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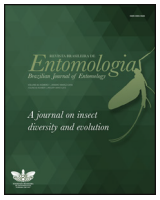

Effect of thiamethoxam (organophosphate) on the flies and beetle visitation and cadaveric decomposition process

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ABSTRACT

Entomofauna associated with decaying cadavers may be useful in criminal investigation, either through the development of immature insects of interest or through entomological succession in corpses. These factors may vary if the insects are exposed to toxic substances that can modify the insect's developmental cycle, as well as its own occurrence, which would imply significant changes in the results of any investigation. However, there are few studies on how contamination by toxic compounds can affect the action of insects on carcasses and their consequence for forensic expertise. Therefore, this study aimed to test the hypothesis that the normal visitation of flies and beetles is altered in insecticide-contaminated carcasses. The experiment was carried out in a sugarcane plantation, using pig carcasses contaminated with insecticide and the same number of carcasses without any type of contamination as a control. In all experiments, the contaminated carcasses reached the final phase of decomposition in a longer time than the uncontaminated carcasses of the control group. A total of 2.767 specimens were collected and identified, 2.103 individuals from the order Diptera and 664 from the order Coleoptera. There was a significant geometric regression adjustment during the decomposition phases only for the control group, different from the contaminated carcasses in which this pattern did not occur. Results show that contamination by insecticide alters decomposition time and phase, altering the action of flies and beetles, affecting the abundance, composition of species as well as their activities, which can alter the data used by experts in criminal experts.

Introduction

Flies are considered the most important group for forensic entomology because their activities are very pertinent to the decomposition process (Mann et al., 1990). Typically, flies are the first to locate and use decomposing matter for their development, either for the maturation of ovaries or for oviposition / larviposition site (Goff and Lord, 1994). Coleoptera is the second most frequent and abundant order in carcasses, particularly during the final phases of decomposition (Smith, 1986; Kulshrestha and Satpathy, 2001).

The entomological fauna associated with decomposing carcasses is diverse and insects seek essential sources for their development and reproduction in this resource (Santos et al., 2014). Usually, the insects that occur in carcasses are classified according to their eating habits, and may be necrophagous, predators, omnivores and accidental (Keh, 1985; Daly et al., 1998).

This direct relationship of insects with decomposing dead matter made them useful to assist in the resolution of several cases of legal

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interest (Magni et al., 2014). Among its uses is the Postmortem Interval Estimation (PMI), that is, the elapsed time from death to the discovery of the body, through data provided by insects, usually by two parameters: the rates of development of larvae or the patterns of succession of insects (Villet and Amendt, 2011). The age of immature specimens collected in cadavers is more useful and accurate to establish the minimum limit of PMI; and the analysis of the pattern of colonization of insects, together with the process of entomologic succession on a corpse, to establish the maximum limit of PMI (Catts and Goff, 1992; Bajerlein et al., 2018).

However, studies have shown that when these insects feed on resources contaminated by toxic substances, various aspects of their life cycles, including the duration time can be changed (Zou et al., 2013; Trivia and Carvalho-Pinto, 2018), thus hindering the conclusions of the expertise (Yan-Wei et al., 2010). Indeed, studies such as Carvalho (2004) and Goff et al. (1997) show that fly larvae used in forensic entomology present in substrate contaminated with licit and illicit drugs have their life cycle accelerated or delayed, depending on the substance, in addition to changes in the weight of immatures and thereby affect the cadaveric decomposition process.

Other types of toxic substances that can eventually contaminate insects at different phases of development are insecticides or other agricultural pesticides used to combat insects considered crop pests or even urban (Gunatilake and Goff, 1989; Wolff et al., 2004; Mahat et al., 2009; Yan-Wei et al., 2010; Zhou et al., 2011; Abd Al Galil et al., 2021a, 2021b; Jales et al., 2021).

Wolff et al. (2004) investigated the presence of the pesticide Methyl parathion in scavenger insects collected in rabbit carcasses. This organophosphate affects attraction, repelling insects from the oral region of the carcass. Yet, this is important in the process of decomposition and investigation of PMI because dipterans normally deposit eggs in the natural cavities of the body (Wolff et al., 2004; Oliveira-Costa, 2013).

Gunatilake and Goff (1989) found differences in oviposition rates and successional pattern in insect larvae that fed on carcasses contaminated with Malathion. According to Yan-Wei et al. (2010), the effect of Malathion on rabbit carcasses under natural conditions alters decomposition rates and the diversity of insect species in carcasses. It also changes in the rate of development enough to change PMI estimates based on the development of fly larvae.

Jales et al. (2021) evaluated immature dipterans associated with decomposing rat carcasses under the effect of different doses of an insecticide from the organophosphate group and observed that the development time and emergence rate of dipterans Calliphoridae and Sarcophagidae were altered, with an increase in mortality and delaying its cycle, in addition to altering the composition and structure of colonizing dipteran species. On the other hand, Abd Al Galil et al. (2021a, 2021b) investigated the effect of different concentrations of the insecticide Dimethoate on the development rate of Calliphoridae and Sarcophagidae, respectively, and observed for both families studied that the duration of the life cycle varied according to the concentration of the insecticide, the higher the concentrations of the insecticide, the longer the estimated time for the PMI.

These data show that toxic substances, such as insecticides and pesticides, can contaminate corpses and carcasses and ultimately affect insects that feed and reproduce in decaying tissue, thereby altering several investigative parameters used by forensic experts. Any type of contamination of a body can alter the normal pattern of colonization by insects of forensic interest.

Thiamethoxam, in particular, is part of a new generation of neonicotinoid insecticides used in many cultures, resulting in increased commercialization worldwide (Maienfisch et al., 2001; Fairbrother et al., 2014). This is a systemic insecticide that acts through contact or ingestion (Di Ilio et al., 2018), and it can be found commercially in different concentrations.

In Brazil, corpses are often found in sugarcane plantations to hide them from the authorities (Campo Grande News, 2018; G1 Piracicaba e Região, 2018; G1 Triângulo Mineiro, 2018), making the accidental contamination of the cadaver possible. Importantly, insecticides are often applied at night because of climatic conditions (Antuniassi and Boller, 2011), making it more difficult for workers to identify the presence of dead bodies among the plants.

Even though it is known that insects may have their action on carcasses altered if contaminated with insecticides, studies that have already investigated this topic are still scarce. Therefore, this study aimed to test the hypothesis that the normal action of flies and beetles is altered in insecticide-contaminated vertebrate carcasses.

Material and methods

Study site and animal model

Experiments were carried out to evaluate the contamination of carcasses in a sugarcane plantation. To accomplish this, a sugarcane plantation was chosen in the municipality of Dourados, Mato Grosso do Sul, Brazil (22º14'08"S; 54º59'13"W), altitude of 434 m. This plantation belonged to a private company that uses sugarcane for the production of sugar and alcohol. During the experiments, no chemical or biological control was performed in these places. We waited until sugarcane plants reached more than 1.50 m in height to simulate the concealment of a corpse. Six pig carcasses were used (*Sus scrofa*) weighing approximately 7.95 ± 0.19 kg each, due to its similarity to humans in relation to factors such as the amount of hair on the skin, food and organ organization (Payne, 1965), not considering the weight, due to the unavailability of animals with the necessary weight.

Three experiments were carried out in three different months, the first in December 2017, the second in February 2018 and the third in March 2018 (N= 3), all in the "hot and humid" season. The collections were carried out only at this season, because the activity of insects and other microorganisms is higher in warmer and more humid periods (Catts and Haskell, 1991). Seasonality was determined using the classification of Marcuzzo (2015), who suggested that the southern region of Mato Grosso do Sul has a humid subtropical climate with two well-defined seasons, the "hot and humid", which comprises the months of September to May, and "cold and dry", which lasts from June to August.

The pigs were euthanized with a blow to the occipital region of the head to avoid undue animal suffering and exposure of injury that causes external bleeding. Proposal #13/2017 was approved by the Comissão de Ética no Uso de Animais – CEUA- of the Universidade Federal da Grande Dourados, always respecting biosafety standards during execution.

Application of Thiamethoxam on the pig carcasses

To evaluate the effect of insecticide contamination on carcasses, two experimental groups were evaluated, one with three carcasses contaminated by insecticides and one with three uncontaminated carcasses, termed as contaminated group and control group. A commercial insecticide containing Thiamethoxam was applied (25%) to the contaminated group with the aid of a 20 L Manual Coastal Sprayer Poison Pump, immediately after exposure of the carcasses in the cane field. The insecticide was applied at a rate of 20 g to 1 L of water, or 1000 g/ha (0.1 g/m^2), resulting in a Thiamethoxam solution in the concentration of 5g/L, as recommended by the manufacturer.

The applications were carried out following the information on the insecticide label Thiametoxam, which indicates an aerial application of

250 L/ha or $25x10^{-5}$ L/cm^2 on sugarcane plants with a length above 1 m. To this, the proportional amount of the commercial product that would be applied in the area of 1961 cm^2 was calculated, wich is equivalent to the tray area where the carcasses were allocated (53x37 cm), calculated by the equation:

$$\mathbf{Ta} \times \mathbf{Tl} = \mathbf{Tt}$$

$$\left(1961\ cm^2\right) \times \left(25 \times 10^{-5}\ L / cm^2\right) = 0.0049025\ L / cm^2 or\ 4.90\ mL / cm^2$$

Where **Ta** is the tray area in cm^2, **Tl** is the amount of Thiamethoxam indicated by the commercial product label for sugarcane plantations in L/cm^2, and **Tt** is the approximate amount of Thiamethoxam applied to the tray area.

To apply an approximate amount to that calculated previously (4.90 mL), was used a costal pump nozzle with a flow rate of 0.30 L/min or $5x10^{-3}$ L/seg (0.005 L/ seg), applying the approximate amount of 5 mL Thiametoxam in the carcasses of each experiment.

The application was standardized in all experiments, making longitudinal movement in relation to a pig's body, always starting from the head to the final portion of the carcass, with the spray nozzle 50 cm away. This process was repeated twice. Commercial Thiamethoxam is from the neonicotinoid chemical group ($C_{23}H_{19}ClF_3NO_3$) and commonly used in cane fields (Di Ilio et al., 2018). As a control group, another three carcasses were exposed under the same conditions without the application of the insecticide.

The exposure points of the carcasses were defined in order to avoid edge effect, always installing them 30 m from the edge of the plantation. In each collection, two carcasses were exposed 50 m distant from each other. Each carcass was packed in a plastic tray (53x37x9 cm), following the methodology of Moretti (2006), the base of which was replaced by a wire mesh with reduced mesh between nodes, allowing water flow and accessibility to terrestrial and / or underground insects. An iron frame (1x1x1 m) covered with one-inch wire mesh was fixed to the ground on the plastic traying by means of four iron hooks, one on each side of the base of the cage, allowing access by arthropods, but preventing access to large carnivores.

The pigs were euthanized always at 1:00 pm in all experiments, and data collection began at 1:30 pm, on the first day of exposure, extending until 3:00 pm. From the second day, the samplings also took place at 1:30 pm in a period of 1 hour and 30 minutes (until 3:00 pm), simultaneously in contaminated and non-contaminated carcasses, by two teams, until the carcasses went through all decomposition phases, as described by Lord and Goff (2003), including (1) fresh, (2) bloated, (3) decay, (4) postdecay and (5) skeletal. The decomposition phases were determined based on the daily observations and based on the characteristic aspects cited by Lord and Goff (2003). Field experiments came to an end when the carcasses completed the five phases of decomposition and apparently were no longer attractive to insects, as described by Rosa et al. (2009).

Temperature and relative humidity of all collection days were obtained from the Instituto Nacional de Meteorologia (INMET), Measuring Station #86858 (Dourados, MS), located at latitude 22º 11'38.11" S and longitude 54º 54'40.88" W, about 9 Km from the collection locations. To assess the entire decomposition process, carcasses were photographed before each collection to document the activity of insects and the phases of decomposition, both in the contaminated group and in the control, as described by Lord and Goff (2003).

Influence of Thiamethoxam on the entomofauna of Diptera and Coleoptera

In order to capture entomofauna, manual collections were performed using tweezers and an aerial scan network. For the collection of soil insects, 8 pitfall traps were installed, using 200 mL plastic containers around the iron frame at a distance of 50 cm from carcasses, two at each end of the cage. The containers were filled with a solution of water and detergent. Preservatives and / or fixatives were not used because of the short period of one day between one collection and another (Sutherland, 1996; Almeida et al., 2003). The solution in the containers was changed daily.

Insects of the order Diptera and Coleoptera were sampling groups to establish entomofauna abundance, richness and composition parameters that visited the carcasses of the contaminated and control group. Dipterans were identified with the aid of identification keys of Carvalho and Mello-Patiu (2008) and coleopterans with the key of Almeida and Mise (2009) and Vaz-de-Mello et al. (2011). In addition, to confirm the identification of the species, specialists were consulted and comparisons were made with the standards of the Entomological Reference Collection of the Masters and Doctoral Program in Entomology and Biodiversity Conservation of the Universidade Federal da Grande Dourados (UFGD).

Influence of contamination on the oviposition and colonization behavior of flies

Behavioral observations were made using the "*ad libitum*" method described by Altmann (1974), in which the observer records all the behavioral acts that the animal performs during the session. With this data, it was possible to establish what type of habit the type of habit the flies exhibited on the carcass. It was also carried out an inspection and observed in the natural holes or on other parts of the carcasses. if there were eggs, larvae and pupae, including empty pupae near the carcasses or in the surrounding ground. As for the eggs, we witnessed the adult to lay the eggs directly and then collected the adults for identification. In addition, it was recorded where and when they laid eggs and whether the eggs hatched and the larvae developed. The number of individuals of each immature stage was not computed, only presence and absence and whether there was enough to form an egg mass or larvae aggregation. An approximate amount of 50 eggs was defined as egg mass and the approximate number of 30 larvae was defined as larval aggregation. As it was not possible to count the amount of larvae and eggs at the site, a visual estimate was made through the photos taken at the site, from the different areas of the carcass.

To determine the species and the larval instar, 10 larvae were collected in different areas of the carcass. In the laboratory, the instars were determined by the morphology of the posterior spiracles (Oliveira-Costa, 2013). Then, the larvae were stored in plastic containers of 350 mL covered with organza and containing fresh ground meat. Subsequently, they were transferred to containers with the same dimensions containing about 250 g of dry sawdust as substrate for pupation that were kept in an incubator chamber under controlled temperature of 25°C±1 and light-dark cycle of 12:12h (protocol of Paula et al., 2018) until the adult stage for subsequent identification with the aid of the identification key of Carvalho and Mello-Patiu (2008).

Statistical analysis

A Chi-square test was used in order to identify whether the treatments (control and contaminated) differed in terms of the duration, in days, of each decomposition phase and also in relation to the total time of the decomposition process. Therefore, each temporal repetition of the experiment was analyzed separately. These analyses were performed using the Statistica 14 (TIBCO Software Inc., 2020).

To assess whether contamination could affect the species richness pattern during the decomposition phases, five different regression models (best fit test) were fitted – linear, exponential, logarithmic, geometric and polynomial – for each of the six combinations between the two experimental treatments (control and contaminated) and the three replications. These analyses were performed using the Statistica 14 (TIBCO Software Inc., 2020).

A Two-Way Multivariate Analysis of Permutational Variance (PERMANOVA) was performed, using the coefficient of Jaccard, to verify whether the treatment and decomposition phase factors influenced the composition of species observed throughout the experiment. This analysis was performed using PAST 3.20 (Hammer et al., 2001).

Finally, in order to complement the previous analysis and be able to identify whether there are species more associated with each treatment and/or decomposition phase, the data on the presence and absence of species were ordered using a Nonmetric Multidimensional Scaling (nMDS), with Jaccard's coefficient. The most representative species of each treatment are closer to their points on the graphic. These analyses were carried out with vegan package from R platform (R Core Team, 2021).

Results

Influence of Thiamethoxam on carcass decomposition pattern

The decomposition phases in the contaminated and control groups can be seen in Fig. 1. The time of each decomposition phase in the contaminated and control carcasses can be seen in Fig. 2 and in the Supplementary material (Table S1 and S2). The three carcasses of the contaminated group reached the end of position after 21, 22 and 28 days respectively. While the three carcasses of the control group finished decomposition after 13, 13 and 12 days respectively. The time that the contaminated carcasses took to pass through the decay, postdecay and skeletal decomposition phases was longer than that of the control carcasses (Fig. 2, Table S1 and S2), although the Chi-square test did not show any difference. Significant difference between these values ($P > 0.05$), nor in the total decomposition time of the carcasses ($p > 0.05$). The abiotic data collected in each of the experiments in the contaminated and control carcass can be seen in the Supplementary material (Table S3 and S4).

Influence of Thiamethoxam on the entomofauna of Diptera and Coleoptera

A total of 2,767 specimens were collected and identified, 2,103 individuals from the order Diptera and 664 from the order Coleoptera (Tables 1 and 2). In the contaminated group, 1,741 insects were identified, 1,462 from the order Diptera and 279 from the order Coleoptera. In the control group, 1,026 insects, 641 Diptera and 385 Coleoptera, were collected.

Eighteen Diptera taxa were collected in both types of carcasses (Table 1) and seventeen Coleoptera taxa were collected in the two types of carcasses (Table 2). Performing tests with the different regression models, it was possible to identify a significant fit of geometric regression in the control group, which is a geometric increase in the number of species throughout the decomposition phases (Fig. 3). In the contaminated group, however, it was not possible to obtain significant

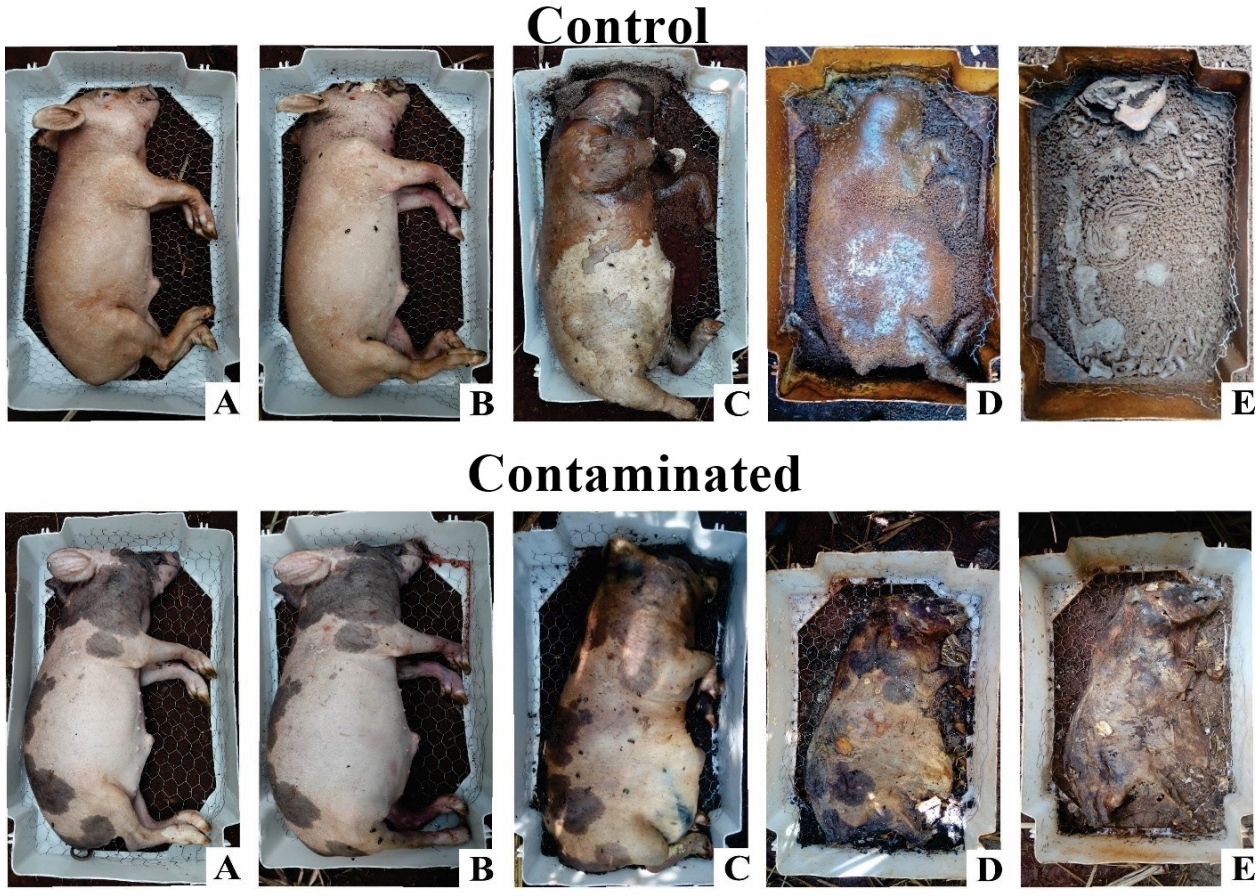


Figure 1 Decomposition phases in the carcasses of control group and the contaminated group with Thiamethoxam: **A** fresh, **B** bloated, **C** decay, **D** postdecay, **E** skeletal.

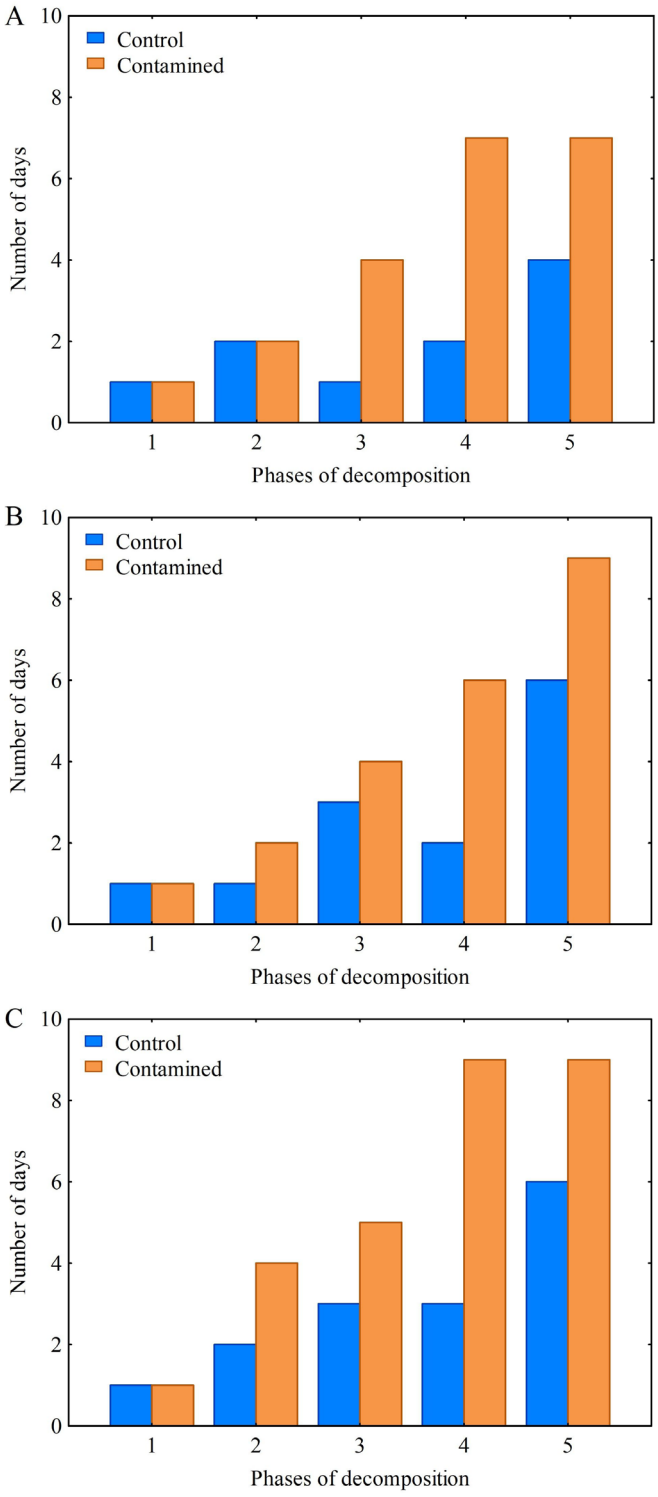


Figure 2 Number of days of each decomposition phase for the contaminated and control group in each of the three developed experiments.

adjustments in species richness during the decomposition phases of the three contaminated carcasses (Fig. 3).

Tables 3 and 4 show the succession of Diptera and Coleoptera that visited the different decomposition phases in control and contaminated group carcasses. In fact, the succession pattern of species that visited the two groups of carcasses was different., with a delay in the arrival of flies of forensic interest in the contaminated group, with individuals of

Table 1
Total abundance of adults of each Diptera taxon collected in the pig carcasses of the contaminated and control groups in a sugarcane plantation in the region of Dourados, MS, Brazil.

Taxon	Control	Contaminated	Total individuals
Chrysomya albiceps (Wiedemann, 1819)	153	521	674
Chrysomya megacephala (Fabricius, 1794)	2	5	7
Chrysomya putoria (Wiedemann, 1818)	1	0	1
Lucilia eximia (Wiedemann, 1819)	6	3	9
Lucilia cuprina (Wiedemann, 1830)	1	0	1
Hemilucilia semidiaphana (Rondani, 1850)	0	8	8
Cochliomyia sp.	2	1	3
Sarcophagidae Macquart, 1834	22	65	87
Musca domestica Linnaeus, 1758	226	389	615
Hermetia illucens (Linnaeus, 1758)	5	12	17
Stratiomyidae sp. Latreille, 1802	3	1	4
Fanniidae Schnabl & Dziedzicki, 1911	29	140	169
Ulidiidae Macquart, 1835	187	307	494
Tachinidae Robineau-Desvoidy, 1830	2	4	6
Ornidia obesa Fabricius, 1775	2	2	4
Syrphidae Latreille, 1802	0	3	3
Asilidae Latreille, 1802	0	1	1
Overall	**641**	**1462**	**2103**

Table 2
Total abundance of adults of each Coleoptera taxon collected in the pig carcasses of the contaminated and control groups in a sugarcane plantation in the region of Dourados, MS, Brazil.

Taxon	Control	Contaminated	Total individuals
Staphylinidae Latreille, 1802	148	70	218
Eulissus chalybaeus Mannerheim, 1830	13	13	26
Histeridae Gyllenhal, 1808	70	74	144
Dermestes maculatus (De Geer, 1774)	30	15	45
Necrobia rufipes De Geer, 1775	36	4	40
Carabidae Latreille, 1802	28	35	63
Tenebrionidae Latreille, 1802	34	18	52
Omorgus suberosus (Fabricius, 1775)	7	23	30
Omorgus persuberosus (Vaurie, 1962)	1	1	2
Coprophaneus horus (d'Olsoufieff, 1924)	0	5	5
Dichotomius bicuspis (Germar, 1824)	6	8	14
Dichotomius bos (Blanchard, 1845)	1	1	2
Dichotomius nisus (Olivier, 1789)	2	0	2
Sulcophaneus menelas (Castelnau, 1840)	6	10	16
Diabrotis mimas (Linnaeus, 1758)	0	1	1
Ontherus appendiculatus (Mannerheim, 1829)	3	0	3
Canthidium hyla Balthasar, 1939	0	1	1
Overall	**385**	**279**	**664**

this order being observed only from the second day of decomposition. In the control group, the flies appeared a few hours after the carcasses were exposed in the field. In addition, the PERMANOVA shows significant differences in the composition of dipteran and coleopteran species among samples from the contaminated and control groups (pseudo-F=0.9425, P≈ 0.0391) and among the five decomposition phases (pseudo-F=1.7858, P≈ 0.0001). Notwithstanding of this, an overlap of species occurrence in several phases.

Results show a consistent separation in species composition between the contaminated and control groups with species occurring at different phases of decomposition, depending on the group (Fig. 4). There was no specificity of occurrence of species in phase 1 of decomposition of

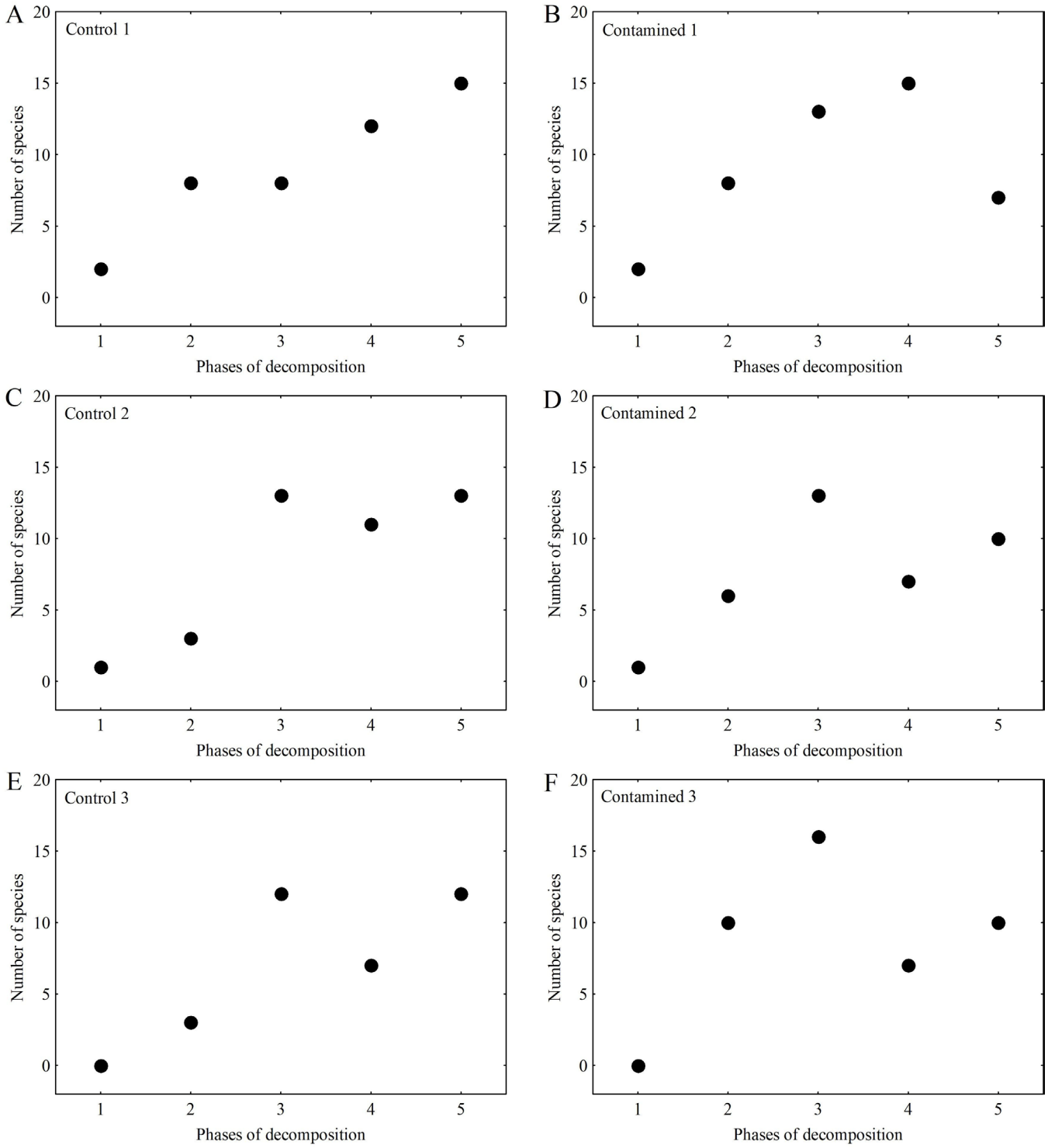


Figure 3 Scatter plot representing the variation in the number of species present in the carcasses at each phase of decomposition, for each treatment (control and contaminated) in each of the three replications of the experiment.

contaminated carcasses. The species/taxa *Chrysomya megacephala* (Fabricius, 1794), *Lucilia eximia* (Wiedemann, 1819), Syrphidae Latreille, 1802 and *Canthidium hyla* Balthasar, 1939 are more related to phase 2, the species/taxa Ulidiidae Macquart, 1835, Staphylinidae Latreille, 1802, Faniidae Schnabl & Dziedzicki, 1911, *Hemilucilia semidiaphana* (Rondani, 1850), *Cochliomya* sp. occurred more frequently in phase 3. and *Dichotomius bicuspis* (Germar, 1824), *Eulissus chalybaeus* Mannerheim, 1830, *Dermestes maculatus* (De Geer, 1774), Tachinidae Robineau-Desvoidy, 1830 occurred more frequently in phases 4 and 5.

In the control carcasses, the species *L. eximia* occurred more frequently in phase 1 of decomposition, while the species *Chrysomya putoria* (Wiedemann, 1818), *Lucilia cuprina* (Wiedemann, 1830), *D. maculatus* and *Chrysomya albiceps* (Wiedemann, 1819) occurred in phase 2. In phases 3, 4 and 5 of these carcasses there was a large overlap of species.

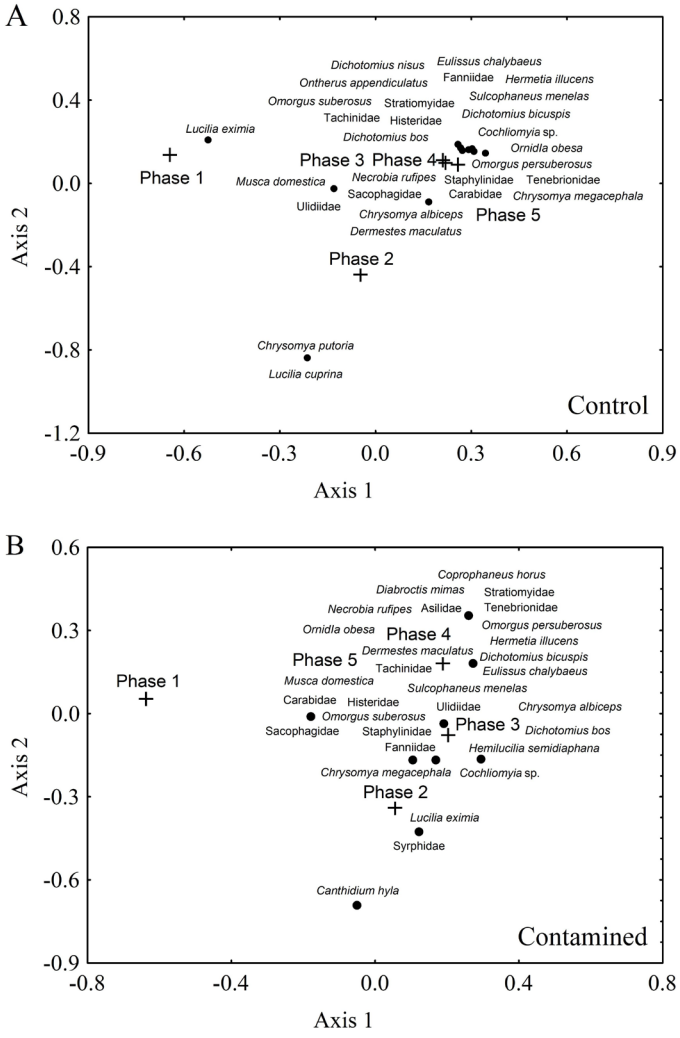


Figure 4 Nonmetric Multidimensional Scaling (nMDS) obtained from data on the presence and absence of species in each stage of decomposition, for the contaminated and control groups.

Influence of contamination on the oviposition and colonization behavior of flies

In the carcasses of the contaminated group, the fly species *C. megacephala* and *L. eximia* were the first to lay eggs, performing oviposition only on the third to fourth day (3.66±0.57) after exposure of the carcasses, however, there was no visual formation of egg mass. In the carcasses of the control group, the first species to lay eggs, one day after the exposure of the carcass, were *C. albiceps* and *C. putoria*, visually forming egg masses. Egg masses were considered to be the agglomerate of eggs with a gelatinous appearance, deposited by the flies.

In the carcasses of the contaminated group, oviposition occurred in the lower portion of the carcass, near the abdomen, with some eggs laid in the head, thorax and lower limbs. In the carcasses of the control group, the flies oviposited mainly in the natural orifices of the facial region, followed by the anogenital region.

It was observed in field that the larval mass behaved differently between the two experimental groups. In the contaminated group, larvae growth was sparse compared to the abundant growth of larvae found in the control group, since the few larvae that hatched from the eggs were able to develop to the second and third instar, while the amount of larvae of these stages of development was very scarce in the carcasses of the contaminated group. Dead adults were always observed near the carcasses of the contaminated group.

Discussion

This is the first study to evaluate the contamination by insecticide pulverization of vertebrate carcasses and investigate the effects on the activity of insects of standard decomposition interest, in Brazil. Only three other studies carried out in China, Malaysia and Colombia (Wolff et al., 2004; Mahat et al., 2009; Yan-Wei et al., 2010) evaluated the action of insects in vertebrate carcasses in field contaminated by insecticide poisoning. Furthermore, Gunatilake and Goff (1989) found traces of Malathion in the larvae of two species of fly found on a decaying human corpse.

According to the Chi-square test, there are no significant differences in the total time and phases of decomposition. In fact, despite the values found, this test has low sensitivity in cases like this. The characteristics of the data collected also contribute to the low sensitivity of the test, since the values of number of days of the decomposition phases are low. Despite this, it is possible to observe that the total decomposition time and between some phases is longer in the contaminated carcasses (Fig. 2), and it is possible to observe that the decomposition pattern was changed since the decay phase (Fig. 1C, Table 1 and 2).

The graphics in Fig. 2 are an indication that the application of insecticide increases the time of the decomposition phases, mainly from the decay phase. This likely happened because in the early phases of decomposition there is mainly the action of bacteria, which consume the decomposing body, mainly the intestines (Lord and Goff, 2003; Oliveira-Costa, 2013), not being affected by the insecticide applied to the epidermis of the carcasses. From the decay phase on, there is greater activity of insects, since there is skin disruption and fluid release on the soil (Lord and Goff, 2003; Oliveira-Costa, 2013). Thus, flies and beetles were affected when in contact with the external region of contaminated carcasses, altering the action of the scavenger fauna, preventing the development of the offspring of individuals and consequently reducing the activities of these groups from the intermediate phases to the final phases of decomposition, causing a cascade effect at all levels of the trophic chain dependent on this ephemeral resource.

Changes observed in decomposition time may have occurred because of changes in the activity of flies and beetles of forensic interest between the two groups of carcasses because of the action of the insecticide. The insecticide had altered the colonization of fauna associated with the contaminated group. Even though individuals from the flies of the most important forensic families were collected, Calliphoridae, Muscidae and Sarcophagidae, they did not manage to complete their developmental cycle in the carcasses of the contaminated group, failing to colonize. When they did manage to oviposit in the carcass, most eggs did not hatch, and even the larvae did not develop into adulthood. On the other hand, in the control group, the same families of flies managed to start the colonization process the carcasses normally and performed oviposition / larviposition.

Another substance such as carbamate, a poison used as rodent control, detected in a cadaver in the state of Rio de Janeiro caused the death of the entomofauna that tried to access the contaminated corpse, also affecting the composition of species and consequently the total decomposition time (Oliveira-Costa and Lopes, 1999). As observed in our study, this high mortality rate generates a low abundance of organisms exploring the resource and consequently affects decomposition. Indeed, other studies have evaluated that contamination by insecticides can change the order of arrival and composition of cadaveric fauna in carcasses (Gunatilake and Goff, 1989; Wolff et al., 2004).

Table 3
Occurrence of Diptera and Coleoptera (Insecta, Arthropoda) by decomposition stage collected in the pig carcasses of the contaminated group in a sugarcane plantation in the region of Dourados, MS, Brazil.

Phases of decomposition	Control											
	Fresh	Bloated		Decay		Postdecay		Skeletal				
Accumulated days	*1st*	*2sd*	*3th*	*4th*	*5th*	*6th*	*7th*	*8th*	*9th*	*10th*	*11th*	*12th*
Chrysomya albiceps												
Chrysomya megacephala												
Chrysomya putoria												
Lucilia eximia												
Lucilia cuprina												
Hemilucilia semidiaphana												
Cochliomyia sp.												
Sarcophagidae												
Musca domestica												
Hermetia illucens												
Stratiomyidae sp.												
Fanniidae												
Ulidiidae												
Tachinidae												
Ornidia obesa												
Syrphidae												
Asiilidae												
Staphylinidae												
Eulissus chalybaeus												
Histeridae												
Dermestes maculatus												
Necrobia rufipes												
Carabidae												
Tenebrionidae												
Omorgus suberosus												
Omorgus persuberosus												
Coprophaneus horus												
Dichotomius bicuspis												
Dichotomius bos												
Dichotomius nisus												
Sulcophaneus menelas												
Diabrotibus mimas												
Ontherus appendiculatus												
Canthidium hyla												

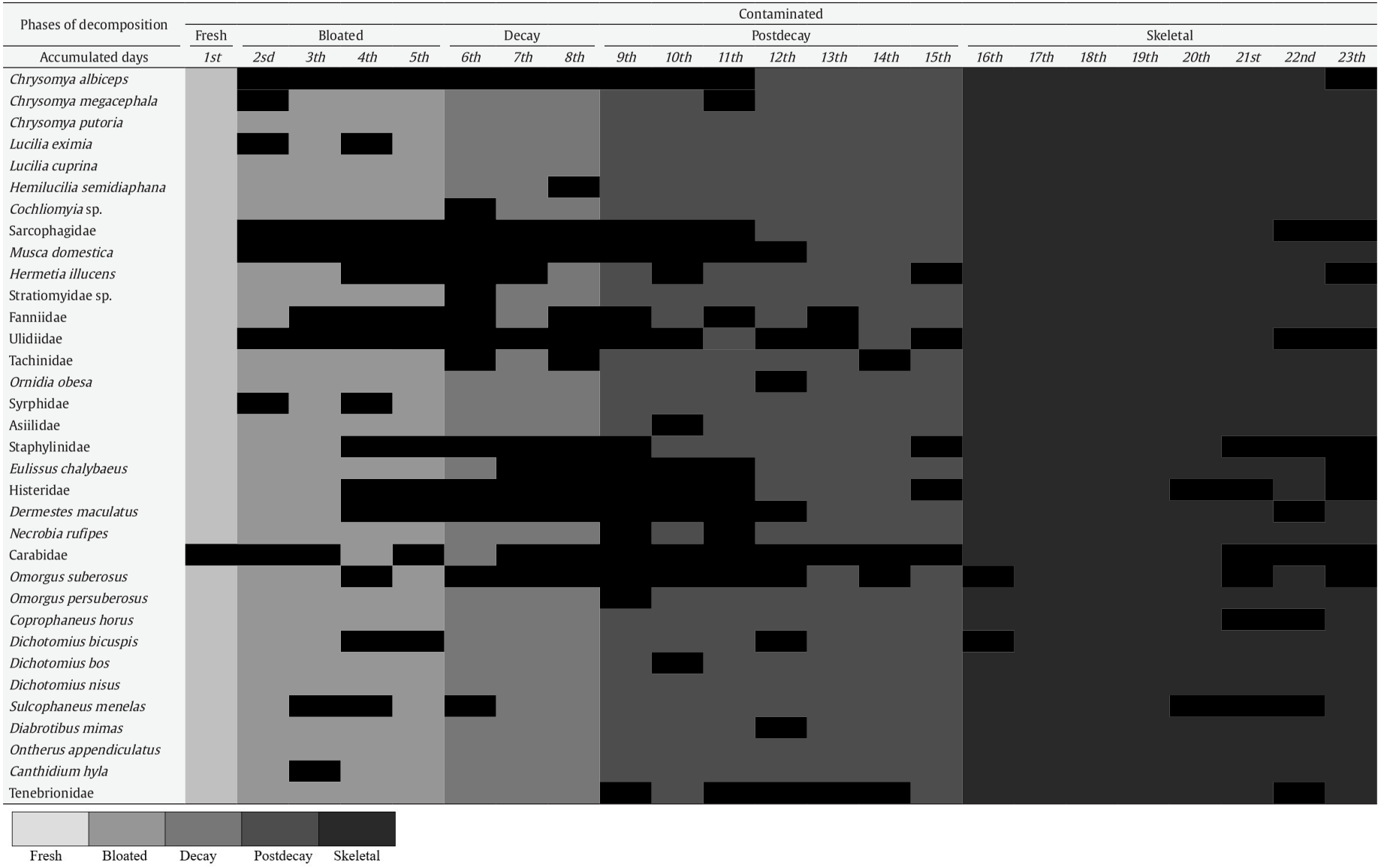
Fresh Bloated Decay Postdecay Skeletal

In addition to the impact on the composition of cadaveric fauna after contact with poisoned carcasses, insecticides can also change the rate of development of individuals that can access contaminated cadavers, for example, in the study by Yan-Wei et al. (2010) the insecticide malathion delayed the development of flies in carcasses within 24 days, delaying the development of larvae and pupae, thus interfering in the decomposition process and delaying the emergence of adults, facts that can generate errors in the calculation of the accumulated degree-day/degree-hour and in the estimation of PMI. Abd Algalil et al. (2021a) and Abd Algalil et al. (2021b) also investigated the effect of the insecticide dimethoate simulating poisoning in fly species of the families of forensic importance Calliphoridae and Sarcophagidae respectively, and both found that the insecticide affected the rate of development of these insects, thus altering the estimate of PMI. Here for the first time, simulating a case of accidental spraying on a hidden corpse in a monoculture environment, we realize the impact this could cause in real cases, as entomological data have been increasingly disseminated and applied to assist in solving real cases.

Tooming et al. (2017) studied the agriculturally important beetle *Platynus assimilis* (Paykull, 1790) subjected to different concentrations of Thiamethoxam and found both hyper- and hypoactivity. These are recurrent characteristics of toxic stress in insects, indicating a reduction in their performance and a reduction in feeding rate, which can lead to changes in fertility and even longevity of infected individuals. Theoretically, this fact could have affected the activity of insects on the carcasses in our study and, consequently, delayed the decomposition process.

The richness of collected species that explored the carcasses vary between the contaminated and control groups (Fig. 3). The insect visit pattern in the three carcasses of the control group showed a significant fit for geometric regression, which indicates an increase in richness during the decomposition phases. During each decomposition phase, changes in species richness can be explained by the carcass supporting only a limited number of species, with few interactions between phases (Paula et al., 2016). On the other hand, none of the three experiments carried out with the contaminated carcasses showed adjustments for the different regression models, which shows that there is no pattern that can

Table 4
Occurrence of Diptera and Coleoptera (Insecta, Arthropoda) by decomposition stage collected in the pig carcasses of the contaminated group in a sugarcane plantation in the region of Dourados, MS, Brazil.



be described by the models used for the contaminated carcasses. These results are an indication of the effects of the insecticide on the visiting species of carcasses, changing the species richness and preventing the presence of species throughout the decomposition phases.

In the carcasses of the contaminated group, we found a greater number of flies (Table 1). In contrast, carcasses of the control group had a greater abundance of coleopterans (Table 2). This difference can be explained by the delay in the phases of decomposition caused by insecticide spraying in contaminated groups (Fig. 2, Table S2), the gases released during decomposition that may have played an important role in attracting entomofauna, since the associated fauna has preference for specific physicochemical characteristics in the carcass (Oliveira-Costa, 2013). Probably, gas release may have occurred over a longer period from the intermediate decomposition phases (bloated and decay), which are more attractive to flies, therefore, even though the contaminated carcass was not colonized by flies, the released odors continued to attract individuals of this order. In addition, most beetles are not attracted by the odors of these phases, but by the odors of the final decomposition process. Moreover, in contaminated carcasses, less food resource is available for some taxa, such as predatory beetles since there were almost no Diptera larvae in the carcass tissues. This fact demonstrates the impact of contamination of an insecticide on insect taxa that commonly colonize carcasses.

Odor is fundamental for attracting insects to carcasses. For instance, flies are more attracted by the compounds released during the initial processes of decomposition, and beetles are, as suggested above, more attracted by odors resulting from the final processes (Verheggen et al., 2017). Furthermore, the composition of fauna in corpses is fundamental to the release of attractive odors. In particular, species of flies and beetles release pheromones that attract individuals to reproduce and feed on these carcasses (von Hoermann et al., 2012; Fockink et al., 2015).

The nMDS shows a difference in the composition of species that occurred in the two types of carcasses (Fig. 4) with some species more frequent, or exclusive, in one group relative to the other. Some species and taxa of flies and beetles occurred exclusively in the carcasses of the contaminated group (Table 1 and 2). Therefore, it is likely that these species may be less sensitive to the insecticide, visiting the carcasses, despite contamination, possibly not even detecting the contaminant. In fact, Reginaldo et al. (2021) performed double-choice laboratory tests in which forensically important flies could choose Thiametoxam-contaminated and non-contaminated meat, and the results showed that these individuals did not detect insecticide concentrations in the contaminated substrate and did not avoid visiting this resource. In addition, competition between different species per dwindling resource in contaminated carcasses can also justify the exclusivity of these species in contaminated carcasses. On the other hand, the abundance of these species in contaminated carcasses was low, being found only once during the three replicates, which may indicate that this occurred at random. Further studies and analyses with a larger scale should be done to corroborate the occurrence of exclusive species and their possibly resistance to this type of insecticide.

In our study, significant differences in the composition of the fauna between the contaminated and control groups were seen in all phases of decomposition, however, in both groups there was an overlap in the occurrence of species with throughout several phases. For Schoenly and Reid (1987), the pattern of succession cannot be sustained based on the decomposition phases, since the insect fauna can change within the same decomposition phase, without specific groups that characterize steps through which cadavers and carcasses pass during the decomposition process.

However, it is known that for an estimate of the maximum PMI to be applied, there must be a good pattern of occurrence of the species. This pattern should be regional, or even local, since the composition of the species will be distinct in different environments, such as urban area (Carvalho et al., 2004), forest (Carvalho and Linhares, 2001), pasture (Faria et al., 2013), and even monoculture (Gomes et al., 2009).

We observed in our study some patterns in the succession of flies and beetles as shown in Tables 3 and 4. The succession in the control group was similar to studies in which collections were performed in rural environments, such as pasture (Horenstein et al., 2012; Ramos-Pastrana et al., 2018) and in plantation areas (Gomes et al., 2009). We found *L. eximia* and *Musca domestica* Linnaeus, 1758 as the first visitors in the carcasses of the control group. These species were also recorded as the first to occur in carcasses in pasture areas (Horenstein et al., 2012; Ramos-Pastrana et al., 2018), which are environments of low vegetation complexity, as well as plantation areas. Moreover, in our study most Coleoptera taxa occurred from the decay phase, with some taxa occurring in the early phases (swelling), which may be related to the emergence of fly larvae in carcasses, which contributes to the increase of predatory beetles such as Histeridae e Staphylinidae.

On the other hand, in the contaminated group the pattern of species composition differed considerably when compared to control. No fly species were collected during the first day (Table 4), different from the control group in which three species were collected in this period, probably due to the volatiles that are released during the first hours after thiamethoxam spraying, which may have influenced the attractiveness of the carcasses, affecting the order of arrival of the species. Indeed, the colonization of corpses by insects can be delayed for several days, by pesticides such as parathion (Wolff et al., 2004) and Malathion (Gunatilake and Goff, 1989; Mahat et al., 2009) or even by the application of some repellent (Charabidze et al., 2009).

It was also possible to observe that the pattern of succession of Coleopterans occurred in a totally different way, when compared to the one between the contaminated group and control group (Table 3 and 4). In general, all beetle taxa that occurred in the contaminated group had a lower frequency without defined pattern (Table 4).

Some scavenger species such as *D. maculatus* and *Necrobia rufipes* (De Geer, 1775) were less abundant in contaminated carcasses. These species are extremely important in the decomposition process, as they consume the dry remains of tissues remaining in the final phases of decomposition, cleaning the bones (Oliveira-Costa, 2013). As there was a reduction in the number of these individuals in the carcasses of the contaminated group compared to the control group, this may explain the delay in decomposition. Carcass contamination seems to have affected the oviposition behavior of flies, especially those of forensic interest. There was a delay of on average 1.66±0.57 to begin to detect visually eggs in these carcasses. In addition, the larvae that hatched from the eggs do not seem to have completed their cycle, since no empty puparia were observed under, near or in the surrounding ground of these carcasses.

Indeed, Gunatilake and Goff (1989) evaluated that insecticides can alter the rate of development in fly larvae. Mahat et al. (2009) also assessed that contamination of carcasses by insecticide can delay the oviposition of flies of forensic interest between one and two days. These changes on the development of insects may be associated with changes in their nervous system. As observed by Abd Algalil et al. (2021a, 2021b) who investigated the effect of the insecticide dimethoate on fly species of the families of forensic importance Calliphoridae and Sarcophagidae respectively, and both found that poisoning by the insecticide had impacts on the rate of development of these organisms, thus altering the determination of PMI. Insecticides such as Thiametoxan can have an inhibitory effect of the enzyme acetylcholinesterase that acts as a neurotransmitter (Nauen et al., 2003) and this toxic stress can cause a reduction in insect performance, affecting all of their systems, which

may result in the death of the insect. The fact that this insecticide has changed the rate of development of the species that were able to perform oviposition is extremely relevant, since if it were to use binomial data, that is, data from the post-embryonic development of flies to reach a minimum PMI, the estimate would be wrong, since these insects would possibly show a later PMI. This fact should be taken into consideration by the expert authorities, since the insecticide will affect all development of the insect and as a result, it can lead to mistakes in the estimation of the minimum PMI.

The feeding pattern of insects can also be affected. In carcasses of the contaminated group, more insects were found visiting and feeding on the surface of the carcass in contact with the soil and thus not directly exposed to insecticide. This means that most insect activity was confined to the unsprayed area. In the final phases of decomposition, Figs. 1C, 1D, and 1E show that the tissues of the upper region were not affected in the carcass of the contaminated group compared the control carcass. To explain, flies seek protected places and that allow the development of their offspring to carry out their oviposition (Smith, 1986), using olfactory clues to search for regions of the carcass that serve for the development of the next generation (Erzinçlioglu, 1996; Gomes et al., 2007).

We found that the activity of Diptera and Coleoptera species was significantly affected by Thiamethoxam contamination, changing the abundance and composition of these insects, the rate of development of the different taxa that occurred in the two treatments, as well as their viability after contamination, causing a significantly different occurrence pattern. Such contamination caused the decomposition time of carcasses to change relative to control, taking longer in comparison.

Here, for the first time, the effects of contamination of pig carcasses sprayed with Thiamethoxam in a sugarcane plantation were investigated. Thiametoxam contamination altered: 1) decomposition patterns across phases; 2) The total decomposition time was longer in the contaminated group in relation to the control group, in the three experiments performed. 3) the activity and composition of fly and beetle species of forensic interest. These results support our hypothesis that Thiametoxam may induce errors in PMI estimates, since exposed carcasses took twice as long to decompose, corroborating other studies in this area. This investigation contributes with key information and can establish useful links in cases of concealment and contamination of corpses exposed in this type of environment.

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Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

ADMME: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. MCPS: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Writing – review & editing. KBM: Investigation, Validation, Writing – review & editing. FCO: Investigation. ACSB: Investigation. SELJ: Formal analysis, Methodology, Writing – review & editing. CALC: Methodology, Writing – review & editing. WFAJ: Conceptualization, Methodology, Project administration, Supervision, Validation, Writing – review & editing.

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Supplementary material

The following online material is available for this article:

Table S1 - Total decomposition time and each decomposition phase according to (Lord and Goff, 2003) of pig carcasses for each of the experiments carried out for the control group.
Table S2 - Total decomposition time and each decomposition phase according to (Lord and Goff, 2003) of pig carcasses for each of the experiments carried out for the contaminated group.
Table S3 - Temperature and humidity data (average, minimum and maximum) and precipitation for each of the experiments carried out for the control group.
Table S4 - Temperature and humidity data (average, minimum and maximum) and precipitation for each of the experiments carried out for the contaminated group.