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Glyphosate residue in honey and impacts on Africanized bee hives under field conditions

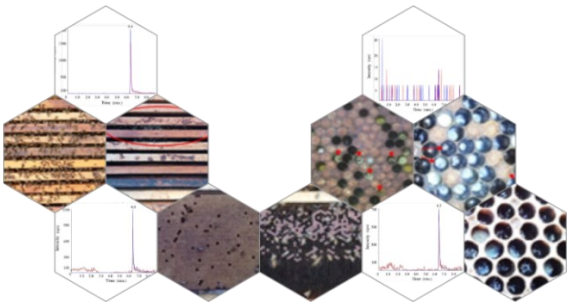
Márcia Regina Faita [a*], Victor Rodrigues Alves [b], Gustavo Amadeu Micke [b], Afonso Inácio Orth [a], Rubens Onofre Nodari [a]

[a] Department of Plant Science, Graduate Program in Plant Genetic Resources, Federal University of Santa Catarina, 88034-001 Florianópolis, SC, Brazil
[b] Department of Chemistry, Federal University of Santa Catarina, 88040-900 Florianópolis, SC, Brazil

Abstract
Honey and other bee products may contain residues of different substances, including pesticides, which is considered a public health problem. In addition, they characterize risks to the health of *Apis mellifera*, which have been showing an increasing decline in their populations. There are many protocols for identifying pesticides in bee products which, in general, are complex matrices whose results of routine investigations in control laboratories are rarely disclosed. In this sense, the objective of the present study was to determine the presence of residues of glyphosate and its metabolite aminomethylphosphonic acid (AMPA) in honey, as well as its effect on the strength of the hive of *A. mellifera*. Samples were collected from hives experimentally exposed to food containing a sublethal dose of Roundup® and conducted by hydrophilic interaction liquid chromatography coupled to tandem mass spectrometry (HILIC-MS/MS). The AMPA content was lower than the method's detection limit in honey samples from hives that received food containing the herbicide. It was possible to quantify glyphosate one week after the last artificial feeding (R1 8.45 ± 1.09 μg g^{-1}; R2 8.15 ± 2.14 μg g^{-1}; R3 23.90 ± 2.95 μg g^{-1}). In a hive sample fed for more than four weeks, glyphosate was present in lower concentrations (3.12 ± 0.89 μg g^{-1}) with no detection of AMPA. From the analysis of the strength of the hives, we observed a decrease in the population of adult individuals and the brood area, the absence of a queen, and no construction of royal cells by the workers in the hives of the Roundup® treatment in comparison to the control group, in which the hives remained with queen size, high adult and brood population, and food stock. Although present, glyphosate did not undergo degradation in honey during the evaluated period. Thus, we could infer that the presence of Roundup® in bee feed may be present in honey, representing a risk to consumers' health and economic damage to beekeepers. This is the first long term study that evaluated the effect on hive strength of glyphosate herbicide-based residues present in pollen offered to honeybees, contributing to the understanding of the Roundup® mode of action in different aspects that affect the survival of colonies under field conditions.

Keywords: Roundup®, contamination, *Apis mellifera*, colony, survival, pesticides.

Graphical Abstract



*Corresponding author: Marcia R. Faita. E-mail address: marcia.faita@gmail.com
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1 Introduction

The presence of residues in bee products, especially in honey, include pesticides, potentially toxic metals, bacteria and radioactive materials (Gérez et al., 2017; Karise et al., 2017; Mitchell et al., 2017; Pacífico da Silva et al., 2015; Wiest et al., 2011; Mullin et al., 2010). The intoxication of pollinators, such as bees, promotes deleterious effects, such as the reduction of populations of these insects, and the production and quality of honey, in addition to compromising the reproduction of different plant species. The presence of contaminants in honey can still be considered a public health problem due to its importance in the food and pharmaceutical industry (Gérez et al., 2017; Al-Waili et al., 2012; Othman, 2012).

Pesticides can be present in sublethal doses, or when bees die, the lethal dose can be under suspicion of envenomation (Kiljanek et al., 2016, 2017). During resource collection, bees may come into contact with contaminants, including pesticides, carrying them into the colony, where they persist for indefinite periods (Sanchez-Bayo et al., 2014). Especially in areas of agricultural cultivation, bees can be exposed to several pesticides simultaneously (Wiest et al., 2011; Mullin et al., 2010), triggering additive effects in terms of toxicity (Zhu et al., 2017; Spurgeon et al., 2016; Sanchez-Bayo et al., 2014) or enhancing susceptibility to parasites and pathogens, compromising the survival of these insects (Faita et al., 2020; Goulson et al., 2015; Pettis et al., 2013). In addition to toxicity, there is a growing number of studies on the presence of pesticides in bee products, such as honey, pollen, propolis and wax (Calatayud-Vernich et al., 2019, 2018; Gérez et al., 2017; Mitchell et al., 2017; Pacífico da Silva et al., 2015; Wiest et al., 2011; Mullin et al., 2010).

Bees of the *Apis mellifera* (Hymenoptera: Apidae) species have a lower number of immunity genes when compared to other insects, such as *Drosophila melanogaster* (Diptera: Drosophilidae) and *Anopheles gambiae* (Diptera: Culicidae) (Evans et al., 2006). The most pronounced differences occur in three superfamilies that encode detoxification enzymes to xenobiotics, showing greater sensitivity of bees to pesticides (Claudians et al., 2006). This condition inherent to bees and their interactions with the environment makes them considered bioindicators of environmental quality (Cadore et al., 2022; Matin et al., 2016). In this sense, the analysis of pesticide residues in honey and other bee products contributes to assessing the risk of these contaminants for bees and human health, in addition to providing data on their application in the environment of apiaries (Al-Waili et al., 2012).

In general, bee products are complex matrices that need special methods for determining pesticide residues. Honey, for example, is a high-sugar matrix for which different protocols to determine pesticide residues have been developed (Gérez et al., 2017). Even so, some substances are not detected, generating the false idea that they are not present and, therefore, are not associated with the weakening and loss of hives (Sánchez-Bayo et al., 2016). This is the case of glyphosate, the most widely used herbicide worldwide (Benbrook, 2016; Giesy et al., 2000), applied before and during the flowering of crops, which may result in residues in honey (De Souza et al., 2020; Karise et al., 2017; Rubio et al., 2014).

Although glyphosate targets plants, in *A. mellifera*, this analytical-grade herbicide has caused impairment of forage sensory learning processes (Farina et al., 2019), disturbances in the intestinal microbiota (Blot et al., 2019; Motta et al., 2018), lower survival rate (Motta et al., 2020) and changes in physiological detoxification, antioxidant, and metabolic markers (Almasri et al., 2022). At the same time, a commercial formulation based on glyphosate caused higher mortality in bees infected with *Nosema* spp. (Faita et al., 2020), ultrastructural alterations of the hypopharyngeal glands (Faita et al., 2018), reduction in royal jelly production and its proteomic profile (Faita et al., 2022; Chaves et al., 2020). Additionally, commercial formulations of herbicides based on glyphosate (HBG) have adjuvants and inert ingredients, which can be even more toxic to bees than the active ingredients (Mullin et al., 2016; Mesnage et al., 2012).

Concerns about the effects of exposure of *A. mellifera* to glyphosate can also be extended to its metabolites, such as aminomethylphosphonic acid (AMPA), which plays a major role in the microbiological degradation of the herbicide, with few studies on its toxicity (El Agrebi et al., 2020). In this way, monitoring degradation products and their precursor pesticides is justified, a common practice performed in multi-residue analyzes for a better understanding of the dynamics and flow of pesticides in biological samples. The determination of glyphosate and AMPA was previously reported in nectar, pollen and bee products by liquid chromatography coupled with sequential mass spectrometry (LC-MS/MS) (Zioga et al., 2022; El

Agrebi et al., 2020). The modality of liquid chromatography by hydrophilic interaction (HILIC) can also be explored due to its advantage in the separation of hydrophilic species, softening the mobile phase conditions for the formation and stability of the electrospray when coupling with mass spectrometry (Saurat et al. al., 2022; Yoshioka et al., 2011).

Given the set of information already known about the action of pure glyphosate or in commercial formulations on *A. mellifera*, we consider it pertinent to address its effects on the hive. Due to the complexity of colonies, the effects of pesticides on their survival in field trials are challenging to establish and evaluate (Rundlöf et al., 2015; Wehling et al., 2009), although toxicity can be demonstrated individually in the laboratory (Rondeau et al., 2014). In this sense, this study aimed to determine the presence of glyphosate and AMPA in honey and associate it with qualitative aspects as well as its effect on the hive strength of *A. mellifera*.

2. Material and methods

The bioassay was conducted between August and December/2017 at the Experimental Apiary of Cidade das Abelhas, Florianópolis, Brazil (27 32012.28 "S, 48 3005.82" W), where Africanized beehives (*A. mellifera*) are kept. The strength and homogeneity of the hives were verified before exposure to the treatments regarding the adult population, brood area and stored food, according to Delaplane et al. (2013). The apiary is surrounded to the east and north by a secondary forest belonging to an environmental conservation unit with an area of 491.5 ha and two small villages to the west and south. As there is no commercial agriculture other than small family gardens, the use of pesticides in areas up to 10 km from the apiary is absent.

2.1. Experimental design

Two treatments were determined based on the feeding of the hives (control and a sublethal dose of Roundup®), in a completely randomized design, with each hive being an experimental unit, according to Faita et al. (2018). The herbicide used was Roundup® Original, in an amount proportional to those recommended by the manufacturer for field application (Concentrated Solution - COMPOSITION: N-phosphonomethyl) isopropylamine salt 480 g L^{-1}) equivalent to N-(phosphonomethyl)glycine acid (GLIFOSATE) 360 g L^{-1}). Briefly, the control treatment consisted of 200 mL of sugar syrup with free pollen input throughout the bioassay, dispensing with the additional supply of pollen mixed with the sugar syrup. The "Roundup®" treatment consisted of 200 mL of sugar syrup mixed with 80 g of pollen and 1.5 µL of Roundup® corresponding to 2.16 $\mu g\ g^{-1}$ of ammonium glyphosate salt. Feeding was provided on two occasions: August 7th to 28th, 2017 and September 12th to October 3rd, 2017, for four consecutive weeks, using internal surface feeders. All food was collected by the bees within 12 h and stored inside the combs. It should be noted that the concentration of glyphosate offered to the hives does not exceed what the bees can find in the field after the application of the herbicide, which corresponds on average to 15.6 mg a.e./kg^{-1} and 310 mg a.e./ kg^{-1} of the residue of glyphosate in nectar and pollen, respectively (Thompson et al., 2014).

2.2. Sample collection

Honey samples were collected in November 2017, directly from the hives, randomly in at least five combs. With the aid of a disposable Pasteur pipette, two samples of honey with 1.5 mL each were collected and transferred to polypropylene tubes with a capacity of 2 mL. The samples were kept under refrigeration (4 ºC) until they were sent for residues analysis at the Capillary Electrophoresis Laboratory of the Federal University of Santa Catarina.

2.3. Detection of the active principle (glyphosate) and its main metabolite (AMPA)

Glyphosate and AMPA standards (Sigma Aldrich, São Paulo, Brazil) were used. For the solutions that make up the mobile phase, HPLC grade acetonitrile (Tedia, São Paulo, Brazil), ultrapure water obtained from a Mili-Q ultra-purification system (Millipore, Bedford, USA) with a minimum resistivity of 18.2 MΩ cm and ammonium carbonate (> 99% - Fluka Analytical, São Paulo, Brazil) were employed.

Solutions of glyphosate and its metabolite AMPA were prepared at a stock concentration of approximately 1000 mg L^{-1}, and working concentrations were prepared from these solutions.

2.3.1. Instrumentation

For the proposed method (Underivatized glyphosate - App ID: 22767, Phenomenex), a high performance liquid chromatograph (HPLC) model 1200 series obtained from Agilent Technologies (Waldbronn, Germany) equipped with a quaternary pump that conducted a mobile phase to a flow of 0.4 mL min^{-1} in a gradient composition of (A) 95% acetonitrile and (B) ammonium carbonate (10 mmol L^{-1}; pH 9.2) in the following proportions: 0 min 5% B; 1.5 min. 5% B; 3.0 min 85% B; 6.5 min 95% B; 7.0 min 95% B; 7.01 min 5% B and 10 min 5% B. The chromatographic separations were carried out at 25 °C on a Luna NH_2 column measuring 150 × 2 mm; 3 μm – 100 Å, obtained from Phenomenex (Torrance, California, USA). In addition, 15 μL of calibration solutions and samples were introduced using an autosampler. The Q trap 3200 triple quadrupole mass spectrometer with ESI ionization source, Turbo V Ion Source/TurboIonSpray, (Applied Biosystems/MDS Sciex, Concord, Canada) was coupled to the HPLC.

Nitrogen (N_2) was used in the processes involved in the ionization source (GS1 and GS2 at 40 psi) and collision gas fragmentation (CAD) at medium flow. Ionization was conducted at 600 °C in negative mode using –4500 V and Unit resolution. The gas curtain (CUR) was maintained at 15 psi. The multiple reaction monitoring method (MRM) was used to follow AMPA and glyphosate through mass transitions (m/z (Q1) → m/z (Q3). For each analyte, two transitions were used, one for quantification and one for confirmation, described in **Table 1**, as well as the optimized energies in the process.

The modification of the LC-MS/MS system instrumental parameters, as well as the data acquisition, were verified using the Analyst 1.6.2 software.

Table 1 Monitored quantification and confirmation transitions for glyphosate, its metabolite AMPA, and the optimized energies for its mass spectrometry analyses.

Analyte	m/z (Q1)	m/z (Q3)	Dwell time (ms)	DP (V)	EP (V)	CEP (V)	CE (V)	CXP (V)
AMPA	110.0	63.0	150.0	–35.0	–6.5	–11.8	–24.0	0.0
	110.0	79.0	150.0	–35.0	–6.5	–1.8	–30.0	0.0
Glyphosate	168.0	63.0	150.0	–30.0	–3.5	–13.7	–32.0	0.0
	168.0	150.0	150.0	–30.0	–3.5	–13.7	–10.0	0.0

2.4. Sample preparation

The employed extraction was based on the method implemented by the European Reference Laboratory for Single Residue Method (EURL-SRM) (Anastassiades et al., 2016). However, it was necessary to adapt to a reduced scale, which consisted of weighing approximately 100 mg of each honey sample exposed to 500 μL of a 60% methanol solution containing 0.1% formic acid. This solution was stirred for 1 min and centrifuged for 5 min at 14000 rpm. Finally, 100 μL was transferred to the container (vial) and analyzed via LC-MS/MS.

2.5. Quantification

The calibration method selected considered the adjustment of the data to the linear model. It was carried out by adding the standard to the free matrix in triplicate and six concentrations ranging from 0.5 to 5 μg mL^{-1} for AMPA and from 0.64 to 6.4 μg mL^{-1} for glyphosate. For this, approximately 100 mg of free sample was weighed for each concentration level. Each of the concentration levels of both analytes was contained in a maximum volume of 500 μL of 60% methanol solution containing 0.1% formic acid.

2.6. Evaluation of hives

The evaluation of the strength of the hives was carried out in all hives of the bioassay and occurred in two moments. The first evaluation occurred before exposing the hives to pesticides (September 2017), while the second was accomplished 90 days after exposure (December 2017). The evaluations were performed according to the subjective method proposed by Delaplane et al. (2013), in which the area of the frame covered by bees, open and closed brood, honey and pollen of each hive was visually estimated by two previously trained human observers, each one assisted by another person who recorded the observations. The percentage values of each parameter were recorded and later added to obtain the number of frames for each parameter. Finally, the obtained results were submitted to the paired *t*-test, at 5% probability.

3. Results and Discussion

3.1. Residue analysis in honey

The HILIC-MS/MS method was used to determine glyphosate and AMPA in honey samples from control hives and those experimentally exposed to food containing Roundup®. The chromatograms resulting from the analysis of one specimen from each of the aforementioned groups are shown in **Figure 1**. The glyphosate and AMPA content in the honey samples can be seen in **Table 2**.

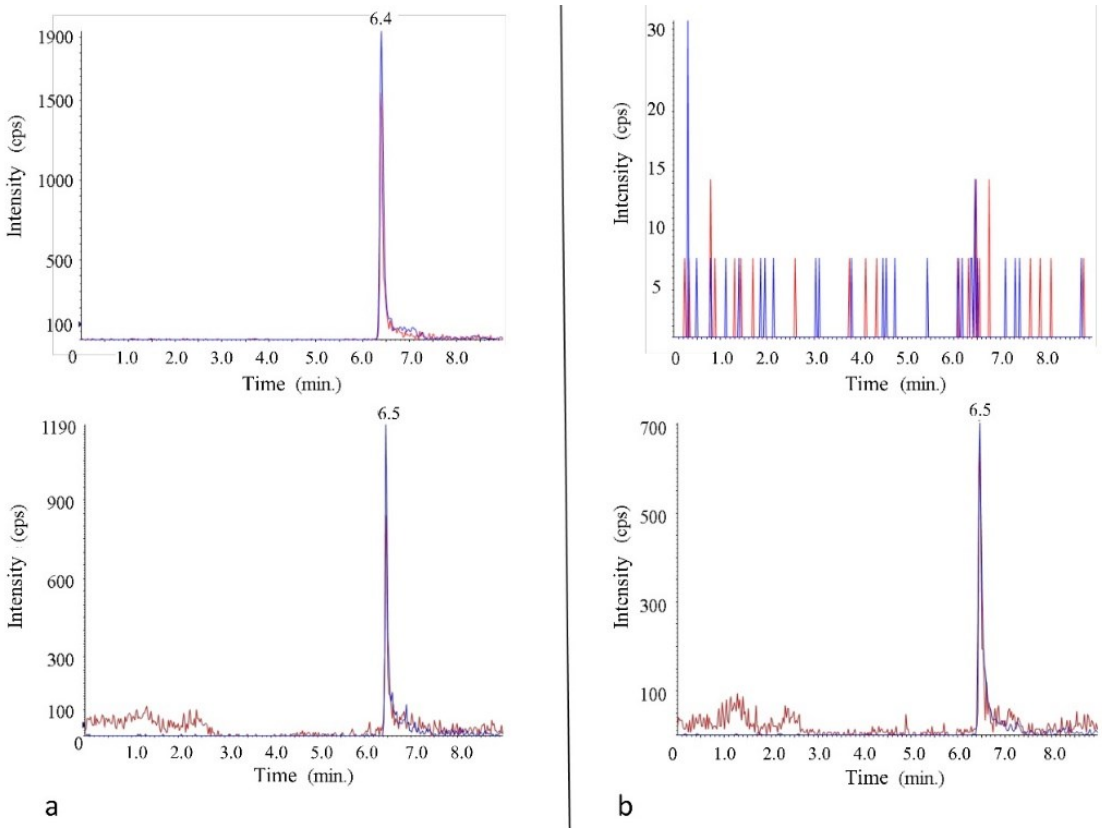

Fig. 1 Chromatograms referring to (a) standard AMPA (top) and glyphosate (bottom) at concentrations 5 and 6.4 mg mL^{-1}, respectively, and (b) code sample C8, AMPA (top) and glyphosate (low).

Glyphosate residues were identified and quantified in all honey samples from the hives that received food containing the herbicide, while for AMPA, there was no signal above the detection limit. The profile for the control samples was lower than the detection limit for both analytes. The average concentration of glyphosate in the honey samples was higher than that present in the Roundup® dose mixed with the hives' food (2.16 μg a.e. g^{-1}), even in the hive that had not received contaminated food for 50 days (3.12 ± 0.89 μg g^{-1}). A possible cause may be associated with the dehydration process that the honey undergoes inside the hives or even the consecutive storage of contaminated food in the same regions of the honeycomb. However, our results were lower than those found in *Phacelia* spp. nectar, collected on the first and third day after spraying the herbicide, which were respectively 31.3 mg a. e./ kg^{-1} and 15.6 mg a.e./kg^{-1} (Thompson et al., 2014).

Table 2 Concentration of glyphosate and aminomethylphosphonic acid (AMPA) in honey collected from hives in the control treatment, without the addition of Roundup® (C1-C3) and hives experimentally exposed to food containing the herbicide (R1-R3).

Analyte	Average concentrations in the samples (mg g^{-1})					
	C1	C2	C3	R1	R2	R3
Glyphosate	< LOD	< LOD	< LOD	8.45 ± 1.09	23.90 ± 2.95	8.15 ± 2.14
AMPA	< LOD	< LOD	< LOD	< LOD	< LOD	< LOD

*number of authentic replicates equal to 3 (n = 3), < LOD – lower than the method's detection limit.

HBG are the most widely used pesticides worldwide (Benbrook, 2016; Giesy et al., 2000), especially in areas of genetically modified crops (Green, 2014), adding to the strong positive correlation between the presence of glyphosate in honey samples in areas with predominant land use for agricultural purposes (Berg et al., 2018). However, it is also used in non-agricultural areas (Benbrook, 2016) and may also be present in organic honey (Rubio et al., 2014). The presence of glyphosate in honey has been detected in samples from all over the world, with variable concentrations reaching up to 300 μg kg^{-1} (Bergero et al., 2021; Raimets et al., 2020; Odemer et al., 2020; Pareja et al., 2019; Thompson et al., 2019; Berg et al., 2018; Zoller et al., 2018; Rubio et al., 2014).

In Brazil, glyphosate residues were quantified in 40 honey samples from five states, of which six had glyphosate levels above the maximum residue limit (MRL) proposed by the European Union (EU) (0.05 μg g^{-1}), and one sample showed AMPA at 0.10 μg g^{-1} (de Souza et al., 2020; Medina-Pastor et al., 2020). The Brazilian regions with the highest presence of glyphosate residues in honey were the same ones that recorded high losses of bee colonies and frequent use of HBG in agriculture, mainly in soybean cultivation (De Souza et al., 2020).

The method used to carry out the analyzes in the present study did not allow the detection of AMPA in the honey samples, indicating that in this matrix, glyphosate is not metabolized in a period of 50 days. Similarly, Karise et al. (2017) and Zioga et al. (2022) did not observe AMPA within detection limits in the honey and pollen samples they analyzed. Glyphosate is highly soluble and considered non-persistent, showing dissipation of 50% of the initial concentration (DT_{50}) in 1.1 and 13.7 days in field studies. However, AMPA presents DT_{50} values of 283.6 and 633.1 days, being classified as persistent (PPDB, 2022). Although Thompson et al. (2014) state that the decline of glyphosate in matrices such as honey and pollen is rapid, no support was found in the literature that elucidates the mode and time of degradation of this herbicide in bee products. Thus, it is possible that glyphosate remains available for long periods inside the hive.

Even though it plays a major role in the microbiological degradation of glyphosate, evaluations on the toxicity of AMPA in *A. mellifera* are scarce (El Agrebi et al., 2020). One of the few examples was the study by Blot et al. (2019), who verified the partial reduction of *Gilliamella apicola* bacteria *in vitro* when exposed to AMPA; however, no significant changes were found in the intestinal microbiota of *A. mellifera*. Although herbicides are not indicated to kill insects, their large-scale use in agricultural systems and the maintenance of vegetation on roads, cities and private gardens configure several routes for glyphosate to be present in the nectar and pollen collected by bees (Karise et al., 2017). These authors also argue that the low concentrations found in their study do not represent a risk to human health and are below acute lethal doses; however, the risks of chronic contamination for bees should not be discarded.

It is also important to highlight that there are practically no studies on the longevity of glyphosate in bee products such as honey, possibly due to the fact that tracking the presence of glyphosate and AMPA requires a separate and costly analysis (Traynor et al., 2016). In addition, there is no monitoring for glyphosate in plant matrices relevant to bees, as the focus for this type of evaluation is usually on insecticides rather than herbicides (Thompson et al., 2014). However, Liao et al. (2017) demonstrated that bees preferred specific concentrations of glyphosate and chlorothalonil present in sugary artificial diets compared to other pesticides, suggesting that the amount of these products in honey is high.

3.2. Evaluation of the strength of the hives

The hives exposed to a sublethal dose of the Roundup® treatment in August 2017 showed a marked reduction in the population of adult bees and brood in the fourth week after the beginning of the bioassay. In the fifth week of the bioassay, when the supply of food containing the herbicide was suspended, two of the three hives in this treatment had about 500 workers bees inside the box, which covered only one side of a comb, with dead brood and without a queen, making it impossible to perform additional assessments.

The set of hives from the second exposure to contaminated food, held in September 2017, showed a significant reduction in the adult population, open and closed brood area between evaluations. In contrast, the hives from the control treatment did not show significant changes in the strength of the hives (**Table 3**).

Table 3 Mean values for hive strength parameters determined in evaluations carried out in September and December 2017, respectively, before and after exposure to control and Roundup® treatments.

	Adult population		Open brood		Closed brood		Food	
	Before	After	Before	After	Before	After	Before	After
Control	7.3	7.3*	2.1	1.9*	2.6	2.6*	2.5	2.9
Roundup®	7.1	4.0*	2.1	0.6*	2.9	0.9*	2.7	1.9

*Values in the column are significantly different by paired *t*-test at 5% probability.

The reduction in the adult bee population and open and closed brood area was visually distinct between the hives of the treatments, as well as the irregularity in the age of larvae and pupae, non-removal of dead pupae and high egg laying by the workers (**Fig. 2**).

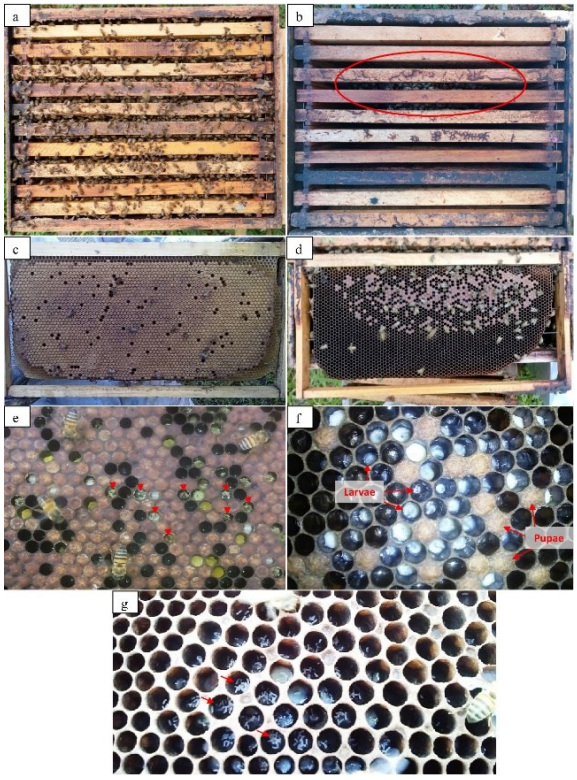

Fig. 2 Images of hives and different areas of the control combs and Roundup® treatments registered in the fourth week after the beginning of the bioassays. a and c – adult bee population and brood area of the control treatment; b and d - adult bee population (indicated by the circle) and brood area of the Roundup® treatment, e - dead pupae not removed by workers, indicated by red arrows; f – larvae and pupae with irregular development, with different ages in the same region of the comb of the Roundup® treatment; g - eggs of *Apis mellifera* workers in an orphan hive, observed 24 h after the attempt to introduce a fertilized queen into a hive of the Roundup® treatment.

The reduction in the population of adult bees and nesting areas in hives exposed to a sublethal dose of the Roundup® treatment (**Fig. 2b** and **2d**) in September was observed 30 days after the end of exposure to the herbicide, corresponding to two months after supplying the first contaminated feed. In this same period, the hives exposed to the herbicide showed the death of 10 to 15% of the pupae on each side of the combs, which were not removed by the workers (**Fig. 2e**) and irregularity in the age of development of larvae and pupae **(Fig. 2f**). The reduction in the population of adult bees is probably related to the reduction in longevity that glyphosate causes in these insects (Faita et al., 2020; Motta et al., 2020) as a result of the physiological changes that compromise the metabolism and ability of bees to detoxify (Almasri et al., 2022).

The reduction in the brood area may be related to different changes in the hive, including: 1) lack of care for the larvae by nursing bees, evidenced by the non-removal of dead pupae and changes in hygienic behavior (Cardozo, 2017); 2) nutritional deficiency due to the reduction of workers to collect resources for the hive, in addition to the early degeneration of the hypopharyngeal glands of the nursing bees (Faita et al., 2018) and reduction in the production of royal jelly (Chaves et al., 2020); 3) impairment of social immunity in the hive due to changes in the proteomic profile of royal jelly with reduced production of MRJP3, the main immunity protein of *A. mellifera* (Faita et al., 2022).

It is relevant to stress that in the present study, in three hives exposed to a sublethal dose of Roundup® it was identified the absence of a queen and the absence of queen cells by the workers. After the attempt to introduce fertilized queens, which were not accepted, it was observed high egg laying by the workers (**Fig. 2g**). Taken together, these changes surpass the ability of bees to maintain hive homeostasis and re-establish their collective functioning as a superorganism (Moritz et al., 1998). However, our results for colony strength estimates differ from the one obtained by Odemer et al. (2020), who did not observe an effect of GBH on the life expectancy of individuals and conditions of the colonies, although in the same study it was identified that chronic exposure to GBH delayed development of the worker's brood. In hives from the control treatment, the queen's absence was not verified during the bioassay.

Residual levels of glyphosate are approximately 10 times higher in pollen than in nectar (Thompson et al., 2014), and this food, which constitutes the protein base of the hive, is provided to the larvae and also consumed by nursing workers for the production of royal jelly (Costa et al., 1999; Huang et al., 1989). Although it has been reported that glyphosate does not negatively affect the survival of larval or adult workers (Herbert et al., 2014; Thompson et al., 2014), these authors have not conducted field colony monitoring studies; thus, their results do not represent the chronic effects of this herbicide on bees. In this sense, chronic effects of exposure of hives to a sublethal dose of fungicides under field conditions were ultrastructural changes in the hypopharyngeal glands of nursing bees and reduced hive strength, with a decrease in the population of adult bees, brood area and stored food (Chaves et al., 2023). Thus, it is likely that the Roundup® residues present in the bee's food, mainly in the pollen, have interfered with the development cycle of larvae and pupae, causing mortality due to intoxication or nutritional deficiency and compromising the survival of the colony.

The results of the present study allow for comparing the effects of a sublethal dose of Roundup® to those caused by chronic exposure of hives to neonicotinoid insecticides. The action of sublethal doses of neonicotinoids in hives includes queen loss 1.5 months after the start of treatments in 60% of colonies, non-replacement of queens and non-swarming in early spring, decrease in the adult bee population and larvae and social immunity compared to hives not exposed to contamination (Tsvetkov et al., 2017; Woodcock et al., 2017; Parrón et al., 2011; Romano et al., 2010; Franco et al., 2010; Dallegrave et al., 2007). The effects of pesticide residues on beehives can be even worse when there are different contaminants, which exert synergistic effects, and the interaction with environmental factors that can amplify the impact of bee losses (Woodcock et al., 2017; Zhu et al., 2017).

4. Conclusions

This work revealed the presence of glyphosate in honey samples and verified that, in an average period of 50 days, its metabolization did not occur, preventing the formation of AMPA. Additionally, the qualitative evaluations of the hives demonstrated that the presence of Roundup® in the bee's diet does not affect the survival of adult individuals but exerts severe deleterious effects on the colony's strength, mainly when present in the pollen. Our results allow inferring that a sublethal dose of the herbicide intoxication caused a reduction in the brood area and, consequently, in the population of adult individuals, compromising work in the hive, in addition to the death and non-replacement of the queen. Together, these factors are consistent with what was described for the event called Colony Collapse Disorder - CCD, a cause of great concern among beekeepers and researchers, due to the disappearance of hives in several countries, without a known specific cause.

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Author contributions

Conceptualization, M. R. F, A. I. O., and R. O. N.; Methodology, M. R. F, A. I. O., and R.O.N.; Investigation, M. R. F. and V. R. A.; Formal Analysis, M. R. F., V. R .A., G. A. M., and A. I. O.; Writing – Original Draft, M. R. F., V. R. A., and R. O. N.; Writing – Review & Editing, M. R. F., V. R. A., A. I. O., G. A. M., and R.O.N. All authors read and approved the final manuscript.

Availability of data and materials

Data are available under request from the corresponding author.

Informed Consent Statement

Not applicable.

Conflicts of Interest

The authors declare no conflict of interest.

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