

Anatomy of *Calliphora vicina* (Diptera: Calliphoridae) larva and evaluation of Roundup Full II® effect on the development and morphology of this forensic species

Anatomía de la larva de *Calliphora vicina* (Diptera: Calliphoridae) y evaluación del efecto de Roundup Full II® sobre el desarrollo y morfología de esta especie forense

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Abstract: Glyphosate formulations have been used in self-poisoning and accidental poisoning. *Calliphora vicina* (Diptera: Calliphoridae) is associated with decomposing human and animal remains. The aims of the study were to describe the anatomy of *C. vicina* larva and changes in it due to Roundup Full® II (RFII). Also, we evaluated the effects of this herbicide on the development and external morphology of *C. vicina*. These flies were exposed to two doses of RFII (7.69 and 4.62 ml/kg) and daily observations were performed to record the duration of life cycles. Larvae of third instar were selected for anatomical studies of the digestive, excretory and fat bodies structures. Also, specimens of all stages were removed to evaluate possible alterations in anatomy, size, external morphology and sex proportion due to RFII. As a result, the microscopic and macroscopic anatomy of the aforementioned structures of the third-stage larva of *C. vicina* is described. Furthermore, the anatomy of the insects exposed to the herbicide did not show any differences compared to the controls, nor were any changes found in the other parameters evaluated. In conclusion, taxonomic determination and PMI estimation could be done as usual.

Keywords: Blue bottle flies, Forensic entomotoxicology, Glyphosate.

Resumen: Formulaciones de glifosato se han usado en autointoxicaciones e intoxicaciones accidentales. *Calliphora vicina* (Diptera: Calliphoridae) está asociada con restos humanos y de animales en descomposición. Los objetivos de este estudio fueron describir la anatomía de la larva de *C. vicina* y cambios en ella debido al Roundup Full® II (RFII). Además, evaluamos los efectos de este herbicida en el desarrollo y la morfología externa de *C. vicina*. Estas moscas se expusieron a dos dosis de RFII (7.69 y 4.62 ml/kg) y se realizaron observaciones diarias para registrar la duración de los ciclos de vida. Larvas de tercer estadio se seleccionaron para los estudios anatómicos de las estructuras digestivas, excretoras y de los cuerpos grasos. También se removieron especímenes de todos los estados para evaluar posibles alteraciones anatómicas, en tamaño, morfología externa, y proporción de sexos debidas al RFII. Como resultado se describe la anatomía microscópica y macroscópica de las estructuras mencionadas de larvas de tercer estadio de *C. vicina*. Además, la anatomía de los insectos expuestos al herbicida no mostró diferencias respecto de los controles, así como tampoco, se encontraron cambios en los otros

parámetros evaluados. En conclusión, la determinación taxonómica y estimación del PMI podrían realizarse de forma habitual.

Palabras clave: Entomotoxicología forense, Glifosato, Moscas azules de la botella.

INTRODUCTION

One of the approaches of forensic entomotoxicology is to study the effect that different xenobiotics have on the development of insects of forensic interest (George et al., 2009; Zanetti et al., 2021; Zanetti & Centeno, 2023). Forensic entomotoxicology can contribute to the estimation of the postmortem interval (PMI), it can determine the cause of death or provide related information, and even verify if the corpse has been moved or its origin (Zanetti et al., 2021; Zanetti & Centeno, 2023).

The Calliphoridae family is capable of being the first colonizer of a corpse (Byrd & Castner, 2009). The genus *Calliphora* includes the cosmopolitan species *Calliphora vicina* Robineau-Desvoidy, 1830 which has a wide distribution in Argentina and is very common in the province of Buenos Aires (Mariluis & Mulieri, 2003). It is usually found from autumn to spring in both rural and synanthropic environments (Battan Horenstein et al., 2007), showing a preference for the latter. In addition to its forensic importance, the species also has veterinary and medical value, since it can cause facultative myiasis in vertebrates and can be a mechanical vector of viruses, bacteria, fungi, protozoa and helminths (Maldonado & Centeno, 2003; Byrd & Castner, 2009; Araghi et al., 2015). The blue bottle fly also fulfills an ecological function due to its role in the decomposition of corpses in the environment and in the recycling of nutrients (Byrd & Castner, 2009). Due to its importance, the development of *C. vicina* has been studied under different variables that could affect its growth rate; one of them are toxic substances or xenobiotics that can cause death (O'Brien & Turner, 2004; Zanetti & Centeno, 2023).

Glyphosate (N-phosphonomethylglycine) is a broad-spectrum post-emergence herbicide. The company Monsanto markets it under the name Roundup® (Agostini et al., 2020) and is currently the most used pesticide worldwide and in Argentina (Mesnage et al., 2015; Anguiano & Ferrari, 2019). There is current evidence of adverse effects of both glyphosate-based herbicides (in addition to the main ingredient, they have other substances such as salts and adjuvants such as ethoxylated alkylamines (POEA), whose function is to facilitate their handling or increase their effectiveness) and glyphosate *per se* on non-target organisms, such as neurological, teratogenic, mutagenic, carcinogenic, immunological, respiratory and reproductive effects (Mesnage et al., 2015; Cortina et al., 2017; Anguiano & Ferrari, 2019; Agostini et al., 2020). Glyphosate poisoning can be accidental or voluntary (Bradberry et al., 2004). There are reports in Asia, Central and South America that indicate that the greatest poisonings with pesticides as a suicidal method occur mainly in countries dedicated to agricultural activities (Ajdacic-Gross et al., 2008; Lee et al., 2015; Anchía-Jiménez et al., 2021). The

symptoms caused by poisoning are very diverse such as vomiting, abdominal pain, gastrointestinal infections, etc., and as pointed above mortality (Chen et al., 2009). It must be taken into account that information on suicides is scarce and/or difficult to access and therefore the real number of cases may be underestimated (Arias & Blanco, 2010; Fritschy, 2012; UNICEF, 2019).

Some works have studied the effects of glyphosate on arthropods. Among them, the chronic and acute toxicity of glyphosate and/or its formulations in crustaceans was evaluated (Gill et al., 2018). Schneider et al. (2009) analyzed in *Chrysoperla externa* Hagen, 1861 (Neuroptera: Chrysopidae) the alterations that glyphosate (at the maximum nominal concentration recorded in the field) could cause in population, reproductive and developmental parameters. In another work, the possible consequences of glyphosate on the life table of *Metopolophium dirhodum* Walker, 1849 (Hemiptera: Aphididae) were studied (Saska et al., 2016). Zanetti & Centeno (2024) studied the effect of glyphosate, using Roundup Full® II (RFII) formulation, on the development of *Dermestes maculatus* DeGeer, 1774 (Coleoptera: Dermestidae) and *Lucilia sericata* Meigen, 1826 (Diptera: Calliphoridae) under controlled conditions. Then, the anatomy of hide beetles under RFII was evaluated by Zanetti (2024).

Morphology is the branch of biology that studies the form and composition of body parts, and is the bases of various fields in biology, such as taxonomy and evolutionary studies (Rocha et al., 2020). Morphology has different divisions and one of them is anatomy. The anatomy of tissues and organs of organisms is part of the species description. Several studies highlight the importance of having detailed knowledge of immature stages in order to correctly identify them, particularly for use in forensic entomology (Grzywacz, 2013; Mendonça et al., 2014), given the applicability of the area or discipline (to use them as the entomological evidence). Moreover, the anatomical study allows evaluating the toxic effect of xenobiotics on the structures (Latif et al., 2013), as well as it is important to be able to understand physiology and carry out studies on it.

The objectives of this work were to characterize the macroscopic and microscopic anatomy of fat bodies, the alimentary canal and the excretory systems of *C. vicina* larva, as well as the effect of RFII on these structures. Also, possible alterations on the development and external morphology of this species under RFII exposure were evaluated.

MATERIALS AND METHODS

Calliphora vicina colony

During the fall and spring, traps baited with porcine or bovine blood, kidney, lung, liver and/or legs of domestic pigs were placed in two sites in the city of Bahía Blanca, Province of Buenos Aires, Argentina (38°43 '00"S 62°16'00"W). Baited traps were checked daily for *C. vicina* eggs. These were transported to the laboratory and placed in 2 kg glass containers with 4 cm of cat health pebbles and covered with *voile*. The containers were placed in an incubator (Ingelab, Argentina) at 22 ± 0.1 °C, a relative humidity of 48 ± 1.2 % and a natural Light/Dark photoperiod of 12:12 h, in the vivarium of the Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur. The temperature was selected because the optimal development range for the species is 20 to 25 °C (Díaz Martín et al., 2014), it is a temperature found in the aforementioned stations, and data on fly development at the aforementioned temperature were not available for the area. The new adults were fed powdered milk, sugar, and distilled water, ad libitum. After 72 h of the emergence, porcine meat and blood were added to allow oviposition (Anderson, 2000). The identification of adult specimens was carried out with taxonomic keys from Mariluis & Schnack (2002) and Florez & Wolff (2009), and the entomological collections of the Laboratorio de Entomología Forense del Instituto de Ciencias Biológicas y Biomédicas del Sur (CONICET-UNS). Of the eggs laid on the meat substrate, a part was used for the tests and the rest was placed in a 2 kg glass container under the same conditions described above, to continue with the colonies that were monitored daily.

Blue bottle flies exposure to RFII

The diets were prepared according to Zanetti & Centeno (2024) and consisted of pork muscle homogenates with doses (7.69 and 4.62, ml/kg) of Roundup Full® II (potassium salt of N-phosphonomethyl glycine 66.2 % w/v, Monsanto Argentina S.A.I.C). These were calculated in order to simulate possible lethal acute overdoses in humans (Zanetti & Centeno, 2024). For the control, the same procedure was applied, but without the addition of RFII. Two experiments were performed, one for life cycle analysis and another for size and external morphology analysis. From this latter, specimens were also selected for microscopic and macroscopic anatomical observations. In the experiments, two replicates of the control were carried out for the highest dose and three for the lowest dose; eight replicates were performed with the RFII (except with L1 and L2 of the highest dose where seven replicates were performed).

Life cycle analysis

In the diets (with RFII and control), 100 *C. vicina* eggs were collected under stereoscopic microscope from bait and transferred to

plastic containers with 3 cm of cat litter stones and closed with *voile*. The containers were placed in the incubator set as previously reported. Observations and quantifications were carried out daily (between 7 and 10 AM) under a stereoscopic microscope (Olympus, Japan) and the duration of each stage and of the total cycle was recorded. To determine if the individuals had reached a certain stage of development, the criterion was established that 50 % or more of the individuals must have molted. This also applied to the molting of the larval instars.

Size and external morphological analysis

Monitoring and selecting specimens

Two hundred and fifty *C. vicina* eggs were transferred to each diet, with and without RFII, and placed in plastic containers. These were maintained in the incubator set as previously reported. Daily observations were carried out to verify the state of development and proceed to randomly extract between 15 and 20 individuals from each larval instar, the pupal stage and the adult stage. The larval specimens were extracted in their last stage of development within each stage, with the exception of L3 (larvae of third instar), in which case the extraction was prior to the post-feeding stage. The pupae were collected 72 h after the start of pupation and were also monitored until adult emergence.

Slaughter and measurements of immature specimens

Larvae were sacrificed with distilled water at 80 °C for 30 s and preserved in 70 % ethanol. Pupae were sacrificed and preserved by immersion in 70 % ethanol. Body width and length were measured with the help of a stereoscopic microscope (Olympus, Japan) with a micrometer eyepiece. The larvae were measured dorsally and the length was recorded from the first cephalic segment to the last abdominal segment. The width was measured at the sixth/seventh segment. In both cases, the following magnifications were used for each stage: 4x (L1), 1.2x (L2), and 0.67x (L3). Pupae were also measured dorsally using 0.8x magnification. The total length was defined from the first cephalic segment to the last abdominal segment and the width was measured in the fifth segment.

Slaughtering, sexing and measuring adults

Adults were sacrificed and preserved by immersion in 70 % ethanol, were sexed through observation of external sexual dimorphism and their length was determined by measuring dorsally from the base of the antennae to the last abdominal segment, and the

width that was examined corresponds to the widest point of the thorax using 0.8x magnification.

External morphological observation

Rough external morphological observation was carried out using a stereoscopic microscope (Olympus, Japan), looking for tissue malformations and color alterations. To do this, immediately after sacrifice, the shades of the specimens were estimated (not quantified) and visually compared (control vs. treatment with RFII) under standardized lighting conditions. Also, comparisons were performed with specimens described in the literature for the species and the collection of the Laboratorio de Entomología Forense del Instituto de Ciencias Biológicas y Biomédicas del Sur.

Anatomical analysis

For each study, macroscopic and microscopic, ten recently molted specimens were randomly extracted from L3 (five for each treatment), sacrificed in 5% ethanol and preserved in 10% formaldehyde. These specimens were selected following the protocol described under the name *Monitoring and selecting specimens*.

Macroscopic anatomy of larva

Control and RFII-treated third instar larvae were removed from formaldehyde 10 % and dissected under stereomicroscope (NXZ, China). Photographs were taken using a digital camera (Optika, Italy) which was mounted to a stereomicroscope (Zeiss, Germany) to observe the digestive, excretory and fat bodies structures. Possible alterations in the form and number of these structures in response to RFII were qualitatively examined. Five replicates (one larva per replicate with three slides each) were performed.

Microscopic anatomy of larva

Control and RFII-treated third instar larvae were removed from formaldehyde 10 % and dissected under stereomicroscope (NXZ, China). Structures were dehydrated in ascending grades of alcohol followed by infiltration and inclusion in paraffin (Ross et al., 1997). 5 µm microtome sections were cut into a rolling ribbon. The ribbon was placed on the glass slide and stained with haematoxylin and eosin (Ross et al., 1997). Finally, slides were mounted, cover slip placed on top and examined under optic microscope (Olympus BX51). This allowed a qualitative comparison of RFII treatment with control. Photographs were taken using an Olympus C-7070 digital camera which was mounted to the microscope to observe any changes in the integrity of the nucleus, cytoplasm, membrane, microvilli, intima, and

cell form, of fat bodies, of the digestive and excretory structures. The magnification was indicated in the figure captions. Five replicates (one larva per replicate with three slides each) were performed.

Statistical analysis

The evaluation of the results involved calculating the mean and/or standard error (SE). A simple ANOVA was used to analyze the parameters related to size. This statistical analysis was chosen because the data were normally distributed. All statistical analyses were carried out using InfoStat.

RESULTS

Macroscopic and microscopic anatomical characterization of digestive structures larvae without exposure to RFII

All the structures of the digestive system are whitish, except when some food remains are present (Fig. 1; Fig. 3a). The crop with contents is shaped like an oval balloon (Fig. 1a; Fig. 3a), consisting of an epithelium with cells with a large nucleus with patches of heterochromatin (Fig. 2a). The inner face of the epithelium is in contact with an intima that borders the lumen and in contact with the outer face of the epithelium; layers of muscles are observed (Fig. 2a). The crop connects with the proventriculus through a duct which has muscles. In the same place but on the opposite margin, the esophagus is inserted into the proventriculus (Fig. 1b). The proventriculus is bulb-shaped and covered with muscle (Fig. 1c). The proventriculus delimits the union of the anterior and midgut, and extends four gastric caeca which also present external muscular layers (Fig. 1c, d). The cells in the gastric diverticula are similar to those in the midgut but without vacuoles (except those near the junction or opening to the midgut), and there are also nidi or clusters of small cells (Fig. 2d, e). In addition, elongated, whitish sometimes transparent salivary glands can be observed (Fig. 1c). The epithelium consists of large rounded or polygonal cells with large central or semibasal nuclei that have patches of heterochromatin (Fig. 2c). The union of the gastric caeca to the proventriculus delimits the beginning of the midgut (Fig. 1b). This is the longest portion of the digestive system (Fig. 3a) and microscopically it is formed by large polygonal cells (sometimes appear a simple cuboidal epithelium) with a large oval-rounded nucleus with patches of heterochromatin and a highly vacuolated cytoplasm (Fig. 3b-d). Towards the lumen, a brush border is recorded along the entire cell margin. The epithelium is surrounded by a layer of internal circular muscle and an external longitudinal muscle layer (Fig. 3b-d). At the base of the epithelium are smaller cells that will form the imaginal intestine and can appear

alone or in clusters along the intestine (Fig. 3d). Midgut tissue contains peritrophic membrane within its lumen (Fig. 3c). The hindgut has a microscopic structure just like the midgut, but with no peritrophic membrane.

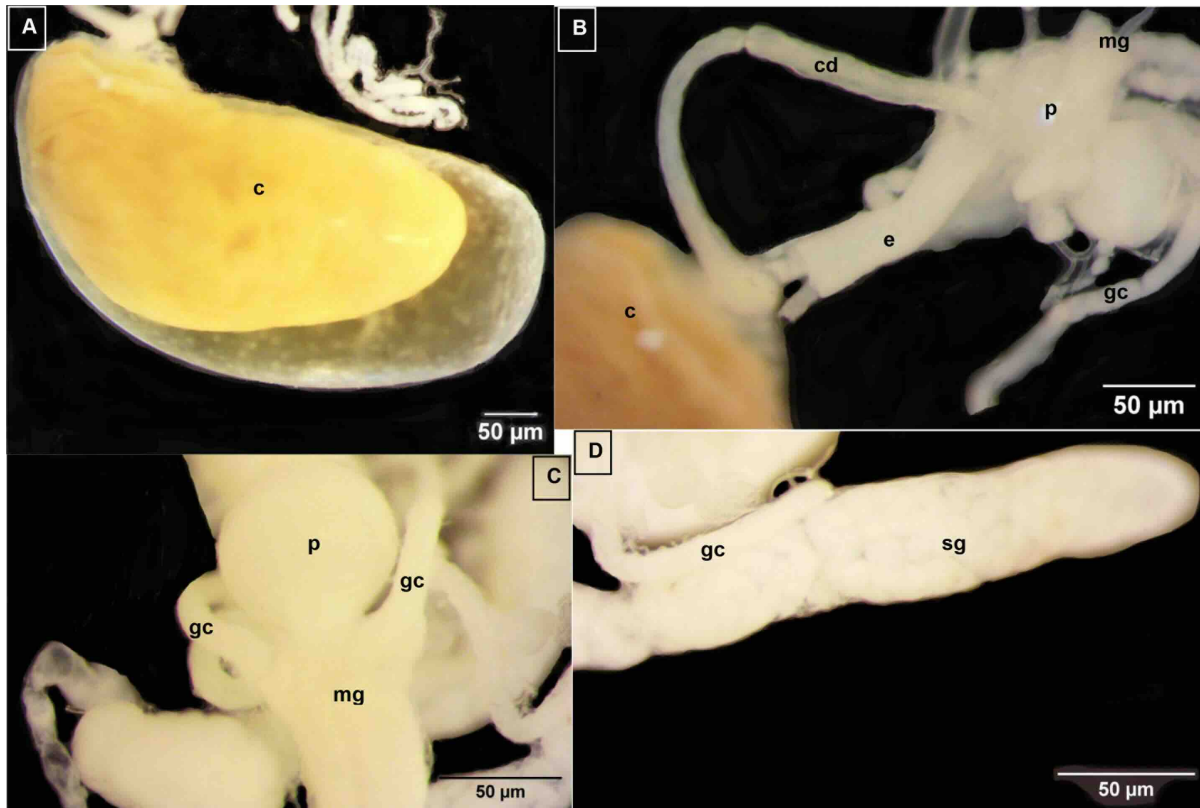


Figure 1

Digestive system of *Calliphora vicina* larva

a. macroscopic anatomy of crop (2x). b. Connection of the crop and esophagus with the proventriculus (3.2x). c. Macroscopic anatomy of proventriculus, gastric caeca and first section of midgut (5x). d. Macroscopic anatomy of salivary gland and gastric caeca (5x). c: crop, cd: crop duct, e: esophagus, gc: gastric caeca, mg: midgut, p: proventriculus, sg: salivary glands

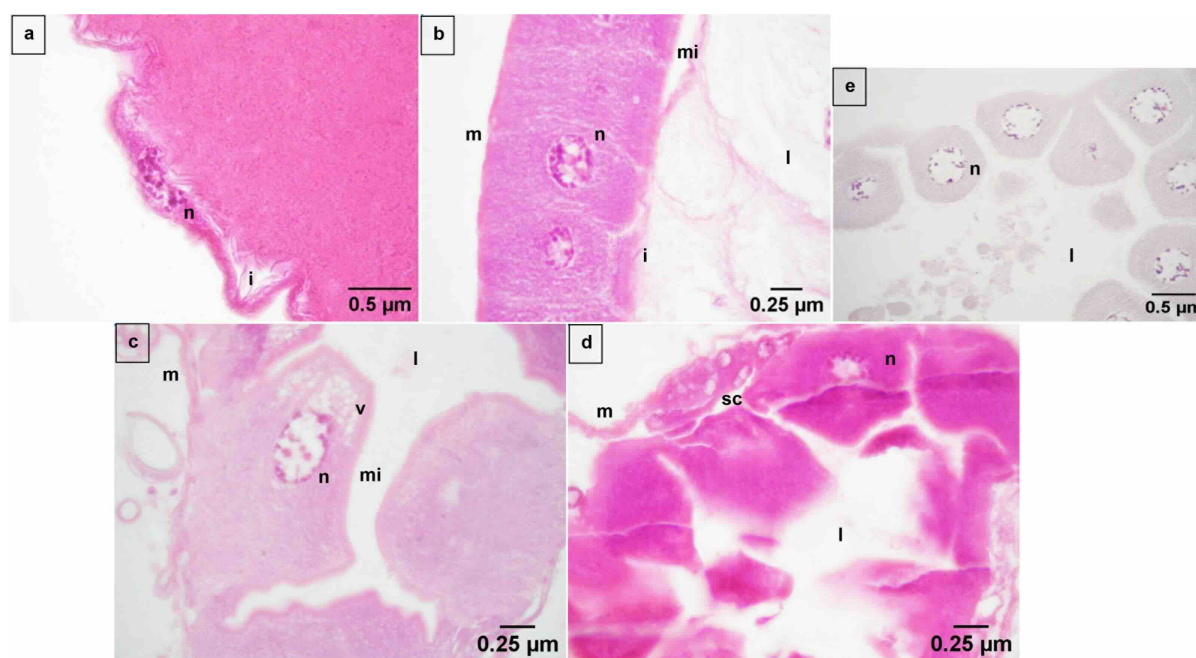


Figure 2

Digestive system of *Calliphora vicina* larva

- a. Longitudinal section of crop (100x). b. Longitudinal section of proventriculus (100x). c. Longitudinal section of gastric caeca (100x). d. Longitudinal section of gastric caeca with emphasis in cluster of small cells (100x). e. Longitudinal section of salivary glands (100x). n: nucleus, i: intima, l: lumen, m: muscle, mi: microtubules, sc: small cells, v: vacuole.

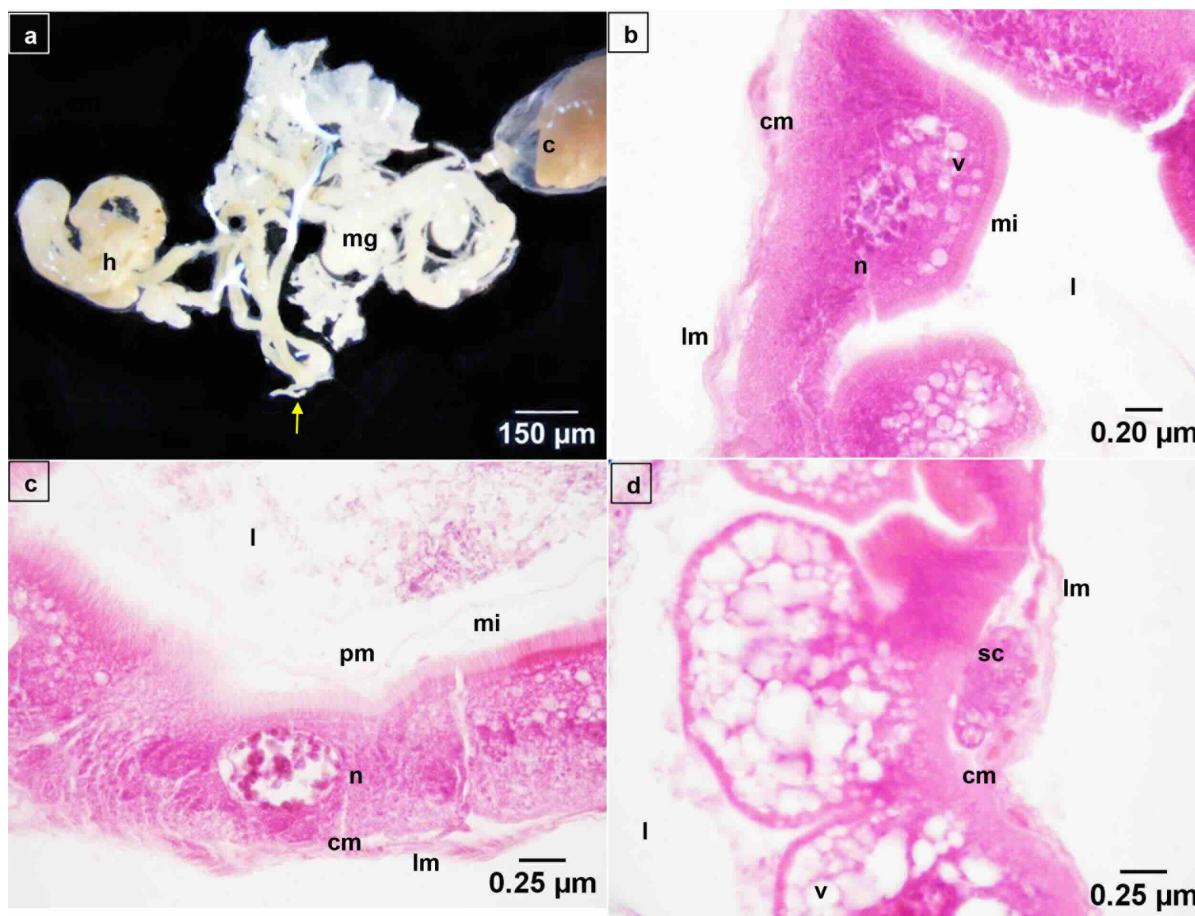


Figure 3

Digestive system of *Calliphora vicina* larva

a. Macroscopic anatomy of alimentary canal (0.8x). b. and c. Longitudinal section of midgut (100x). d. Longitudinal section of midgut with emphasis in cluster of small cells (100x). c: crop, cm: circular muscle, h: hindgut, l: lumen, lm: longitudinal muscle, mg: midgut, mi: microvilli, n: nucleus, pm: peritrophic membrane, sc: small cells, v: vacuole. The yellow arrow shows the insertion of the Malpighian tubules into the intestine and the division of the midgut and hindgut

Macroscopic and microscopic anatomical characterization of Malpighian tubules larvae without exposure to RFII

The Malpighian tubules are long, rosary-shaped structures located at the midgut–hindgut junction, composed of cells containing large oval-rounded nuclei with heterochromatin patches (Fig. 4a, b). Towards the lumen, the apex of the cells has a brush border and bordering the external margin there is a layer of circular and longitudinal muscles, and then the basement membrane (Fig. 4b, c).

Macroscopic and microscopic anatomical characterization of fat bodies larvae without exposure to RFII

Fat bodies have a macroscopic appearance that resembles united islets (Fig. 5a). Microscopically, they are formed by large polygonal

cells with a large oval-rounded nucleus with patches of heterochromatin and a vacuolated cytoplasm (Fig. 5b, c). The cells are delimited by a basal lamina or membrane (Fig. 5b).

Larval anatomy under RFII

No changes (for example, loss of the brush border, damage to epithelial cell nuclei, damage to cell membrane, etc.) were evident in the alimentary canal, in the fat bodies or in the Malpighian tubules of *C. vicina* larvae when the microscopic anatomy of such structures was analyzed in the presence of RFII.

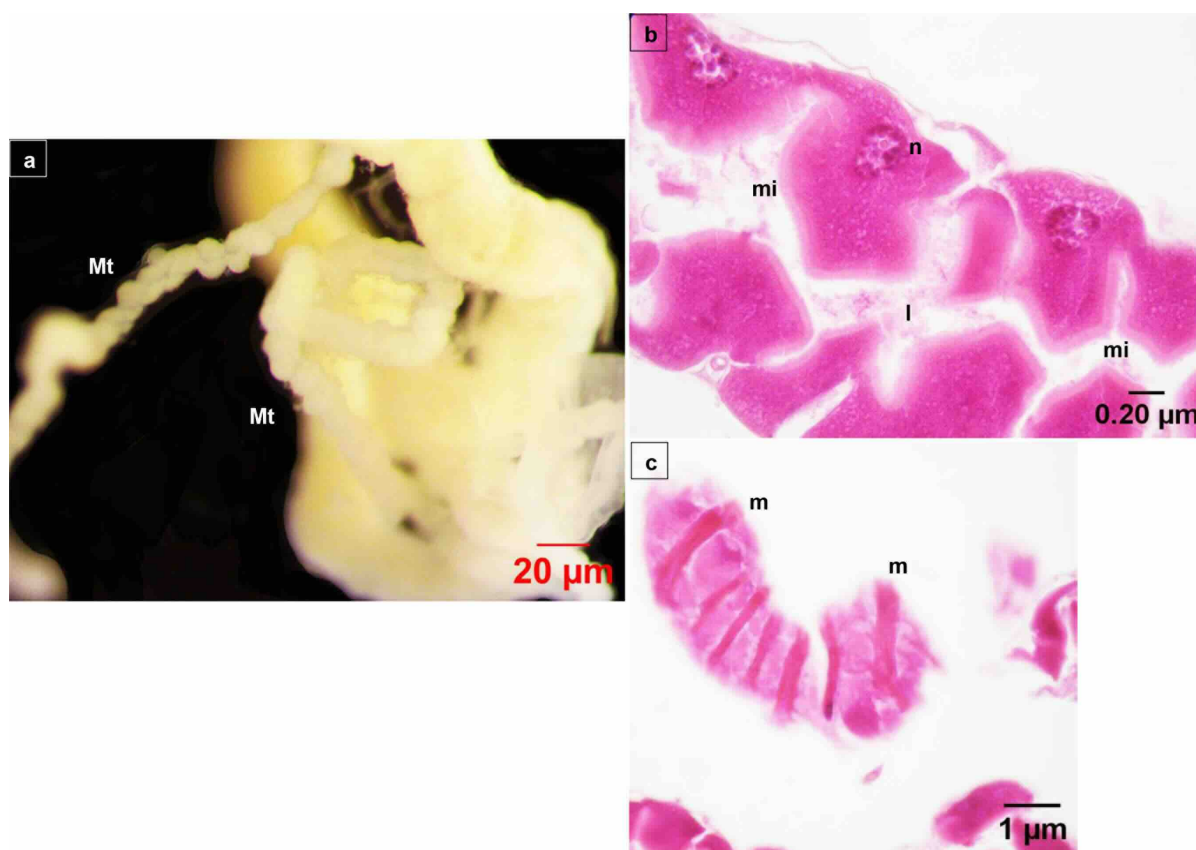


Figure 4

Malpighian tubules of *Calliphora vicina* larva

a. Macroscopic anatomy (5x). b. Longitudinal section of Malpighian tubules (100x). c. Idem b (40x). l: lumen, m: muscle, mi: microvilli, Mt: Malpighian tubule, n: nucleus

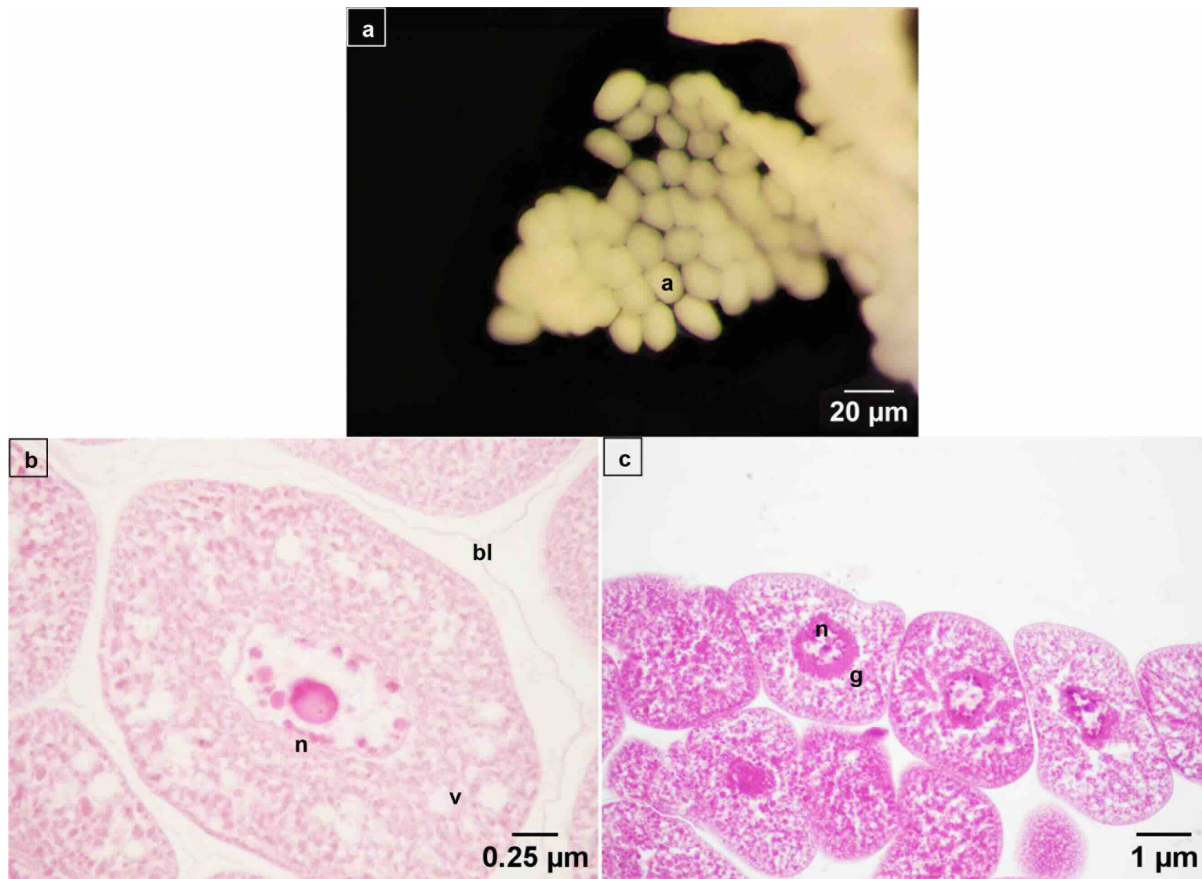


Figure 5

Fat bodies of *Calliphora vicina* larva

a. Macroscopic anatomy (5x). b. Longitudinal section of fat bodies (100x). c. Idem b (40x). a: adipocytes, bl: basal lamina, g: granules, n: nucleus, v: vacuole

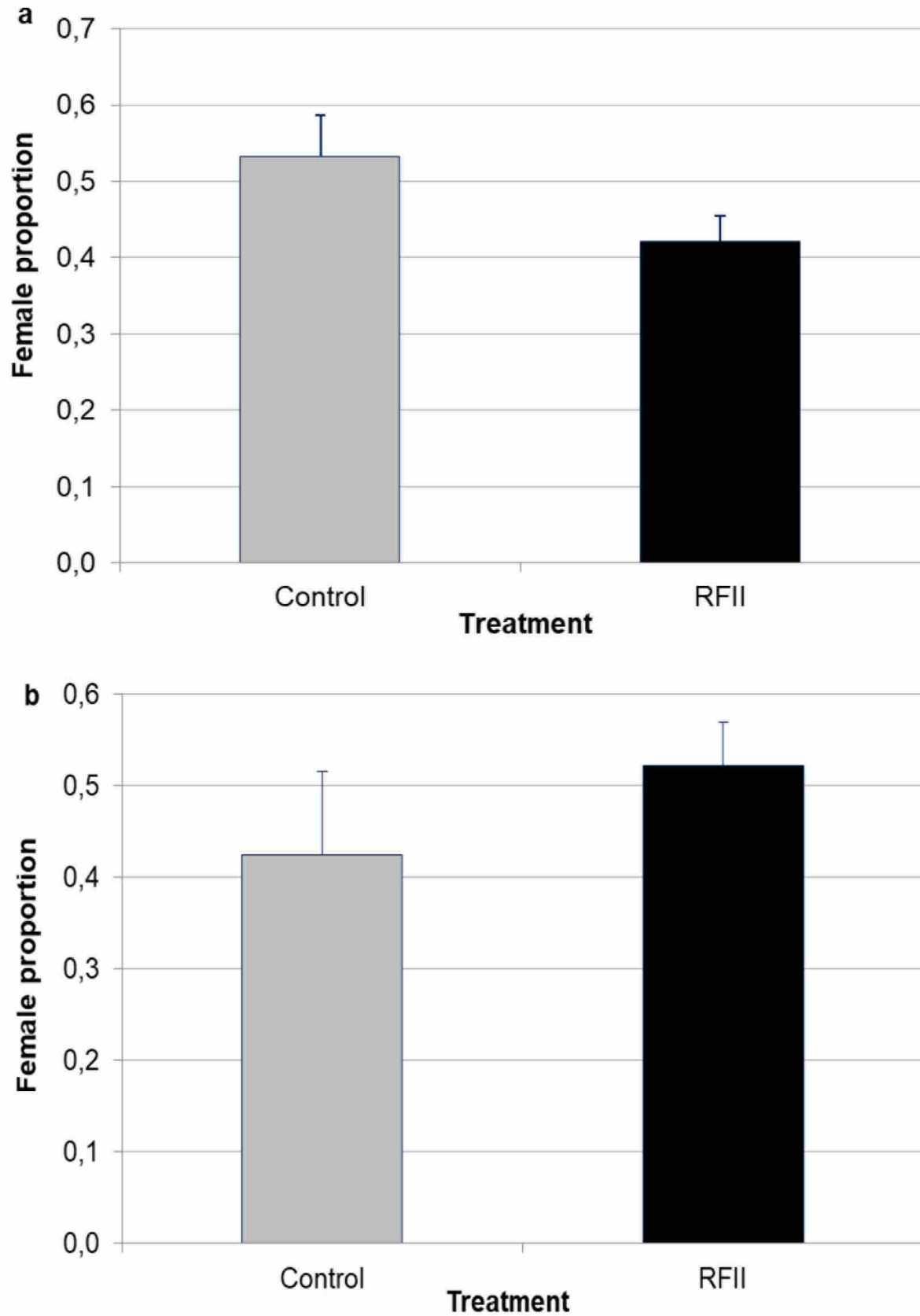


Figure 6

Female proportion in *Calliphora vicina* obtained after exposure of larvae with Roundup Full® II

a. 4.62 ml/kg. b. 7.69 ml/kg. Error bars are SEs

Blue bottle flies development under RFII

The duration of each developmental stage of *C. vicina* did not show differences between the control specimens and those treated with RFII at any dose. Table I shows the durations of stages of development after exposure to RFII. Neither the body length in any stage (L1: $F = 1.89$, d.f.= 8, $p = 0.21$; L2: $F = 0.04$, d.f.= 8, $p = 0.86$; L3: $F = 0.19$, d.f.= 9, $p = 0.68$; Pupa: $F = 0.02$, d.f.= 9, $p = 0.89$; Adult: $F = 0.06$, d.f.= 8, $p = 0.82$) nor the body width of the immature stages and the thoracic width of adults were altered (L1: $F = 1.45$, d.f.= 8, $p = 0.27$; L2: $F = 0.57$, d.f.= 8, $p = 0.48$; L3: $F = 0.02$, d.f.= 9, $p = 0.89$; Pupa: $F = 0.67$, d.f.= 9, $p = 0.44$; Adult: $F = 2.49$, d.f.= 10, $p = 0.66$) due to RFII treatments (Table II). The external morphology *grosso modo* did not exhibit structural or color changes in the presence of RFII. Figure 6 shows the proportion of females unchanged between controls and RFII treatments ($F = 0.88$, d.f.= 8, $p = 0.38$).

Table I

Duration of immature stages in hours of *Calliphora vicina* under Roundup Full® I

Treatment	Egg	L1	L2	L3	Pupa
Control	24	24	24	120	338±8.00
4.62 ml/kg RFII	24	24	24	120	318±3.95
Control	24	24	24	120	312±1.00
7.69 ml/kg RFII	24	24	24	120	306±0.31

Duration is presented as Mean. SE is reported for pupae because means were equal between replicates of egg and larval instars. SE was ± 24 h for egg and larval stages

Table II

Size of *Calliphora vicina* exposed to Roundup Full® II

Instar/stage	Length (mm) Control	RFII (4.62 ml/kg)	Width (mm) Control	RFII (4.62 ml/kg)
L1	5.18±0.04	5.19±0.04	0.56±0.03	0.54±0.02
L2	7.28±0.20	7.31±0.12	1.23±0.05	1.25±0.03
L3	17.58±0.29	17.94±0.16	2.96±0.08	3.10±0.04
Pupa	8.70±0.11	8.71±0.07	3.72±0.08	3.69±0.05
Adult	9.96±0.06	10.03±0.04	3.74±0.07	3.71±0.04

Instar/stage	Length (mm) Control	RFII (7.69 ml/kg)	Width (mm) Control	RFII (7.69 ml/kg)
L1	5.65±0.07	5.53±0.04	0.65±0.02	0.62±0.01
L2	7.96±0.26	7.96±0.14	1.51±0.06	1.47±0.03
L3	18.36±0.32	18.20±0.16	3.19±0.20	3.16±0.10
Pupa	10.64±0.11	10.02±0.06	4.09±0.06	4.15±0.03
Adult	10.74±0.14	10.78±0.08	4.72±0.04	4.65±0.02

Size is reported as Mean \pm SE. There were no statistically significant differences between the control and both RFII concentrations in any of the comparisons

DISCUSSION

It is relevant to claim the use and study of *C. vicina* as a species of forensic interest. *Calliphora vicina* larvae have been collected from corpses resulting from drug-related suicides (Sadler et al., 1995). Half

of the collected larvae were used as such and the other half were incubated until pupation, to carry out toxicological studies that provided scientific information that also served to resolve forensic cases (Introna et al., 2001).

The microscopic anatomical characteristics of *C. vicina* presented similarities with those of *Lucilia cuprina* Wiedemann, 1830 and *Chrysomya megacephala* Fabricius, 1794 (Diptera: Calliphoridae). In these species four gastric caeca were found at the base of the proventriculus and the beginning of the midgut (Waterhouse & Stay, 1955; Boonswrong et al., 2011; Yasmeen & Amir, 2018). This digestive region in these species is formed by a layer of large cells with a notable basal membrane and a brush border on its surface surrounded by an internal layer of circular muscle and an external layer of longitudinal muscle. A peritrophic membrane was also found in the midgut of *L. cuprina* and *Ch. megacephala* (Waterhouse & Stay, 1955; Boonswrong et al., 2011; Yasmeen & Amir, 2018). The mentioned characteristics were also recorded in *C. vicina*. Also, in this species, small cells were also found alone or in clusters along the midgut at the base of the epithelium. These were also observed in *L. cuprina* by Waterhouse & Stay (1955) who suggested that those cells made up the imaginal midgut, although no regenerative cells were found (Waterhouse & Stay, 1955). Contrary to what was reported by these authors for *L. cuprina*, in *C. vicina* no difference was observed in the cell types or in the characteristics of such cells (presence of vacuoles, arrangement of the nucleus, the border in brush, etc.) depending on the midgut region. Yasmeen & Amir (2018) described the epithelium as cuboidal, with vacuolization and a large nucleus in the center of the cytoplasm. Hobson (1931) reported the absence of vacuoles in the midgut; they were only found in this intestine in the transition zone to the hindgut. The difference observed could also be a result of the methodology used by the latter author.

Regarding the foregut and hindgut, large highly vacuolated cells were also observed in *L. sericata* (Hobson, 1931), as was observed in *C. vicina*. According to Hobson (1931), the presence of fat coincided with vacuolization and only occurred in the anterior and posterior regions of fed larvae and could be a temporary storage product. Hobson (1931) also reported that in *Lucilia* the cells in the gastric diverticula are similar to those in the anterior segment but without vacuoles; however, the cells immediately adjoining the opening into the lumen of the gut are vacuolated. These results matched with what was observed in *C. vicina*. Salivary glands of *C. vicina* are similar to those in *Ch. megacephala* which are large long tubes (Boonswrong et al., 2011).

The excretory system in *L. cuprina* and *Ch. megacephala* is made up of two pairs of Malpighian tubules near the end of the midgut (Waterhouse & Stay, 1955; Boonswrong et al., 2011; Yasmeen & Amir, 2018). The same was found in *C. vicina*. Likewise, Vieira &

Lello (1996) indicated that the rosary appearance of the tubules is due to the alternating arrangement of giant cells that are located on the monolayer wall of cobblestones. In *L. cuprina*, the tubules have a series of circular and longitudinal muscles and the epithelial cells have microvilli and a notable basement membrane (Begum et al., 2012). These characteristics were also recorded in *C. vicina*.

The duration of the development stages was not affected by RFII, similar results were reported in a previous work where the effect of RFII on *L. sericata* was evaluated (Zanetti & Centeno, 2024). On the contrary, the duration of dermestids exposed to RFII showed a reduction in larval development that translated into a reduction in total development (Zanetti & Centeno, 2024). A study performed on a Chrysopidae species found that development from third larval instar to pupae was shorter in glyphosate-treatment than in the control (Schneider et al., 2009). In contrast, other organophosphates that have been studied in various insects of relevance in forensic cases can delay their life cycle. Abd Al Galil et al. (2021a, b) reported that dimethoate presents a significant negative correlation with the larval and pupal development rates of *Chrysomya megacephala* Fabricius, *C. saffrana* Bigot, *C. rufifacies* Macquart, *Sarcophaga peregrine* Robineau-Desvoidy, *S. dux* Thomson, and *S. ruficornis* Fabricius. Other authors obtained similar results when working with malathion and *C. megacephala* larvae and pupae (Rashid et al., 2008; Liu et al., 2009). This organophosphate also affected the larval development rate in a species of dipteran of the Phoridae family (Castillo-Alanis et al., 2022). When studying the effect of glyphosate on a species of Chrysopidae, it was observed that the duration of the third larval stage to pupa was shorter in the presence of the herbicide than in the control (Schneider et al., 2009). There are different factors such as the animal species, the concentration of the xenobiotic, dose, stage of development, variability between individuals, pharmacokinetics, etc., which may be the cause of the differences mentioned above (Introna et al., 2001; Avigliano et al., 2014; Zanetti et al., 2021; Castillo-Alanis et al., 2022; Zanetti & Centeno, 2024).

There were also no changes in size due to the RFII in *C. vicina*. The same was found in *D. maculatus* (Zanetti & Centeno, 2024). Likewise, there were no external morphological changes in these species caused by RFII. In contrast, in eggs of *Crysoperla externa* Hagen, 1861 treated with glyphosate abnormalities were evident and adults also developed tumors (Schneider et al., 2009). An explanation similar to that given for cycle length can be applied to size.

When comparing with Schneider et al. (2009), in addition to the fact that the species evaluated are distinct, the preparation of the herbicide was different since authors made solutions considering the maximum nominal concentration recorded in the field, with which they sprayed the food source or diet. In our work, no solutions were prepared but the RFII was purely added to the meat and water

mixture. The concentration and doses used were also different from those of Schneider et al. (2009) since we calculated doses taking into account forensic cases (doses that could cause the death of a person, for example a suicide). Furthermore, some aims differed between studies, as well as the behavior of the evaluated insect, its habitat, etc.

Regarding the glyphosate detoxification process in *C. vicina*, it is likely to occur through the Malpighian Tubules. Some studies that analyzed larvae of Calliphoridae fed on corpses resulting from suicides with different drugs such as amitriptyline, temazepam, among others or a combination of them, indicated that the drugs are efficiently eliminated through the Malpighian Tubules of the dipteran larvae (Introna et al., 2001). Likewise, Farina et al. (2022) indicated that Malpighian Tubules can mitigate the toxic activity of insecticides. Another possible way of drug elimination is through the intestine (Gosselin et al., 2011). Maybe this also occurs with glyphosate.

In conclusion, this work provides novel anatomical data on these forensically relevant fly species and demonstrates that exposure to the RFII compound does not affect lifespan or alter morphological structures in these organisms. Understanding internal morphology is not only important for studying physiological and evolutionary aspects of the species, but also highlights the potential of these flies as model organisms for testing other chemical compounds and exploring toxicological responses in Diptera.

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COMPETING INTERESTS

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The authors have declared that no competing interests exist

AUTHORS CONTRIBUTIONS

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Noelia I. Zanetti: conceptualization, methodology, investigation, supervision, visualization, project administration, funding acquisition, writing-original draft preparation, review & editing. Aylín M. Varela Villa: investigation, review of the original draft. All authors approved final draft

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evaluation of Roundup Full II® effect on the development and
morphology of this forensic species

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Calliphoridae) y evaluación del efecto de Roundup Full II® sobre
el desarrollo y morfología de esta especie forense

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