Biomass degradation after harvest of genetically modified products compared to conventional counterparts

Hallison Vertuan[a,1], Marcia Jose[a], Augusto Crivellari[a], Gustavo G. Belchior[a], Luciana Verardino[a], Daniel J. Soares[a], Fabiana Bacalhau[a], Marcos Barancelli[a], Daniel Sordi[a], Geraldo U. Berger[a]

[a] Regulatory Science, Bayer Crop Science, São Paulo, SP, Brazil

Abstract

Conventional breeding and modern biotechnology tools have been successfully combined over the years to generate GM crops. Single events have been crossed to generate stacked products and these combinations have proven to be an effective way to combine different gene products and associated characteristics which are agronomically relevant and result in a yield increase. Regulatory agencies around the world still require risk assessment data for these products while no evidence-based additional biosafety concerns have emerged in over 20 years of global use. As part of the environmental risk assessment to evaluate the biosafety of GMOs, the Brazilian regulatory agency requests biomass degradation analyses of GM plants compared to their conventional counterparts. Here we present results on the evaluation of biomass degradation of GM and non-GM crops for soybean, maize and cotton, including single events and stacked products. Field trials were performed in representative cultivated areas in Brazil to generate biomass samples after harvest. The degradation studies were conducted on the plant consisting of stalks, senescent leaves and stems after harvest. Collected samples in different growing seasons were used in degradation studies conducted in a greenhouse. For each product, data was subjected to analysis of variance and pairwise differences between GM and conventional counterparts were assessed with a 5% significance level. Our results show that single events and stacked products of soybean, maize and cotton presented no significant differences from their conventional counterparts for biomass degradation. This adds to the existing weight of evidence that indicates that single and stacked GM crops follow the same pattern of biomass degradation compared to conventional counterparts.

Keywords: Biomass degradation, genetically modified products, environmental risk assessment, soybean, maize, cotton.

1. Introduction

Humankind has been selecting for better agronomic plant characteristics over 10,000 years and conventional breeding has played a pivotal role in this process (McCouch, 2004). Even though traditional plant breeding has been crucial to ensure the genetic diversity and improve varieties of domesticated species (Swarup et al., 2021), genetic modification can introduce new agronomic traits that would not naturally occur. Thus, genetic modification has been an important tool of modern biotechnology for the specific introduction of desired traits in agricultural crops (Glenn et al., 2017; Halpin, 2005; James, 2010; Raman, 2017), leading to substantial improvements in insect and weed control and allowing for other desired characteristics.

Genetically modified (GM) crops are indeed credited to result in increased yields without compromising food/feed security (ISAAA, 2018), also bringing environmental benefits associated with cuts in fuel use and tillage changes which resulted in a significant reduction

[1] Corresponding author: Hallison Vertuan- hallison.vertuan@bayer.com

in the release of greenhouse gas emissions, while still providing production growth and food security (Brookes, 2020; Brookes and Barfoot, 2018).

The use of conventional breeding and modern biotechnology in order to generate multiple GM traits in the same plant (stacked products) with two or more single events results in a convenient way to combine distinct characteristics to improve flexibility, performance and productivity (Glenn et al., 2017; James, 2010; Smyth, 2020; Vertuan et al., 2017).

However, the commercial release of GM crops involves a considerable investment of resources (time and money) and robust data packages. From trait discovery to the commercial approval of a single product, developers invest approximately US$ 136 million and 13 years of research and data generation (McDougall, 2011). Extensive studies prior to commercialization are needed to attend specific requirements from biosafety legislations in different countries, where generated data is presented to regulatory agencies for risk evaluation and approval in order to a product to reach the market (Craig et al., 2008; De Schrijver et al., 2007; Kramer et al., 2016).

Studies have demonstrated that single events and stacked products represent no greater risk compared to their conventional counterparts when different characteristics were evaluated (Bell et al., 2018; Berman et al., 2011; de Cerqueira et al., 2017; Gampala et al., 2017; Goodwin et al., 2021; Herman et al., 2017; Marques et al., 2018; Raybould et al., 2012; Ridley et al., 2011; Venkatesh et al., 2014) and there is scientific consensus that genetic engineering methods are safe (Herman et al., 2017). Jose et al. (2020) demonstrated there were no differences in agronomic and phenotypic plant characteristics between single-event and stacked products when compared to their conventional counterparts, for soybean, maize and cotton. Finally, Clawson et al. (2019) provided evidence showing that risk assessment outcomes were consistent between single-event and conventionally-bred stacked maize products.

As part of the environmental risk assessment (ERA) to evaluate the biosafety of genetically modified organisms (GMOs), the Brazilian regulatory agency CTNBio (National Technical Biosafety Commission) requests biomass degradation analyses of GM plants compared to their conventional counterparts to be presented as part of the commercial approval process for these technologies (CTNBio, 2008). Research presented in this manuscript includes multiple years of greenhouse data assessment on biomass degradation in samples collected from conventional, single-event, and stacked products of soybean (*Glycine max*), maize (*Zea mays*) and cotton (*Gossypium hirsutum*) cultivated in Brazil.

Table 1 Field trial locations where samples were generated and associated characteristics

Location	State; Region	Altitude (m)	Climate	Crop cultivation[a]
Não-Me-Toque	Rio Grande do Sul; South	500	Subtropical	Soybean, maize
Rolândia	Paraná; South	600	Tropical	Soybean, maize, cotton
Santa Cruz das Palmeiras	São Paulo; Southeast	650	Tropical	Soybean, maize, cotton
Cachoeira Dourada	Minas Gerais; Southeast	450	Tropical	Soybean, maize, cotton
Luís Eduardo Magalhães	Bahia; Northeast	825	Tropical	Soybean, maize, cotton
Sorriso	Mato Grosso; Central-West	360	Tropical	Soybean, maize, cotton

[a] Cultivation of crops in each region. Soybean and maize are cultivated in all locations where trials were performed. Cotton is not cultivated in Rio Grande do Sul state.

2. Materials and methods

2.1 Field trials and sample production

Biomass samples were collected in field trials performed in 5 to 6 locations for maize and 5 locations for soybean and cotton (Table 1) in different growing seasons. Figure 1 demonstrates the different ecoregions where samples were collected, representing a diverse range of geography, climate classes and wide range of soil properties (Agriculture & Food Systems Institute, 2020). The field trials were set up in a randomized complete block design (RCBD) with four replications. For all crops and, in each location, GM materials, conventional controls (with the same genetic background) and four commercial references were cultivated in the trials. Variation in planting, collection and harvesting dates were expected, since the trial locations are widespread across Brazil. Crop maintenance in each location followed the most adopted commercial practices for each region. All studies were conducted with prior authorization from CTNBio, the local federal regulatory body responsible for handling matters related to genetically modified organisms, including the GM crops that were under regulated status when the studies were conducted. The biomass samples were collected in the following growth stages that represents harvest maturity: R8 for soybeans, R6 for maize and, after boll collection for cotton. The plants consisting of stalks, senescent leaves and stems after harvest were considered the biomass assessed and 40 plants per material were collected (10 plants per repetition) and placed together. After collection, samples were chopped with a tractor-mounted thresher (model JF 80, JF Máquinas, Itapira-SP, Brazil) through a 0.5 mm sieve and packed in pre-labeled containers. Between samples processing, compressed air was used to clean the equipment to avoid cross contamination. Samples in each station were dried in a kiln at 60 °C for 72 hours to prevent mold spreading and stored and shipped in ambient conditions prior to incubation. Samples from different locations were shipped to Santa Cruz das Palmeiras research station. The biomass samples were used in degradation studies conducted in a greenhouse at the Santa Cruz das Palmeiras (SP) field station after each harvest. The single-event and stacked products tested are listed in Table 2. The sampling location, growing season where samples were collected, and number of plots are presented in Supplementary Material (Table S1). The single-event, stacked product, conventional control and commercial references were grown at each location for biomass production.

2.2 Incubation and sampling

After sample production in each season across locations, biomass containers of each material were shipped to the Santa Cruz das Palmeiras field station for preparation. Before incubation with biomass, soil samples were collected in Santa Cruz das Palmeiras, air-dried, sieved and stored in an open container with a room temperature to maintain the natural microbial and fungal population. Soil samples were classified as red latosol with the following characteristics: pH (H_2O) 5.3, 63.2% of clay, 15.5% of silt, 21.3% of sand, 2.8% of organic matter, 2.1 $cmol_c/dm^3$ of Ca, 0.5 $cmol_c/dm^3$ of Mg, 0.57 $cmol_c/dm^3$ of K and 23.2 mg/dm^3 of P (Melich). Soil samples analyzed for microbiology attributes reveled a range of $2.2\times10^6 – 5.3\times10^6$ for bacteria, $3.2\times10^4 – 4.5\times10^4$ for fungi and $2.7\times10^5 – 1.6\times10^6$ for actinomycetes. The biomass samples from GM crops, conventional and commercial references were mixed with soil at a proportion of 10% (10 g of biomass sample and 90 g of soil sample) and deposited in previously labeled containers. A rigid polysterene cup without cap was used as a container. Before mixing, biomass and soil moisture were determined by weighing aliquots of each material and drying them in a kiln at 60 °C for 72 hours or until constant mass was observed based on a similar methodology proposed by Calonego et al. (2012). The average of crop biomass moisture observed prior drying was between 7.3 to 9.4% in average. This allowed for correct mass calculation, considering the initial soil and biomass moisture that could have been added to materials due to storage before incubation. The containers with soil and biomass mixture were incubated in greenhouse under controlled conditions (temperature between 26-28 °C, approximately 70% of air humidity and natural light). The experiments

were set up in a randomized complete block design (RCBD) with three/four replicates (depending on year/product). The number of replications were described in Table S1. Water was applied weekly to sustain moisture based on field moisture capacity. Samples were collected at 30, 60, and 90 days after incubation (DAI). After collection, samples (soil + biomass) in each collection time were dried in a kiln at 60 °C for 72 hours or until constant mass. Dried samples were weighted on a calibrated scale. The mass difference between initial incubated sample (day-zero) and collected sample at 30, 60 and 90 DAI was considered as degraded biomass. The percentage of degradation was estimated using the following equation:

$$\%\, Degradation = (\Delta i - \Delta f / \Delta i) \times 100$$

where Δi is the initial mass and Δf is the final mass. Initial soil and biomass moisture values were subtracted from the respective total initial mass values to adjust the final sample mass. This study aimed to understand if the biomass of GM and conventional plants follow the same pattern of degradation in the same condition under a controlled environment. No mechanistic elements were explored.

Fig. 1 Locations where samples were collected. Ecoregions defined by Agriculture & Food Systems Institute (2020)

Table 2 Single and stacked events assessed for biomass degradation

Crop	Single/stacked products	Trait	Corresponding transgenic gene product
Soybean	MON 87751	Insect resistance	IR: Cry1A.105, Cry2Ab2
	MON 87708	Herbicide tolerance	HT: DMO
	MON 87701	Insect resistance	IR: Cry1Ac
	MON 89788	Herbicide tolerance	HT: CP4 EPSPS
	MON 87751 × MON 87701 × MON 87708 × MON 89788	Insect resistance	IR: Cry1A.105, Cry2Ab2, Cry1Ac
		and herbicide tolerance	HT: DMO, CP4 EPSPS
Maize	MON 87411	Insect resistance	IR: Cry3Bb1, *DvSnf7*
		and herbicide tolerance	HT: CP4 EPSPS
	MON 87427	Herbicide tolerance	HT: CP4 EPSPS
	MON 89034	Insect resistance	IR: Cry1A.105, Cry2Ab2
	MON 89034 × MIR162	Insect resistance	IR: Cry1A.105, Cry2Ab2, Vip3Aa
	MON 87427	Herbicide tolerance	HT: CP4 EPSPS
	MON 89034	Insect resistance	IR: Cry1A.105, Cry2Ab2
	MON 87411	Insect resistance	IR: Cry3Bb1, *DvSnf7*
		and herbicide tolerance	HT: CP4 EPSPS
	MON 87427 × MON 89034 × MIR162 × MON 87411	Insect resistance and herbicide tolerance	IR: Cry1A.105, Cry2Ab2, Vip3Aa, Cry3Bb1, *DvSnf7* HT: CP4 EPSPS
	MON 95379	Insect resistance	IP: Cry1B.868, Cry1Da_7
	MON 87429	Herbicide tolerance	HT : CP4 EPSPS, DMO, FT_T, PAT
Cotton	MON 15985	Insect resistance	IR: Cry1Ac, Cry2Ab2
	MON 88913	Herbicide tolerance	HT: CP4 EPSPS
	COT102 × MON 15985 × MON 88913	Insect resistance	IR: Vip3Aa, Cry1Ac, Cry2Ab2
		and herbicide tolerance	HT: CP4 EPSPS
	MON 88701	Herbicide tolerance	HT: DMO, PAT
	MON 88913 × MON 88701	Herbicide tolerance	HT: DMO, CP4 EPSPS, PAT
	COT102 × MON 15985 × MON 88913 × MON 88701	Insect resistance	IR: Vip3Aa, Cry1Ac, Cry2Ab2
		and herbicide tolerance	HT: CP4 EPSPS, DMO, PAT

Products are indicated by their event codes. Each biotechnology-derived trait (IR: insect resistance; HT: herbicide tolerance) is indicated per single or stacked product, as well as corresponding transgenic gene product. CP4 EPSPS: *Agrobacterium tumefaciens* (strain CP4) 5-enolpyruvylshikimate-3-phosphate synthase (tolerance to glyphosate herbicide); Cry (various proteins): *Bacillus thuringiensis* (different strains), Cry δ-endotoxins (resistance to lepidopteran/coleopteran insects); DMO: *Stenotrophomonas maltophilia* (strain DI-6), dicamba mono-oxygenase (tolerance to dicamba herbicide); *DVSnf7*: *Diabrotica virgifera virgifera* double-stranded RNA transcript containing a 240 bp fragment of the *Diabrotica* species *Snf7* gene (resistance to specific Coleopteran insects); PAT: *Streptomyces hygrosopicus* phosphinothricin N-acetyltransferase (tolerance to glufosinate herbicide); Vip3Aa: *Bacillus thuringiensis* (strain AB88) vegetative insecticidal protein (lepidopteran insect resistance); FT_T: *Sphingobium herbicidovorans* dioxygenase protein (tolerance to 2,4-d and FOPs). All gene products are proteins, except for the double-stranded RNA molecule *DvSnf7*.

2.3 Variance components analyses

Combined site analyses between GM materials and their conventional control were performed. Analysis of variance (ANOVA) was performed and pairwise differences between GM and their conventional counterparts were tested by *t-test* at the 5% level of significance ($\alpha = 0.05$).

The combined site analysis was conducted according to the following model for a randomized complete block design, using JMP® 12 software:

$$Y_{ijk} = \mu + S_i + R(S)_{j(i)} + M_k + (SM)_{ik} + \varepsilon_{ijk}$$

where:
Y_{ijk} is the observed response for the k^{th} material in the j^{th} replicate of the i^{th} site;
μ is the overall mean;
S_i is the random effect of the i^{th} site;
$R(S)_{j(i)}$ is the random effect of the j^{th} replicate nested with the i^{th} site;
M_k is the fixed effect of the k^{th} treatment (GM and conventional control);
$(SM)_{ik}$ is the random effect of the interaction between the i^{th} site and k^{th} treatment (GM and conventional control);
ε_{ijk} is the residual error.

Reference ranges were obtained from the combined-site of minimum and maximum overall mean values observed for reference materials across locations. When significant differences between GM and conventional control were detected, the GM mean value was compared to the reference range and observed if the value was within the range. The commercial references cultivated in these studies represented the natural variability of biomass degradation.

ERA studies typically make use of pairwise comparisons between GM products and their conventional counterparts to evaluate mean values. Some authors used this statistical analysis approach to compare the means between GM crops *versus* conventional control (Clawson et al., 2019; Díaz et al., 2017; Jose et al., 2020).

3. Results

3.1 Soybean biomass degradation

Biomass samples were collected in Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada and Luís Eduardo Magalhães field stations. The comparisons between GM soybean (single and stacked) events and the corresponding conventional counterparts revealed no significant differences (Table 3) among 15 comparisons that were made.

Table 3 Percentage of biomass degradation in soybean single-event and stacked products compared to conventional control

Material	30 DAI[b]	60 DAI	90 DAI
	Average % (SE)[c]		
MON 87751	24.6 (2.3)	34.0 (3.3)	40.0 (4.0)
MON 87708	26.0 (2.3)	33.9 (3.7)	45.8 (4.6)
MON 87701	23.5 (2.8)	27.9 (4.5)	37.4 (5.5)
MON 89788	23.0 (3.2)	25.9 (4.1)	31.7 (4.5)
MON 87751 × MON 87701 × MON 87708 × MON 89788	15.6 (2.5)	20.8 (3.7)	32.3 (5.2)
Conventional control	19.6 (2.5)	24.9 (2.7)	39.6 (4.0)
Reference range (Min-Max)[d]	4.5 – 39.1	1.4-62.3	13.6 – 66.7

[a] Number of locations where materials were cultivated and sampled. [b] DAI = days after incubation. [c] SE = standard error. [d] Minimum and maximum reference range average.

Table 4 Percentage of biomass degradation in maize single-event and stacked products compared to conventional control

Material	30 DAI[b]	60 DAI	90 DAI
	Average % (SE)[c]		
MON 87411	33.3 (6.2)	38.3 (5.8)	45.5 (6.3)
Conventional control	30.6 (5.9)	37.1 (5.8)	46.9 (6.5)
Reference range (Min-Max)[d]	4.3-55.1	11.6-63.8	19.4-69.6
MON 87427	15.0 (2.7)	23.1 (5.2)	17.4 (3.2)
Conventional control	16.1 (2.5)	26.6 (4.2)	22.6 (4.2)
Reference range (Min-Max)[d]	3.0-34.7	4.5-45.8	7.6-39.1
MON 89034	45.4 (3.4)	51.2 (1.5)	56.7 (1.2)
MON 89034 × MIR162	42.0 (2.6)	49.1 (1.6)	55.5 (1.3)
Conventional control	43.4 (2.4)	51.9 (1.3)	57.1 (0.7)
Reference range (Min-Max)[d]	24.2-62.5	37.6-59.1	44.7-61.6
MON 87427	29.0 (1.5)	51.9 (2.7)	58.1 (2.3)
MON 89034	25.5 (1.4)*	48.2 (2.6)	57.1 (2.6)
MON 87411	32.4 (1.5)	52.9 (2.5)	60.4 (2.5)
MON 87427 × MON 89034 × MIR162 × MON 87411	30.9 (1.7)	54.7 (2.3)	57.1 (2.1)
Conventional control	33.0 (2.0)	49.3 (2.2)	60.8 (2.2)
Reference range (Min-Max)[d]	22.9-57.8	38.3-60.6	50.5-70.7
MON 95379	30.0 (1.1)	40.1 (1.7)	47.3 (1.6)
Conventional control	30.2 (1.1)	41.5 (1.5)	47.5 (1.5)
Reference range (Min-Max)[d]	21.3-42.7	27.9-55.5	30.5-58.2
MON 87429	26.3 (1.1)	33.0 (1.2)	34.2 (1.6)
Conventional control	26.4 (1.1)	32.9 (1.3)	35.3 (1.5)
Reference range (Min-Max)[d]	17.0-31.4	20.5-38.7	21.9-40.8

*Indicates significant difference ($p<0.05$) between GM and conventional control. [a] Number of locations where materials were cultivated and sampled. [b] DAI = days after incubation. [c] SE = standard error. [d] Minimum and maximum reference range average.

3.2 Maize biomass degradation

Biomass samples were collected in Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Sorriso and Luís Eduardo Magalhães field stations, except for the stacked product MON 87427 × MON 89034 × MIR162 × MON 87411 and it singles that biomass samples were collected in Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada and Sorriso field stations. The comparisons between GM maize (single and stacked) events and the corresponding conventional counterparts revealed no significant differences (Table 4) among 29 comparisons that were made. The only exception was the biomass degradation for MON 89034 at 30 days after incubation, which presented a lower mean value when compared to the conventional control. However, this value was within the reference range.

3.2 Cotton biomass degradation

Biomass samples were collected in Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Sorriso and Luís Eduardo Magalhães locations.

Table 5 shows the comparisons between GM cotton (single and stacked) events and the corresponding conventional counterparts. No significant differences were found for any of the comparisons between materials.

Table 5 Percentage of biomass degradation in cotton single-event and stacked products compared to conventional control

Material	30 DAI[b]	60 DAI	90 DAI
	Average % (SE)[c]		
MON 15985	34.5 (3.9)	56.1 (5.5)	57.9 (4.0)
MON 88913	35.5 (4.4)	54.2 (4.7)	53.4 (6.4)
COT102 × MON 15985 × MON 88913	36.9 (6.4)	54.2 (3.6)	59.0 (3.7)
Conventional control	31.3 (3.9)	55.3 (5.0)	54.2 (5.2)
Reference range (Min-Max)[d]	15.3-61.9	33.9-80.6	21.9-81.9
MON 88701	11.9 (2.9)	14.7 (3.9)	24.3 (6.4)
Conventional control	16.4 (4.1)	16.9 (4.3)	26.1 (7.7)
Reference range (Min-Max)[d]	4.5-29.2	1.5-38.9	6.1-47.2
MON 88701 × MON 88913	23.3 (1.1)	31.5 (1.3)	36.8 (1.6)
COT102 × MON 15985 × MON 88913 × MON 88701	22.1 (1.3)	29.6 (1.7)	33.8 (2.1)
Conventional control	22.4 (1.2)	29.4 (1.2)	34.7 (1.5)
Reference range (Min-Max)[d]	15.7-28.8	21.1-38.9	23.9-44.5

[a] Number of locations where materials were cultivated and sampled. [b] DAI = days after incubation. [c] SE = standard error. [d] Minimum and maximum reference range average.

4. Discussion

Different aspects of products derived from modern biotechnology may be taken into consideration from a biosafety standpoint. The commercial approval process of GM crops in Brazil requires applicants to address environmental risk assessment questions considering different safety aspects and the data package undergoes a rigorous scientific evaluation before CTNBio grants commercial release approval. The possible modifications of the biodegradability of the GM plant compared to conventional counterpart is one of the questions asked by the Brazilian authority.

As soon as biomass gets to the soil, the decomposition process initiates as the biomass is incorporated into, or is kept over the soil, leading to the transformation of plant residues into organic matter. It is understood that organic matter transformation is essentially mediated by a plethora of soil microorganisms that act upon plant residues (Becker et al., 2014). A large portion of the biomass is degraded by the soil microbiota, and the previously published literature demonstrates that the cultivation of GM crops does not impact microbial soil activity when compared to conventional counterparts (de Souza et al., 2008; de Souza, 2013; Fernandes et al., 2019; Li et al., 2011; Ma et al., 2011; Miethling-Graff et al., 2010), indicating no increased environmental risk. The Cry1Ab protein in root exudates and biomass of *Bt* maize appears not to be toxic to earthworms, nematodes, protozoa, bacteria, and fungi (Saxena and Stotzky, 2001).

Furthermore, the field research has demonstrated that gene products produced by GM crops do not persist in soil following cultivation. Dubelman et al. (2005) and Gruber et al. (2012) assessed the

GM maize producing the Cry1Ab protein in different soils in a long-term cultivation over 3 and 9 growing seasons, respectively. No experimental evidence for accumulation and persistence and insect toxicity in soils were observed across years. A similar result obtained by Shan et al. (2014) after 3 years of continuous planting of GM maize expressing Cry1F protein was observed, representing no accumulation of protein in the soil. The amount of protein accumulated as a result of continuous use of transgenic *Bt* cotton expressing Cry1Ac protein, and subsequent incorporation of plant residues into the soil, does not result in detectable biological activity (Head et al., 2002) after 6 years of cultivation. Sims & Ream (1997) observed that CryIIA insecticidal protein within transgenic cotton tissue remains less than 25% of the initial bioactivity after 120 days in the soil. Joaquim et al. (2019) characterized the environmental fate of DvSnf7 double-stranded RNA (dsRNA) produced by MON 87411 maize in Brazilian soils. The study shows that DvSnf7 dsRNA dissipated rapidly in tropical soils and is unlikely to persist in soil following cultivation in tropical environments.

Becker et al. (2014) concluded that the multi-insect resistant GM maize (MON 89034 × MON 88017) did not present an adverse impact on straw decomposition neither an impact on the involved microbial communities when compared to the isogenic control. A similar result was found by Lehman et al. (2008), working with GM maize. No significant differences in decomposition rates between *Bt* and non-*Bt* maize residue were observed over a period of 22 months under field conditions.

The DNA present in the soil is susceptible to rapid cleavage by endonucleases generating smaller DNA fragments, which results in a loss of genetic information. Subsequently, the DNA fragments are degraded into single nucleotides by DNases, making the accumulation of genetic information in the soil unlikely (Blum et al., 1997; England et al., 1997; England and Trevors, 2003; Levy-Booth et al., 2007). For example, in a field study with GM soybean and corn performed by Gulden et al. (2008), the persistence of transgenes was investigated using quantitative real-time PCR assays and no accumulation of DNA from GM plants in the soil was observed. The authors mentioned that transgenic DNA can persist in rotation at detectable levels for up to 2 years, but the vast majority of plant target DNA was degraded shortly after harvest.

5. Conclusions

Our results show that the genetic modification of soybean, maize, and cotton by the introduction of genes that confer insect resistance and/or herbicide tolerance does not affect the degradability of these crops biomass in soil after harvest, considering 5-6 locations representing different environments and various seasons in Brazil. These results corroborate the current weight of evidence from the previously published literature, which support the biosafety profile of GM crops from an ERA perspective, demonstrating yet again that products derived from modern biotechnology are as safe as their conventional counterparts and do not pose an increased risk or concern from a biosafety standpoint.

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7. Competing interests

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Supplementary Material

Table S1 Field design of biomass production, sampling location, season where samples were harvested, number of repetitions and number of plots in each location

Crop	Entry number	Cultivation and sampling location	Growing season[a]	Number of plots in each location (entry × rep)
Soybean	1. MON 87751 2. MON 87708 3. MON 87701 4. MON 89788 5. MON 87751 × MON 87708 × MON 87701 × MON 89788 6. Conventional control 7. Commercial reference 8. Commercial reference 9. Commercial reference 10. Commercial reference	Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães	2014	10 × 4 = 40 plots
Maize	1. MON 87411 2. Conventional control 3. Commercial reference 4. Commercial reference 5. Commercial reference 6. Commercial reference	Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2013	6 × 4 = 24 plots
	MON 87427 2. Conventional control 3. Commercial reference 4. Commercial reference 5. Commercial reference 6. Commercial reference	Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2014	6 × 4 = 24 plots
	1. MON 89034 2. MON 89034 × MIR162 3. Conventional control 4. Commercial reference 5. Commercial reference 6. Commercial reference 7. Commercial reference	Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2015	7 × 4 = 28 plots
	1. MON 87427 2. MON 89034 3. MON 87411 4. MON 87427 × MON 89034 × MIR162 × MON 87411 5. Conventional control 6. Commercial reference	Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Sorriso	2016	9 × 4 = 36 plots

Crop	Entry number	Cultivation and sampling location	Growing season[a]	Number of plots in each location (entry × rep)
	7. Commercial reference			
	8. Commercial reference			
	9. Commercial reference			
	MON 95379	Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2018	6 × 4 = 24 plots
	2. Conventional control			
	3. Commercial reference			
	4. Commercial reference			
	5. Commercial reference			
	6. Commercial reference			
	1. MON 87429	Não-Me-Toque, Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2018	6 × 4 = 24 plots
	2. Conventional control			
	3. Commercial reference			
	4. Commercial reference			
	5. Commercial reference			
	6. Commercial reference			
Cotton	1. MON 15985	Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2012	8 × 4 = 32 plots
	2. MON 88913			
	3. COT102 × MON 15985 × MON 88913			
	4. Conventional control			
	5. Commercial reference			
	6. Commercial reference			
	7. Commercial reference			
	8. Commercial reference			
	1. MON 88701	Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2013	6 × 4 = 24 plots
	2. Conventional control			
	3. Commercial reference			
	4. Commercial reference			
	5. Commercial reference			
	6. Commercial reference			
	1. MON 88913 × MON 88701	Rolândia, Santa Cruz das Palmeiras, Cachoeira Dourada, Luís Eduardo Magalhães Sorriso	2016	6 × 4 = 24 plots
	2. COT102 × MON 15985 × MON 88913 × MON 88701			
	3. Conventional control			
	4. Commercial reference			
	5. Commercial reference			
	6. Commercial reference			

[a] Growing season = Brazilian season where the materials were grown for biomass sampling.

Table S2 Greenhouse study design for biomass degradation assessment

Crop	Entry number	Number of locations for sample production	Number of repetitions[a]	Number of experimental units assessed in each collection period		
				30 DAI[b]	60 DAI	90 DAI
Soybean	1. MON 87751	5	3	15	15	15
	2. MON 87708	5	3	15	15	15
	3. MON 87701	5	3	15	15	15
	4. MON 89788	5	3	15	15	15
	5. MON 87751 × MON 87708 × MON 87701 × MON 89788	5	3	15	15	15
	6. Conventional control	5	3	15	15	15
	7. Commercial reference	5	3	15	15	15
	8. Commercial reference	5	3	15	15	15
	9. Commercial reference	5	3	15	15	15
	10. Commercial reference	5	3	15	15	15
Maize	1. MON 87411	6	3	18	18	18
	2. Conventional control	6	3	18	18	18
	3. Commercial reference	6	3	18	18	18
	4. Commercial reference	6	3	18	18	18
	5. Commercial reference	6	3	18	18	18
	6. Commercial reference	6	3	18	18	18
	MON 87427	6	3	18	18	18
	2. Conventional control	6	3	18	18	18
	3. Commercial reference	6	3	18	18	18
	4. Commercial reference	6	3	18	18	18
	5. Commercial reference	6	3	18	18	18
	6. Commercial reference	6	3	18	18	18
	1. MON 89034	6	3	18	18	18
	2. MON 89034 × MIR162	6	3	18	18	18
	3. Conventional control	6	3	18	18	18
	4. Commercial reference	6	3	18	18	18
	5. Commercial reference	6	3	18	18	18
	6. Commercial reference	6	3	18	18	18
	7. Commercial reference	6	3	18	18	18
	1. MON 87427	5	3	15	15	15
	2. MON 89034	5	3	15	15	15
	3. MON 87411	5	3	15	15	15
	4. MON 87427 × MON 89034 × MIR162 × MON 87411	5	3	15	15	15
	5. Conventional control	5	3	15	15	15
	6. Commercial reference	5	3	15	15	15
	7. Commercial reference	5	3	15	15	15
	8. Commercial reference	5	3	15	15	15
	9. Commercial reference	5	3	15	15	15

Crop	Entry number	Number of locations for sample production	Number of repetitions[a]	Number of experimental units assessed in each collection period		
				30 DAI[b]	60 DAI	90 DAI
Cotton	MON 95379	6	4	24	24	24
	2. Conventional control	6	4	24	24	24
	3. Commercial reference	6	4	24	24	24
	4. Commercial reference	6	4	24	24	24
	5. Commercial reference	6	4	24	24	24
	6. Commercial reference	6	4	24	24	24
	1. MON 87429	6	4	24	24	24
	2. Conventional control	6	4	24	24	24
	3. Commercial reference	6	4	24	24	24
	4. Commercial reference	6	4	24	24	24
	5. Commercial reference	6	4	24	24	24
	6. Commercial reference	6	4	24	24	24
	1. MON 15985	5	3	15	15	15
	2. MON 88913	5	3	15	15	15
	3. COT102 × MON 15985 × MON 88913	5	3	15	15	15
	4. Conventional control	5	3	15	15	15
	5. Commercial reference	5	3	15	15	15
	6. Commercial reference	5	3	15	15	15
	7. Commercial reference	5	3	15	15	15
	8. Commercial reference	5	3	15	15	15
	1. MON 88701	5	3	15	15	15
	2. Conventional control	5	3	15	15	15
	3. Commercial reference	5	3	15	15	15
	4. Commercial reference	5	3	15	15	15
	5. Commercial reference	5	3	15	15	15
	6. Commercial reference	5	3	15	15	15
	1. MON 88913 × MON 88701	5	3	15	15	15
	2. COT102 × MON 15985 × MON 88913 × MON 88701	5	3	15	15	15
	3. Conventional control	5	3	15	15	15
	4. Commercial reference	5	3	15	15	15
	5. Commercial reference	5	3	15	15	15
	6. Commercial reference	5	3	15	15	15

[a] Number of repetitions sampled after sample collection and mixing. [b] DAI = days after incubation.