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Article

Tolerance of DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 Soybeans to 2,4-D and Glyphosate in the Cerrado Region of Brazil

Tolerância da Soja DAS-444Ø6-6 e DAS-444Ø6-6 x DAS-81419-2 ao 2,4-D e Glifosato na Região do Cerrado Brasileiro

KALSING, A.[1]*
LUCIO, F.R.[1]
ROSSI, C.V.S.[1]
RAMPAZZO, P.E.[1]
GONÇALVES, F.P.[1]
VALERIANO, R.[1]

ABSTRACT - The DAS-444Ø6-6 soybean expresses AAD-12, 2mEPSPS and PAT enzymes to provide, respectively, tolerance to 2,4-D, glyphosate and ammonium glufosinate. This event is also associated with DAS-81419-2 expressing Cry1Ac and Cry1F proteins to provide protection against a broad spectrum of soybean lepidopteran insect species. Successful adoption by growers of these technologies relies on the traits performing in the diverse environments of Brazil, especially under adverse weather conditions. The objective of this study was to characterize the tolerance of DAS 444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans to 2,4-D and glyphosate in the Cerrado region of Brazil. Eight trials across four sites were conducted in the 2015/2016 summer rainy season using a randomized complete block design, with a factorial arrangement of treatments. Factor A was 2,4-D choline salt (456 g a.e. L^{-1}) + glyphosate DMA salt (480 g a.e. L^{-1}) at 975 + 1,025 and 1,950 + 2,050 g a.e. ha^{-1}, respectively and ready-mix of (2,4-D choline salt 195 g a.e. L^{-1} + glyphosate DMA salt 205 g a.e. L^{-1}) at 2,000 (975 + 1,025) and 4,000 (1,950 + 2,050) g a.e. ha^{-1}. Factor B was application timing at V3, V6 and R2 soybean growth stages. No visible symptoms of leaf chlorosis, epinasty, or growth inhibition were observed. Slight leaf droop was observed at 1 day after treatment (DAT), but it was null at 7 DAT. Less than 6% and 13% of leaf necrosis or injury were observed at 7 DAT, respectively, at 975 + 1,025 and 1,950 + 2,050 g a.e. ha^{-1} of 2,4-D choline salt + glyphosate DMA salt, regardless of applied as a ready-mix or not, but it was always 2% or less at 28 DAT. Crop grain yield was not influenced by herbicide treatments, application timing and their interaction, regardless of the soybean event and experimental site. DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans showed high tolerance to 2,4-D choline salt and glyphosate DMA in the Cerrado region of Brazil.

Keywords: 2,4-Dichlorophenoxyacetic acid, aryloxyalkanoate dioxygenase-12, Conkesta™ + Enlist E3™, Enlist E3™, *Glycine max*, injury.

RESUMO - A soja DAS-444Ø6-6 expressa as enzimas AAD-12, 2mEPSPS e PAT, conferindo, respectivamente, tolerância aos herbicidas 2,4-D, glifosato e glufosinato de amônio. Esse evento também pode ser combinado à soja DAS-81419-2, que expressa as enzimas Cry1Ac e Cry1F e confere resistência a amplo espectro de insetos lepidópteros. A adoção bem-sucedida dessas tecnologias requer eventos biotecnológicos que atuem nas diferentes regiões climáticas do país, sobretudo sob condições ambientais adversas. O objetivo deste estudo foi caracterizar a tolerância das sojas DAS 444Ø6-6 e DAS-444Ø6-6 x DAS-81419-2 aos herbicidas 2,4-D e glifosato na região do cerrado brasileiro. Oito ensaios foram realizados

*** Corresponding author:**
<Akalsing@dow.com>

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[1] Field Scientist, Crop Protection Research and Development, Dow AgroSciences Industrial Ltda., São Paulo-SP, Brazil.

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em campo em quatro locais durante a safra 2015/2016, usando-se delineamento de blocos ao acaso com tratamentos em esquema fatorial. O fator A foi 2,4-D sal colina (456 g e.a. L^{-1}) + glifosato sal DMA (480 g e.a. L^{-1}) nas doses de 975 + 1.025 e 1.950 + 2.050 g e.a. ha^{-1}, e a mistura formulada de 2,4-D sal colina (195 g e.a. L^{-1}) + glifosato sal DMA (205 g e.a. L^{-1}) nas doses de 2.000 (975 + 1.025) e 4.000 (1.950 + 2.050) g e.a. ha^{-1}. O fator B foi a aplicação nos estádios V3, V6 e R2. Não se observou clorose, epinastia e inibição de crescimento nas plantas de soja. Foi observado leve murchamento um dia após o tratamento (DAT), mas não aos 7 DAT. O 2,4-D sal colina + glifosato sal DMA nas doses de 975 + 1.025 e 1.950 + 2.050 g e.a. ha^{-1} causou, respectivamente, menos de 6 e 13% de necrose ou injúria aos 7 DAT, e menos de 2% na avaliação realizada aos 28 DAT, independentemente da formulação aplicada. O rendimento de grãos não foi afetado pelos fatores testados ou pela sua interação, independentemente da composição de eventos da soja e da localização da área experimental. A soja DAS-444Ø6-6, combinada ou não com a DAS-81419-2, mostrou elevada tolerância ao 2,4-D sal colina e glifosato sal DMA na região do cerrado no Brasil.

Palavras-chave: 2,4-Diclorofenoxiacetato, ácido, ariloxialcanoato dioxigenase-12, Conkesta™ + Enlist E3™, Enlist E3™, *Glycine max*, injúria.

INTRODUCTION

Soybean, *Glycine max* (L.) Merrill, has considerable economic importance in the Cerrado region of Brazil, where it was grown in 20 million hectares in the 2015/2016 season (USDA, 2016). In this region, glyphosate-resistant cultivars were highly adopted by soybean growers, gaining widespread acceptance by providing a convenient weed control system. However, extensive use of this herbicide as a "one-size-fits-all" approach led to selection by eight glyphosate-resistant weeds from 2003/2004 to 2017/2018 seasons (Heap, 2018). In addition, the same approach has induced significant biotype shifts to weed species with inherent tolerance to glyphosate (Marchi et al., 2013; Takano et al., 2013). Glyphosate-resistant and tolerant weeds are increasingly prevalent in soybean fields, and a diverse weed control system is needed to sustain soybean cropping in the Cerrado.

The 2,4-D (2,4-Dichlorophenoxyacetic) was the first organic and selective herbicide developed in the world, and since the 1970s it has been used in agriculture in more than 100 countries. Over the last decade, the discovery of genes from soil bacteria confering tolerance to 2,4-D and their transfer to crops have enabled the development of crops tolerant to this herbicide. 2,4-D-tolerant soybeans cultivars have been developed by insertion of the aryloxyalkanoate dioxygenase-12 (*aad-12*) gene from *Delftia acidovorans* (Wright et al., 2010). The AAD-12 enzyme can metabolize 2,4-D by a rapid, single step, metabolic detoxification mediated by an Fe(II)/α-ketoglutarate-dependent dioxygenase (Griffin et al., 2013). The introduction of AAD-12 soybean will cause changes in 2,4-D using and allow its pre and post-emergence spray to control broadleaf weeds on soybean (Robinson et al., 2012).

The DAS-444Ø6-6 soybean is the first triple-gene herbicide tolerant technology (Enlist E3™) that expresses AAD 12, 2-mutant 5-enolpyruvyl shikimate-3-phosphate synthase (2mEPSPS) and phosphinothricin acetyltransferase (PAT) enzymes, which provide, respectively, tolerance to 2,4-D, glyphosate and ammonium glufosinate herbicides (Lepping et al., 2013). In Brazil, this novel herbicide trait package is also associated with the soybean event DAS-81419-2 (Conkesta™), which consists of insect-resistant technology that expresses Cry1Ac and Cry1F proteins and provides protection against a broad spectrum of soybean lepidopteran insect species (Fast et al. 2015; Marques et al., 2016). The combination of these advanced technologies will provide Brazilian soybean growers with a convenient and more diverse system for management of both weeds and insects.

Herbicide-resistant crops treated with the associated herbicides at situations of high doses or in adverse weather conditions have eventually shown reduction on their performance parameters. Glyphosate-resistant soybean had a significant reduction in root, shoot, and nodule biomass when glyphosate was applied at 1,200 or 2,400 g a.e. ha^{-1} (Zobiole et al., 2012). Moreover, drought stress condition following two 1,680 g a.e. ha^{-1} glyphosate treatments to glyphosate-resistant soybean reduced crop grain yield by 12 to 25% (King et al., 2001). Thus, successful

adoption by growers of DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans events relies on the traits performing in the diverse climates conditions. In particular, these technologies need to be evaluated in the Cerrado region of Brazil, where drought and high temperature might often cause intense plant stress on soybean crops.

Therefore, the objective of this research was to characterize the tolerance of DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans after foliar application of 2,4-D and glyphosate in the Cerrado region of Brazil.

MATERIALS AND METHODS

Eight trials were conducted under conventional field conditions during the 2015/2016 summer rainy season, at four different sites distributed across the Cerrado region of Brazil (Table 1). In each site, two trials were equally conducted side by side, testing the same list of herbicide treatments, with the composition of soybean events being the only difference between trials. The selection of sites was based on the commercial importance of soybean production and reflected the environmental conditions normally found in the Brazilian Cerrado. Air temperature and rainfall rate were monitored by automated remote weather stations, and Figure 1 shows the data collected daily for each site throughout the season. The trials always followed strict adherence to regulatory requirements of Brazil and were conducted at accredited certified sites containing Quality Certificate Biosafety.

The soybean cultivars used in the trials come from breeding programs of Sementes Don Mário (GDM) and Tropical Melhoramento e Genética Ltda. (TMG) for the Brazilian Cerrado (Table 2). These cultivars contained the soybean event DAS-444Ø6-6 (Enlist E3™) and its stack with DAS-81419-2 (Conkesta™) (Dow AgroSciences LLC, Indianapolis, IN). The fields were planted in all situations at row spacing of 45 cm, and in-row spacing from 6 to 7 cm, with seed rate ranging from 320,000 to 360,000 seeds ha^{-1}. Agronomic practices and inputs used for crop establishment and maintenance were those recommended for the crop in the Central region of Brazil (Embrapa, 2011). The trials relied on natural rainfall coming from the summer rainy season, and artificial irrigation was not implemented even in periods of severe water deficit stress.

The trials were arranged in a complete randomized block design and the treatments were arranged as a factorial (4 x 3), using four replications, except one case, which had three replications. Factor A was four post-emergence herbicide treatments: 2,4-D choline salt

Table 1 - Experimental sites and three soil characteristics

Site	Latitude	Longitude	Soil Type	Organic matter	pH
Indianópolis, MG	18°57'08.60"	47°51'08.90"	Clay	3.4%	6.0
Rio Verde, GO	17°45'33.80"	51°02'04.60"	Sandy	2.2%	5.1
Rondonópolis, MT	16°23'17.23"	54°30'39.74"	Clay	4.2%	6.3
Sorriso, MT	12°27'31.50"	55°49'40.20"	Clay	4.2%	5.5

Table 2 - Soybean cultivars and their maturation group

Site	Soybean event	Cultivar code[(1)]	Maturate group
Indianópolis, MG	DAS-444Ø6-6	GDM 1	5.8
	DAS-444Ø6-6 x DAS-81419-2	GDM 2	6.0
Rio Verde, GO	DAS-444Ø6-6	TMG 1	5.6
	DAS-444Ø6-6 x DAS-81419-2	TMG 2	5.6
Rondonópolis, MT	DAS-444Ø6-6	TMG 3	5.8
	DAS-444Ø6-6 x DAS-81419-2	TMG 4	5.8
Sorriso, MT	DAS-444Ø6-6	TMG 5	5.8
	DAS-444Ø6-6 x DAS-81419-2	TMG 6	5.8

[(1)] GDM (Sementes Don Mário); TMG (Tropical Melhoramento e Genética Ltda.).

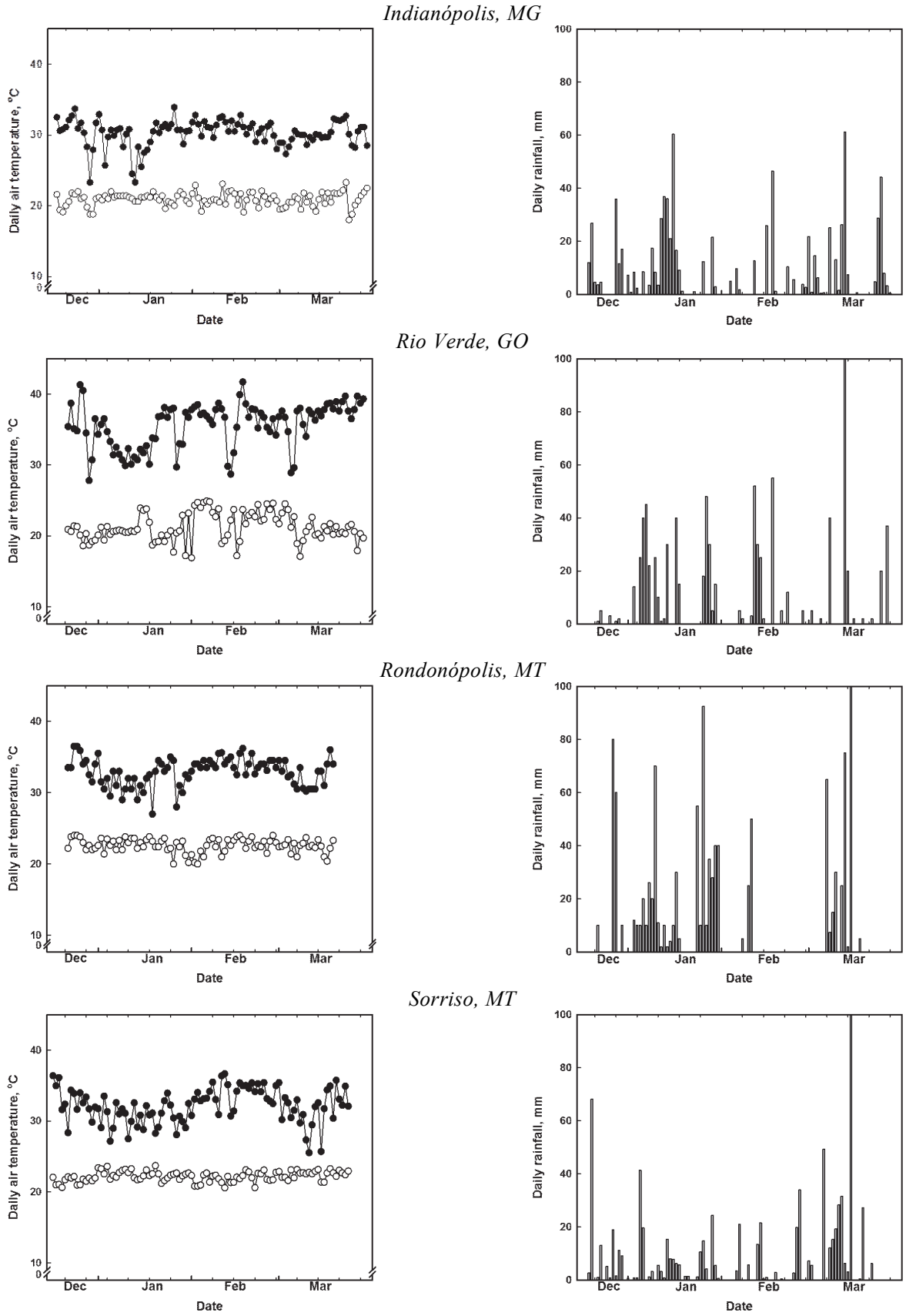

Figure 1 - Daily air temperature[1] and natural rainfall rate along the season observed in Indianópolis (MG), Rio Verde (GO), Rondonópolis (MT) and Sorriso (MT).

(456 g a.e. L^{-1}) + glyphosate dimethylamine (DMA) salt (480 g a.e. L^{-1}) at 975 + 1,025 and 1,950 + 2,050 g a.e. ha^{-1}, respectively; and, ready-mix formulation of (2,4-D choline salt 195 g a.e. L^{-1} + glyphosate DMA salt 205 g a.e. L^{-1}) at 2,000 (975 + 1,025) and 4,000 (1,950 + 2,050) g a.e. ha^{-1}. In addition, an untreated-check plot was added as a standard of comparison of the herbicides, which was permanently maintained weed free through periodic manual weeding. The twice rate (1,950 + 2,050 g a.e. ha^{-1}) was included to determine the extent of tolerance enabled by DAS-444Ø6-6 event in a field situation in which overlap application occurs. Factor B was three application timings at V3 (three trifoliate leaves), V6 (six trifoliate leaves) and R2 (full flowering) growth stages, according to Fehr and Caviness (1977). The treatments were always applied in a water carrier with volume of 100 L ha^{-1} at 25-40 PSI using a CO_2 backpack sprayer with a 3.0 m boom using AIXR 110.015 flat-fan nozzles.

Visible leaf chlorosis, epinasty, necrosis, growth inhibition, drooping and overall injury were assessed separately, from 0 (no injury) to 100% (complete plant death), according to the scale of Camper (1986). These herbicide symptoms were assessed separately to improve the assessments of the evaluators and detect eventual differential behavior in their magnitude along the evaluations. Visible drooping symptom was assessed at 1 and 7 days after herbicide treatment (DAT), while the other explanatory variables of injury were assessed at 7, 14 and 28 DAT. Also, crop grain yield and grain moisture were assessed at soybean maturity by harvesting the two central rows sprayed and adjusting grain mass to 13% of moisture. Data were subjected to analysis of variance by the F-test for significant effects ($p<0.05$) and Tukey's pairwise comparison test for mean separation of the tested treatments. The tested factors and their interaction were considered as fixed effects and locations as a random variable because there was homogeneity of error variances (Zimmermann, 2004).

RESULTS AND DISCUSSION

The data collected on weather conditions indicate that plants possibly suffered periods of stress during the season, especially high air temperature (>35 °C) at the four experimental sites (Figure 1). Nevertheless, crop injuries were null or low regardless of herbicide treatment and application timing, as well as soybean event and experimental site. In fact, no leaf chlorosis, epinasty, or growth inhibition were observed, while there was slight leaf droop at 1 day after treatment (DAT), but it was null at 7 DAT (Table 3). The 2,4-D choline salt + glyphosate DMA salt at 975 + 1,025 g a.e. ha^{-1} caused up to 6, 4, and 1% of necrosis or injury, respectively, in the assessments at 7, 14, and 28 DAT. The 2,4-D choline salt + glyphosate DMA salt at 1,950 + 2,050 g a.e. ha^{-1} caused up to 13, 10, and 2% of necrosis or injury, respectively, at 7, 14, and 28 DAT (Tables 4 and 5).

The safe use of 2,4-D and glyphosate on different soybean cultivars containing the DAS-444Ø6-6 event was confirmed, even in situations with plant stress, as in the Brazilian Cerrado region. In other studies, conducted in *in vitro* conditions, the tolerance to 2,4-D of AAD-12 soybean had been very effective and consistent (Wright et al., 2010; Griffin et al., 2013). Also, in a field trial, the foliar application of 2,4-D at 1,120 or 2,240 g a.e. ha^{-1} at V5 and R2 growth stages caused ≤ 3% injury on all evaluation dates (Robinson et al., 2015). The injury was typically small necrotic spots on the treated leaves, as seen in our study – Figure 2, which were gradually covered and reduced by new leaf growth. Leaf necrosis might have been caused by a high concentration of active 2,4-D, or inert ingredients of the formulation might have caused the damage (Robinson et al., 2015).

Crop grain yield of both soybean events was not significantly influenced by herbicide treatments, herbicide application timing and their interaction, regardless of the experimental site (Table 5). Thus, there was no yield reduction on the soybean cultivars expressing the DAS-444Ø6-6 event because of post emergence applications of 2,4-D choline salt and glyphosate DMA salt. Also, the cultivars containing this event showed equal level of tolerance to glyphosate, compared to the glyphosate-tolerant cultivars currently available to soybean growers. The spray of 2,4-D at 1,120 or 2,240 g a.e. ha^{-1} at V5 and R2 growth stages of soybean with AAD-12 trait did not influence the yield or its components, either (Robinson et al., 2015). This research found that minimal soybean injury occurred after 2,4-D treatments were applied and did not reduce the grain yield or yield components of AAD-12 soybean.

Table 3 - Visible leaf drooping (0 to 100%) at 1 and 7 days after treatment (DAT) for the soybean events DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 observed in Indianópolis (MG), Rio Verde (GO), Rondonópolis (MT) and Sorriso (MT) (mean of four trials by soybean event) in the 2015/2016 summer rainy season

Treatment		Visible drooping (%)			
2,4-D + glyphosate (g a.e. ha^{-1})	Application timing	DAS-444Ø6-6		DAS-444Ø6-6 x DAS-81419-2	
		1 DAT	7 DAT	1 DAT	7 DAT
0	Untreated	0 c	0	0 c	0
975 + 1,025	V3[2]	1 c	0	1 bc	0
2,000[1]		1 c	0	1 bc	0
1,950 + 2,050		2 bc	0	2 b	0
4,000[1]		3 b	0	3 ab	0
975 + 1,025	V6	2 bc	0	2 b	0
2,000[1]		3 b	0	3 b	0
1,950 + 2,050		5 a	0	5 a	0
4,000[1]		5 a	0	5 a	0
975 + 1,025	R2	1 c	0	1 bc	0
2,000[1]		1 c	0	1 bc	0
1,950 + 2,050		3 b	0	3 ab	0
4,000[1]		4 ab	0	4 a	0
F		26.9	0	24.2	0
VC (%)		26.1	0	29.2	0

[1] Ready-mix formulation (2,4-D choline salt 195 g a.e. L^{-1} + glyphosate DMA salt 205 g a.e. L^{-1}). [2] Soybean phenological stages according to Fehr and Caviness (1977). Means within a column followed by the same letter are not significantly different according to Tukey's pairwise comparison ($p<0.05$).

Table 4 - Visible leaf necrosis (0 to 100%) at 7, 14, and 28 days after treatment (DAT) for the soybean events DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 observed in Indianópolis (MG), Rio Verde (GO), Rondonópolis (MT) and Sorriso (MT) (mean of four trials by soybean event) in the 2015/2016 summer rainy season

Treatment		Visible Leaf Necrosis (%)					
2,4-D + glyphosate (g a.e. ha^{-1})	Application timing	DAS-444Ø6-6			DAS-444Ø6-6 x DAS-81419-2		
		7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
0	Untreated	0 c	0 c	0 b	0 c	0 c	0 b
975 + 1,025	V3[2]	4 bc	4 b	1 b	4 bc	4 bc	1 b
2,000[1]		4 bc	3 bc	1 b	4 bc	3 c	1 b
1,950 + 2,050		9 ab	5 b	1 b	9 a	5 b	1 b
4,000[1]		8 b	6 ab	1 b	9 a	6 b	1 b
975 + 1,025	V6	6 b	4 b	1 b	6 b	4 bc	1 b
2,000[1]		5 b	3 bc	0 b	5 b	4 bc	0 b
1,950 + 2,050		12 a	7 ab	1 b	12 a	8 ab	2 a
4,000[1]		13 a	8 a	2 a	13 a	8 ab	2 a
975 + 1,025	R2	2 c	0 c	0 b	2 c	1 c	0 b
2,000[1]		2 c	0 c	0 b	2 c	0 c	0 b
1,950 + 2,050		8 b	9 a	1 b	9 a	10 a	1 b
4,000[1]		8 b	8 a	1 b	9 a	9 a	1 b
F		64.7	90.6	121.2	49.9	79.1	96.5
VC (%)		14.1	16.6	19.4	14.2	22.7	30.9

[1] Ready-mix formulation (2,4-D choline salt 195 g a.e. L^{-1} + glyphosate DMA salt 205 g a.e. L^{-1}). [2] Soybean phenological stages according to Fehr and Caviness (1977). /Means within a column followed by the same letter are not significantly different according to Tukey's pairwise comparison ($p<0.05$).

Table 5 - Visible overall injury (0 to 100%) at 7, 14, and 28 days after treatment (DAT) for the soybean events DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 observed in Indianópolis (MG), Rio Verde (GO), Rondonópolis (MT) and Sorriso (MT) (mean of four trials by soybean event) in the 2015/2016 summer rainy season

Treatment		Overall Injury (%)					
2,4-D + glyphosate (g a.e. ha^{-1})	Application timing	DAS-444Ø6-6			DAS-444Ø6-6 x DAS-81419-2		
		7 DAT	14 DAT	28 DAT	7 DAT	14 DAT	28 DAT
0	Untreated	0 c	0 d	0 b	0 d	0 c	0 b
975 + 1,025	V3[2]	4 b	4 c	1 b	4 c	4 b	1 b
2,000[1]		4 b	3 c	1 b	4 c	3 bc	1 b
1,950 + 2,050		9 a	5 b	1 b	9 b	5 b	1 b
4,000[1]		9 a	6 b	1 b	9 b	6 b	1 b
975 + 1,025	V6	6 b	4 c	1 b	6 c	4 b	1 b
2,000[1]		4 b	3 c	0 b	4 c	4 b	0 b
1,950 + 2,050		12 a	7 b	1 b	12 a	8 a	2 a
a4,000[1]		13 a	8 a	2 a	13 a	8 a	2 a
975 + 1,025	R2	2 bc	0 d	0 b	3 cd	1 c	0 b
2,000[1]		2 bc	0 d	0 b	2 d	0 c	0 b
1,950 + 2,050		9 a	9 a	1 b	9 b	10 a	1 b
4,000[1]		8 ab	8 a	1 b	9 b	9 a	1 b
F		64.9	86.4	109.5	47.3	73.3	90.7
VC (%)		14.0	15.4	21.7	14.1	19.3	28.8

[1] Ready-mix formulation (2,4-D choline salt 195 g a.e. L^{-1} + glyphosate DMA salt 205 g a.e. L^{-1}). [2] Soybean phenological stages according to Fehr and Caviness (1977). Means within a column followed by the same letter are not significantly different according to Tukey's pairwise comparison ($p<0.05$).

Table 6 - P-values of the analysis of variance through the F-test for grain yield of soybean events DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 in Indianópolis (MG), Rio Verde (GO), Rondonópolis (MT) and Sorriso (MT) in the 2015/2016 summer rainy season

Site	Soybean event	2,4-D + glyphosate[1]	Application timing[2]	Interaction
		P value		
Indianópolis, MG	DAS-444Ø6-6	0.1824	0.9315	0.8409
	DAS-444Ø6-6 x DAS-81419-2	0.2707	0.6782	0.4914
Rio Verde, GO	DAS-444Ø6-6	0.1341	0.7575	0.7388
	DAS-444Ø6-6 x DAS-81419-2	0.5547	0.9694	0.0667
Rondonópolis, MT	DAS-444Ø6-6	0.6741	0.1269	0.3162
	DAS-444Ø6-6 x DAS-81419-2	0.2676	0.3346	0.5884
Sorriso, MT	DAS-444Ø6-6	0.5247	0.6570	0.9210
	DAS-444Ø6-6 x DAS-81419-2	0.3285	0.6666	0.8335

[1] 2,4-D + glyphosate: 975 + 1,025, 2,000 (ready-mix), 1,950 + 2,050 and 4,000 (ready-mix) g a.e. ha^{-1}. [2] Application timings: V3, V6 and R2 soybean growth stages, according to Fehr and Caviness (1977).

The triple-herbicide tolerance to 2,4-D, glyphosate and glufosinate provided by DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans will offer advances over single-herbicide technologies. In fact, several glyphosate-resistant weeds such as *Conyza* spp. and *Amaranthus palmeri*, as well as glyphosate-tolerant weeds such as *Commelina* spp., *Euphorbia* spp., *Ipomoea* spp., *Richardia* spp., *Spermacoce* spp., among others, can be well controlled by 2,4-D (Kruger et al., 2010; Marchi et al., 2013; Takano et al., 2013; Trezzi et al., 2016). In addition to providing more efficient and convenient control of a broad weed spectrum, chemical diversity will deliver lower probability of weed resistance to herbicides. However, DAS-444Ø6-6 soybean will only be sustainable when used as part of an integrated weed management program and by implementing good agricultural practices. The utilization of no-tillage systems, residual herbicides and good agricultural practices is still important to prevent the evolution of weed resistance to herbicides.

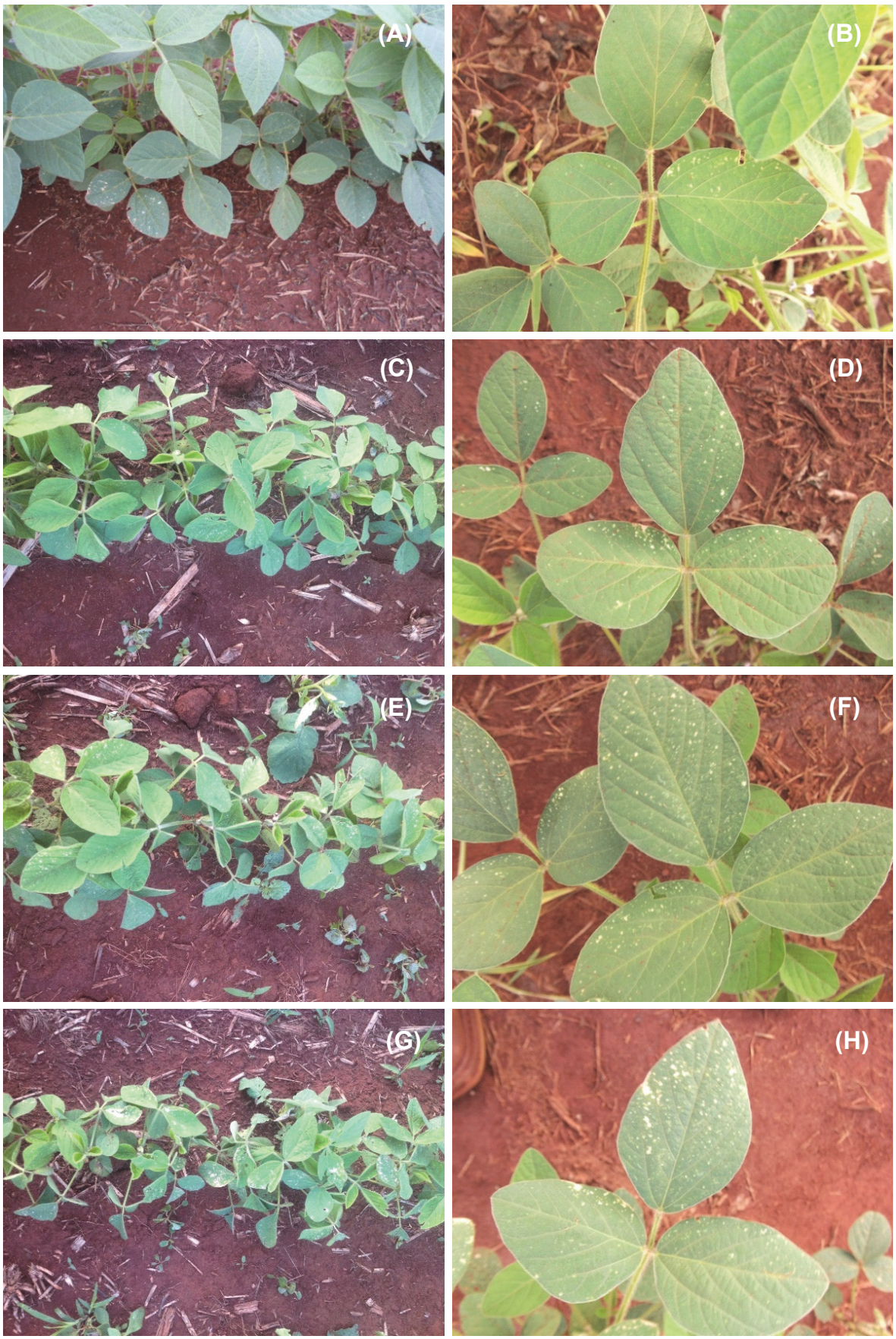

Figure 2 - Examples of DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans exhibiting 1-2% (A and B), 4-5% (C and D), 7-8% (E and F) and 10-11% (G and H) of leaf necrosis at 7 days after 2,4-D choline salt and glyphosate DMA treatments.

In our research, the DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans showed high tolerance to 2,4-D choline salt and glyphosate DMA in four different sites across the Cerrado region of Brazil. The foliar application of these herbicides caused very low levels of visual necrosis and injury and did not reduce the potential of grain yield of both soybean events evaluated. The DAS-444Ø6-6 and DAS-444Ø6-6 x DAS-81419-2 soybeans will allow the safe use of 2,4-D and glyphosate for postemergence weed control in soybean in the Brazilian Cerrado.

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