Differential Diptera succession patterns onto partially burned and unburned pig carrion in southeastern Brazil

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Abstract

In the present contribution we compared the entomological succession pattern of a burned carcass with that of an unburned one. For that, we used domestic pig carcasses and focused on Calliphoridae, Muscidae and Sarcophagidae flies, because they are the ones most commonly used in Postmortem Interval estimates. Adult and immature flies were collected daily. A total of 27 species and 2,498 specimens were collected, 1,295 specimens of 26 species from the partially burned carcass and 1,203 specimens of 22 species from the control carcass (unburned). The species composition in the two samples differed, and the results of the similarity measures were 0.875 by Sorensen and 0.756 by Bray-Curtis index. The results obtained for both carcasses also differ with respect to the decomposition process, indicating that the post mortem interval would be underestimated if the entomological succession pattern observed for a carcass under normal conditions was applied to a carbonized carcass.

Keywords: Forensic Entomology, *post mortem* interval, burnt carcass, Caliptratae, flies.

Diferenças no padrão de sucessão de Diptera em carcaças de porco parcialmente carbonizadas e não carbonizadas

Resumo

Nesta contribuição, nós comparamos o padrão de sucessão entomológica de uma carcaça carbonizada com outra não carbonizada. Para tal, nós usamos carcaças de porcos domésticos e focamos nos dípteros Calliphoridae, Muscidae e Sarcophagidae, por estes serem os grupos de insetos mais comumente usados na estimativa do intervalo pós-morte. Moscas adultas e seus imaturos foram coletados diariamente. Um total de 27 espécies e 2.498 espécimes foi coletado, 1.295 espécimes de 26 espécies na carcaça parcialmente carbonizada e 1.203 espécimes de 22 espécies na carcaça controle (não carbonizada). A composição específica das duas amostras foi diferente e os cálculos de similaridade resultantes foram 0,875 pelo índice de Sorensen e 0,756 pelo índice de Bray-Curtis. Os resultados obtidos para ambas as carcaças também diferiram em relação ao processo de decomposição, indicando que o intervalo pós-morte poderia ser subestimado se o padrão de sucessão entomológica observado na decomposição de uma carcaça sob condições normais fosse aplicado a uma carcaça carbonizada.

Palavras-chave: Entomologia Forense, intervalo pós-morte, carcaça carbonizada, Caliptratae, moscas.

Introduction

The *Postmortem* interval (PMI) is an important forensic tool used in homicide investigations to estimate the time between death and the discovery of a corpse. The PMI is influenced by many variables, including the circumstances in which the crime was committed (Catts, 1992). Some insect species are associated with decomposing corpses, playing a particularly important role in the estimation of the PMI. These species use the carrion for feeding, breeding, mating and hiding. Entomological succession varies according to different factors but, under similar situations, it follows an expected and relatively well-known pattern, which can be used to estimate the time elapsed since death (Smith, 1985).

Among the insects associated with cadaver decomposition, flies (Diptera) play a special role, because they are present from the beginning to the end of the process. More specifically, muscoid flies are considered the most important group of insects to forensics. Typically, blowflies (Calliphoridae), flesh flies (Sarcophagidae), and house flies (Muscidae) are the primary indicators of the PMI (Catts and Haskell, 1991).

In order to be able to use entomological methods and techniques in death investigations, it is necessary to have local data on insect succession (Amendt et al., 2007). This is because the sequence of species on a corpse differs among places according to factors such as the local climate (Goff, 2000). Within the same region, the pattern of species succession may also vary according to the circumstances of the crime, and the conditions that the body has been subjected to, which may alter the duration of the decay stages (Micozzi, 1986; Mann et al., 1990). These changes may alter the frequency of each species actively participating in this process, from the first visit to the colonization of the entire corpse (Avila and Goff, 1998).

The attraction exerted by a carbonized corpse on the forensically important Diptera and the consequent species succession patterns are probably different from the attraction exerted by a corpse that has not been burnt. To make the estimation of the IPM possible for carbonized bodies, it is essential to know the specific pattern of species succession on them. However, few comparative studies of the entomological succession patterns in burned and unburned carrion have been published (Avila and Goff, 1998; Pai et al., 2007; Heo et al., 2008). The aim of the present contribution is to investigate the duration of the decomposition stages, as well as the frequency and abundance of calyptrate flies (Calliphoridae, Muscidae and Sarcophagidae) associated with a carbonized carcass, compared with those obtained for a corpse that has not been carbonized.

Material and Methods

Site description

The experiment was carried out in the city of Rio de Janeiro, state of Rio de Janeiro, Brazil, inside a military base, the 26° Batalhão de Infantaria Pára-Quedista, in the neighborhood of Vila Militar. This site is in the urban perimeter, but the experimental area has no human activity.

Rio de Janeiro has a tropical savanna climate that closely borders a tropical monsoon climate, according to the Köppen climate classification, and is characterized by long periods of heavy rain from December to March. In inland areas of the city, high temperatures are common during the summer, though rarely for long periods. Because of its geographical location, cold fronts advancing from Antarctica frequently reach the city often, especially during autumn and winter, causing frequent weather changes. The temperature varies according to factors such as elevation, distance from the coast, and type of vegetation. The average annual minimum temperature is 21 °C (70 °F), the average annual maximum temperature is 27 °C (81 °F), and the average annual temperature is 23 °C (73 °F). The average yearly precipitation is 1,175mm. According to INMET (2012), the lowest temperature ever registered in the 21st century was 8.1 °C (47 °F) in Vila Militar, July 2011.

Experimental procedures

The experiments were performed using two domestic pigs (*Sus scrofa* Linnaeus, 1758), weighing about 18 kg each. The animals were killed by the commercial slaughter method, which is commonly used in slaughterhouses. In order to simulate a crime scene, the animals were dressed after the sacrifice. One of them was partially burned with two liters of gasoline soon after its death (see Figure 1), while the other was maintained in natural conditions for posterior comparison (unburned). The project was approved by the ethics in research on animals of the Universidade Castelo Branco and biosecurity rules were respected during the execution (Process number 044/11).

Each carcass was put inside an iron cage in order to avoid the attack of vertebrate scavengers. A distance of approximately 100 m separated the cages from each other. The cage had a pyramidal roof with a collecting jar on its upper portion and was positioned at the center of a Shannon trap (3 x 3 m). A tray with sawdust was placed underneath the cage to allow the collection of the immature flies that abandoned the carcass (see Figure 2) (Oliveira-Costa, 2011). Six pitfall traps surrounded each cage to capture the immature flies in dispersion (Schoenly et al., 2006).

Figure 1. Pig carcass partially burned.

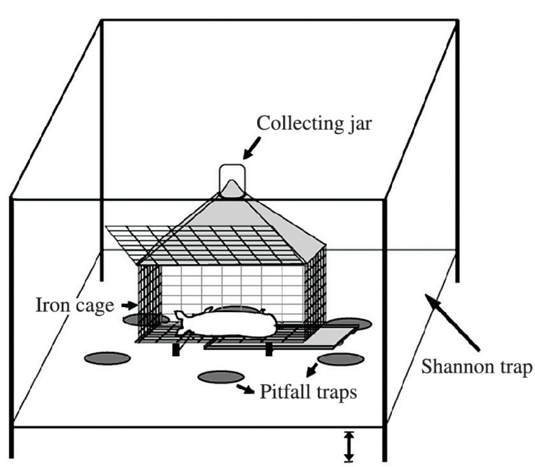

Figure 2. Collecting traps (cage, Shannon, and pitfall traps).

Pitfall traps were built with 2.5 liter plastic buckets, buried with their edges parallel to the floor and placed 1.0 m away from the cage and 1.0 m between them. One quarter of the bucket capacity was filled with water with detergent soap (for breaking the surface tension), but no preservative liquid (as alcohol) was used, to avoid interfering with the attractiveness of the corpse (see Figure 2).

Adult flies were captured with a collecting jar that was removed daily and substituted by an empty jar (Oliveira-Costa, 2011). Specimens were also collected directly from inside Shannon trap with an entomological net and/or killing jar, and were euthanized with ethyl acetate. We conducted daily sampling until the flies stopped visiting the carcass. Therefore, the total collecting period was a function of the interval of time until the complete decomposition of the carcass. The maggots and eggs were collected using delicate tongs and brushes, the material in the pitfalls was sifted and the pupae in the soil were collected with a gardener spoon, within a radius of 6 meters (Byrd and Castner, 2001). According to the visible changes in the carcass, the decomposition stages were classified as in Goff (2000): fresh, bloated, decay, post decay and skeletal.

The material collected was transported to the forensic entomology laboratory at Universidade Castelo Branco, Rio de Janeiro. The adults were killed by freezing, pinned and identified using taxonomic keys (Carvalho and Couri, 2002; Couri and Carvalho, 2002; Mello, 2003; Carvalho and Mello-Patiu, 2008). A portion of the maggots (10%) was preserved in commercial ethyl alcohol 70%, whereas the remaining specimens were reared with an artificial diet until the emergence of the adults, in order to confirm larval identification (Oliveira-Costa, 2011). The voucher material is deposited at the Forensic Entomology Laboratory of the "Instituto de Criminalística Carlos Éboli", Rio de Janeiro.

Data analysis

The succession pattern of species on the two carcasses was tabulated associating the different taxa with decomposition stages. The species richness was analyzed by the Margalef Index and by the Rarefaction curve. The diversity was compared by the Simpson and Shannon Indexes and Hill ratio (dominance, heterogeneity and uniformity) using PAST (Harmer, 2009). To estimate the similarity between the samples, the Estimate S (Colweel, 2009) was used to calculate the Sorensen index, whereas the Bray-Curtis index was used as a quantitative modification of the Sorensen Index (Magurran, 1988). Student's t test was applied with a significance level of $p<0.05$.

Results

Carcass decomposition and consequently the sampling period lasted 74 days. More individuals and a greater number of species visited the partially burned carcass. A total of 1,295 specimens, corresponding to 26 species, were collected from the partially burned carcass. By contrast, 1,203 specimens corresponding to 22 species were collected from the control carcass (unburned). Species and their abundance are resumed in Table 1; species richness and a comparison among diversity indexes in Table 2. The dominance was low on both substrates, indicating that species diversity was high. Comparing the richness values estimated by the rarefaction curve (see Figure 3), the standard deviations of 1.22 for the control carcass (unburned) and of 0.65 for partially burned carcass were found. The similarity in species composition between the two samples was 0.875, as calculated by the Sorensen index, and 0.756 according to Bray-Curtis index. There was no significant statistical difference regarding the carcasses.

Among the 27 species collected (as shown in Table 1), 21 colonized both carcasses. *Sarcophaga ruficornis* (Fabricius) (Sarcophagidae) was collected only from the control carcass, whereas five species were present only on the partially burned carcass: *Atherigona orientalis* Schiner (Muscidae), *Titanogrypa larvicida* (Lopes), *Helicobia aurescens* (Townsend), *Helicobia morionella* (Aldrich) and *Sarcodexia lambens* (Wiedemann) (Sarcophagidae). Tables 3 and 4 show the frequency of each taxon through the decay stages of both carcasses.

Five decomposition stages were observed. Differences between the two carcasses were observed since the first stage of decomposition. The fresh stage was observed in the first three days of decomposition in the control carcass (unburned), attracting 10 species (see Table 3). Maggots of three species were collected during this stage: *Lucilia eximia* (Wiedemann), *Peckia chrysostoma* (Wiedemann) and *P. intermutans* (Walker) (2^{nd} and 3^{rd} days of PMI). A different situation was observed for the partially burned carcass, where the fresh stage lasted only one day and no specimen was attracted to it (see Table 4).

The bloated stage was also longer in the control carcass, lasting five days, contrasting with two days in the other carcass. The first eggs and larvae of *Chrysomya albiceps* (Wiedemann) were also observed on the unburned carcass. In this stage, the first specimens began to appear on the partially burned carcass. In the last carcass, the insects totaled nine species, still less than the number of species found on the control, which was visited by 16 species. The first eggs of *L. eximia* and first larvae of *P. chrysostoma* were also observed on the burned carcass at this stage.

As with the two first stages, the decay stage of the control carcass lasted about three times longer, thirteen days more than in the partially burned carcass, where it

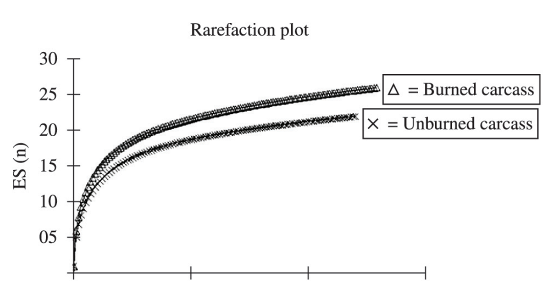

Figure 3. Rarefaction curve comparing the richness between the control (unburned) and the partially burned carcasses.

Table 1. Abundance of Calliphoridae, Muscidae and Sarcophagidae (Diptera) collected in the control (unburned) and in the partially burned carcasses in Rio de Janeiro.

FAMILY	TAXA	UNBURNED	BURNED
Calliphoridae	*Chrysomya albiceps* (Wiedemann)	397	268
	Chrysomya megacephala (Fabricius) 1794)	72	202
	Chrysomya putoria (Wiedemann)	10	12
	Cochliomyia macellaria (Fabricius)	8	10
	Hemilucilia segmentaria (Fabricius) 1805)	8	11
	Lucilia eximia (Wiedemann)	116	36
Muscidae	*Atherigona orientalis* Schiner	0	8
	Graphomyia sp. Robineau-Desvoidy 1830	4	9
	Musca domestica Linnaeus	27	51
	Ophyra aenescens (Wiedemann)	379	338
	Ophyra sp. Robineau-Desvoidy	10	26
	Synthesiomyia nudiseta (Wulp)	2	6
Sarcophagidae	*Titanogrypa larvicida* (Lopes)	0	1
	Helicobia aurescens (Townsend)	0	1
	Helicobia morionella (Aldrich)	0	1
	Microcerella erythropyga (Lopes)	1	1
	Oxysarcodexia amorosa (Schiner)	1	1
	Oxysarcodexia diana (Lopes)	8	12
	Oxysarcodexia fluminensis Lopes	65	173
	Oxysarcodexia thornax (Walker)	46	66
	Oxysarcodexia timida (Aldrich)	2	3
	Peckia chrysostoma (Wiedemann)	18	18
	Peckia intermutans (Walker)	5	2
	Ravinia belforti (Prado & Fonseca)	1	3
	Sarcodexia lambens (Wiedemann)	0	2
	Sarcophaga ruficornis (Fabricius)	1	0
	Tricharaea occidua (Fabricius)	22	34
TOTAL		1,203	1,295

Table 2. Species richness and comparative diversity indexes of Calliphoridae, Muscidae and Sarcophagidae (Diptera) collected in unburned and burned carcasses in Rio de Janeiro.

INDEX	UNBURNED CARCASS	BURNED CARCASS
Species richness	22	26
Dominance	0.2268	0.1598
Shannon – H	1.894	2.179
Eveness – E	0.302	0.3399
Simpson	0.7732	0.8402
Margalef	2.961	3.489
Equitability	0.6127	0.6688

lasted four days. However, it was in the decay stage that an inversion in species number began to occur, 16 species were observed in the burnt carcass and only 12 in the control. Maggots of *C. albiceps, Chrysomya megacephala* (Fabricius)*, L. eximia,* and *Ophyra aenescens* (Wiedemann) were found at this stage on both carcasses and *P. chrysostoma* maggots only in the burnt carcass.

The post decay stage of the control carcass was very long, lasting 38 days, more than three times longer than in the burnt carcass, in which it lasted 12 days. Also in this stage, the greatest number of species, 21, was found in the burnt carcass, contrasting with only16 species in the unburned carcass.

The skeletal stage of the control carcass (unburned) lasted 15 days, whereas that of the burned carcass lasted 55 days. Less species visited the control carcass (six) than the burnt carcass (seventeen). Only the muscid *O. aenescens* was collected as a maggot colonizing the

Table 3. Species succession related to the decomposition stages in the unburned control carcass (**C** = Calliphoridae; **M** = Muscidae; **S** = Sarcophagidae; E = eggs; L = Larvae; P = Pupae; Ad = Adults)

Species	Fresh	Bloated	Decay	Post Decay	Skeletal
P. intermutans **S**	L, Ad	L, P, Ad	Ad		
O. thornax **S**	Ad	Ad	Ad	Ad	
P. chrysostoma **S**	L, Ad	L, P, Ad	Ad	Ad	
T. occidua **S**	Ad	Ad	Ad	Ad	
C. albiceps **C**	Ad	E, L, Ad	L, P, Ad	L, P, Ad	
C. megacephala **C**	Ad	Ad	E, L, Ad	L, P, Ad	
H. segmentaria **C**	Ad	Ad	Ad	Ad	
L. eximia **C**	E, L, Ad	E, L, Ad	L, P, Ad	P, Ad	
S. nudiseta **M**	Ad				
O. fluminensis **S**	Ad	Ad	Ad	Ad	Ad
M. domestica **M**		Ad	Ad	Ad	Ad
O. aenescens **M**		Ad	L, Ad	L, Ad	L, P, Ad
C. macellaria **C**		Ad		Ad	
O. timida **S**		Ad		Ad	
C. putoria **C**		Ad			
M. erythropyga **S**		Ad			
O. amorosa S		Ad			
Graphomyia sp. **M**			Ad	Ad	Ad
O. diana **S**				Ad	Ad
Ophyra sp. **M**				Ad	
R. belforti **S**				Ad	
L. ruficornis **S**					Ad

Table 4. Species succession related to the decomposition stages in the partially burned carcass (**C** = Calliphoridae; **M** = Muscidae; **S** = Sarcophagidae; E = eggs; L = Larvae; P = Pupae; Ad = Adults).

Species	Fresh	Bloated	Decay	Post Decay	Skeletal
C. albiceps **C**		Ad	E, L, Ad	L, P, Ad	Ad
M. domestica **M**		Ad	Ad	Ad	Ad
O. fluminensis **S**		Ad	Ad	Ad	Ad
P. chrysostoma **S**		L, Ad	L, Ad	L,P, Ad	Ad
T. occidua **S**		Ad	Ad	Ad	Ad
L. eximia **C**		E, L, Ad	L, P, Ad	P, Ad	
Graphomyia sp**. M**		Ad		Ad	
P. intermutans **S**		Ad	Ad.		
M. erythropyga **S**		Ad			
C. megacephala **C**			E, L, Ad	L, P, Ad	Ad
C. putoria **C**			Ad	Ad	Ad
H. segmentaria **C**			Ad	Ad	Ad
O. aenescens **M**			L, Ad	L, P, Ad	L,P, Ad
Ophyra sp. **M**			Ad	Ad	Ad
O. diana **S**			Ad	Ad	Ad
O. thornax **S**			Ad	Ad	Ad
C. macellaria **C**			Ad	Ad	
O. timida **S**			Ad	Ad	
R. belforti **S**				Ad	Ad
S. nudiseta **M**				Ad	Ad
A. orientalis **M**				Ad	
O. amorosa **S**				Ad	
S. lambens **S**				Ad	
C. larvicida **S**					Ad
H. aurescens **S**					Ad
H. morionella **S**					Ad

carcasses in the two experiments; moreover, this species was present in almost the entire decomposition process of both pig carrions.

Discussion

Five decomposition stages were observed in contrast with those of Avila and Goff (1998), who did not record the last stage (skeletal). On the other hand, Heo et al. (2008) also observed five stages, although they classified them differently.

The fresh stage was observed in the first three days of decomposition in the control carcass and only one day for the partially burned carcass. These results were different from those of Avila and Goff (1998) and Heo et al. (2008), who obtained a similar duration of this stage in both carcasses. Nevertheless, the composition of the fauna at this stage was different in both last investigations. Heo et al. (2008) found adults and eggs only on the control carcass, whereas Avila and Goff (1998) immediately observed egg-laying on both carcasses.

The carbonization of the carcass probably contributed to make the fresh stage shorter. Fire facilitates tissue disruption, especially in the abdominal region. However, other factors might have repelled the insects in this initial stage. The remains of the freshly burned carcass were much drier than those of the unburned carcass, which looked softer and more attractive to pioneering species. Wardle (1921) observed that pieces of liver singed by the flame of a Bunsen burner were not attractive to flies. From that he concluded that humidity in the tissues, not the denaturation of their proteins, is essential for the oviposition. Another factor that must have delayed the pioneering colonization was the smoke and the gasoline odor exhaled for some hours by the burnt carcass. These data corroborate Heo et al. (2008), who concluded that the lower incidence of insects at this stage was a consequence of these factors and of the temperature of the carcass.

The bloated stage was also longer in the control carcass. It is possible that the combustion also contributed to the reduction in the duration of this stage in the burned carcass due to the disruption of the tissues that provoked the evisceration of the organs. This was also observed by Avila and Goff (1998) who had concluded that the exhalations of gases contributed to the reduction of this period. According to these authors, the abundant fluids exuded in this stage, due to the premature exposure of the viscera, may attract flies to oviposit.

Even though more species visited the burnt carcass in the decay stage, the number of individuals on it was smaller, so as result from Heo et al. (2008), who observed the least number of specimens on the burnt carcass at this stage. The greatest abundance observed during all the process of decomposition of the burned carcass, in the present investigation, can also be related to the premature exposure of the viscera. Avila and Goff (1998) collected Calliphoridae, Muscidae and Sarcophagidae only until the bloat stage, which makes difficult the comparison of their results with ours.

The post decay stage of the control carcass was very long because a change called saponification (adipocera) was observed, a factor that might have influenced its duration, also contributing to turn the unburnt substrate less attractive.

Avila and Goff (1998) did not find any difference in the fauna that visited the two types of carcass, but found differences in the pattern of succession and decomposition process, which was 1 to 4 days faster in the burned carcass. They pointed out that this difference may alter the PMI estimate. Differently, although the first four stages were faster on burned carcass, the results showed a delay in the rhythm of total decomposition process (from fresh to skeletal stage), and in the consequent insect succession. However, this delay may also make an accurate PMI estimate difficult. Pai et al. (2007) stated that, if the succession pattern of an unburned carcass is used to estimate the PMI for a burned carcass, the PMI will be overestimated. However, Heo et al. (2008) believe that burning does not affect either the rate of decomposition or the insect succession pattern, and therefore does not influences the PMI estimation.

Byrd and Castner (2001) stated that the effects of burning depend on the level of incineration. Therefore, it would be necessary to know the incineration level to better compare these four investigations. The different conclusions showed by different experiments instigate more studies on this matter.

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References

AMENDT, J., CAMPOBASSO, CP., GAUDRY, E., REITER, C., LEBLANC, HN., HALL, MJ. and European Association for Forensic Entomology, 2007. Best practice in forensic entomology—standards and guidelines. *International Journal of Legal Medicine*, vol. 121, no. 2, p. 90-104. http://dx.doi.org/10.1007/s00414-006-0086-x. PMid:16633812

AVILA, FW. and GOFF, ML., 1998. Arthropod succession patterns onto burnt carrion in two contrasting habitats in the Hawaiian Islands. *Journal of Forensic Sciences*, vol. 43, no. 3, p. 581-586. PMid:9608693.

BYRD, JH. and CASTNER, JL., 2001. *Forensic entomology: the utility of arthropods in legal investigations*. Boca Raton: CRC Press. 418 p.

CARVALHO, CJB. and COURI, MS, 2002. Part I. Basal groups. In CARVALHO, CJB. *Muscidae (Diptera) of the neotropical region: taxonomy*. Curitiba: Editora Universidade Federal do Paraná. p. 17-132.

CARVALHO, CJB. and MELLO-PATIU, CA., 2008. Keys to the adults of the most common forensic species of Diptera in South

America. *Revista Brasileira de Entomologia*, vol. 52, no. 3, p. 390-406. http://dx.doi.org/10.1590/S0085-56262008000300012.

CATTS, EP., 1992. Problems in estimating the post-mortem interval in death investigations. *Journal of Agricultural Entomology*, vol. 9, p. 245-255.

CATTS, EP. and HASKELL, NH., 1991. *Entomology and death: a procedural guide*. Clemson: Joyce's Print Shop. 182 p.

COLWEEL, RK., 2009. *EstimateS: Statistical Estimation of Species Richness and shared species from samples*. Software version 8.2.

COURI, MS. and CARVALHO, CJB., 2002. Part II: apical groups. In CARVALHO, CBJ. *Muscidae (Diptera) of the Neotropical Region: taxonomy*. Curitiba: Editora Universidade Federal do Paraná. p. 133-286.

GOFF, ML., 2000. *A fly for the prosecution: how insect evidence helps solve crimes*. Cambridge: Harvard University Press. 225 p.

HARMER, O., 2009. *PAST: Paleontological Statistical*. Software version 1.94.

HEO, CC., MOHAMAD, AM., AHMAD, FM., JEFFERY, J., KURAHASHI, H. and OMAR, B., 2008. Study of insect succession and rate of decomposition on a partially burned pig carcass in an oil palm plantation in Malaysia. *Tropical Biomedicine*, vol. 25, no. 3, p. 202-208. PMid:19287358.

Instituto Nacional de Meteorologia - INMET, 2012. Brasília. Available from: <http://www.inmet.gov.br/>. Access in: 13 Jun. 2012.

MAGURRAN, AE., 1988. *Ecological diversity and its measurement*. Princeton: Princeton University.

MANN, RW., BASS, WM. and MEADOWS, L., 1990. Time since death and decomposition of the human body: variables and observations in case and experimental field studies. *Journal of Forensic Sciences*, vol. 35, no. 1, p. 103-111. PMid:2313251.

MELLO, RP., 2003. Chave para identificação das formas adultas das espécies da família Calliphoridae (Diptera, Brachycera, Cyclorrhapha) encontradas no Brasil. *Entomología y Vectores*, vol. 10, p. 255-268.

MICOZZI, MS., 1986. Experimental study of postmortem change under field conditions: effects of freezing, thawing, and mechanical injury. *Journal of Forensic Sciences*, vol. 31, no. 3, p. 953-961. PMid:3734745.

OLIVEIRA-COSTA, J., 2011. *Entomologia Forense: quando os insetos são vestígios*. 3. ed. Campinas: Millennium. 502 p.

PAI, CY., JIEN, MC., LI, LH., CHENG, YY. and YANG, CH., 2007. Application of forensic entomology to postmortem interval determination of a burned human corpse: a homicide case report from southern Taiwan. *Journal of the Formosan Medical Association*, vol. 106, no. 9, p. 792-798. http://dx.doi.org/10.1016/S0929-6646(08)60043-1. PMid:17908671

SCHOENLY, KG., HASKELL, NH., MILLS, DK., BIEME-NDI, C., LARSEN, K. and LEE, Y., 2006. Recreating death's acre in the school yard: using pig carcasses as model corpses to teach concepts of forensic entomology and ecological succession. *The American Biology Teacher*, vol. 68, no. 7, p. 402-410. http://dx.doi.org/10.1662/0002-7685(2006)68[402:RDAITS]2.0.CO;2.

SMITH, KGV., 1985. *A manual of forensic entomology*. Oxford: University Printing House. 205 p.

WARDLE, RA., 1921. The protection of meat commodities against blowflies. *Annals of Applied Biology*, vol. 8, no. 1, p. 1-9. http://dx.doi.org/10.1111/j.1744-7348.1921.tb05529.x.