

Formicidae fauna in pig carcasses contaminated by insecticide: implications for forensic entomology

Giovanna Silva Viana¹ , Michele Castro de Paula^{1,2,*} ,
Aylson Dailson Medeiros de Moura Eulalio^{1,3} , Poliana Galvão dos Santos¹ ,
Sidnei Eduardo Lima-Junior⁴ , William Fernando Antonialli-Junior^{1,4}

¹Universidade Estadual de Mato Grosso do Sul, Laboratório de Ecologia Comportamental, Dourados, MS, Brasil.

²Universidade Estadual de Mato Grosso do Sul, Naviraí, MS, Brasil.

³Universidade Federal da Grande Dourados, Programa de Pós-graduação em Entomologia e Conservação da Biodiversidade, Dourados, MS, Brasil.

⁴Universidade Estadual de Mato Grosso do Sul, Centro de Estudos em Recursos Naturais, Programa de Pós-graduação em Recursos Naturais, Dourados, MS, Brasil.

ARTICLE INFO

Article history:

Received 07 August 2021

Accepted 14 March 2022

Available online 04 April 2022

Associate Editor: Rodrigo Kruger

Keywords:

Entomotoxicology

Forensic science

Thiametoxam

Monoculture

Ants

ABSTRACT

Corpses in Brazil are commonly hidden in sugarcane plantations in the attempt to delay their finding and hinder the solution of the crime. On the other hand, these plantations are regularly sprayed with insecticides for pest control. Until now no study has reported the effects of insecticides on ant fauna. This study aimed to test the hypothesis that if a body hidden in a monoculture is accidentally contaminated by an insecticide, both the carcass decomposition pattern and the Formicidae fauna will be affected. To accomplish this, pig carcasses contaminated and non-contaminated were placed in a sugarcane monoculture environment and subsequently examined for data collection every 24 hours. The concentration used to contaminate the carcasses was 20 grams per liter of thiamethoxam. The decomposition patterns of contaminated carcasses were changed, in turn affecting the behavior of Formicidae fauna. A total of 5318 ants were collected, 3397 in contaminated carcasses and 1919 in non-contaminated carcasses, and 30 species of 11 genera were identified. According to the analysis, there are no differences between the composition of species between contaminated and non-contaminated carcasses, however, a significant difference was observed in the composition of species along the stages of decomposition between the two types of carcasses. Therefore, our hypothesis has been confirmed, contaminated carcasses undergo changes in their normal pattern of decomposition and the fauna of ants that act on them. As this group of insects has great importance for forensic sciences, the analysis of the experts should take these results into account.

Introduction

Diptera is the most abundant order of insects found in a decomposing carcass and, hence, the most frequently used for minimum postmortem interval (PMI_{min}) estimation because these insects are the first to locate and lay eggs on the carcass (Smith, 1986). The second most abundant order in carcasses is Coleoptera; this group is present in all phases of decomposition, especially in the final phases (Mise et al., 2007). The third most numerous order of insects in carcasses is Hymenoptera, especially ants (Oliveira-Costa, 2011), generally occurring during all decomposition phases (Paula et al., 2016). For Early and Goff (1986), vertebrate carcasses are an additional source of food for insects, and ants are particularly abundant in this type of resource, acting as predators

of the eggs, larvae, and pupae of Diptera and Coleoptera, and/or acting as necrophagous feeders on decomposing tissues and exudates.

Ants directly influence the decomposition process, and studies, such as Singh et al. (2020), show that ants can use carcasses as a resource and also disrupt the activity of other insects of forensic importance, such as flies and beetles. It can act in different ways on carcasses. Necrophagous ants, such as the species *Camponotus sericeiventris* (Guérin-Méneville, 1838) rip out pieces of the corpse and carry them to their nest, thereby accelerating the decomposition process and causing injuries to the body in a way that ends up being a doorway for Diptera larvae to get access to wet areas, facilitating their feeding. Predator ant species such as *Camponotus* (*Tanaemyrmex*) sp. can delay the decomposition process because they carry a great number of eggs of flies to their nest for later feeding, thus interfering with decomposition (Paula et al., 2016).

*Corresponding author.

E-mail: michelecastro.ufgd@gmail.com (M.C. Paula).

Some species, such as ants of the genus *Pheidole* exhibit omnivorous behavior preying on part of the cadaverous fauna and additionally removing small pieces of superficial skin (Paula et al., 2016). In all cases, ants are keystone species for forensic experts because they can accelerate or delay decomposition and cause injuries that can resemble artifacts caused before death and thus lead to erroneous inferences (Patel, 1994). Park and Moon (2020a) observed the presence of some species of ants, especially *Tetramorium tsushimae* Emery, 1925, making scars on the skin in a way that interferes with the succession process of larvae. The ants occur in the carcass soon after death and during all phases of decomposition, even after fly larvae leave the body, often being the dominant group in abundance on the corpse (Campobasso et al., 2009) since they have a large number of individuals per colony (Hölldobler and Wilson, 1990).

Forensic entomology also studies the application of necrophagous insects in toxicological analysis to identify drugs and toxins present in tissue (Introna et al., 2001), or their effects on the rate of insect development (Yan-Wei et al., 2010; Al-Galil et al., 2021a, 2021b), which would have direct implication in this forensic analysis, termed Entomotoxicology. The increase of death related to the use of drugs, mainly heroin and cocaine, or deaths related to accidental or purposeful consumption of poisons or other toxic substances, justifies the great interest in this new area (Oliveira-Costa, 2011). In addition to illicit drugs, entomotoxicologists also consider the effects of contamination on corpses and carcasses by other substances, such as insecticides, since there are documented cases of poisoning by ingesting this type of substance (Wolff et al., 2004; Yan-Wei et al., 2010; Zhou et al., 2011). Wolff et al. (2004) investigated and qualitatively detected the presence of the insecticide parathion in necrophagous insects collected in rabbit carcasses, it was determined that this insecticide attracted insects to the carcass.

In addition, Yan-Wei et al. (2010) studied the effect of malathion on rabbit carcasses under natural conditions and discovered that the insecticide alters decomposition rates and the diversity of insect species in the carcass. They also found differences in the development rates, enough to alter PMI estimates, based on the development of fly larvae. These data show the importance of studying toxic substances, such as insecticides, because it is necessary to consider their effects on decomposing corpses when estimating PMI based on entomological evidence (Yan-Wei et al., 2010).

In Brazil, the number of corpses concealed in monoculture, such as sugarcane plantations, has been increasing to evade detection by authorities (Campo Grande News, 2018; G1 Piracicaba e Região, 2018; G1 Triângulo Mineiro, 2018). Since such monocultures are routinely sprayed with insecticides, it is important to determine their effects on insect activity in the context of forensic examination. Therefore, this study first assumed a model in which a body is hidden in a sugarcane field and then accidentally contaminated by insecticide application. Under these conditions, this study hypothesized that both the pattern of decomposition of the carcass and the ant fauna would be significantly affected, changing its patterns of activity on corpses, which would, in turn, skew forensic results.

Material and Methods

Experiments were carried out to simulate the contamination of a corpse in a sugarcane plantation, using pig carcasses (*Sus scrofa* Linnaeus, 1758) as a model. To accomplish this, experiments were performed in a sugarcane plantation in the municipality of Dourados, Mato Grosso do Sul, Brazil (22°14'08"S; 54°59'13"W), altitude of 434 m. These experiments began as soon as sugarcane plants reached more than 1.50 m in height to simulate the concealment of a corpse.

Six pig carcasses were used (*S. scrofa*) weighing approximately 7.95 ± 0.19 kg each, as a model for comparison with human corpses (Payne, 1965). Three experiments were carried out (N= 3), all in the "hot and humid" season, from December to May 2018/2019. According to Marcuzzo (2014) the southern region of Mato Grosso do Sul has a humid subtropical climate with two well-defined seasons, the "hot and humid" period, which comprises the months from September to May, and "cold and dry", which lasts from June to August. Higher insect activity was expected since warmer and more humid periods stimulate their activity and consequently intensify the action of decomposition (Catts and Haskell, 1990).

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; as it can increase attractiveness and alter the colonization of insects (Oliveira-Costa, 2011). 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 sacrifice.

The locations where carcasses were placed were defined 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. The carcasses were deposited in places where there were no ant nests to avoid overestimating the occurrence of some species, potentially skewing the results of this study. Each carcass was placed in a plastic tray (53x37x9 cm), following the methodology of Moretti (2006), the base of which was replaced by a mesh, allowing water flow and accessibility to terrestrial and/or underground insects. The plastic trays allowed the carcasses to be lifted more easily, for the collection of individuals who were under them in the soil. An iron frame (1x1x1 m) covered with one-inch wire mesh was fixed to the ground on the plastic packaging using four iron hooks, one on each side of the base of the cage, allowing access by arthropods, but preventing access to large carnivores.

Pigs were euthanized at 1:00 pm in all experiments, and data collection started at 1:30 pm, right after the first insects appeared, extending until 3:00 pm. The collections continued in the following days at the same time until the carcasses went through all decomposition phases, as described by Goff (2000), including (1) fresh, (2) swelling, (3) deterioration, (4) post-deterioration, and (5) skeletonization of the body.

Insecticide was applied with the aid of a Manual Coastal Sprayer Poison Pump with a flat fan nozzle that produces average drops from 201 to 400 microns immediately after exposure of the carcasses in the cane field. The application was standardized in all experiments, making longitudinal movement 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. The commercial product used was from the neonicotinoid chemical group. Its main reagent is thiamethoxam ($C_{23}H_{19}ClF_3NO_3$), a compound that is commonly used in sugarcane fields, containing a 25% concentration of this active ingredient (Di Ilio et al., 2018). The proportion used was 20 g of thiamethoxam (25%) to 1 L of water, and 1000 g/ha (0,1 g/m²) was applied, as recommended by the manufacturer. As a control group, another carcass was exposed under the same conditions without the application of the insecticide.

To capture the ant fauna that acted on the carcasses, manual collections were performed using tweezers and Eppendorf tubes with ethanol 70%. Eight pitfall traps were also 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 (Almeida et al., 2003; Sutherland, 2006) and were changed daily. Before collecting each ant, it was recorded how it acted on the carcass, along with other visitors.

The Formicidae fauna was identified at species level when possible with the aid of identification keys of Bolton (2003). Also, 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).

Air temperature and relative humidity for all collection days were obtained from the Instituto Nacional de Meteorologia (INMET), Measuring Station #86858 (Dourados, MS), located at latitude 22° 11'38.11" S and longitude 54° 54'40.88" W, about 9 Km from the collection locations.

Statistical analysis

To assess the significant difference between the decomposition time of the contaminated group and the control, a Student's *t*-test was performed. To assess whether climatic factors varied significantly over the period in which the experiment was carried out, we applied temperature and relative humidity measurements using an Analysis of Variance (ANOVA). To assess whether contamination could affect the species richness of ants during the decomposition phases, a Wilcoxon matched pairs test was performed, considering the mean richness at each phase as a separated sample. To compare the species richness along the stages of decomposition, a Kruskal-Wallis test was used for each treatment (non-contaminated and contaminated). All analyses were performed using the Statistica 13.3 software (TIBCO Software Inc., 2017).

Detrended Correspondence Analysis was also performed (DCA) and applied to the species abundance data so that the graph obtained from this analysis would show the distribution of the species according to their abundance in the contaminated and control groups, in which the most representative species of each treatment are closer to their

points on the graph. The intermediate numbers represent the species composition that occurred in the two types of carcasses. These analyses were carried out with Past 3.20 software (Hammer et al., 2001).

Finally, to complement this analysis and visualize the species ordering according to the treatment and the decomposition phases, a non-metric multidimensional scaling (nMDS) was performed, using the Bray-Curtis coefficient (R Core Team, 2017).

Results

The total time of decomposition of the carcasses showed significant differences between contaminated and non-contaminated carcasses ($p=0.024$), with an average decomposition time of 23.33 (± 4.15) days for contaminated carcasses and 12.67 (± 2.5) days for non-contaminated carcasses. However, when the decomposition time of each phase was compared between the two treatments, only the deterioration phase ($p=0.037$) and post-deterioration phase ($p=0.018$) showed significant differences with an average duration in the deterioration and post-deterioration of 4.33 (± 0.58) days and 2.33 (± 0.57) days for the non-contaminated group, respectively.

The ANOVA results show that temperature averages ($p=0.366$) and humidity ($p=0.591$) did not vary significantly over the entire experimental period.

During the experiments, 5318 ants were collected, 3397 in the contaminated carcasses and 1919 in the non-contaminated carcasses (Table 1). Thirty-two species of the genus *Acromyrmex*, *Anochetus*, *Atta*,

Table 1 Absolute number of occurrences of different species of ants in contaminated and noncontaminated carcasses.

Genus	Species	Representation in the Fig. 2 and Fig. 3	Non-contaminated carcass	Contaminated carcass
Acromyrmex	<i>Acromyrmex</i> sp.	1	0	1
Anochetus	<i>Anochetus mayri</i> Emery, 1884	2	0	1
	<i>Anochetus</i> sp.	3	1	0
Atta	<i>Atta sexdens</i> (Linnaeus, 1758)	4	606	35
Brachymyrmex	<i>Brachymyrmex</i> sp. 1	5	100	13
	<i>Brachymyrmex</i> sp. 2	6	0	1
Camponotus	<i>Camponotus fastigatus</i> Roger, 1863	7	13	22
	<i>Camponotus renggeri</i> Emery, 1894	8	34	8
	<i>Camponotus crassus</i> Mayr, 1862	9	1	0
	<i>Camponotus melanoticus</i> Emery, 1894	10	4	0
	<i>Camponotus punctulatus</i> Mayr, 1868	11	0	1
	<i>Camponotus rufipes</i> (Fabricius, 1775)	12	3	0
	<i>Camponotus</i> sp. 1	13	7	5
	<i>Camponotus</i> sp. 2	14	1	2
	<i>Camponotus</i> sp. 3	15	1	3
	<i>Camponotus termitarius</i> Emery, 1902	16	5	1
Dorymyrmex	<i>Dorymyrmex amazonicus</i> Cuzzo and Guerrero, 2012	17	177	1
	<i>Dorymyrmex brunneus</i> Forel, 1908	18	563	2781
Odontomachus	<i>Odontomachus brunneus</i> (Patton, 1894)	19	0	2
	<i>Odontomachus meinerti</i> Forel, 1905	20	3	3
Pachycondyla	<i>Pachycondyla</i> sp.	21	0	2
Pheidole	<i>Pheidole</i> sp. 1	22	377	433
	<i>Pheidole</i> sp. 2	23	1	19
	<i>Pheidole</i> sp. 3	24	10	44
	<i>Pheidole</i> sp. 4	25	0	2
	<i>Pheidole</i> sp. 5	26	2	5
	<i>Pheidole</i> sp. 6	27	10	4
	<i>Pheidole</i> sp. 7	28	1	3
Pseudomyrmex	<i>Pseudomyrmex gracilis</i> (Fabricius, 1804)	29	1	0
Solenopsis	<i>Solenopsis</i> sp. 1	30	0	2
	<i>Solenopsis</i> sp. 2	31	0	1
Total abundance	-	-	1021	3395
Total richness	-	-	45	55

Brachymyrmex, *Camponotus*, *Dorymyrmex*, *Odontomachus*, *Pachycondyla*, *Pheidole*, *Pseudomyrmex* and *Solenopsis* were identified (Table 1).

According to the results, we can notice that some species like, *Dorymyrmex brunneus* Forel, 1908, *Pheidole* sp. 1, were more abundant, both in contaminated and non-contaminated carcasses (Table 1). On the other hand, species such as *Brachymyrmex* sp. 1, *Camponotus fastigatus* Roger, 1863 occurred even though in low abundance in all phases of decomposition, except in the first. Some species occurred only once, in both carcass types, such as *Anochetus mayri* Emery, 1884 and *Brachymyrmex* sp. 2 (Table 1).

The richness and abundance of the species changed according to the decomposition phases, both in contaminated and non-contaminated carcasses. In contaminated carcasses, the phase in which the greatest number of species occurred was the deterioration with 16 species. In non-contaminated carcasses, the phase in which the greatest number of species occurred was the last phase of skeletonization, with 13 species. The greatest abundance in contaminated carcasses was found in the swelling phase, with 2513 ants, while in non-contaminated carcasses the greatest abundance was found in the post-deterioration phase with 809 ants. The greatest abundance of ants was found in contaminated carcasses, mainly by the occurrence of *D. brunneus*.

According to the Wilcoxon matched pairs test, no significant differences occurred in the richness of species between the contaminated and non-contaminated carcasses ($p=0.8927$). However, a significant difference was noted in the richness of species along the stages of decomposition between the two types of carcasses ($p<0.05$) (Fig. 1).

On the other hand, Fig. 2 shows a characteristic composition of species in each of the carcass types. Some species, as indicated in Fig. 2, were found in an intermediate position in both types of carcasses. In addition, the nMDS ordering analysis (Fig. 3) shows that some species appear more often in some stages of decomposition, both in non-contaminated and contaminated carcasses.

Discussion

To the best of our knowledge, this is the first study of its kind to investigate the effects on ant fauna from carcasses contaminated by insecticides and the resultant forensic implications. Previous work also carried out on sugarcane plantations presented only Formicidae fauna associated with non-contaminated carcasses (Gomes et al., 2007). On the other hand, Yan-Wei et al. (2010) evaluated the effects of contamination of malathion on cadaveric entomofauna, including Formicidae, but this was carried out in rabbit carcasses contaminated by enema.

Carcass decomposition time differed significantly between the two experimental groups, mostly in the contaminated group, the decomposition time of which was considerably extended, explaining the highest relative abundance and richness of ant species in those carcasses, since these carcasses were exposed longer to the action of these insects. In the study of Paula et al. (2016), the authors did not analyze carcasses under the effect of any contamination, however, they discovered that pig carcasses exposed longer also attracted a greater variety of species. It is worth highlighting that the non-contaminated decomposition time of the carcasses was similar to that described by Paula et al. (2016), a hot and humid station in the same region where our study was conducted.

The changes observed in the decomposition time between the two types of carcasses may be explained by the fact that the insecticide may have affected the action of the cadaveric entomofauna in general, mainly flies and beetles. The activity of insects occurs mainly after the rupture of the skin and the opening of the natural orifices caused by pioneer insects, such as flies that allow easier access to soft tissues in

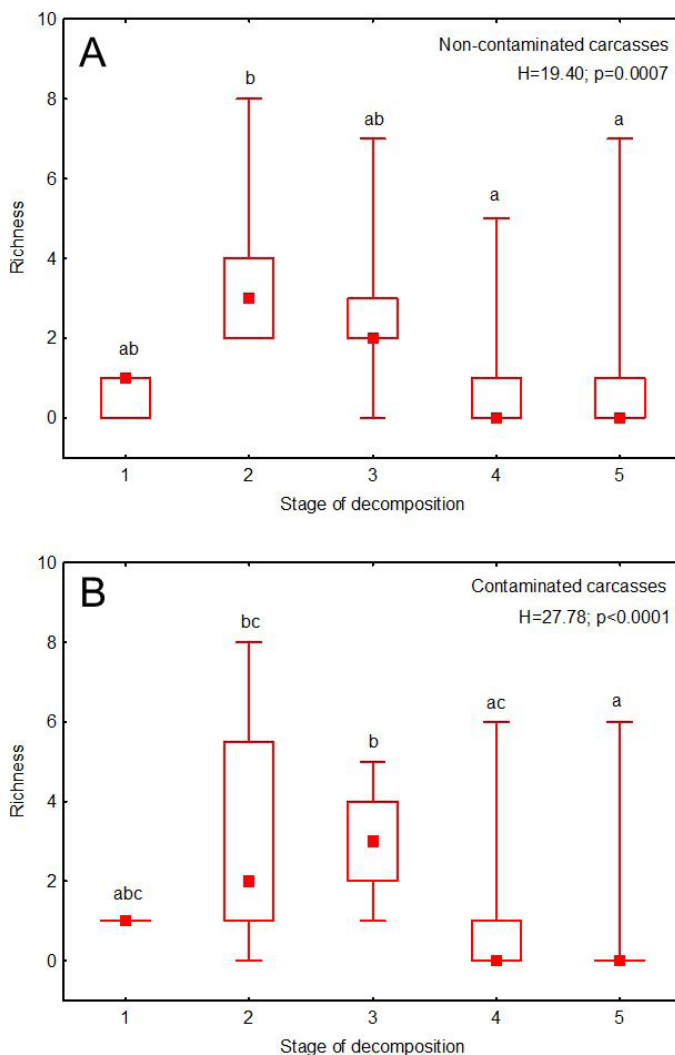


Figure 1 Median (square), quartile range (box) and total range (vertical line) of the species richness in each stage of decomposition in non-contaminated carcasses (A) and in contaminated carcasses (B). Different lowercase letters (a, b, c) indicate significant differences between stages in each treatment ($p < 0.05$). The stages are 1= Fresh; 2= bloated; 3= deterioration; 4= post-deterioration; 5= skeletonization.

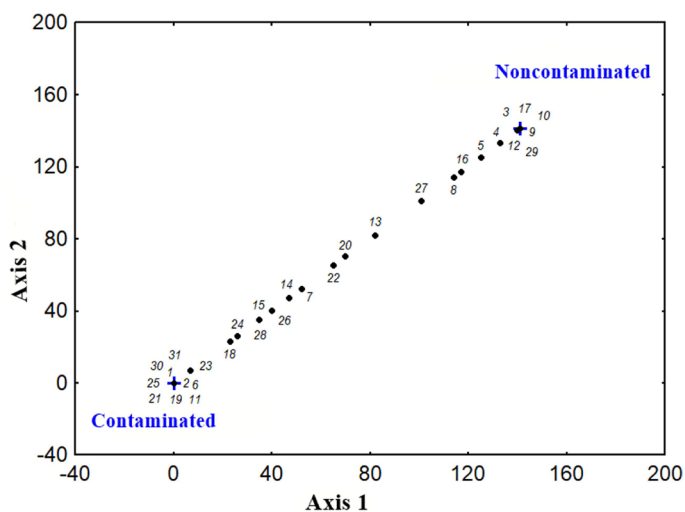


Figure 2 Detrended Correspondence Analysis (DCA) using species occurrence to assess the change in the composition of ant species that occurs in both types of carcasses. The numbers correspond to the species appear in Table 1. On the right are those that occur more effectively in noncontaminated carcasses, and on the left are those that occurred in contaminated carcasses.

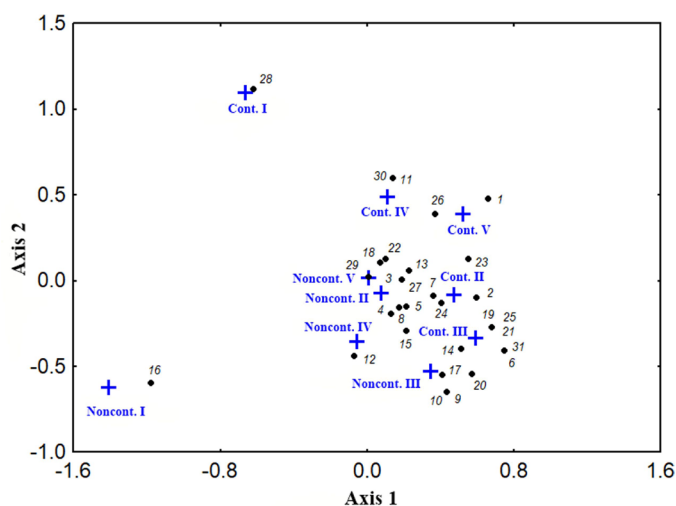


Figure 3 Nonmetric multidimensional scaling analysis (nMDS), using species occurrence to assess the change in the composition of ant species that act on different types of carcasses, along the different stages of decomposition. The numbers correspond to the species appear in Table 1. On the right are those that occur in contaminated carcasses, and on the left are those that occurred more effectively in non-contaminated carcasses. Noncont. = Non-contaminated and Cont. = Contaminated, I= Fresh; II= bloated; III= deterioration; IV= post-deterioration; V= skeletonization.

the deterioration and post-deterioration phase (Goff, 2000; Oliveira-Costa, 2011).

In particular, the change in ant activity may have also contributed to the changes found between carcass decomposition times since ants were considered as one of the most active groups on this type of resource (Paula et al., 2016). In general, we observed that the ants did not use the contaminated carcass tissues, but preyed on both living and dead visitors, such as flies and beetles. Therefore, the absence of necrophagous activity by ants and other insects in the contaminated carcasses may have contributed to the delay in decomposition. In fact, Paula et al. (2016) and Meyer et al. (2020) reported that the behavior of necrophagous ants can accelerate the decomposition process in carcasses.

The richness of species found in this study, both in contaminated and non-contaminated carcasses, was relatively smaller than that found in studies done with the same type of carcasses in a forest environment (Paula et al., 2016). The Monoculture environment shows a smaller diversity of species as mentioned in the work of Gomes et al. (2009) who found only three ant genera. The range of species is directly related to vegetation complexity because greater complexity correlates with a greater diversity of resources used by ant colonies (Morrison, 1998).

The ants species generally occurred in contaminated and non-contaminated carcasses in the 2nd or 3rd decomposition phase. This is possibly because in these phases there is a greater release of gases by the putrefaction process, attracting a larger insect fauna (Anderson, 2020). In addition, species of predatory and omnivorous ants occur in the carcass when there is greater availability of food, however, in the 1st phase, there is oviposition of various species of dipterans, and in subsequent phases, there is a large larval mass that serves as food for ants (Oliveira-Costa, 2011). These results agree with those of Paula et al. (2016) and Park and Moon (2020b) who reported an increase in the richness of ant species after the fresh phase in exposed carcasses during the hotter season of the year. These results seem to indicate the preference of Formicidae for initial phases of decomposition, even in different environments: forest in Paula et al. (2016), anthropized areas in Park and Moon (2020b) or in monoculture plantations in this study. It can also be related to the capacity of ants to detect the presence of carcasses in the first moments after exposure, as described by Paula et al. (2016).

In the last phase of decomposition of contaminated carcasses, there was a decrease in the richness of ant species (Fig. 1). An aspect that may have contributed to the reduction in the number of species, at least in contaminated carcasses, is the lack and/or impracticality of the resource.

The composition of ant species differed between contaminated and non-contaminated carcasses (Fig. 2) and stage of decomposition (Fig. 3). In fact, in the same carcass throughout the stages of decomposition, species composition tended to differ, most likely because of the characteristics, activity and resources available at each stage (Paula et al., 2016; Meyer et al., 2020). Therefore, the cadaveric stages of the process of decomposition also affect the composition of ant species attracted to the contaminated, as well as the non-contaminated, resource.

Between the two types of carcasses (contaminated and non-contaminated), the difference in the composition of the species can be explained by the presence of the contaminant that affected the action of the necrophagous species and also by the greater carcass decomposition time that must have altered the pattern of occurrence of species. In this sense, although ants with omnivorous and necrophagous habits were found in both types of carcasses, they were more frequent in non-contaminated carcasses, probably because they did not consume contaminated carcass tissue, which made them less attractive to ants with this type of habit.

On the other hand, ants with omnivorous and predatory habits occurred in both types of carcasses, and since they can prey on necrophagous insects like flies, they can slow down the decomposition process (Early and Goff, 1986; Wells and Greenberg, 1994; Lindgren et al., 2011; Paula et al., 2016), increasing the differences in the decomposition times between the two types of carcasses. This fact, then, should not be ignored by forensic experts since hidden corpses can be contaminated by insecticides in sugarcane plantations, and this can alter the decomposition pattern by the changing behavior of ants, impacting all cadaveric fauna causing, in turn, PMI mistakes.

Ants of the genus *Dorymyrmex*, especially *D. brunneus*, was the most abundant, both in contaminated and non-contaminated carcasses. These ants are considered generalist predators (Baccaro et al., 2015), but they acted differently according to the resource available. On non-contaminated carcasses, they feed themselves with extracorporeal liquids, especially from the eyes, removing the cornea. In contaminated carcasses, they feed on entomofauna attracted to the resource (Fig. 4A). Horenstein et al. (2012) classified *Dorymyrmex* as omnivorous because those ants were observed feeding on various resources found in the carcass like eggs and larvae of dipteran and other ants. Andrade-Silva et al. (2015) and Pereira et al. (2017) also found this genus in vertebrate carcasses in Brazil. The abundance can be related to the type of environment where the carcasses were exposed since ants of this genus build nests in environments with rare or non-existent vegetation cover; therefore, they are generally collected in anthropized environments exposed to the soil, such as monoculture areas (Ribas, 2018). *Dorymyrmex* and *Pheidole* are often found co-occurring in carcasses (Horenstein et al., 2012; Andrade-Silva et al., 2015; Eubanks et al., 2019; Sharif and Kamar, 2021). In this study, ants of the genus *Pheidole* were also frequently observed in the carcasses of the two types. These ants present omnivorous behavior in carcasses, both feeding of the carcass (Fig. 4B), but also preying on immature and/or adults of dipterans (Fig. 4C) and coleopterans. However, in contaminated carcasses, they only prey on other living or dead insects. According to Rossi and Fowler (2004), ants of this genus were observed preying and feeding on protein bait, so they can be considered as opportunistic omnivores and also often found in monocultures.

Ants of the *Atta* genus were abundant in non-contaminated carcasses in the final phases of "post-deterioration". However, specimens were not observed foraging or performing any behavior on the carcasses at any time. This is because some species can adjust the foraging period

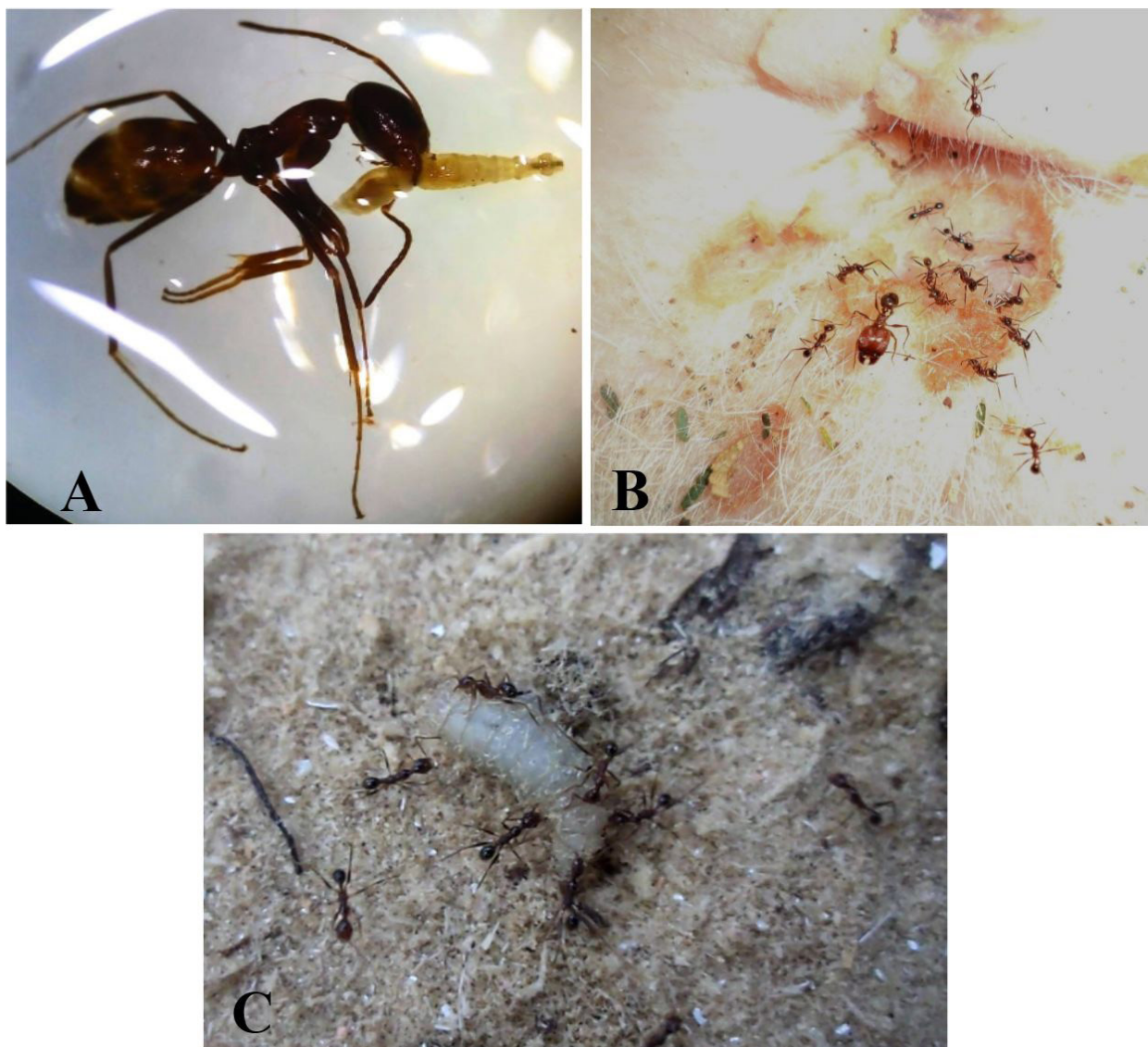


Figure 4 Ant *Dorymyrmex brunneus* feeding of the entomofauna attracted to the carcass (A); *Pheidole* ants feeding of the carcass (B); *Pheidole* ants preying immature of dipterans (C).

from day to night in order to prevent parasites like forids (Hölldobler and Wilson, 2010). According to the literature, these ants can perform necrophagous habits on carcasses because they cut pieces of tissue from the carcasses and take them to their nests (Paula et al., 2016), even though they are leaf-cutting ants. Although the numbers indicate that they occur in greater abundance in non-contaminated carcasses, we cannot discard the hypothesis that the place where the carcasses were installed could be close to the ants nesting area, even though it was verified before the carcass installations, and no nest could be found.

The least common genera were *Acromyrmex* and *Pseudomyrmex*, with only one specimen of each genus, probably because of their lifestyle habits, the first being that of a leaf cutter and considered an insect pest of monocultures controlled through the use of neonicotinoids, changing the diversity of *Acromyrmex* in monocultures (Camargo et al., 2006) and also possibly, because their colonies are much less numerous than the ants of the genus *Atta*, making it more difficult for these ants to occur in the carcasses. *Pseudomyrmex* is an arboreal species that nests in branches and pre-existing cavities in the tree trunk. Since anthropized environments are inappropriate to shelter these ants (Ribas, 2018), the

low number of specimens can be explained by the distance between the nests and the locations of carcasses.

Conclusion

According to the results, it is possible to conclude that if the carcasses were contaminated by insecticide, their decomposition pattern can be changed, affecting the habit and feeding behavior of Formicidae fauna, as well as the composition of species in the different stages of decomposition. Another important fact is ants do not seem to occur in the early stages of decomposition. Therefore, these results have important forensic implications.

Acknowledgments

The authors thank the college students Danielly Fidelis de Souza (UFGD), Leticia da Silva Reginaldo (UEMS), Ivana Benevides Rissato (UEMS), Viviane dos Santos Simplício (UEMS) and Fabiane Cassari de

Oliveira (UFGD) for their help in some steps of the experiments during their internship at Labeco.

Funding

Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) by granting research grants to authors WFAJ [grant number 308182/2019-7] and PGS [grant number 380795/2020-5]. Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT) by granting research grant to author MCP [grant number 71/700.088/2020].

Conflicts of interest

The authors declare no conflicts of interest.

Author contribution statement

GSV: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Data curation, Writing – review & editing. MCP: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Supervision, Validation, Data curation, Writing – review & editing. ADMME: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. PGS: Data curation, Investigation, Methodology, Project administration, Validation, Visualization, Writing – original draft. SELJ: Formal analysis, Methodology, Writing – review & editing. WFA-J: Conceptualization, Methodology, Project administration, Supervision, Validation, Funding acquisition, Writing – review & editing.

References

- Al-Galil, F. M. A., Zambare, S. P., Al-Mekhlafi, F. A., Al-Keridis, L. A., 2021a. Effect of dimethoate on the developmental rate of forensic importance Calliphoridae flies. *Saudi J. Biol. Sci.* 28 (2), 1267–1271. <http://dx.doi.org/10.1016/j.sjbs.2020.12.022>.
- Al-Galil, F. M. A., Zambare, S. P., Al-Mekhlafi, F. A., Wadaan, M. A., Al-Khalifa, M. S., 2021b. Effects of insecticide dimethoate on the developmental rate of forensic importance sarcophagid flies. *Journal of King Saud. Univ. Sci.* 3, 101349.
- Almeida, L., Ribeiro-Costa, C., Marinoni, L., 2003. Manual de coleta, conservação, montagem e identificação de insetos, 1ª ed. Holos, Ribeirão Preto.
- Anderson, G. S., 2020. Factors that influence insect succession on carrion. In: Byrd, J.H., Tomberlin, J.K. (Eds.), *Forensic Entomology: The Utility of Arthropods in Legal Investigations*, 3rd ed. CRC Press, Florida, pp. 103–132.
- Andrade-Silva, J., Pereira, E. K. C., Silva, O., Delabie, J. H. C., Rebelo, J. M. M., 2015. Ants (Hymenoptera: Formicidae) associated with pig carcasses in an urban area. *Sociobiology* 62 (4), 527–532. <http://dx.doi.org/10.13102/sociobiology.v62i4.795>.
- Baccaro, F. B., Feitosa, R. M., Fernandez, F., Fernandes, I. O., Izzo, T. J., Souza, J. L. P., Solar, R., 2015. Guia para os gêneros de formigas do Brasil, 1ª ed. Editora INPA, Manaus.
- Bolton, B., 2003. *Synopsis and classification of Formicidae*, 1st ed. American Entomological Institute, Gainesville.
- Camargo, R. S., Forti, L. C., Lopes, J. F. S., Nagamoto, N. S., 2006. Studies on leaf-cutting ants, *Acromyrmex* spp. (Formicidae, Attini): Behavior, reproduction and control. *Recent Res. Dev. Entomol.* 5, 1–21.
- Campo Grande News, 2018. Moradora da reserva indígena é encontrada morta em milharal na Panambizinho. Available in: <https://www.campograndenews.com.br/cidades/interior/moradora-da-reserva-indigena-e-encontrada-morta-em-milharal-na-panambizinho> (accessed 3 August 2019).
- Campobasso, C. P., Marchetti, D., Introna, F., Colonna, M. F., 2009. Postmortem artifacts made by ants and the effect of ant activity on decompositional rates. *Am. J. Forensic Med. Pathol.* 30 (1), 84–87. <http://dx.doi.org/10.1097/PAF.0b013e318187371f>.
- Catts, E. P., Haskell, N. H., 1990. *Entomology and Death: a Procedural Guide*, 1st ed. Forensic Entomology Assocs., South Carolina.
- Di Ilio, V., Metwaly, N., Saccardo, F., Caprio, E., 2018. Adult and egg mortality of *Rhynchophorus ferrugineus* Oliver (Coleoptera: Curculionidae) induced by Thiamethoxam and Clothianidin. *J. Agric. Vet. Sci.* 11, 59–67. <https://doi.org/10.9790/2380-1102025967>.
- Early, M., Goff, M. L., 1986. Arthropod succession patterns in exposed carrion on the Island of O'ahu, Hawaiian Islands, USA1. *J. Med. Entomol.* 23 (5), 520–531. <http://dx.doi.org/10.1093/jmedent/23.5.520>.
- Eubanks, M. D., Lin, C., Tarone, A. M., 2019. The role of ants in vertebrate carrion decomposition. *Food Webs* 18, e00109. <http://dx.doi.org/10.1016/j.fooweb.2018.e00109>.
- G1 Piracicaba e Região, 2018. Homem de 22 anos é encontrado morto em canal de Santa Bárbara d'Oeste. Available in: <https://g1.globo.com/sp/piracicaba-regiao/noticia/2018/10/13/homem-de-22-anos-e-encontrado-morto-em-canal-de-santa-barbara-doeste.ghtml> (accessed 18 August 2019).
- G1 Triângulo Mineiro, 2018. Corpo de idoso desaparecido é encontrado em canal na cidade de Delta. Available in: <https://g1.globo.com/mg/triangulo-mineiro/noticia/2018/12/17/corpo-de-idoso-desaparecido-e-encontrado-em-canal-na-cidade-de-delta.ghtml> (accessed 15 March 2019).
- Goff, M. L., 2000. *A Fly for the Prosecution: How Insect Evidence Helps Solve Crimes*, 2nd ed. Harvard University Press, Cambridge.
- Gomes, L., Gomes, G., Desuó, I. C., 2009. A preliminary study of insect fauna on pig carcasses located in sugarcane in winter in southeastern Brazil. *Med. Vet. Entomol.* 23 (2), 155–159. <http://dx.doi.org/10.1111/j.1365-2915.2009.00796.x>.
- Gomes, L., Gomes, G., Oliveira, H. G., Morlin Junior, J. J., Desuo, I. C., Queiroz, M. M. C., Giannotti, E., Von Zuben, C. J., 2007. Occurrence of Hymenoptera on *Sus scrofa* carcasses during summer and winter seasons in southeastern Brazil. *Rev. Bras. Entomol.* 51 (3), 394–396. <http://dx.doi.org/10.1590/S0085-56262007000300019>.
- Hammer, Ø., Harper, D. A. T., Ryan, P. D., 2001. Past: paleontological statistics software package for education and data analysis. *Palaeontol. Electronica* 4, 1–9.
- Hölldobler, B., Wilson, E. O., 1990. *The Ants*, 1st ed. Harvard University Press, Cambridge.
- Hölldobler, B., Wilson, E. O., 2010. *The Leaf Cutter Ants: Civilization by Instinct*, 1st ed. Norton & Company, New York.
- Horenstein, M. B., Rosso, B., García, M. D., 2012. Seasonal structure and dynamics of sarcosaprophagous fauna on pig carrion in a rural area of Cordoba (Argentina): their importance in forensic science. *Forensic Sci. Int.* 217 (1–3), 146–156. <http://dx.doi.org/10.1016/j.forsciint.2011.10.043>.
- Introna, F., Campobasso, C., Goff, M. L., 2001. Entomotoxicology. *Forensic Sci. Int.* 120 (1–2), 42–47. [http://dx.doi.org/10.1016/S0379-0738\(01\)00418-2](http://dx.doi.org/10.1016/S0379-0738(01)00418-2).
- Lindgren, N. K., Bucheli, S. R., Archambeault, A. D., Bytheway, J. A., 2011. Exclusion of forensically important flies due to burying behavior by the red imported fire ant (*Solenopsis invicta*) in southeast Texas. *Forensic Sci. Int.* 204 (1–3), e1–e3. <http://dx.doi.org/10.1016/j.forsciint.2010.05.016>.

- Marcuzzo, F. F. N., 2014. Distribuição espacial da sazonalidade da precipitação pluviométrica no Mato Grosso do Sul e estudo de anomalias. *Acta Geografica*. 8, 22-39. <https://doi.org/10.5654/actageo2014.0004.0015>.
- Meyer, F., Monroe, M. D., Williams, H. N., Goddard, J., 2020. *Solenopsis invictax richteri* (Hymenoptera: Formicidae) necrophagous behavior causes post-mortem lesions in pigs which serve as oviposition sites for Diptera. *Forensic Sci. Int. Rep.* 2, 1-8. <http://dx.doi.org/10.1016/j.fsir.2020.100067>.
- Mise, K. M., Almeida, L. M., Moura, M. O., 2007. Levantamento da fauna de Coleoptera que habita a carcaça de *Sus scrofa* L., em Curitiba, Paraná. *Rev. Bras. Entomol.* 51 (3), 358-368. <http://dx.doi.org/10.1590/S0085-56262007000300014>.
- Moretti, T. D. C., 2006. Artrópodes associados às carcaças de pequenos roedores expostas em área de formação vegetal secundária no município de Campinas, SP. *Biota Neotrop.* 6 (3), 1. <http://dx.doi.org/10.1590/S1676-06032006000300027>.
- Morrison, L. W., 1998. The spatiotemporal dynamics of insular ant metapopulations. *Ecology* 79 (4), 1135-1146. [http://dx.doi.org/10.1890/0012-9658\(1998\)079\[1135:TSDOIA\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(1998)079[1135:TSDOIA]2.0.CO;2).
- Oliveira-Costa, J., 2011. *Entomologia forense: quando os insetos são vestígios*, 3rd ed. Millennium, Campinas.
- Park, S., Moon, T., 2020a. Forensic implication of cadaveric behaviors of the Japanese pavement ant (*Tetramorium tsushimae* Emery) attending to rabbit cadavers. *Entomol. Res.* 50 (4), 177. <http://dx.doi.org/10.1111/1748-5967.12416>.
- Park, S., Moon, T., 2020b. Positive and negative impact to decomposition of pig cadavers by ants (Hymenoptera: Formicidae) at the Islet Yeongdo in Busan, South Korea. *Entomol. Res.* 50 (2), 124-127. <http://dx.doi.org/10.1111/1748-5967.12419>.
- Patel, F., 1994. Artefact in forensic medicine: postmortem rodent activity. *J. Forensic Sci.* 39 (1), 257-260. <http://dx.doi.org/10.1520/JFS13594J>.
- Paula, M. C., Morishita, G. M., Cavarson, C. H., Gonçalves, C. R., Tavares, P. R. A., Mendonça, A., Suárez, Y. R., Antoniali-Junior, W. F., 2016. Action of ants on vertebrate carcasses and blow flies (Calliphoridae). *J. Med. Entomol.* 53 (6), 1283-1291. <http://dx.doi.org/10.1093/jme/tjw119>.
- Payne, J. A., 1965. A summer carrion study of the baby pig *Sus Scrofa* Linnaeus. *Ecology* 46 (5), 592-602. <http://dx.doi.org/10.2307/1934999>.
- Pereira, E. K. C., Andrade-Silva, J., Silva, O., Santos, C. L. C., Moraes, L. S., Bandeira, M. C. A., Silva, C. R. R., Rebêlo, J. M. M., 2017. *Solenopsis saevissima* (Smith) (Hymenoptera: Formicidae) activity delays vertebrate carcass decomposition. *Sociobiology* 64 (3), 369-372. <http://dx.doi.org/10.13102/sociobiology.v64i3.1266>.
- R Core Team, 2017. R: a language and environment for statistical computing. R foundation for statistical computing, Vienna. Available in: <https://www.r-project.org/> (accessed 15 March 2019).
- Ribas, C. R. (Ed.), 2018. *Cartilha para avaliação de impactos ambientais e reabilitação de áreas degradadas baseada em comunidade de formigas e suas interações com plantas*. Universidade Federal de Lavras, Lavras.
- Rossi, M. N., Fowler, H. G., 2004. Predaceous ant fauna in new sugarcane fields in the state of São Paulo, Brazil. *Braz. Arch. Biol. Technol.* 47 (5), 805-811. <http://dx.doi.org/10.1590/S1516-89132004000500017>.
- Sharif, S., Kamar, A., 2021. Insect faunal succession on buried goat carcass in Aligarh Region of Uttar Pradesh, India, with implications in forensic entomology. *Egip. J. Forens. Sci.* 11 (1), 21. <http://dx.doi.org/10.1186/s41935-021-00235-5>.
- Singh, S., Abdullah, N. A. B., Carbaugh, J., Heo, C. C., 2020. Ants associated with a rat carcass: its implications in forensic entomology with special emphasis on *Carebara diversa* (Hymenoptera: Formicidae). *Int. J. Trop. Insect Sci.* 40 (3), 703-706. <http://dx.doi.org/10.1007/s42690-020-00110-1>.
- Smith, K. G. V., 1986. *A Manual of Forensic Entomology*, 1st ed. Trustees of the British Museum (Natural History), London.
- Sutherland, W. J., 2006. *Ecological census techniques: a Handbook*, 2nd ed. Cambridge University Press, Cambridge.
- TIBCO Software Inc., 2017. *Statistica (Data Analysis Software System)*, Version 13. Available in: <http://tibco.com> (accessed 15 March 2019).
- Wells, J. D., Greenberg, B., 1994. Effect of the red imported fire ant (Hymenoptera, Formicidae) and carcass type on the daily occurrence of postfeeding carrion-fly larvae (Diptera, Calliphoridae, Sarcophagidae). *J. Med. Entomol.* 31 (1), 171-174. <http://dx.doi.org/10.1093/jmedent/31.1.171>.
- Wolff, M., Builes, A., Zapata, G., Morales, G., Benecke, M., 2004. Detection of Parathion (O, O-diethyl O-(4-nitrophenyl) phosphorothioate) by HPLC in insects of forensic importance in Medellín, Colombia. *Anil Aggrawal's Internet J. Forensic Med. Toxicol.* 5, 6-11.
- Yan-Wei, S., Xiao-Shan, L., Hai-Yang, W., Run-Jie, Z., 2010. Effects of Malathion on the insect succession and the development of *Chrysomya megacephala* (Diptera: Calliphoridae) in the field and implications for Estimating *postmortem* interval. *Am. J. Forensic Med. Pathol.* 31 (1), 46-51. <http://dx.doi.org/10.1097/PAF.0b013e3181c215b4>.
- Zhou, L., Liu, L., Chang, L., Li, L., 2011. Poisoning deaths in Central China (Hubei): a 10-year retrospective study of forensic autopsy cases. *J. Forensic Sci.* 56, 234-237. <http://dx.doi.org/10.1111/j.1556-4029.2010.01625.x>.