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Plant Resistance

Efficacy of Soybean's Event DAS-81419-2 Expressing Cry1F and Cry1Ac to Manage Key Tropical Lepidopteran Pests Under Field Conditions in Brazil

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Abstract

Bacillus thuringiensis (*Bt*) event DAS-81419-2 (Conkesta technology) in soybean, *Glycine max* (L.) Merrill, expresses Cry1F and Cry1Ac proteins to provide protection from feeding by several lepidopteran pests. A total of 27 field experiments across nine locations were conducted from 2011 to 2015 in southern and central Brazil to characterize the efficacy of DAS-81419-2 soybean infested with *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae), *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), *Heliothis virescens* (F.) (Lepidoptera: Noctuidae), and *Spodoptera cosmioides* (Walker) (Lepidoptera: Noctuidae) during vegetative (V4) and reproductive (R2 and R4) crop developmental stages. The efficacy of DAS-81419-2 was compared to that of a non-*Bt* isogenic variety managed with or without applications of commercial foliar insecticides for lepidopteran control. DAS-81419-2 soybean consistently experienced defoliation levels of 0.5% or less (compared with 20.05–56.74% in the non-*Bt*, nonsprayed treatment) and larval survival of $< 0.1\%$ in all four species across the vegetative and reproductive plant stages evaluated. The efficacy of DAS-81419-2 was significantly higher than commercial foliar insecticides applied to the non-*Bt* variety. DAS-81419-2 soybeans containing two highly effective *Bt* proteins are expected to be a more robust IRM tool compared to single-trait *Bt* technologies. The consistent efficacy of DAS-81419-2 soybeans across years, locations, and crop stages suggests that it will be a valuable product for management of hard-to-control key lepidopteran pests in South American soybean production.

Key words: Conkesta, *Anticarsia gemmatalis*, *Chrysodeixis includens*, *Heliothis virescens*, *Spodoptera cosmioides*

Soybean, *Glycine max* (L.) Merrill (Fabaceae: Phaseoleae), is the world's most important legume crop, with 90% of its global production grown in the United States, Brazil, Argentina, China, and India (Chang et al. 2015). Brazil is the second largest soybean producer with an estimated production of 100 million metric tons during the 2015–2016 growing season, following the United States with an estimated 107 million metric tons (USDA 2016). Economic losses to soybean production caused by a wide number of arthropod pests are experienced every year (Higley and Boethel 1994, Funderburk et al. 1999). Almost all major soybean-producing areas suffer significant crop losses from a complex of lepidopteran pests. Brazil is no exception and important pests include the velvetbean caterpillar, *Anticarsia gemmatalis* Hübner (Lepidoptera: Erebidae), and the soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) (Panizzi 1990, Funderburk 1994, Sullivan and Boethel 1994, Walker et al. 2000, Moscardi et al. 2012, Sosa-Gómez et al. 2014). Additional lepidopteran species such as *Spodoptera cosmioides* (Walker, 1858), *Spodoptera eridania* (Stoll, 1782), and *Heliothis virescens* (F., 1781) (Lepidoptera: Noctuidae) are becoming notorious in Brazil for their ability to inflict significant crop damage (Tomquelski and Maruyama 2009, Bueno et al. 2011, Moscardi et al. 2012, Bortolotto et al. 2014).

Synthetic insecticides are commonly used for controlling lepidopteran infestations in soybeans, often with limited success on target pests due to differences in intrinsic activity of products, developing insecticide tolerance, and sheltered feeding habits within the plant canopy (Thomas and Boethel 1994, Aragón et al. 1997, di Oliveira et al. 2010, Martins and Tomquelski 2015).

Alternate control strategies such as biological insecticides and the use of natural enemies are available (Luttrell et al. 1998, Moscardi 1999, Bueno et al. 2012). However, they are difficult to successfully and consistently implement on commercial large-scale production systems. Technological advances and commercial implementation of crop biotechnology for lepidopteran control offer a promising alternative and an additional tool to complement chemical insecticides in soybean (Bernardi et al. 2012, 2014a). Since commercially introduced in 1996, transgenic crops expressing δ-endotoxins (Cry proteins) from the soil bacterium *Bacillus thuringiensis* Berliner (*Bt*) have become important tools for effective pest control (Kumar et al. 2008). The successful adoption of *Bt* biotechnology in cotton, *Gossypium* spp., has enabled more effective management of lepidopteran pests and reduced synthetic chemical insecticide use (Perlak et al. 2001, Qaim and de Janvry 2005, Wu et al. 2008, Krishna and Qaim 2012). Similarly, the adoption of *Bt* biotechnology in corn, *Zea mays* L., has reduced the economic impact of lepidopteran pests while protecting yield potential (Pilcher et al. 2002, Fernandes et al. 2003, Buntin 2008, Siebert et al. 2008, Burkness et al. 2010, Siebert et al. 2012). Single-trait *Bt* soybeans were first commercialized in Brazil, Paraguay, Argentina and Uruguay in 2013 (Brookes and Barfoot 2015, Yano et al. 2015), demonstrating good efficacy on *A. gemmatalis*, *C. includens*, and *H. virescens* (Bernardi et al. 2012, 2014a). However, this single-trait *Bt* soybean has showed poor control against the *Spodoptera* complex (Yu et al. 2013, Bernardi et al. 2014b). To date, there are no commercially available multi *Bt*-trait soybeans in South America.

Dow AgroSciences has developed soybean event DAS-81419-2 (trademark Conkesta technology) via *Agrobacterium*-mediated transformation to express Cry1Ac, Cry1F, and phosphinothricin acetyltransferase (PAT) proteins derived from *Bacillus thuringiensis* subspecies *kurstaki*, *Bacillus thuringiensis* subspecies *aizawai*, and *Streptomyces viridochromogenes*, respectively, where Cry1Ac and Cry1F provides protection against lepidopteran insect species, and PAT confers tolerance to the herbicide glufosinate as a selectable marker (Fast et al. 2015). Event DAS-81419-2 is a dual *Bt* technology for soybeans developed to provide South American agricultural producers with wide-spectrum control of key lepidopteran pests.

The objective of this study was to demonstrate the field performance of DAS-81419-2 (Conkesta) and to assess its comparative efficacy against a non-*Bt* isogenic line with and without the use of insecticidal sprays to manage a complex of lepidopteran pests including *A. gemmatalis*, *C. includens*, *H. virescens*, and *S. cosmioides* in Brazil.

Materials and Methods

Field Sites and Growing Seasons

Field experiments were conducted from 2011 to 2015. All field experiments were conducted during the summer rainy season (October to March) in Brazil for commercial soybean production. Field sites were distributed across southern and central Brazil (Fig. 1). These areas were selected based on the commercial importance of soybean production and reflected a range of different environmental and agronomic conditions normally observed in soybean-producing areas of Brazil. All trials followed strict adherence to Brazilian regulatory requirements and were conducted at accredited certified field research sites which included Dow AgroSciences, Coodetec—Desenvolvimento, Produção e Comercialização Agrícola LTDA, and SGS Gravena field stations (Table 1).

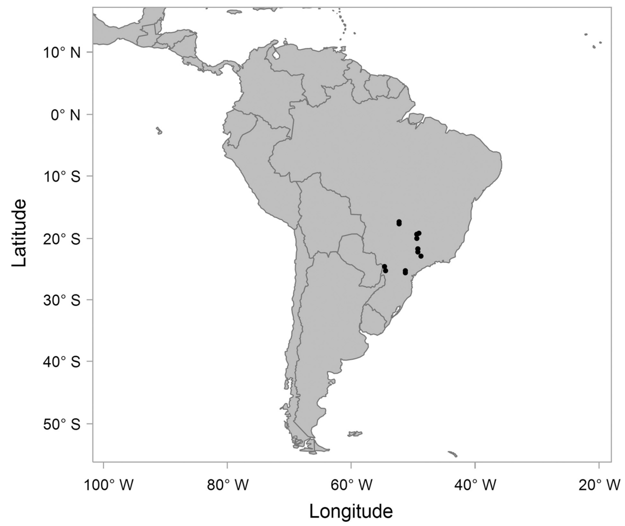

Fig. 1. Locations of field experiments in Brazil from 2011 to 2015. Location markers are slightly staggered for a better perception of the number of trials per region; see Table 1 for GPS coordinates.

Experimental Design

All field experiments were arranged in a randomized complete block design (RCBD) with four replications, except in four trials which had three replications. Plots sizes across locations ranged from four to 20 rows wide (45.0- to 50.0-cm row centers) by 5.0 to 20.0 m in length. The germplasm used for these experiments consisted of Maverick (University of Missouri, Columbia, MO) or Maverick crossed with DM16 (Don Mario Seeds, Buenos Aires, Argentina). All fertilization and weed control programs followed locally recommended practices to grow the crop. No foliar applied insecticides were used on the test plots, except in the plots for the non-*Bt* isogenic variety managed with insecticides. Most experimental sites relied on natural rainfall. However, artificial irrigation was available and occasionally used to avoid water stress during times of drought.

Treatments

The efficacy of a soybean variety expressing the *Bt* proteins Cry1F and Cry1Ac (DAS-81419-2 event, Conkesta technology, Dow AgroSciences LLC, Indianapolis, IN) was compared to a non-*Bt* isogenic soybean variety sprayed with insecticides (Brazil commercial insecticide program). The third treatment consisted of the non-*Bt* isogenic, and received no pest control treatments.

Insecticide Applications

The specific products used for the commercial insecticide treatment were selected based on standard soybean IPM recommendations in Brazil in the year in which the trials were conducted. The commercial insecticide-treated plots always used three sequential insecticide applications. To avoid drift, all adjacent plots were covered with tarps during the insecticide application. The insecticide program during the 2011 season consisted of chlorpyrifos at 480 g a.i./ha (Lorsban 480BR EC insecticide, Dow AgroSciences LLC, Indianapolis, IN), methomyl at 215 g a.i./ha (Lannate BR SL, DuPont, Wilmington, US), and imidacloprid + beta-cyfluthrin at 112.5 g a.i./ha (Connect SC, Syngenta, Basel, Switzerland). These products were sprayed during the vegetative (V4) and reproductive (R2 and R4) soybean crop stages (Ritchie et al. 1985), respectively. The insecticide program used during the 2012 to 2015 growing

Table 1. Trial locations and pests artificially infested in each year, from 2011 to 2015 in Brazil

	Trial location			
City, state	Latitude	Longitude	Year	Pest infested
Cravinhos, SP	21° 18′05.53″ S	47° 44′27.66″ W	2011	*A. gemmatalis*, *C. includens*, *S. cosmioides*, *H. virescens*
Indianópolis, MG	18° 57′39.19″ S	47° 51′12.95″ W	2011	*A. gemmatalis*, *C. includens*, *H. virescens*
Uberlândia, MG	19° 02′28.16″ S	48° 11′52.01″ W	2011	*A. gemmatalis*, *C. includens*, *H. virescens*
Cascavel, PR	24° 53′20.84″ S	53° 32′37.36″ W	2012	*A. gemmatalis*
Castro, PR	24° 47′32.33″ S	49° 53′56.97″ W	2012	*A. gemmatalis*, *C. includens*, *S. cosmioides*
Cravinhos, SP	21° 18′02.98″ S	47° 44′26.47″ W	2012	*A. gemmatalis*, *C. includens*
Cravinhos, SP	21° 18′02.60″ S	47° 44′25.40″ W	2012	*A. gemmatalis*, *C. includens*, *H. virescens*
Indianópolis, MG	18° 57′55.00″ S	47° 51′28.00″ W	2012	*A. gemmatalis*, *C. includens*, *H. virescens*
Indianópolis, MG	18° 57′54.00″ S	47° 51′32.00″ W	2012	*A. gemmatalis*, *C. includens*, *H. virescens*
Indianópolis, MG	18° 57′23.54″ S	47° 51′22.81″ W	2012	*A. gemmatalis*, *C. includens*, *S. cosmioides*, *H. virescens*
Montividiu, GO	17° 22′33.15″ S	51° 23′46.36″ W	2012	*A. gemmatalis*, *C. includens*, *S. cosmioides*, *H. virescens*
Montividiu, GO	17° 22′37.46″ S	51° 23′31.08″ W	2012	*A. gemmatalis*, *C. includens*, *S. cosmioides*, *H. virescens*
Uberlândia, MG	19° 02′30.40″ S	48° 11′49.90″ W	2012	*A. gemmatalis*, *C. includens*, *H. virescens*
Uberlândia, MG	19° 02′33.40″ S	48° 11′50.00″ W	2012	*A. gemmatalis*, *C. includens*, *H. virescens*
Uberlândia, MG	19° 02′30.31″ S	48° 11′44.27″ W	2012	*A. gemmatalis*, *C. includens*
Cravinhos, SP	21° 18′02.11″ S	47° 44′16.15″ W	2013	*S. cosmioides*, *H. virescens*
Indianópolis, MG	18° 57′28.08″ S	47° 51′22.09″ W	2013	*S. cosmioides*, *H. virescens*
Cascavel, PR	24° 53′13.30″ S	53° 32′24.05″ W	2014	*S. cosmioides*
Montividiu, GO	17° 22′38.08″ S	51° 23′44.62″ W	2014	*A. gemmatalis*, *C. includens*, *S. cosmioides*, *H. virescens*
Montividiu, GO	17° 22′37.57″ S	51° 23′44.02″ W	2014	*S. cosmioides*, *H. virescens*
Castro, PR	24° 47′34.42″ S	49° 53′57.18″ W	2015	*S. cosmioides*
Conchal, SP	22° 24′11.69″ S	47° 06′52.03″ W	2015	*A. gemmatalis*, *C. includens*, *S. cosmioides*, *H. virescens*
Mogi Mirim, SP	22° 26′49.81″ S	47° 04′14.79″ W	2015	*H. virescens*
Palotina, PR	24° 21′19.42″ S	53° 45′15.69″ W	2015	*A. gemmatalis*, *C. includens*, *S. cosmioides*, *H. virescens*
Palotina, PR	24° 21′18.49″ S	53° 45′15.56″ W	2015	*C. includens*, *S. cosmioides*, *H. virescens*
Indianópolis, MG	18° 57′27.85″ S	47° 51′22.35″ W	2015	*S. cosmioides*
Indianópolis, MG	18° 57′28.00″ S	47° 51′20.97″ W	2015	*A. gemmatalis*, *C. includens*, *S. cosmioides*

seasons consisted of chlorantraniliprole at 10 g a.i./ha (Premio SC, DuPont, Wilmington, US), thiamethoxam + lambda-cyhalothrin at 49.4 g a.i./ha (Engeo Pleno SC, Bayer CropScience, Monheim, Germany), and imidacloprid + beta-cyfluthrin at 112.5 g a.i./ha and were sprayed at V4, R2, and R4 crop stages, respectively.

Artificial Infestations

All treatments were evaluated against *A. gemmatalis*, *C. includens*, *H. virescens*, and *S. cosmioides*. Field trials were subjected to artificial pest infestations to ensure uniform pest pressure across plots in all locations. All larvae were obtained from insect colonies maintained by SGS—Gravena at its location near the city of Jaboticabal in the state of São Paulo in Brazil. Colonies were maintained either in a room with controlled temperature or in an incubator at 25 ± 3 °C, 60 ± 5% RH, at a photoperiod of 14:10 (L:D) h and reared on artificial diet. Vigor in all insect colonies was boosted by introducing new field-collected larvae every year. Larvae of *A. gemmatalis*, *C. includens*, *H. virescens*, and *S. cosmioides* were infested at V4, R2, and R4 stages of soybean growth. A total of 10–20 plants per plot at each growth stage, randomly selected from one of the mid four rows of each plot, were marked and infested with 10–20 first-instar larvae per plant. Infestations were performed manually using a camel's hair brush. Upon infestation, plants were covered with a fine-mesh cage to prevent larval escape. Field evaluations were conducted ~10 d after each artificial infestation at the three selected plant growth stages. Variables evaluated included the percentage of live larvae per plot and a visual estimation of feeding defoliation expressed in percentage of leaf surface. The number of surviving larvae was recorded from each of the artificially infested plants per plot. Prior to removing each cage, the infested plant was gently shaken inside the cage to recover all surviving larvae. The fine-mesh cages were removed from the plants and all larvae inside the cage were counted. Any remaining live larva from the plant was also counted. To estimate the percent defoliation, the plant foliage that was enclosed within the cage was observed for lepidopteran feeding damage. The amount of tissue consumed by the larvae was visually estimated and expressed in percentage. Defoliation values were estimated for each artificially infested plant per plot.

Statistical Analyses

Mean defoliation (%) values, for each insect species and growth stage, were analyzed with the linear mixed model:

$$\eta_{ijk} = \eta + Treatment_i + Trial_j + Treatment \times Trial_{ij} + Block_{k(j)} + nlarvaeX_{ijk}$$

with observations normally distributed, $y_{ijk} \sim N\left(\mu_{ijk}, \sigma^2\right)$, and identity link function $\eta_{ijk} = \mu_{ijk}$; $nlarvaeX_{ijk}$ is a covariate to account for the number of larvae used in the artificial infestation on each plant. In order to improve the normality and homogeneity of variance of the dataset, both requirements for linear model application, values were transformed using $\sqrt{x + 0.5}$. Least square means presented in tables are back-transformed values and their standard errors were estimated with the delta method (Stroup 2012).

Percentage of live larvae, binomial response, was analyzed with the generalized linear mixed model:

$$\eta_{ijk} = \eta + Treatment_i + Trial_j + Treatment \times Trial_{ij} + Block_{k(j)}$$

with observations binomially distributed, $y_{ijk} \sim Binomial(N_{ijk}, \pi_{ijk})$, where N_{ijk} and π_{ijk} are the number of larvae and proportion of live larvae in each experimental unit, respectively. The link function for the binomial distribution is the logit function $\eta_{ijk} = log\left[{}^{\pi_{ijk}}/_{1-\pi_{ijk}}\right]$.

In both models, treatment is modeled as a fixed factor and trial, block (trial), and the interaction treatment × trial are modeled as random factors. Significance of treatment effect was evaluated with F-approximate test ($\alpha = 0.05$) and least square means from different treatments were compared with Tukey's test. In the case of linear mixed model, the estimation method was restricted maximum likelihood and Kenward Rodgers for degrees of freedom; for the generalized linear mixed model the estimation method was maximum likelihood with Laplace approximation. The proportions of variance explained by random factors were calculated; corresponding significance levels were determined with the likelihood ratio test at $\alpha = 0.05$ (Stroup 2012). Linear mixed models were estimated with Proc MIXED and generalized linear mixed models were estimated with Proc GLIMMIX (SAS Institute 2011).

Results

Bt soybean event DAS-81419-2 significantly reduced the level of defoliation caused by *A. gemmatalis* (Fig. 2A) compared to the non-*Bt* isogenic treatment with and without insecticide sprays across all growth stages evaluated (V4, $F_{2,34.0} = 115.36$, $P < 0.0001$; R2, $F_{2,23.8} = 35.13$, $P < 0.0001$; R4, $F_{2,19.2} = 40.50$, $P < 0.0001$). The percent defoliation ranged from 30.8 to 56.7% in the non-*Bt*, nonsprayed treatment, which is above the economic injury threshold level adopted in Brazil of 30% defoliation during the vegetative stage and 15% during the reproductive stage of soybeans. The use of insecticides reduced defoliation to ~8.2 to 19.3%. However, event DAS-81419-2 consistently reduced the percentage of defoliation to 0.1% or less (Table 2). DAS-81419-2 provided almost 100% mortality to *A. gemmatalis* larvae and was significantly better than all other treatments across different plant phenological stages (V4, $F_{2,34.0} = 47.91$, $P < 0.0001$; R2, $F_{2,24} = 34.65$, $P < 0.0001$; R4, $F_{2,20} = 30.20$, $P < 0.0001$). The percent of surviving larvae was 0.01% or less on DAS-81419-2, compared to 14.6 to 25.7% survival on the non-*Bt*, nonsprayed soybeans and around 1% when the non-*Bt* soybeans were managed with a commercial spray program (Table 3).

Chrysodeixis includens was also highly susceptible to the DAS-81419-2 event throughout the vegetative and reproductive soybean crop stages evaluated (Fig. 2B). Defoliation levels observed in DAS-81419-2 soybeans were significantly lower compared to all other treatments (V4, $F_{2,33.9} = 99.45$, $P < 0.0001$; R2, $F_{2,21.9} = 30.88$, $P < 0.0001$; R4, $F_{2,17.8} = 56.55$, $P < 0.0001$). Defoliation in the non-

Table 2. Percentage of defoliation by *A. gemmatalis*, *C. includens*, *H. virescens*, and *S. cosmioides* at V4, R2, and R4 soybean growth stage

Pest species	Treatment	% Defoliation					
		Growth Stage V4		Growth Stage R2		Growth Stage R4	
		No. of trials	Lsq mean +SE	No. of trials	Lsq mean +SE	No. of trials	Lsq mean +SE
A. gemmatalis	DAS-81419-2 (Cry1F+Cry1Ac)	18	0.13± 0.58c	13	0.06± 0.73c	11	0.04± 0.71c
	Non-*Bt* isoline sprayed		19.30± 3.24b		9.63± 3.12b		8.26± 2.86b
	Non-*Bt* isoline nonsprayed		56.74± 5.52a		30.81± 5.48a		33.64± 5.64a
C. includens	DAS-81419-2 (Cry1F+Cry1Ac)	18	0.21± 0.61c	12	0.07± 0.65b	10	0.09± 0.61c
	Non-*Bt* isoline sprayed		21.54± 3.45b		13.53± 3.22a		13.54± 2.98b
	Non-*Bt* isoline nonsprayed		49.07± 5.04a		26.21± 4.44a		27.81± 4.24a
H. virescens	DAS-81419-2 (Cry1F+Cry1Ac)		—	8	0.01± 0.61c	14	0.68± 0.65c
	Non-*Bt* isoline sprayed		—		10.01± 2.92b		15.35± 2.39b
	Non-*Bt* isoline nonsprayed		—		27.88± 4.80a		23.45± 2.93a
S. cosmioides	DAS-81419-2 (Cry1F+Cry1Ac)	9	0.42± 0.95c	5	0.14± 0.67c	14	0.48± 0.63c
	Non-*Bt* isoline sprayed		10.74± 3.57b		7.35± 2.38b		11.03± 2.15b
	Non-*Bt* isoline nonsprayed		31.02± 5.59a		20.05± 3.85a		21.57± 2.98a

Values with the same letter in each column, within species, are not significantly different (Tukey's test, $P > 0.05$).

Table 3. Percentage of live larvae of *A. gemmatalis*, *C. includens*, *H. virescens*, and *S. cosmioides* at V4, R2, and R4 soybean growth stages

Pest species	Treatment	% of Live larvae					
		Growth stage V4		Growth stage R2		Growth Stage R4	
		No. of trials	Lsq mean +SE	No. of trials	Lsq mean +SE	No. of trials	Lsq mean +SE
A. gemmatalis	DAS-81419-2 (Cry1F+Cry1Ac)	18	0.002 ± 0.003c	13	0.014 ± 0.012c	11	0.004± 0.005c
	Non-*Bt* isoline sprayed		1.01 ± 0.46b		0.78 ± 0.38b		1.16± 0.52b
	Non-*Bt* isoline nonsprayed		25.74 ± 8.25a		18.97 ± 6.99a		14.63± 5.33a
C. includens	DAS-81419-2 (Cry1F+Cry1Ac)	18	0.017 ± 0.012c	12	0.008 ± 0.008c	10	0.007± 0.009c
	Non-*Bt* isoline sprayed		4.54 ± 1.64b		2.69 ± 1.20b		2.80± 1.31b
	Non-*Bt* isoline nonsprayed		20.20 ± 5.79a		12.57 ± 4.83a		10.64± 4.37a
H. virescens	DAS-81419-2 (Cry1F+Cry1Ac)		—	8	0.018 ± 0.021c	14	0.005± 0.006c
	Non-*Bt* isoline sprayed		—		4.91 ± 2.03b		2.09± 1.07b
	Non-*Bt* isoline nonsprayed		—		19.90 ± 6.77a		6.27± 2.98a
S. cosmioides	DAS-81419-2 (Cry1F+Cry1Ac)	9	0.047 ± 0.042c	5	0.029 ± 0.036c	14	0.077± 0.047c
	Non-*Bt* isoline sprayed		1.81 ± 1.21b		1.82 ± 1.27b		2.67± 1.18b
	Non-*Bt* isoline nonsprayed		16.35 ± 8.28a		19.64 ± 10.59a		8.58± 3.52a

Values with the same letter in each column, within species, are not significantly different (Tukey's test, $P > 0.05$).

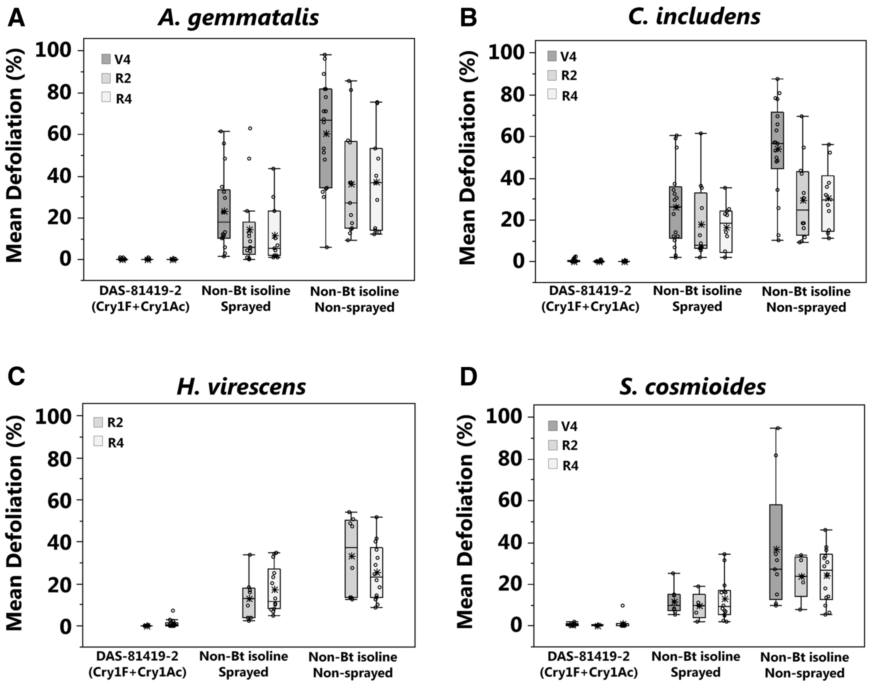

Fig. 2. Mean defoliation (%) caused by *A. gemmatalis*, *C. includens*, *H. virescens*, and *S. cosmioides* at V4, R2 and R4 soybean growth stages.

Bt, nonsprayed treatment was 49.0% during the vegetative stage and ranged from 26.2 to 27.8% in the reproductive stages, both of which are above the economic threshold levels (Table 2). The insecticide program brought defoliation levels down to a 13.5 to 21.5% range. Significant differences were also observed on the percentage of surviving larvae on DAS-81419-2 soybean compared to the sprayed and nonsprayed non-*Bt* isogenic treatments at V4, R2, and R4 stages (V4, $F_{2,33}=47.42$, $P<0.0001$; R2, $F_{2,22}=24.30$, $P<0.0001$; R4, $F_{2,18}=21.74$, $P<0.0001$; Table 3). Larval survival on event DAS-81419-2 was 0.01% or less across all trials and throughout the different crop stages evaluated. Surviving larvae on the non-*Bt*, nonsprayed treatment ranged from 10.6 to 20.2% and was reduced to 2.6 to 4.5% when the non-*Bt* crop was maintained with a commercial insecticide program.

DAS-81419-2 soybean significantly reduced (R2, $F_{2,19.9}=27.24$, $P<0.0001$; R4, $F_{2,25.9}=92.10$, $P<0.0001$) the defoliation injury caused during reproductive crop stages by another important pest, *H. virescens*, compared to both sprayed and nonsprayed non-*Bt* isogenic soybeans at the R2 and R4 stages evaluated (Fig. 2C). The levels of defoliation measured on event DAS-81419-2 ranged from 0.01 to 0.68% compared to 23.4 to 27.8% in the non-*Bt*, nonsprayed soybean, and compared to 10.0 to 15.3% in non-*Bt* soybeans managed with insecticides (Table 2). DAS-81419-2 caused significant levels of mortality to *H. virescens* compared to those observed on the non-*Bt* soybeans with and without insecticides (R2, $F_{2,14}=24.64$, $P<0.0001$; R4, $F_{2,26}=22.57$, $P<0.0001$). The percent of surviving *H. virescens* on DAS-81419-2 was near zero (0.01% or less) compared to 6.2 to 19.9% on the non-*Bt*, nonsprayed treatment, and to 2.0 to 4.9% on the non-*Bt* soybeans managed with insecticides (Table 3).

Defoliation injury caused by *S. cosmioides* was significantly reduced by event DAS-81419-2 (Fig. 2D) and was successful in keeping defoliation damage to an average of less than 0.5% (Table 2), which was significantly lower (V4, $F_{2,15.7}=22.94$, $P<0.0001$; R2, $F_{2,7.2}=37.49$, $P=0.0002$; R4, $F_{2,25.8}=65.48$, $P<0.0001$) compared to non-*Bt* isogenic soybeans with and without insecticide sprays in which defoliation levels ranged from 7.3 to 11.0% and from 20.0 to 31.0%, respectively. Similarly, DAS-81419-2 inflicted significant mortality to *S. cosmioides* larvae compared to all other treatments across the different crop vegetative and reproductive stages evaluated (V4, $F_{2,15}=16.45$, $P=0.0002$; R2, $F_{2,8}=22.9$, $P=0.0005$; R4, $F_{2,26}=25.24$, $P<0.0001$). Survival on DAS-81419-2 was less than 0.1% (Table 3) and was significantly lower than non-*Bt* isogenic soybeans with insecticides, which was significantly less damaged than the non-*Bt* soybeans without insecticides.

For all pest species, random variation was mainly explained by treatment × trial and trial effects, both for percentage of defoliation and percentage of live larvae. The proportion of variance explained by treatment × trial effect ranged from 31 to 75% for percentage of defoliation and from 21 to 75% for percentage of live larvae (all χ^2_{1df} likelihood ratio tests were significant, $P<0.05$). The proportion of variance explained by trial effect ranged from 3 to 37% for percentage of defoliation (only 2 out of 11 χ^2_{1df} likelihood ratio tests were significant, $P<0.05$) and from 18 to 68% for percentage of live larvae (only 3 out of 11 χ^2_{1df} likelihood ratio tests were significant, $P<0.05$). The proportion of variance explained by block (trial) was not relevant, with values ranging from 0 to 12%. The significance of the interaction treatment × trial and trial effects were due to the variation in performance of the insecticide applications across trials and also due to the variation in the response of the non-*Bt*, nonsprayed treatment. Performance of the event DAS-81419-2 was consistent across trials, with very low levels of defoliation and surviving larvae.

Discussion

Results from our multiyear and cross-geographic studies show that *Bt* soybean event DAS-81419-2 provides high efficacy against *A. gemmatalis*, *C. includens*, *H. virescens*, and *S. cosmioides* during vegetative and reproductive stages of crop development. These extremely low levels of defoliation were consistently and significantly observed in DAS-81419-2 soybeans compared to a non-*Bt* soybean managed with commercial insecticides. The percent of defoliation injury levels averaged around 0.5% or less in soybeans with DAS-81419-2 event, which will represent an important field attribute under South American conditions. Our results also indicate that DAS-81419-2 soybeans cause high levels of mortality to all four lepidopteran species evaluated, suggesting that survival to adult in the field will be extremely low. High levels of mortality are indicative of effective IRM when the product is planted with a suitable non-*Bt* refuge (Gould 1998). The very limited survival of key target pest larvae tested in these studies supports the durability of DAS-81419-2 soybean when used in this manner.

Bernardi et al. (2012, 2014a) discussed results from recent studies evaluating the efficacy of events MON 87701 × MON 89788 soybean against key lepidopteran pests of soybeans in Brazil. MON 87701 × MON 89788 soybeans express a single *Bt* insecticidal protein, Cry1Ac, and was first commercially available in South America in 2013. The authors concluded that MON 87701 × MON 89788 soybeans provides a high-level efficacy against *A. gemmatalis*, *C. includens* (Bernardi et al. 2012), and *H. virescens* (Bernardi et al. 2014a). However, lower mortality was reported against species of the *Spodoptera* complex in laboratory and greenhouse trials, revealing low mortality on *S. cosmioides* and *S. eridania* of <13%, and ∼50% mortality against *Spodoptera frugiperda* (J.E. Smith, 1797) (Lepidoptera: Noctuidae) (Bernardi et al. 2014b). While *Bt* soybean events DAS-81419-2 and MON 87701 × MON 89788 provided excellent levels of control on three important lepidopteran pests (*A. gemmatalis*, *C. includens*, and *H. virescens*), the additional and consistent high level control of *S. cosmioides* demonstrated with DAS-81419-2 brings a much needed solution to South American soybean production, as this insect is gaining notoriety for becoming more damaging to soybean production in key commercial soybean-producing areas of Brazil.

The expression of two *Bt* insecticidal proteins in DAS- 81419-2 soybeans combines the efficacy of Cry1Ac and Cry1F. It provides dual control potential and is a significant advance over single-trait products. This is important because the ability to control damaging pests is enhanced within the plant resulting in broad and high level efficacy, and also because the dual protein expression is an effective strategy for delivering increased product durability.

Our results also demonstrate that DAS-81419-2 soybean is significantly more efficient in controlling key lepidopteran pests compared to the non-*Bt* isogenic variety managed with a standard foliar insecticide program representative of the region in which these tests were conducted. The difficulties in achieving high levels of efficacy with foliar spray programs in soybeans is explained in part by the different degrees of susceptibility of key pests to commercial foliar sprays and also by the well-known behavior of some of these pests to feed deep within the plant canopy, which makes it difficult to be reached by lethal doses of foliar sprays. When managed properly, DAS-81419-2 soybeans is expected to reduce the number of foliar insecticides sprays to manage lepidopteran larvae. When commercially available, DAS-81419-2 soybeans will be a valuable and highly effective new tool to manage hard-to-control lepidopteran infestations in soybeans. As such, the durability of DAS-81419-2 soybean can only be maximized when used as part of both IPM and IRM programs and with the implementation of best management practices for *Bt* crop production that are relevant to South America conditions including planting of refuge areas consistent with manufacturer guidelines. Pest monitoring is still important to deploy on-time additional control tactics and help manage heavier than expected lepidopteran pest infestations or to control other insects not targeted by this technology.

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