurrent parents, it is noteworthy that the overall abundance of nymphs was statistically lower on respective genetically modified lines, i.e., by about 40% in Trial #1, and 14 to 21% in Trial #2 (Table 6).

## Discussion

Earlier we hypothesized that the Cowpea mild mottle virus Carla-virus in common bean plants may have been misdiagnosed as Bean golden mosaic begomovirus, and that the presence of Bean golden mosaic begomovirus-resistant bean plants may provide a pathway for the spread of Cowpea mild mottle virus (Faria et al. 2016; Souza et al. 2018).

The present study showed that while genetically modified elite lines were resistant to Bean golden mosaic begomovirus, most were not resistant to Cowpea mild mottle virus. While Cowpea mild mottle virus was observed in all genetically modified elite lines under high whitefly pressure, there were differences among varieties. In particular, higher infection rates occurred in genetically modified elite lines derived from BRS Pontal versus Pérola. It is possible that Cowpea mild

**Table 5.** Mean number of adults of *Bemisia tabaci* biotype B on transgenic Bean golden mosaic virus-resistant common bean elite lines (CNFCT 16201 to 16210) and on conventional common bean lines (Pérola, BRS Pontal, IPR Eldorado, BRB 169, CNFP 15882) at 4 sampling dates. Field Trial #2, Santo Antonio de Goiás, Goiânia, Goiás, Brazil.

Bean lines	Nymphs per leaflet <sup>1</sup>												Adults pooled <sup>1</sup>	Nymphs pooled <sup>1</sup>
	Adult whitefly per leaflet <sup>1</sup>				D after emergence				Nymphs per leaflet <sup>1</sup>					
	5	11	17	25	11	17	25	31	25	31	31	31		
16201	2.13	1.23 b	0.90	0.03 b	22.83 c	67.40 b	76.30 b	58.53 b	1.07 b	56.27 b				
16202	1.77	0.83 b	0.70	0.13 b	22.33 c	44.27 c	73.17 b	57.07 b	0.85 b	49.21 b				
16203	1.57	1.80 a	1.03	0.13 b	22.20 c	50.53 c	92.20 a	64.00 b	1.13 b	57.23 b				
16204	1.93	1.17 b	1.30	0.23 b	27.93 c	82.47 a	98.93 a	67.63 b	1.16 b	69.24 a				
16205	2.07	1.80 a	1.43	0.40 a	17.77 c	74.13 b	101.27 a	110.73 a	1.42 a	75.97 a				
16206	1.63	1.20 b	1.27	0.47 a	24.30 c	54.73 c	100.47 a	79.40 b	1.14 b	64.72 b				
16207	1.44	1.97 a	1.57	0.30 a	20.37 c	38.73 c	106.27 a	102.50 a	1.32 a	66.97 b				
16208	1.80	1.40 b	1.53	0.43 a	30.40 b	44.83 c	93.63 a	73.47 b	1.29 a	60.58 b				
16209	2.07	2.23 a	1.57	0.37 a	32.33 b	59.33 b	103.23 a	86.60 a	1.56 a	70.37 a				
16210	1.77	1.90 a	1.77	0.47 a	32.97 b	35.33 c	82.63 b	58.00 b	1.47 a	52.23 b				
Pérola	2.47	1.60 b	1.20	0.30 a	39.87 a	60.77	89.03 a	104.30 a	1.39 a	73.49 a				
BRS Pontal	2.60	1.40 b	1.77	0.33 a	43.73 a	49.40	5.63 d	114.47 a	1.52 a	75.81 a				
IPR Eldorado	2.10	1.57 b	1.20	0.17 b	33.83 b	94.67 a	87.17 a	68.23 b	1.26 b	70.97 a				
BRB 169	2.00	2.03 a	1.73	0.47 a	26.17 c	78.43 a	103.40 a	126.20 a	1.56 a	83.55 a				
CNFP 15882	1.87	1.13 b	1.33	0.33 a	21.77 c	46.83	57.40 c	79.77 b	1.17	51.74 b				
CV (%)	34.55	37.41	38.21	30.71	28.89	29.34	21.31	32.09	41.17	36.88				
F	1.10	1.99	1.66	1.82	6.28	8.26	4.22	5.89	2.47	5.39				
P	0.3521	0.017	0.06	0.0342	0.0001	0.0001	0.0001	0.0001	0.0018	0.0001				

<sup>1</sup>Day after plant emergence.

<sup>2</sup>Means followed by the same letter in the table columns are not significantly different by the Scott-Knott method ( $P < 0.05$ ).



**Table 6.** Whiteflies, viruses (% Cowpea mild mottle virus and Bean golden mosaic virus), seed yield (kg ha<sup>-1</sup>), and yield performance for transgenic (Bean golden mosaic virus-resistant) common bean elite lines compared to conventional recurrent parents (Pérola and BRS Pontal), observed in 2 field trials in Brazil.

Recurrent parent	Trial #1 — Goiânia					Trial #2 — Santo Antônio de Goiás				
	Adults <sup>2,3</sup>	Nymphs <sup>2,3</sup>	Virus incidence <sup>3,4</sup>	Yield (kg ha <sup>-1</sup> ) <sup>3</sup>	Relative yield increase (%) <sup>5</sup>	Adults <sup>2,3</sup>	Nymphs <sup>2,3</sup>	Virus incidence <sup>3,4</sup>	Yield (kg ha <sup>-1</sup> ) <sup>3</sup>	Relative yield increase (%) <sup>5</sup>
Genetically modified lines derived from Pérola	1.7 a	242.5 b	11.7 c	720.1 b	1,045	1.0 b	58.0 c	6.1 c	1,896 b	372
Genetically modified lines derived from BRS Pontal	1.8 a	262.2 b	67.9 b	1023.0 a	325	1.4 a	65.4 b	30.7 b	2,187 a	199
Pérola (conventional)	2.0 a	403.1 a	100.0 a	68.9 d	—	1.4 a	73.5 a	76.0 a	510 d	—
BRS Pontal (conventional)	1.9 a	416.1 a	97.4 a	315.2 c	—	1.5 a	75.8 a	78.2 a	1,100 c	—
F	1.3 ns*	9.3**	6.8**	14.7**	—	7.1**	7.7**	37.0**	35.3**	—
CV (%)	57.6	68.5	34.42	25.5	—	41.5	37.2	25.9	15.8	—

<sup>1</sup>Three genetically modified lines derived from Pérola (CNFCT16201, CNFCT16203, CNFCT16204) and 5 genetically modified lines derived from BRS Pontal (CNFCT16205, CNFCT16206, CNFCT16207, CNFCT16208, CNFCT16209).

<sup>2</sup>Mean number of whitefly nymphs or adults per leaflet on 4 samplings dates.

<sup>3</sup>Means followed by the same letter in the column are not different (Scott-Knott,  $P \leq 0.05$ )

\*Not significant. \*\*Significance  $P \leq 0.001$ .

<sup>4</sup>Only Cowpea mild mottle virus was determined in genetically modified elite lines because they are resistant to Bean golden mosaic virus. For conventional cultivars, the incidence of Bean golden mosaic virus and Cowpea mild mottle virus was grouped.

<sup>5</sup>Relative yield performances estimated based on the yield performances of the recurrent parents BRS Pontal or Pérola.

mottle virus incidence in Pérola lines may have been underestimated due to their milder mosaic symptoms. In BRS Pontal-derived lines, Cowpea mild mottle virus is easily visible from leaf crinkling symptomatology. Among conventional varieties, the IPR Eldorado and the black seeded lines are claimed to be partially tolerant to golden mosaic (Bianchini 2010). This claim was substantiated in our trial although seed yield and weight for IPR Eldorado were similar to BRS Pontal.

Two genetically modified lines (16205 and 16210) were consistently the most productive (seed yield) in our trials. Similar findings were reported in 31 field trials carried out in different growing areas in Brazil (Souza et al. 2018). Line CNFCT 16205 has been registered in Brazil as cultivar BRS FC401 RMD based on seed quality traits for yield and virus resistance (Souza et al. 2018).

Most virus epizootics are initiated soon after whiteflies enter the crop (Hirano et al. 1993). Therefore, many growers in Brazil apply seed treatments and foliar insecticides for whitefly control when plants are at the V3–V4 phenological stage and preferred by adults. However, use of insecticides alone for vector control does not avoid virus transmission. One reason for the failure of insecticides to prevent non-persistently transmitted viruses is that the vector can transmit the virus more quickly than it succumbs to the insecticide (Cohen & Berlinger 1986). Thus, the aim of insecticides is to limit the increase in number of vectors in the crop. However, insecticides may fail when there is continuing migration of new viruliferous vectors. This latter point was shown in 5 previous field trials with common bean (Santos 2019). In trials with BRS FC401 RMD, seed treatment (neonicotinoid) followed by 5 or 6 insecticide applications starting at 80% seedling emergence delayed Cowpea mild mottle virus transmission but did not avoid virus transmission (100% of infected plants in Jan to Feb planting dates, the dry season).

Field surveys in central and southern Brazil indicate that whitefly transmitted viruses are an increasing threat (Quintela et al. 2016). Whitefly populations may be most severe in common bean following the harvest of soybean or dry bean host crop, resulting in “clouds of adult whiteflies” which contain viruliferous individuals. In recent yr, whiteflies and viruses in common beans have been observed in both the summer (Nov to Dec) and winter (Apr to Jul) crop seasons (Faria et al. 2016). Whiteflies may have benefited from increased soybean cultivation and the recent observation of whitefly reproduction in corn (in Feb to Mar) that aids the season-long survival of the pest (Quintela et al. 2016).

Differences in whitefly preference have been reported among common bean cultivars (Jesus et al. 2010; Pinheiro et al. 2014; Silva et al. 2014). In our trials, fewer adult whiteflies were observed on some genetically modified elite lines and conventional cultivars, which may have resulted in fewer nymphs and virus-infected plants. Moreover, some studies show a preference and performance (fitness) benefit for *B. tabaci* feeding on Begomovirus-infected plants (Jiu et al. 2007; Luan et al. 2013). In our experiments, we observed preference for *B. tabaci* on conventional cultivars infected by both Bean golden mosaic begomovirus and Cowpea mild mottle virus when compared with genetically modified lines that were infected only by Cowpea mild mottle virus. Further studies are needed to determine these various interactions.

In summary, this research highlights that tested elite bean cultivars resistant for Bean golden mosaic begomovirus are susceptible to Cowpea mild mottle virus. Because conventional breeding programs have developed and identified carioca germplasm with potential resistance to Cowpea mild mottle virus (Faria et al. 2016; Souza et al. 2018), specific crosses were carried out at Embrapa Rice and Beans Station aiming to develop a “second generation” of genetically modified cultivars with multiple resistance to viruses (Bean golden mosaic begomovirus, Bean golden mosaic begomovirus + Cowpea mild mottle virus). As a first and

solid result, 10 genetically modified carioca seeded elite lines were obtained and have been being tested since 2019 for general agronomic performance in final field trials carried out in the principal Brazilian growing regions. These lines already have shown grain commercial quality according to the requirement of Brazilian consumer market, high yield potential and stability, in addition to adaptation to direct mechanical harvesting.

From these lines will be selected the next cultivar to be released and used as an important tool for the integrated management of the viruses transmitted by the whitefly in the common bean crop.

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