INTRODUCTION

The commercialization of agricultural products derived from biotechnology, or Genetically Modified (GM) crops, started in 1996, and in 2017 the cumulative area planted in the World was of approximately 2.3 billion hectares, including the main agricultural crops (soybean, corn, cotton and canola). In the period between 1996 and 2016, GM crops were adopted by more than 18 million farmers, 90% of them small farmers in emerging countries. In 2017, 24 countries planted GM crops, of which 19 are emerging countries. In the same year, the area cultivated with GM crops in the World grew 3% and totaled approximately 190 million hectares, and the main countries have adopted this technology are the United States, Brazil and Argentina, which had more than 75% of their agriculture area planted with GM crops (ISAAA, 2017).

In Brazil, the total of 50.2 million hectares planted with GM crops in 2017, approximately 34 million hectares were GM soybean, being 40% of them were for herbicide tolerant cultivars (HT) and 60% to insect resistant and herbicide tolerant cultivars (IR/HT). GM corn was cultivated at approximately 15.6...
millions of hectares with most of the area (75%) being cultivated with IR/HT hybrids. GM cotton; conversely, was cultivated in 0.94 million hectares distributed among cultivars IR, HT and IR/HT, with 11%, 30% and 59% of area, respectively. Based on these data, Brazil has cultivated approximately 400 million hectares of GM crops cumulatively since the approval of the first GM crop in the country (ISAAA, 2017).

Glyphosate is a broad-spectrum herbicide capable of controlling a great diversity of weed, including dicotyledons, monocotyledons and perennials. Its lack of residual activity, low cost and safe environmental and toxicological profile (EPA, 1993; EPA, 2013b; EPA, 2017; EFSA, 2017; PMRA, 2017) make this herbicide the most used in the world (DUKE & POWLES, 2008; JAN et al. 2009; DILL et al. 2010; POLLAK, 2011; SZÉKÁCS & DARVAS, 2012; BENBROOK, 2016).

Glyphosate could leave residues after its use for weed control, whether in conventional crops or GM crops, and for this reason, international and national regulatory agencies have registered agrochemicals products and use its safety to environment and human and animal health. The CODEX ALIMENTARIUS (2018), for example, established MRLs (Maximum Residue Limits) for soybean at 20.0 mg kg\(^{-1}\), corn at 5.0 mg kg\(^{-1}\) and cotton at 40.0 mg kg\(^{-1}\). In Brazil, the National Agency of Sanitary Surveillance – ANVISA established the following MRLs: 10 mg kg\(^{-1}\) in soybean grains; 1.0 mg kg\(^{-1}\) in corn grains; and 3.0 mg kg\(^{-1}\) in cotton seeds (ANVISA MRLs pattern were used as the main threshold in this study). The quantification of agrochemicals residues levels is relevant in GM soybean, corn and cotton crops mainly to economic importance that these biotechnology products have in Brazil. Together with glyphosate residues data assessment, residues evaluations of its metabolite AMPA (aminomethylphosphonic acid) are commonly performed (EPA, 2013b), being these levels assessment equally important.

The aim of this study was to measure the glyphosate and AMPA metabolite residues in GM glyphosate tolerant soybean and corn and cotton seeds sampled in commercial areas in Brazil. The monitoring of GM glyphosate tolerant cotton, corn and soybean were conducted between 2012/2013 and 2017/2018 seasons.

MATERIALS AND METHODS

Experimental design

The samples (grains and seeds) were collected in commercial producing areas of soybean, corn and cotton located in different Brazilian agricultural regions. The experimental design is described in figure 1 and table 1.

Two approaches were considered: the first to monitor events expressing Bt proteins (MON 531, MON 15985, MON 89034, MON 88017 and MON 87701); and the second to monitor glyphosate tolerant events (MON 1445, MON 88913, MON 89788, MON 88017). But, the term “control treatment” does not imply the non-adoption of the herbicide glyphosate to weed control. In general, it means having a treatment without insect resistance (IR) technology and; therefore, controls 1, 2, 3, 4 and 5 are here designated as non-Bt cultivars or hybrids.

The studies with GM soybean and corn were composed of two treatments distributed in a randomized complete block design (RCBD) with three replicates, evaluated during four to five consecutive seasons. The management for weed control in MON 87701 × MON 89788 soybean used from a single application of the herbicide in the pre-planting (PP) phase up to four applications of this herbicide, including PP and post-emergency (PE) phase and with great variability of applied doses (Table 2).

In the corn study, treatments MON 89034 × NK603, control 1, MON 89034 × 88017 and control 2, the glyphosate application was carried out in PP and PE of the crop (Tables 3 and 4).

The GM cotton technologies studies were conducted in single block trials per treatment. Sampling was done in two areas for MON 15985 × MON 88913 (Table 5) and three areas separately for MON 531 × MON 1445 in the five monitored seasons (Table 6). Glyphosate application was carried out in PP and PE of cotton crops (Tables 5 and 6).

The studies were conducted in areas with more than 12 hectares, with individual plots of 2.0 to 7.0 hectares, depending on the study site and the crop. Both the production area of the farm and the experimental area of the study received the same agricultural practices and inputs. In each plot, the monitored technologies and control treatments, three collection points were defined. From each point, a radius of 20.0 meters was established for random plant sampling to be performed. In this way, matured soybean plants and corn ears were collected to compose a sample of 2.0 kg of grains/plot. For cotton crop, seeds (fiber + seed) were collected to compose the same sample of 2.0 kg of seeds/plot. The three subsamples collected for each treatment were homogenized, allowing the subsequent withdrawal of two composite aliquots of 1.0 kg for cotton seeds and 0.5 kg for corn and soybean grains. Each sample received in the
laboratory was individually packaged in sealed plastic bags and identified with coded labels. Subsequently, the samples were stored and conditioned at -20 °C ± 5 °C until the laboratory analysis.

Table 1 - Information about the treatments, crops and study sites.

| GM Technology       | Variable                              | Treatments                                      |
|---------------------|---------------------------------------|-------------------------------------------------|
| MON 89034 × NK603   | Residues glyphosate - AMPA            | MON 89034 × NK603 Control 1                     |
| MON 89034 × MON 88017 | Residues glyphosate - AMPA           | MON 89034 × MON 88017 Control 2                 |
| MON 531 × MON 1445  | Residues glyphosate - AMPA            | MON 531 × MON 1445 Control 3                    |
| MON 15985 × MON 88913 | Residues glyphosate - AMPA         | MON 15985 × MON 88913 Control 4                 |
| MON 87701 × MON 89788 | Residues glyphosate - AMPA        | MON 87701 × MON 89788 Control 5                 |

Figure 1 - Schematic presentation of data sets of the following GM products: MON 89034 × NK603 (VT PRO2®), MON 89034 × MON 88017 (VT PRO3®), MON 531 × MON 1445 (Bollgard RR®), MON 15985 × MON 88913 (Bollgard II RR FLEX®) and MON 87701 × MON 89788 (Intacta RR2 Pro®).

Glyphosate and AMPA residue analysis

Glyphosate and AMPA reference standards were purchased from Sigma-Aldrich (USA), with purity 99.8 and 98.7%, respectively. Individual stock

Table 1 - Information about the treatments, crops and study sites.

| GM Technology       | Variable                              | Treatments                                      |
|---------------------|---------------------------------------|-------------------------------------------------|
| MON 89034 × NK603   | Conventional Hybrids/glyphosate tolerant (Control 1)* | Corn 2012/2013 to 2016/2017 ROL and MVD       |
| MON 89034 × MON 88017 | Hybrids glyphosate tolerant (Control 2)    | Corn 2014/2015 to 2017/2018 ROL and MCT         |
| MON 531 × MON 1445  | Conventional Cultivars/glyphosate tolerant (Control 3)* | Cotton 2012/2013 to 2016/2017 LEM, CAV and PVL |
| MON 15985 × MON 88913 | Cultivars glyphosate tolerant (Control 4)  | Cotton 2013/2014 to 2016/2017 LEM, CAV and PVL |
| MON 87701 × MON 89788 | Cultivars glyphosate tolerant (Control 5)  | Soybean 2013/2014 to 2017/2018 MCT and PVL      |

* MCT: Mato Castelhano, RS; SLG: São Luiz Gonzaga, RS; ROL: Rolândia, PR; PGR: Ponta Grossa, PR; LON: Londrina, PR; MVD: Montividiú, GO; SHG: Santa Helena de Goiás, GO; LEM: Luís Eduardo Magalhães, BA; BAR: Barreiras, BA; RON: Roncador, MT; LEM: Luís Eduardo Magalhães, BA; BAR: Barreiras, BA; RON: Rondonópolis, MT; SOR: Sorriso, MT; PVL: Primavera do Leste, MT; CAV: Campo Verde, MT; SGO: São Gabriel do Oeste, MS.

* control 1: conventional hybrids were used in the 2012/2013 and 2013/2014 seasons, RR hybrids were used as control 1 in the other seasons; control 3: conventional cultivar was used in the 2012/2013 season, RR cultivars were used as control 3 in the other seasons.

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Table 2 - MON 87701 × MON 89788 soybean: study sites, seasons, treatments, application number, time of application and doses.

| Study sites/Seasons | Treatments | Applications number | Times | Doses (g a.e ha\(^{-1}\)) |
|---------------------|------------|---------------------|-------|---------------------------|
| MCT 2013/2014       | MON 87701 × MON 89788 soybean | 3 | PP; PE | 1016; 1080 e 360 |
|                     | Control 5 |                      |       |                           |
| PVL 2013/2014       | MON 87701 × MON 89788 soybean | 3 | PP; PE | 1650; 1364 e 909 |
|                     | Control 5 |                      |       |                           |
| MCT 2014/2015       | MON 87701 × MON 89788 soybean | 1 | PE | 1189 |
|                     | Control 5 |                      |       |                           |
| PVL 2014/2015       | MON 87701 × MON 89788 soybean | 2 | PP; PE | 975; 911 |
|                     | Control 5 |                      |       |                           |
| MCT 2015/2016       | MON 87701 × MON 89788 soybean | 2 | PP; PE | 654; 654 |
|                     | Control 5 |                      |       |                           |
| PVL 2015/2016       | MON 87701 × MON 89788 soybean | 4 | PP; PE | 1016; 1080 e 360 |
|                     | Control 5 |                      |       |                           |
| MCT 2016/2017       | MON 87701 × MON 89788 soybean | 2 | PP; PE | 1000; 1080 |
|                     | Control 5 |                      |       |                           |
| PVL 2016/2017       | MON 87701 × MON 89788 soybean | 2 | PP; PE | 975; 911 |
|                     | Control 5 |                      |       |                           |
| MCT 2017/2018       | MON 87701 × MON 89788 soybean | 2 | PP; PE | 576; 1030 |
|                     | Control 5 |                      |       |                           |

\(^{a}\) MCT: Mato Castelhano, RS; PVL: Primavera do Leste, MT.
\(^{b,c,d}\) PP: pre-planting; PE: post-emergence; the management used with glyphosate was decided by each farmer responsible for the area in which MON 87701 × MON 89788 soybean study was installed. (g a.e ha\(^{-1}\)): grams of equivalent acid per hectare.

Table 3 - MON 89034 × NK603 corn: study sites, seasons, treatments, application number, time of application and doses.

| Study sites/Seasons | Treatments | Applications number | Times | Doses (g a.e ha\(^{-1}\)) |
|---------------------|------------|---------------------|-------|---------------------------|
| ROL 2012/2013       | MON 89034 × NK603 corn | 2 | PP; PE | 722; 981 |
|                     | Control 1\(^{*}\) |                      |       |                           |
| MVD 2012/2013       | MON 89034 × NK603 corn | 2 | PP; PE | 711; 872 |
|                     | Control 1 |                      |       |                           |
| ROL 2013/2014       | MON 89034 × NK603 corn | 2 | PP; PE | 1081; 893 |
|                     | Control 1 |                      |       |                           |
| MVD 2013/2014       | MON 89034 × NK603 corn | 1 | PP | 667 |
|                     | Control 1 |                      |       |                           |
| ROL 2014/2015       | MON 89034 × NK603 corn | 2 | PP; PE | 1440; 1440 |
|                     | Control 1 |                      |       |                           |
| MVD 2014/2015       | MON 89034 × NK603 corn | 2 | PP; PE | 1636 |
|                     | Control 1 |                      |       |                           |
| ROL 2015/2016       | MON 89034 × NK603 corn | 2 | PP; PE | 785; 1011 |
|                     | Control 1 |                      |       |                           |
| MVD 2015/2016       | MON 89034 × NK603 corn | 2 | PP; PE | 1200; 1470 |
|                     | Control 1 |                      |       |                           |
| ROL 2016/2017       | MON 89034 × NK603 corn | 1 | PE | 2774 |
|                     | Control 1 |                      |       |                           |
| MVD 2016/2017       | MON 89034 × NK603 corn | 1 | PE | 960 |
|                     | Control 1 |                      |       |                           |

\(^{a}\) ROL: Rolândia, PR; MVD: Montividiú, GO.
\(^{b,c,d}\) PP: pre-planting; PE: post-emergence; the management used with glyphosate was decided by each farmer responsible for the area in which MON 89034 × NK603 corn study was installed. (g a.e ha\(^{-1}\)): grams of equivalent acid per hectare.
\(^{*}\) Control 1: conventional hybrids were used in the 2012/2013 and 2013/2014 seasons, RR hybrids were used as control 1 in the other seasons.
solutions were prepared by weighing 20 mg of each standard in 20 mL volumetric flasks. The volume flask was complete with water and the solutions were stored at -20 ± 5 °C. Standard working solutions were prepared by diluting mobile stock solutions in the concentration range of 0.05 to 75 μg mL\(^{-1}\).

Methanol and HPLC grade chloroform and hexane grade PA were purchased from JT Baker.

| Study sites/Seasons\(^a\) | Treatments | Applications number\(^b\) | Times\(^c\) | Doses (g a.e ha\(^{-1}\))\(^d\) |
|--------------------------|------------|---------------------------|------------|-------------------------------|
|                          | MON 89034 × MON 88017 corn | 2 | PP, PE | 1025; 793 |
|                          | Control 2 | 2 | PP, PE | 1025; 793 |

Table 4 - MON 89034 × MON 88017 corn: study sites, seasons, treatments, application number, time of application and doses.

\(\text{a MCT: Mato Castelhano, RS; ROL: Rolândia, PR.}
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\(\text{b,c,d PP: pre-planting; PE: post-emergence; the management used with glyphosate was decided by each farmer responsible for the area in which MON 89034 × MON 88017 corn study was installed. (g a.e ha\(^{-1}\)): grams of equivalent acid per hectare.}
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Water was obtained from a Milli-Q water system (Millipore, Bradford, MA, USA) with a resistivity of 18.2 MΩ cm\(^{-1}\). o-Phthaldialdehyde (oPA) was purchased from Sigma (USA); monopotassium phosphate, calcium hypochlorite, 2-mercaptoethanol, sodium chloride, sodium hydroxide and hydrochloric acid were purchased from Merck (Germany). Chloride resins AG1 X8 mesh 200-400 and in the sodium form Chelex-100 100-200 mesh were purchased from Bio-Rad (USA).

Samples were processed, homogenized and conditioned in double plastic bags, and then kept in freezer -20 ± 5 °C until the moment of the analysis. Sample extraction was based on CoWELL et al. (1986). Aliquots of 25.0 g of soybean and corn grains and cotton seeds were transferred to 250 mL centrifuge tubes. The soybean grains were prepared with 50 mL of hexane for 30 seconds and centrifuged for 5 min at 16000 g (18 to 23 °C). This step was carried out to remove the oils and fat in soybean grains. Then, aliquots of 25.0 g of soybean grains, cotton seeds and corn seeds were transferred to 250 mL centrifuge tubes. The soybean grains were prepared with 50 mL of hexane for 30 seconds and centrifuged for 5 min at 16000 g (18 to 23 °C). This step was carried out to remove the oils and fat in soybean grains.

Twenty grams of Chelex-100 resin impregnated with Fe\(^{3+}\) solution (COWELL et al. 1986) were placed in the glass column. The collected extracts containing glyphosate and AMPA (120 mL for corn and 80 mL for cotton and soybean) were percolated through the column and washed with 100 mL of 0.1 mol L\(^{-1}\) HCl solution. Then, glyphosate and AMPA were eluted with 5 aliquots of 5 mL of 6 mol L\(^{-1}\) HCl. At this point, 10 mL of concentrated HCl was added to the eluate and the extract was homogenized and applied to another glass column with 15 grams of AG1-X8 resin. After eluting the cleaned extract free of impurities or interferences, it was vacuum dried and re suspended in a suitable volume of mobile phase, filtered through a Millex HV filter (0.45 μm, Millipore) and analyzed by HPLC-FD.

Analyzes were performed on a Shimadzu LC 20A HPLC system with RF10 AxL fluorescence detector (Tokyo, Japan). The chromatographic separation was performed using two ion exchange columns in the potassium form A-9 (300 x 4.6 mm, and 150 x 4.6 mm, 5μ) obtained from Bio Rad (USA). The temperature of the column oven was maintained at 50 °C. An isocratic flow of 0.6 mL min\(^{-1}\) was employed with the composition of the mobile phase in 0.005 mol L\(^{-1}\) of monopotassium phosphate (KH\(_2\)PO\(_4\)) with 4% in methanol and pH adjusted to 1.9. The injection volume was 50 μL. The post-column reaction was performed using a solution of OPA with 2-mercaptoethanol (MERC) and calcium hypochlorite, prepared according COWELL et al. (1986). Fluorescent derivatives were formed at 38 °C in post-column oven and detected at excitation wavelength of 330 nm and emission of 465 nm.

For the soybean and cotton analyzes the Shimadzu FCV-20AH2 valve (Tokyo, Japan) was also used in conjunction with an LC 20 AD pump to perform the cleaning of the analytical column.

The previously described method was validated for corn, soybean and cotton crops (SANTE, 2016) for the detection of glyphosate and AMPA in these crops.

### Table 6 - MON 15985 × MON 88913 cotton: study sites, seasons, treatments, application number, time of application and doses.

| Study sites/Seasons\(^{a}\) | Treatments | Applications number\(^{b}\) | Times\(^{c}\) | Doses (g a.e ha\(^{-1}\))\(^{d}\) |
|-----------------------------|-------------|-----------------------------|-------------|-----------------------------|
| LEM 2013/2014               | MON 15985 × MON 88913 cotton | 1 | PP | 797 |
| Control 4                   | 2 | PP, PE | 797; 960 |
| LEM 2015/2016               | MON 15985 × MON 88913 cotton | 2 | PP, PE | 1500; 1250 |
| Control 4                   | 2 | PP, PE | 1500; 1250 |
| CAV 2015/2016               | MON 15985 × MON 88913 cotton | 2 | PP, PE | 900; 900 |
| Control 4                   | 2 | PP, PE | 900; 900 |
| CAV 2016/2017               | MON 15985 × MON 88913 cotton | 2 | PP, PE | 1296; 1440 |

\(^{a}\) LEM: Luís Eduardo Magalhães, BA; PVL: Primavera do Leste, MT; CAV: Campo Verde, MT.

\(^{b,c,d}\) PP: pre-planting; PE: post-emergence; the management used with glyphosate was decided by each farmer responsible for the area in which MON 15985 × MON 88913 cotton study was installed. (g a.e ha\(^{-1}\)): grams of equivalent acid per hectare.
RESULTS

Analyzing the different experimental sites, with different numbers of applications and doses, most of the samples showed no herbicide residues. Results regarding corn grains showed residual concentrations of glyphosate ranging from ND up to 0.15 mg kg\(^{-1}\) (Table 7). The average levels of glyphosate residues were 0.05 mg kg\(^{-1}\), 0.02 mg kg\(^{-1}\), 0.07 mg kg\(^{-1}\) and 0.07 mg kg\(^{-1}\), corresponding to treatments MON 89034 × NK603, control 1, MON 89034 × 88017 and control 2, respectively (Figure 2). The AMPA metabolite levels varied between ND at 0.49 mg kg\(^{-1}\) for the period between last application and harvest (Figure 3b).

In general, considering the averages of all treatments for glyphosate residues and its metabolite AMPA, higher values were evidenced for MON 87701 × MON 89788 and control 5 (soybean crop), but lower than the MLR standardized for this crop (10 mg kg\(^{-1}\)). In the remaining treatments averages lower than 0.15 mg kg\(^{-1}\) for Glyphosate and 0.06 mg kg\(^{-1}\) for AMPA (Figure 2) were observed.

DISCUSSION

The MRL established by ANVISA in Brazil to glyphosate in corn is 1 mg kg\(^{-1}\) (ANVISA, 2015). Considering the results presented for this crop, glyphosate levels residues in the MON 89034 × NK603 treatment were 20 times below the MRL, as were control 1 (50 times), MON 89034 × 88017 (14 times) and control 2 (14 times). For cotton, which the glyphosate MRL established by ANVISA is 3 mg kg\(^{-1}\), the treatments MON 531 × MON 1445, control 3, MON 15983 × MON 88913 and control 4 present results were 23, 20, 38 and 50 times below the MRL, respectively. For soybeans, ANVISA has established the MRL of 10 mg kg\(^{-1}\), then the averages were 11.5 times below the MRL for MON 87701 × MON 89788 and 13 times below of this value for control 5. In a study conducted by ABAKERLI et al. (2014), eight areas from commercial soybean areas with SCV/HCV + SRR/HCV (Roundup Ready and conventional soybean with application of conventional herbicides) treatment and SRR/HRR (Roundup Ready soybean with application of Roundup Ready herbicide) treatment were monitored during five seasons. The
results were below the ANVISA’s soybean MRL in 200 and 33 times to SCV/HCV + SRR/HCV and SRR/HRR treatment, respectively. DUKE et al. (2003) also showed residues below the MRL in soybeans, within the range of 0.08 to 3.08 mg kg⁻¹. Similarly, BOHN et al. (2014) reported glyphosate residues levels between 0.4 and 8.8 mg kg⁻¹ in GM soybean plants, considering in this case the use of soybean for silage (SEFER et al. 2004; GOBETTI et al. 2011; RIGUEIRA et al. 2015). The studies conducted by DUKE et al. (2003) and BOHN et al. (2014) followed the MRL established by the Codex Alimentarius (20 mg kg⁻¹). Considering the MRLs recommended by the Codex Alimentarius for soybean, corn and cotton crops, the glyphosate residues levels detected in the present study are at

| Treatments              | Study sites/Seasons | Glyphosate | AMPA  |
|-------------------------|---------------------|------------|-------|
| MON 89034 × NK603       | ROL 2012/2013       | ND         | ND    |
| Control 1*              |                     | ND         | 0.16  |
| MON 89034 × NK603       | MVD 2012/2013       | ND         | ND    |
| Control 1               |                     | ND         | <LOQ  |
| MON 89034 × NK603       | ROL 2013/2014       | 0.11       | 0.49  |
| Control 1               |                     | ND         | 0.05  |
| MON 89034 × NK603       | MVD 2013/2014       | ND         | ND    |
| Control 1               |                     | ND         | ND    |
| MON 89034 × NK603       | ROL 2014/2015       | <LOQ       | <LOQ  |
| Control 1               |                     | <LOQ       | <LOQ  |
| MON 89034 × NK603       | MVD 2014/2015       | ND         | ND    |
| Control 1               |                     | ND         | ND    |
| MON 89034 × NK603       | ROL 2015/2016       | <LOQ       | ND    |
| Control 1               |                     | <LOQ       | ND    |
| MON 89034 × NK603       | MVD 2015/2016       | 0.06       | <LOQ  |
| Control 1               |                     | ND         | <LOQ  |
| MON 89034 × NK603       | ROL 2016/2017       | <LOQ       | 0.05  |
| Control 1               |                     | 0.07       | <LOQ  |
| MON 89034 × NK603       | MVD 2016/2017       | ND         | ND    |
| Control 1               |                     | ND         | ND    |
| MON 89034 × MON 88017   | MCT 2014/2015       | 0.09       | <LOQ  |
| Control 2               |                     | 0.11       | <LOQ  |
| MON 89034 × MON 88017   | ROL 2014/2015       | <LOQ       | ND    |
| Control 2               |                     | <LOQ       | ND    |
| MON 89034 × MON 88017   | MCT 2015/2016       | ND         | ND    |
| Control 2               |                     | ND         | ND    |
| MON 89034 × MON 88017   | ROL 2015/2016       | <LOQ       | <LOQ  |
| Control 2               |                     | 0.05       | <LOQ  |
| MON 89034 × MON 88017   | ROL 2016/2017       | <LOQ       | ND    |
| Control 2               |                     | <LOQ       | ND    |
| MON 89034 × MON 88017   | ROL 2017/2018       | 0.06       | 0.01  |
| Control 2               |                     | 0.09       | 0.02  |
| MON 89034 × MON 88017   | ROL 2017/2018       | 0.15       | <LOQ  |
| Control 2               |                     | 0.14       | <LOQ  |

* MCT: Mato Castelhano, RS; ROL: Rolândia, PR; MVD: Montividiu, GO.
LOQ: Limit of Quantification (0.05 mg kg⁻¹); ND: Not Detected.
*control 1: conventional hybrids were used in the 2012/2013 and 2013/2014 seasons; RR hybrids were used as control 1 in the other seasons.
Table 8 - Glyphosate and AMPA residues in cotton seeds from treatments MON 531 × MON 1445, control 3, MON 15985 × MON 88913 and control 4.

| Treatments          | Study sites/Seasons | Glyphosate (mg kg\(^{-1}\)) | AMPA (mg kg\(^{-1}\)) |
|---------------------|---------------------|-----------------------------|------------------------|
| MON 531 × MON 1445  | LEM 2012/2013       | 1.78                        | 0.06                   |
| Control 3\(^a\)     |                     |                             |                        |
| MON 531 × MON 1445  | PVL 2013/2014       | 0.08                        | ND                     |
| Control 3            |                     |                             |                        |
| MON 531 × MON 1445  | PVL 2014/2015       | <LOQ                        | <LOQ                   |
| Control 3            |                     |                             |                        |
| MON 531 × MON 1445  | LEM 2015/2016       | <LOQ                        | <LOQ                   |
| Control 3            |                     |                             |                        |
| MON 531 × MON 1445  | CAV 2015/2016       | 0.46                        | <LOQ                   |
| Control 3            |                     |                             |                        |
| MON 531 × MON 1445  | LEM 2016/2017       | <LOQ                        | ND                     |
| Control 3            |                     |                             |                        |
| MON 531 × MON 1445  | CAV 2016/2017       | 0.11                        | ND                     |
| Control 3            |                     |                             |                        |
| MON 15985 × MON 88913 | LEM 2013/2014     | ND                          | ND                     |
| Control 4            |                     |                             |                        |
| MON 15985 × MON 88913 | LEM 2015/2016     | 0.07                        | <LOQ                   |
| Control 4            |                     |                             |                        |
| MON 15985 × MON 88913 | CAV 2015/2016     | 0.13                        | <LOQ                   |
| Control 4            |                     |                             |                        |
| MON 15985 × MON 88913 | CAV 2016/2017     | <LOQ                        | ND                     |
| Control 4            |                     |                             |                        |

\(^a\) LEM: Luís Eduardo Magalhães, BA; CAV: Campo Verde, MT; PVL: Primavera do Leste, MT.

LOQ: Limit of Quantification (0,05 mg kg\(^{-1}\)); ND: Not Detected.

\(^*\) Control 3: conventional cultivar was used in the 2012/2013 season; RR cultivars were used as control 3 in the other seasons.
least 23 times below the recommended MRL for soybean (20 mg kg\(^{-1}\)), 71 times below the MRL for corn (5 mg kg\(^{-1}\)) and 267 times below the established MRL for cotton (40 mg kg\(^{-1}\)).

Currently, in glyphosate tolerant GM crops, weed control with this herbicide can also be performed after the emergence of the main crop, varying the number of applications from one to three times (BEENBROK, 2016). In addition, in soybean it is considered that sequential application of glyphosate (two applications) should be used in the PE of RR (Roundup Ready) soybean in areas with high infestation and/or uneven germination of weed. Thus, it was verified that in a single site (PVL - soybean 2015/2016) this criterion was not followed, and three PE applications were performed in treatments MON 87701 × MON 89788 and control 5 by decision of the producer due to particularities of the area, techniques and high density of weed. Despite all the management carried out for weed control, the variation in the number of herbicide applications during the development of the soybean crop had a low correlation (r=0.39) with the herbicide residues in grains. The same results were observed for corn (r=0.28) and cotton (r=0.18). Because it is a positive correlation, the increase in applications number should result in an increase in the residue variable of glyphosate; however, this increase was very low or almost null, even decreasing in some cases (Figure 3a).

Considering the relationship between AMPA and glyphosate average concentrations, three analysis can be exploited: i) the correlation between the averages was high (r=0.94) (Figure 2), it means and reinforce that AMPA came from glyphosate metabolism; ii) the ratio were 1.35 in soybean, 0.90 in maize and 0.11 in cotton. The ratio greater than 1 for soybean can be explained due to the higher average of AMPA instead of glyphosate (Figure 2), which in turn is related with slow metabolization of AMPA, compared with glyphosate metabolization (ABAKERLI et. al., 2014). iii) an additional result inferred by this study goes through the correlations between the glyphosate residues and interval of application until the harvest. Analyzing the results for soybean (r = -0.48), maize (r = -0.25) and cotton (r = -0.21), they showed that the greater amount of interval between applications and harvests, the values of soybean and corn glyphosate residues and cotton seeds are lower (Figure 3b), which indicated the existence of an inversely proportional relation, validating the metabolism of both substances over time.

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Table 9 - Glyphosate and AMPA residues in soybean grains from treatments MON 87701 × MON 89788 and control 5.

| Treatments         | Study sites/Seasons | Glyphosate (mg kg\(^{-1}\)) | AMPA (mg kg\(^{-1}\)) |
|--------------------|---------------------|-----------------------------|------------------------|
| MON 87701 × MON 89788 | MCT 2013/2014       | 0.25                        | 0.5                    |
| Control 5          |                      | 0.23                        | 0.53                   |
| MON 87701 × MON 89788 | PVL 2013/2014       | 0.72                        | 1.02                   |
| Control 5          |                      | 0.36                        | 0.61                   |
| MON 87701 × MON 89788 | MCT 2014/2015       | 0.33                        | 0.45                   |
| Control 5          |                      | 0.35                        | 0.5                    |
| MON 87701 × MON 89788 | PVL 2014/2015       | 0.75                        | 0.76                   |
| Control 5          |                      | 1.02                        | 0.59                   |
| MON 87701 × MON 89788 | MCT 2015/2016       | 0.17                        | 0.59                   |
| Control 5          |                      | 0.20                        | 0.44                   |
| MON 87701 × MON 89788 | PVL 2015/2016       | 2.81                        | 3.38                   |
| Control 5          |                      | 1.42                        | 2.21                   |
| MON 87701 × MON 89788 | MCT 2016/2017       | 0.42                        | 0.29                   |
| Control 5          |                      | 0.46                        | 0.55                   |
| MON 87701 × MON 89788 | PVL 2016/2017       | 1.88                        | 2.69                   |
| Control 5          |                      | 2.26                        | 1.69                   |
| MON 87701 × MON 89788 | MCT 2017/2018       | 0.50                        | 0.80                   |
| Control 5          |                      | 0.82                        | 1.12                   |

a MCT: Mato Castelhano, RS; PVL: Primavera do Leste, MT.
CONCLUSION

Considering the variation of factors such as the different experimental sites, number of applications and the interval between the last application of the herbicide and the harvest studied in corn, cotton and soybeans crops, all results were below the Maximum Residue Limits (MRLs) established by ANVISA for glyphosate to each one of these crops in Brazil. In this study the observation of AMPA residue values indicated a correlation with glyphosate residues.

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DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The funding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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