Research Article

Received: 28 August 2015 Revised: 13 November 2015 Accepted article published: 19 November 2015 Published online in Wiley Online Library: 29 December 2015

(wileyonlinelibrary.com) DOI 10.1002/ps.4191

High susceptibility and low resistance allele frequency of *Chrysodeixis includens* (Lepidoptera: Noctuidae) field populations to Cry1Ac in Brazil

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Abstract

BACKGROUND: The soybean looper (SBL), *Chrysodeixis includens* (Walker), is one of the most important soybean pests in Brazil. MON 87701 × MON 89788 soybean expressing Cry1Ac has been recently deployed in Brazil, providing high levels of control against the primary lepidopteran pests. To support insect resistance management (IRM) programmes, the baseline susceptibility of SBL to Cry1Ac was assessed, and the resistance allele frequency was estimated on the basis of an F_2 screen.

RESULTS: The toxicity (LC_{50}) of Cry1Ac ranged from 0.39 to 2.01 μg mL^{-1} diet among all SBL field populations collected from crop seasons 2008/09 to 2012/13, which indicated approximately fivefold variation. Cry1Ac diagnostic concentrations of 5.6 and 18 μg mL^{-1} diet were established for monitoring purposes, and no shift in mortality was observed. A total of 626 F_2 family lines derived from SBL collected from locations across Brazil during crop season 2014/15 were screened for the presence of Cry1Ac resistance alleles. None of the 626 families survived on MON 87701 × MON 89788 soybean leaf tissue (joint frequency 0.0004).

CONCLUSIONS: SBL showed high susceptibility and low resistance allele frequency to Cry1Ac across the main soybean-producing regions in Brazil. These findings meet important criteria for effective IRM strategy.

Keywords: soybean looper; Cry1Ac; baseline susceptibility; resistance allele frequency

1 INTRODUCTION

Soybean [*Glycine max* (L.) Merr.] fields in Brazil have been consistently damaged by several insect species, requiring control tactics such as biological and chemical products.[1] The current no-tillage cultivation system has affected integrated pest management (IPM) owing to the use of cover crops (e.g. millet, grasses) during the intercropping season and the presence of crop residues, both of which provide a favourable environment for insect pests.[2] Moreover, the overuse of non-selective insecticides, often associated with herbicides and fungicides, has contributed to the challenges faced by IPM programmes in Brazil. For instance, the increasing use of fungicides to control soybean rust after the first occurrence in Brazil in 2001 has reduced the prevalence of naturally occurring entomopathogenic fungi that are major mortality factors of soybean looper (SBL), *Chrysodeixis includens* (Walker).[1−3]

All of these factors have changed the status of SBL to a primary soybean pest widely distributed across the main soybean-producing areas in Brazil.[4−6] SBL management based on chemical insecticides raises concerns owing to the history of SBL resistance to different compounds.[7,8] Additionally, SBL typically infests the soybean plants at early reproductive stages, when adjacent plant rows overlap, and prefers lower plant parts,[6] which weakens the efficacy of chemical control.

Transgenic crops expressing insecticidal proteins from *Bacillus thuringiensis* (*Bt*) have been used worldwide with relative success.[9,10] The first commercial *Bt* soybean (MON 87701 × MON 89788), expressing Cry1Ac protein, was recently approved for cultivation in Brazil[11] and launched in crop season 2013/14 as a promising alternative for control of the main lepidopteran pests

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Table 1. Populations of *Chrysodeixis includens* field collected in soybean

Season	State[a]	Location/code	Latitude	Longitude	Date
2008/09	BA	Luis Eduardo Magalhães (LemBA)	11° 37′ 23.0″	45° 35′ 66.1″	Jan. 14, 2009
	MT	Rondonópolis (RonMT)	16° 28′ 15″	54° 38′ 15″	Dec. 10, 2008
	GO	Santa Helena (ShGO)	17° 51′ 17.0″	50° 51′ 74.8″	Jan. 26, 2009
	PR	Londrina (LonPR)	23° 14′ 10.1″	51° 08′ 43.5″	Feb. 12, 2009
2009/10	BA	Luis Eduardo Magalhães (LemBA)	11° 28′ 41.1″	45° 34′ 99.7″	Jan. 15, 2010
	MT	Campo Verde (CvMT)	15° 24′ 50.1″	55° 06′ 54.8″	Dec.18, 2009
	GO	Santa Helena (ShGO)	17° 51′ 17.0″	50° 51′ 74.8″	Jan. 22, 2010
	SP	Santa Cruz das Palmeiras (ScpSP)	21° 49′ 04.8″	47° 16′ 11.1″	Jan. 11, 2010
2010/11	BA	Roda Velha (RvBA)	12° 48′ 10.7″	46° 07′ 49.52″	Jan. 13, 2011
	MT	Campo Verde (CvMT)	15° 38′ 19,7″	55° 13′ 36.11″	Dec. 28, 2010
	GO	Rio Verde (RvGO)	17° 47′ 27.1″	50° 57′ 33.7″	Jan. 16, 2011
	PR	Cambé (CamPR)	21° 07′ 52.0″	51° 23′ 26.2″	Dec. 29, 2010
	RS	Selbach (SelRS)	28° 61′ 52″	52° 95′ 33″	Jan. 15, 2011
2011/12	BA	Luis Eduardo Magalhães (LemBA12)	12° 05′ 35″	45° 50′ 42″	Feb. 12, 2012
	MT	Sinop (SinMT12)	11° 26′ 30.7″	55° 31′ 23.93″	Dec. 20, 2011
	GO	Rio Verde (RvGO)	17° 47′ 18.2″	52° 00′ 16.4″	Jan. 05, 2012
	PR	Mauá da Serra (MauPR)	23° 52′ 55.4″	51° 17′ 19.2″	Jan. 06, 2012
	RS	Pontão (PonRS)	28° 3′ 36.96″	52° 35′ 13.85″	Mar. 05, 2012
2012/13	MT	Sinop (SinMT13)	11° 39′ 58″	55° 47′ 53.93″	Dec. 18, 2012
	BA	Barreiras (BarBA13)	12° 01′ 37.38″	46° 16′ 38.7″	Dec. 30, 2012
	MT	Rondonopolis (RonMT13)	16° 37′ 38.63″	54° 41′ 58.04″	Jan. 11, 2013
	GO	Montividiu (MonGO13)	16° 54′ 04.5″	51° 03′ 17.0″	Dec. 12, 2012
	GO	Inaciolândia (InaGO13)	18° 30′ 33.3″	49° 57′ 50.6″	Jan. 25, 2013
	MS	Chapadão do Sul (ChaMS13)	18° 48′ 28.59″	52° 30′ 49.07″	Jan. 18, 2013
	SP	Jaboticabal (JabSP13)	21° 16′ 46.7″	48° 16′ 42″	Jan. 25, 2013
	PR	Rolandia (RolPR13)	23° 16′ 57.6″	51° 20′ 56.7″	Jan. 25, 2013
	RS	Ciríaco (CirRS13)	28° 17′ 21.41″	51° 58′ 56.61″	Mar. 13, 2013
	RS	Rio Pardo (RioRS13)	29° 61′ 3.33″	52° 46′ 7.5″	Mar. 15, 2013
2013/14	BA	Luis Eduardo Magalhães (LemBA14)	11° 57′ 27.89″	46° 05′ 26,64″	Jan. 16, 2014
	MT	Sorriso (SorrisoMT14)	12° 31′ 56″	55° 37′ 33″	Dec. 16, 2013
	GO	Montividiu (MontivGO14)	17° 22′ 35.9″	51° 23′ 39.4″	Dec. 30, 2013
	GO	Inaciolândia (InaciolGO14)	18° 29′ 25.5″	49° 57′ 28.3″	Jan. 8, 2014
	MS	Chapadão do Sul (ChapadMS14)	18° 51′ 06.43″	52° 29′ 09.01″	Jan. 16, 2014
	MG	Uberlândia (UberlMG14)	19° 02′ 33.4″	48° 11′ 44.5″	Jan. 16, 2014
	PR	Castro (CastroPR14)	24° 46′ 44″	50° 03′ 23″	Mar. 7, 2014
2014/15	BA	Barreiras (BarBA15)	11° 31′ 27.5″	45° 23′ 26.2″	Jan. 29, 2015
	BA	Formosa do Rio Preto (FRPBA15)	11° 20′ 11″	45° 40′ 0.1″	Jan. 29, 2015
	MT	Campo Verde (CvMT15)	15° 7′ 48″	55° 1′ 55.6″	Jan. 21, 2015
	MG	Araguari (AraMG15)	18° 43′ 28.55″	47° 41′ 19.68″	Jan. 9, 2015
	MS	Rio Brilhante (RiBMS15)	21° 45′ 2.2″	54° 31′ 15.6″	Jan. 21, 2015
	PR	Londrina (LonPR15)	23° 17′ 30.5″	51° 12′ 31″	Feb. 11, 2015
	PR	Campo Mourão (CaMPR15)	24° 5′ 38.8″	52° 11′ 35.2″	Jan. 12, 2015
	RS	Não me Toque (NmtRS15)	28° 18′ 47.5″	52° 26′ 8.8″	Feb. 25, 2015

[a] BA = Bahia; GO = Goiás; MS = Mato Grosso do Sul; MG = Minas Gerais; MT = Mato Grosso; PR = Paraná; RS = Rio Grande do Sul; SP = São Paulo.

of soybean. MON 87701 × MON 89788 soybean has been rapidly adopted by growers[12] owing to the high levels of control provided against the main lepidopteran pests, especially SBL, velvetbean caterpillar (*Anticarsia gemmatalis* Hübner), tobacco budworm [*Heliothis virescens* (F.)] and old-world bollworm [*Helicoverpa armigera* (Hübner)].[13–16]

As with other *Bt* crops, the primary threat to the continued success of *Bt* soybean is the evolution of resistance by target pests.[17] Proactive insect resistance management (IRM) programmes for *Bt* traits must be designed and implemented to delay resistance evolution.[18] The most effective IRM strategy for *Bt* crops, the high-dose/refuge strategy, is based on the assumptions that *Bt* proteins have high toxicity against the target pest, resistance is functionally recessive, resistant alleles are rare and refuge areas with non-*Bt* plants are cultivated to provide a source of susceptible insects.[17,19] A previous study reported high toxicity of MON 87701 × MON 89788 soybean against SBL and assessed it as near high dose against this species.[13] To refine the proactive IRM programme for SBL in MON 87701 × MON 89788 soybean in Brazil, we describe in this paper the baseline susceptibility of several field SBL populations to Cry1Ac and the implementation of a resistance monitoring programme. Moreover, we estimate the resistance allele frequency to MON 87701 × MON 89788 soybean by screening F_2 isoline families on *Bt* plant tissue.

Table 2. Toxicity (LC_{50}) of Cry1Ac protein and effective concentration (EC_{50}) causing growth inhibition to *Chrysodeixis includens* populations sampled in soybean fields across Brazil

Location	n^a	$LC_{50}{}^b$(FL 95%)	Slope ± (SE)^c	χ^2	$EC_{50}{}^d$(FL 95%)
Crop season 2008/09					
Sus	640	1.34 (1.13–1.55)	2.88 ± 0.28	5.34	–
LonPR	1152	1.12 (0.61–1.58)	2.08 ± 0.19	12.29	0.596 (0.532–0.667)
LemBA	1472	0.82 (0.47–1.12)	2.34 ± 0.19	13.54	0.742 (0.542–11.042)
RonMT	1712	0.88 (0.47–1.23)	2.27 ± 0.18	13.42	0.500 (0.297–0.835)
Crop season 2009/10					
ShGO	768	1.39 (0.57–1.92)	2.75 ± 0.31	15.24	0.615 (0.402–1.002)
BvpPR	1008	1.24 (0.95–1.53)	2.27 ± 0.16	7.77	0.711 (0.556–0.929)
ScpSP	1728	0.81 (0.65–0.96)	4.23 ± 0.26	4.98	0.687 (0.546–0.892)
CvMT	1232	1.53 (0.92–2.08)	2.05 ± 0.19	13.67	0.932 (0.466–1.843)
Crop season 2010/11					
CamPR	1152	2.01 (1.65–2.37)	1.93 ± 0.18	4.44	0.924 (0.458–1.689)
RvGO	1152	1.62 (1.18–2.02)	2.57 ± 0.24	7.56	0.856 (0.524–1.363)
RvBA	1152	0.99 (0.53–1.41)	2.22 ± 0.22	8.80	–
CvMT	1280	1.64 (0.65–2.38)	2.36 ± 0.25	16.48	0.919 (0.719–1.169)
SelRS	1024	1.53 (1.31–1.73)	2.78 ± 0.25	1.80	0.830 (0.630–1.117)
Crop season 2012/13					
JabSP13	896	0.74 (0.59–0.91)	2.56 ± 0.18	4.26	0.335 (0.262–0.437)
SinMT13	1008	0.71 (0.52–0.91)	1.75 ± 0.12	4.25	0.224 (0.132–0.395)
RonMT13	768	0.39 (0.32–0.47)	1.60 ± 0.12	2.98	0.216 (0.175–0.270)
RolPR13	896	0.96 (0.62–1.28)	2.31 ± 0.21	6.53	0.784 (0.406–1.918)
ChaMS13	1120	0.52 (0.41–0.65)	2.36 ± 0.14	5.71	0.413 (0.303–0.591)
MonGO13	1008	0.78 (0.65–0.92)	2.17 ± 0.16	2.61	0.278 (0.140–0.607)
BarBA13	896	0.99 (0.81–1.17)	2.06 ± 0.17	3.95	0.252 (0.114–0.603)
InaGO13	768	0.70 (0.36–1.03)	2.22 ± 0.23	5.83	0.154 (0.147–0.161)
CirRS13	1008	0.92 (0.55–1.29)	2.17 ± 0.18	9.89	0.638 (0.474–0.859)
RioRS13	864	0.72 (0.60–0.84)	2.12 ± 0.17	1.57	0.447 (0.239–0.990)

[a] *n* = number of insects tested.
[b] Lethal concentration (CL); fiducial limits (FL) in μg Cry1Ac mL^{-1} diet.
[c] SE = standard error.
[d] Effective concentrations (EC_{50}) required to cause 50% growth inhibition after 1 week and fiducial limits (FL) expressed in μg Cry1Ac mL^{-1} diet.

2 MATERIALS AND METHODS

2.1 Insect collection

Forty-three SBL field populations were sampled from soybean-growing seasons 2008/09 to 2014/15 from multiple regions across Brazil to establish Cry1Ac baseline susceptibility, validate a diagnostic concentration for a resistance monitoring programme and estimate the frequency of Cry1Ac resistance allele through an F_2 screen (Table 1). Approximately 800–2000 larvae were collected in each sampling area. Adults obtained from these larvae were maintained in oviposition cages. The final number of adults per population ranged from 400 to 900. Insects were reared on the artificial diet used by Greene *et al.*[20] in a climatic chamber at 26 ± 2 °C and 70 ± 10% relative humidity (RH) with a 14 h photoperiod. A susceptible SBL colony (Sus) maintained at Embrapa Soybean was used as a reference in bioassays. Most of the bioassays were conducted using larvae (neonates) derived from the first generation (F_1) of field collections in the lab, which minimised any impact that inbreeding and genetic drift might have on the susceptibility of the tested colony. Owing to limited insect availability, there were instances when it was necessary to carry out bioassays until collections reached the third generation in the lab.

2.2 *In vitro* bioassays with Cry1Ac

A synthetic Cry1Ac protein formulated product (MVP II, *Pseudomonas* encapsulated Cry1Ac from Dow Chemicals, San Diego, CA, containing 11.14% of active Cry1Ac protein) was incorporated into the artificial diet, without formalin and antibiotics, when the diet temperature reached 56 °C. To establish the baseline curves, a total of eight concentrations were used: 0, 0.056, 0.18, 0.56, 1.8, 3.14, 5.6 and 18 μg active protein mL^{-1} diet. A 1 mL aliquot of diet containing the protein was poured into each cell of a 16-cell division square of a bioassay tray (128 cells). The trays were sealed with self-adhesive plastic sheets (BIO-CV-16; CD International Inc., Pitman, NJ) that allowed gas exchange with the external environment, and then placed in a climatic chamber (temperature 26 ± 2 °C, 70 ± 10% RH, 14 h photoperiod). The experimental design was completely randomised, with 6–16 replicates per concentration and 16 larvae per replicate at each concentration.

Mortality and the weight of the surviving larvae[21] were recorded after 7 days. The mean weight of survivors was subjected to non-linear regression analysis to estimate the EC_{50} and EC_{90} effective concentrations and the respective confidence intervals [fiducial limit (FL) 95%] using JMP v.8.0.2.[22] The LC_{50} and LC_{90} lethal concentrations and the respective confidence intervals (FL 95%) were estimated using POLO-PC statistical software.[23] Resistance monitoring bioassays were conducted as described above, using diagnostic concentrations of 5.6 and 18 μg protein mL^{-1} diet. At least 100 insects per replicate were used in each concentration.

2.3 F_2 screen for Cry1Ac resistance alleles

The F_2 screen was conducted according to Huang *et al.*[24] Pupae of SBL derived from non-*Bt* soybean plants were sexed and individually placed in cylindrical plastic cages (24 cm height × 14.5 cm diameter) until emergence. Adults were matched in smaller plastic cages (24 cm height × 10 cm diameter) and allowed to mate and oviposit. Each single-pair mating represented an insect family line. Sixty-four F_1 progeny larvae of each single-pair mating were then reared on artificial diet to the pupal stage, as described above. F_1 adults (20 couples) from each single-pair mating were sib-mated in plastic cages (24 cm height × 14.5 cm diameter) to produce F_2 offspring. Offspring produced from a single-pair mating were considered as a two-parent family line. The F_2 screen was conducted in 128-well trays (BIO-BA-128; CD International Inc.). Leaf tissue was excised from leaves of MON 87701 × MON 89788 soybean plants at V4–V6 stages and placed in each well. The leaf tissue was placed on a non-gelled mixture of agar–water at 2% in 128-well trays and then placed in a climatic chamber (temperature $26 \pm 2\,^\circ$C, $70 \pm 10\%$ RH, 14 h photoperiod). The soybean leaf tissue was separated from the agar–water layer by a filter paper disc. For each insect family line, 128 F_2 neonates were screened. The number of surviving larvae was recorded at 4 days after larval inoculation. The joint frequency (*P*) and its corresponding 95% credibility interval were estimated as described by Andow and Alstad.[25,26]

3 RESULTS

3.1 Baseline susceptibility and resistance monitoring

The toxicity of Cry1Ac protein (LC_{50}) to SBL ranged from 0.39 to 2.01 µg mL^{-1} diet across all populations tested from crop seasons 2008/09 to 2012/13, a range of approximately fivefold (Table 2). Comparisons between field populations and the susceptible reference (Sus) showed no statistical difference. Because growth inhibition is also considered to be a valuable parameter for evaluating the response of lepidopteran larvae to *Bt* proteins,[27] the median effective concentration causing growth inhibition (EC_{50}) was calculated. EC_{50} values estimated for the SBL field populations ranged from 0.154 to 0.932 µg mL^{-1}, a range of approximately sixfold (Table 2).

Based on the baseline susceptibility curves, two diagnostic concentrations, 5.6 and 18 µg Cry1Ac mL^{-1} diet, have been used to monitor SBL susceptibility to Cry1Ac protein since crop season 2009/10. With the exception of BvpPR and CvMT, across all SBL field populations tested, the mortality in the control treatment was consistently below 10% (data not presented). The concentration of 5.6 µg Cry1Ac mL^{-1} diet caused high mortality of the SBL neonates tested. Mortality at this concentration ranged from 88.2 to 99.89% throughout the years of testing (Table 3). Therefore, any shift in susceptibility might be indicative of initial resistance evolution. The second concentration used (18 µg Cry1Ac mL^{-1} diet) caused near-complete mortality of the insects tested. Both concentrations have proved to be useful for monitoring purposes and have been used since the commercial launch of *Bt* soybean in Brazil in crop season 2013/14. SBL field populations across different geographical regions and years did not show noticeable differences in Cry1Ac susceptibility (Table 3).

Table 3. *Chrysodeixis includens* protein monitoring using diagnostic concentrations of Cry1Ac protein

Insect population	% Mortality[a]	
	5.6 µg Cry1Ac mL^{-1} diet	18 µg Cry1Ac mL^{-1} diet
Season 2009/10		
BvpPR	97.30 (512)	100 (496)
CvMT	93.70 (448)	99.50 448)
ScpSP	95.40 (448)	99.80 (448)
ShGO	88.20 (448)	100 (448)
Season 2010/11		
RvBA	99.78 (448)	99.78 (448)
RvGO	98.19 (496)	100 (496)
SelRS	96.96 (560)	100 (560)
CvMT	97.92 (480)	99.58 (480)
CamPR	94.76 (496)	99.40 (496)
Season 2011/12		
LemBA12	–	100 (912)
SinMT12	–	100 (912)
RvGO	–	100 (912)
MauPR	–	100 (952)
PonRS	–	100 (952)
Season 2012/13		
SinMT13	95.80 (160)	100 (160)
BarBA13	96.90 (160)	100 (160)
RonMT13	100 (160)	100 (160)
MonGO13	97.90 (144)	100 (144)
InaGO13	100 (160)	100 (160)
ChaMS13	99.37 (144)	100 (144)
RolPR13	99.22 (144)	100 (144)
Season 2013/14		
LemBA14	99.67 (896)	100 (896)
SorrisoMT14	99.67 (896)	100 (896)
MontivGO14	99.40 (891)	99.89 (891)
InaciolGO14	99.78 (896)	100 (896)
ChapadMS14	99.30 (896)	100 (896)
UberlMG14	99.22 (896)	100 (896)
CastroPR14	99.89 (896)	100 (896)
Season 2014/15		
BarBA15	98.50 (560)	99.80 (560)
FRPBA15	95.80 (448)	99.80 (448)
CvMT15	95.75 (896)	99.80 (896)
AraMG15	97.10 (448)	100 (448)
RiBMS15	98.70 (896)	100 (896)
LonPR15	99.10 (672)	99.90 (672)
CaMPR15	97.20 (896)	99.70 (896)
NmtRS15	96.40 (896)	99.90 (896)

[a] In parentheses, the number of insects tested.

3.2 Frequency of Cry1Ac resistance alleles

A total of 626 F_2 two-parent lines (originating from 1252 feral individuals) of SBL were established from larvae collected from seven locations in Brazilian soybean fields in crop season 2014/15 (Table 4). These F_2 lines were screened for resistance to MON 87701 × MON 89788 soybean leaf tissue expressing Cry1Ac. Among these lines, no surviving larvae were found after 4 days, indicating that none of the 626 families possessed resistance alleles to MON 87701 × MON 89788 soybean. The joint frequency of the resistance allele was 0.0004 (95% credibility interval 0–0.0014) (Table 4).

4 DISCUSSION

The SBL field populations investigated in this work were highly susceptible to Cry1Ac protein, and very low natural variation was

Table 4. F_2 screen for detecting resistance alleles to Cry1Ac in Brazilian populations of *Chrysodeixis includens*

Insect population	Single pairs (F_0)	F_1 parental isoline families	F_2 parental isoline families: Screened	F_2 parental isoline families: Positive	Estimated R frequency
Araguari, MG	270	120	100	0	0.0024 (0.0000–0.0091)
Correntina, BA	240	130	100	0	0.0024 (0.0000– 0.0091)
Campo Grande, MS	103	99	99	0	0.0024 (0.0000–0.0091)
Campo Verde, MT	100	85	85	0	0.0029 (0.0000–0.0106)
Casa Branca, SP	184	100	100	0	0.0024 (0.0000–0.0091)
Londrina, PR	250	138	100	0	0.0024 (0.0000–0.0091)
Pitanga, PR	60	42	42	0	0.0057 (0.0001–0.0209)
Total	1207	714	626	0	0.0004 (0.0000–0.0014)

found across seasons and geographic populations. The diagnostic concentration of 5.6 µg Cry1Ac mL^{-1} diet caused near-complete mortality of SBL neonates. The high susceptibility of SBL to Cry1Ac was previously reported through protein assays[13,28] and plant assays using soybean expressing Cry1Ac protein.[13,15] A similar range of variation in susceptibility to Cry1Ac was observed in populations of *H. virescens* and *H. armigera* in Brazil and India respectively.[29,30] Using a reference susceptible strain or setting up a tiered approach to test putative resistance-carrying insects is a best practice that should be incorporated in the monitoring programme for Cry1Ac in SBL. Results of the F_2 screen testing SBL on MON 87701 × MON 89788 soybean leaf tissue showed that no Cry1Ac resistance alleles were detected in 626 two-parent family lines of seven SBL field populations during crop season 2014/15. The joint allele frequency for this product in the combined populations was estimated to be 0.0004, similar to the Cry1A resistance allele frequency observed in 545 two-parent family lines of *Helicoverpa punctigera* (Wallengren) and 826 lines of *H. armigera* in Australia.[31,32] The Cry1Ac resistance allele frequency obtained for SBL (0.0004) was lower than 10^{-3}, the frequency found in *Diatraea saccharalis* (F.) to Cry1Ab and in *Ostrinia nubilalis* (Hübner) to Cry1F in the United States and in *H. armigera* to Cry1Ac in China.[33–36]

The high toxicity of *Bt* soybean against SBL and the low resistance allele frequency found in SBL meet important requirements for a successful IRM strategy, as the high-dose/refuge strategy is expected to be more effective if resistance is conferred by rare recessive alleles, nearly all the heterozygous insects are controlled and most of the resistant adults emerging from *Bt* crops mate with susceptible adults from refuge areas.[17,37] Dominant resistance to Cry1Ac has not been detected in SBL, and recessive inheritance of resistance to Cry1Ac was previously observed for the cabbage looper *Trichoplusia ni* (Hübner).[38] Additionally, a key factor to the success of the high-dose/refuge strategy is having RR individuals that mate almost exclusively with common SS individuals; therefore, non-random mating would compromise the strategy.[39] Several research initiatives have aimed to understand more about the movement of the major target pests of *Bt* crops in Brazil. Vilarinho *et al.*[40] used mark–release–recapture techniques to evaluate the dispersal capacity of fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] adults and found maximum recapture distances of 806 m for males and 608 m for females. Caixeta,[41] using the same techniques to evaluate velvetbean caterpillar (*A. gemmatalis*), noted that more than 10% of moths were recaptured between 800 and 900 m from the release point. Both study cases supported the 800 m maximum distance currently recommended between refuge areas and *Bt* crops in Brazil.

Although the attributes of MON 87701 × MON 89788 soybean and SBL, such as high toxicity and low resistance allele frequency, respectively, contribute to minimising the risk of resistance evolution, it is worth mentioning that the level of risk is intrinsically related to the exposure of the insect to the *Bt* trait.[17] The potential for SBL exposure to crops expressing Cry1Ac is significant in Brazil, given the extensive acreage cultivated with soybean and cotton (30 million and 1 million ha respectively).[12] In addition, SBL will be exposed to the recently deployed MON 87701 × MON 89788 soybean and to *Bt* cotton fields expressing Cry1Ac singly or stacked with other *Bt* traits. It is worth mentioning, however, that *Bt* cotton has been commercialised in Brazil since crop season 2006/07, and no shift in Cry1Ac susceptibility has been observed in this study from populations collected since crop season 2008/09. Moreover, SBL has a wide range of plant hosts distributed in almost 30 families,[42] which could work as alternative hosts during the intercropping season, with unknown impacts on the evolution of resistance to *Bt* crops.

All things considered, three key factors favour success of a refuge strategy: recessive inheritance of resistance, low resistance allele frequency and abundant refuges of non-*Bt* host plants near *Bt* crops.[17,43] Reports on field-evolved resistance to *Bt* crops indicate that in each case at least one of these criteria was not met: examples include *Busseola fusca* (Füller) resistance to Cry1Ab maize,[44] *S. frugiperda* resistance to Cry1F maize,[45,46] *Pectinophora gossypiella* (Saunders) resistance to Cry1Ac cotton[47] and *Diabrotica virgifera virgifera* LeConte resistance to Cry3Bb1 maize.[48] Because the product attributes of MON 87701 × MON 89788 soybean and the main target pest (SBL) are likely to meet the first two of these three criteria, the refuge implementation is the last key component of a successful IRM strategy. Low compliance with non-*Bt* refuge recommendations has been observed for maize in Brazil, the first *Bt* crop widely adopted by Brazilian growers.[49] Based on this previous experience, joint efforts of growers, consultants, industry, academic researchers and government will be necessary to implement an effective IRM strategy for *Bt* soybean in Brazil, including the planting of refuge areas of at least 20% non-*Bt* soybean.

ACKNOWLEDGEMENTS

This study was partially supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (Process No. 503555/2011-8 and 308947/2014-2). We would like to thank Fábio E Paro for performing the baseline susceptibility and resistance monitoring bioassays. We thank SGS Gravena (SISBIO License No.

10018-1) and PROMIP (SISBIO License No. 40380-2) for collecting insect samples.

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