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Influence of insecticide on insect fauna of forensic importance in rat carcasses (*Rattus norvegicus*) exposed in a suburban area near Atlantic rainforest fragments of southeastern Brazil

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Abstract. Forensic entomology is the science that applies the study of insects, in combination with other forensic disciplines, in investigations. Chemical compounds, common in deaths linked to accidental or deliberate use of poisons or other toxic substances may alter the succession pattern and development of insects presents in corpses, leading to errors in estimating the postmortem interval. Carcasses of rats (*Rattus norvegicus*) were sprayed with insecticides of pyrethrins and pyrethroids group and exposed in a suburban area in the Atlantic Rainforest domain. The results indicated that pesticides alter the entomological succession, affecting the activity and development of insects found in dead bodies, modifying the duration of stages of decomposition and may lead to an error in estimating the postmortem interval.

Keywords: Forensic entomology, entomotoxicology, pirethrins and pirethroids.

Resumo. Influência de inseticidas sobre a entomofauna de importância forense em carcaças de ratos (*Rattus norvegicus*) expostos em área suburbana no Domínio da Mata Atlântica, no sudeste do Brasil. A entomologia forense é a ciência que aplica o estudo dos insetos, em associação a outros procedimentos criminalísticos, em investigações de âmbito cível e criminal. Compostos químicos, frequentes em mortes ligadas ao uso acidental ou proposital de venenos, ou outras substâncias tóxicas, podem alterar o padrão de sucessão e o desenvolvimento da entomofauna presente nos cadáveres, levando a erros na estimativa do intervalo pós-morte. Carcaças de ratos (*Rattus norvegicus*) foram pulverizadas com inseticidas do grupo das piretrinas e piretróides e expostas em área suburbana no Domínio da Mata Atlântica. Os resultados indicaram que inseticidas alteram a sucessão entomológica, prejudicando a atividade e o desenvolvimento dos insetos presentes nos cadáveres, modificando a duração dos estágios da decomposição e podendo levar a um erro na estimativa do intervalo pós-morte.

Palavras-chave: Entomologia forense, entomotoxicologia, piretrinas e piretróides.

Introduction

The study of cadaveric fauna is the most important application of forensic entomology in forensic medicine. Studies of decomposition and life cycles of insects helps to improve the accuracy of postmortem interval estimation, and contributes to streamline the time required for analysis in the medical-legal investigations (Goff *et al.*, 1988; Oliveira-Costa, 2003).

Pyrethrins are natural compounds with insecticidal properties found in the extract of pyrethrum flowers of certain species of chrysanthemum

(*Chryanthemum* spp). Pyrethroids are synthesized chemicals with a structure very similar to pyrethrins. Both are widely used in household insecticides and products to control insects on pets and gardens (Agencia para Sustancias Tóxicas y el Registro de Enfermedades, 2003). Workers applicators of these substances are always badly protected, without personal protective equipment, and often exposed to residues that are generally responsible for cases of sub-acute or chronic intoxications which in some cases, are so high that cause acute poisonings and deaths (Almeida, 1985).

Different types of chemicals can act in tissues of various ways and produce changes in the pattern of the life cycle of insects, speeding up or slowing the development, and altering the pattern of succession in corpses (Oliveira-Costa, 2003). In this context, the present work examines the insects of forensic importance in carcasses of rats (*Rattus norvegicus*) treated with insecticides of pyrethrins and pyrethroids group. We investigated the influence of pesticides on species richness and in the decomposition stages, and verified the occurrence of changes that could lead to an error in estimating the post-mortem interval.

Materials and Methods

The study was conducted from July to August 2006 in the interior of Campus Planalto of Universidade Metodista de São Paulo (23 ° 42’22, 7 “S, 46 ° 34’14, 8”W), located in a suburban area surrounded by forest fragments at São Bernardo do Campo, São Paulo state, southeastern of Brazil.

The area can be characterized by a topography of hills and ridges disposed in intermediate quotas varying between 60 a 986.5 meters of altitude, occupying the slopes of Serra do Mar to the Atlantic plateau. The region was originally covered by Atlantic forest, of which less than 7% remain today in the country (Tabarelli *et al*., 2005). The average annual temperature is 20ºC and the average temperatures in the warmer and colder months of the year are 23ºC and 16ºC, respectively. The climate corresponds to Cwa of Koeppen’s classification, presenting dry winters extending from April to September, and rainy summers from October to March; the annual average precipitation is 1473.5 mm (Leitão-Filho, 1993; CEPAGRI/UNICAMP, 2006).

Four carcasses of adult male laboratory rats (*Rattus norvegicus*) weighing about 300 g and killed mechanically by a blow to the head were used to perform the experiment. The animals were supplied by the biotherium of the Núcleo de Pesquisas Biológicas of Universidade Metodista de São Paulo, and ethical approval for this study was obtained from the ethics committee of the university.

After being sacrificed, two of the animals (T1 and T2) were sprayed with a multiple-action insecticide from chemical group of pyrethrins and pyrethroids, class IV, the other two animals formed the control groups (C1 and C2).

In order to ensure that only insects and other arthropods had access to carcasses, making these inaccessible to other animals like birds and mammals, each carcass was placed alone in a cage with 36 cm of high, 42 cm long and 47 cm depth, which had 1.5 cm screen of mesh between nodes (Fig. 1). The cages were placed on two campus buildings at a height of 9 meters above ground, with a distance of approximately 10 meters between them.

The collection of the specimens, observations of the decomposition stages durations and registration of the environmental conditions (temperature and relative humidity) were carried out daily. The verification of the temperature and humidity values was made using a minimum and maximum thermometer. Measurements of the external and internal temperature of the carcasses were made with a digital thermometer.

Figure 1. Trap for collection of insects from carcasses exposed in a suburban area of the Atlantic Rainforest Domain.

The organisms were manually collected in each carcass. After the collection, the specimens were taken to the laboratory for identification. The larvae collected were stored in polyethylene bottles containing an artificial diet previously prepared. All insects were identified to species or family (Hymenoptera) level using dichotomous keys published by Borror and Delong (1969), Carvalho & Ribeiro (2000), and Mello (2003).

Results

Composition and abundance of taxa: total fauna - A total of 326 specimens of 10 species from orders Diptera (n = 180 or 55.0% of the total specimens), Coleoptera (n = 19 or 5.8% of the total) and Hymenoptera (n = 127 or 38.4% of the total) were collected on the four carcasses (Table 1).

Table 1. Total of insects collected from carcasses exposed in in a suburban area of the Atlantic Rainforest Domain from southeastern Brazil, July to August of 2006. C1 = Control 1; C2 = Control 2; T1 = Treatment 1; T2 = Treatment 2.

Order	**Family**	**Genera/Species**	**Number of individuals**				
			C1	C2	T1	T2	TOTAL
Diptera	Calliphoridae	*Chrysomya megacephala*	12	1	1	-	14
		Chrysomya putoria	3	7	2	1	13
		Chrysomya albiceps	8	-	2	2	12
		Chrysomya sp.	-	-	-	3	3
		Paralucilia sp.	1	-	-	-	1
	Sarcophagidae	*Sarcophaga (Liopygia) ruficornis*	26	10	5	6	47
		Peckia (Peckia) chrysostoma	6	-	2	10	18
		Peckia (Pattonella) intermutans	-	-	1	-	1
		Peckia sp.	-	2	1	1	4
		Not Identified	2	2	2	2	8
	Muscidae	Not Identified	-	2	-	-	2
	Pupae not emerged		20	28	7	2	57
Coleoptera	Dermestidae	*Dermestes maculatus*	3	1	3	2	9
		Dermestes peruvianus	-	1	-	-	1
		Dermestes sp.	7	-	-	-	7
	Silphidae	*Oxelytrum discicolle*	1	-	-	-	1
	Cleridae	*Necrobia rufipes*	1	-	-	-	1
Hymenoptera	Figitidae	Not Identified	4	3	3	-	10
	Not Identified	Not Identified	2	-	-	-	2
	Pteromalidae	Not Identified	-	3	79	33	115
Total			**96**	**60**	**108**	**62**	**326**

In order Diptera, the collected species belonging to Calliphoridae (23.9% of total flies) were *Chrysomya megacephala* (4.3% of total fauna), *Chrysomya putoria* (4.0%); *Chrysomya albiceps* (3.7%), *Chrysomya* sp. (0.9%) and a specimen of *Paralucilia* sp. (0.3% of total fauna). *Sarcophaga* (*Liopygia*) *ruficornis* was the most abundant species found in Sarcophagidae (43.3% of the total fauna of Diptera), accounting for 14.4% of the fauna collected in the four carcasses, followed by *Peckia* (*Peckia*) *chrysostoma* (5.5% of total fauna).

Coleoptera was represented by 19 specimens (5.8% of total fauna), distributed in families Cleridae and Silphidae, each contributing with 0.3% of the fauna, and Dermestidae, which accounted for 5.2% of total fauna. Among the total number of collected beetles, Dermestidae was the most dominant (89.5%) and *Dermestes maculatus* was the species of higher frequency (2.6% of total fauna). Silphidae and Cleridae were accounted to the small number of beetles collected (5.3% each), being represented by only one individual *Oxelytrum discicolle* and *Necrobia rufipes*, respectively.

Hymenoptera (38.8% of the total fauna), was the second most order in abundance, being dominated in terms of frequency, by dipteran parasitoids, Pteromalidae (90.6% of the Hymenoptera, 35.2% of total fauna) and Figitidae (7.8% of the Hymenoptera, 3.1% of total fauna).

Composition and abundance of taxa in control and treatment carcasses - In carcass control 1 (C1) a total of 96 specimens of insects were collected, 81.2% of Diptera, 12.5% of Coleoptera and 6.2% of Hymenoptera. Sarcophagidae (43.6%) contributed with the largest percentage of Diptera, followed by Calliphoridae (30.8%). Carcass C1 also had the highest richness in Coleoptera, with the occurrence of Dermestidae (83.3%), Silphidae (8.3%) and Cleridae (8.3%). Figitidae accounted for 66.7% of the Hymenoptera fauna.

The carcass control 2 (C2) totaled 61 insects collected, 85.2% of Diptera, 9.8% of Hymenoptera and 3.3% of Coleoptera. The dominance of Diptera was the same as C1, with Sacophagidae (26.9%) followed by Calliphoridae (15.4%) but adding in C2 the occurrence of Muscidae (3.8%). Dermestidae constituted 100.0% of Coleoptera and Hymenoptera was equally composed by Figitidae (50.0%) and Pteromalidae (50.0%).

The carcass treatment 1 (T1), showed the greatest richness of specimens, totaling 108 insects, with dominance of Hymenoptera (75.9%), followed by Diptera (21.3%) and Coleoptera (3.3%). The frequency of Diptera was similar to that obtained in carcasses control, Sarcophagidae was more frequent (47.8%) followed by Calliphoridae (21.7%). Dermestidae was the predominant family in Coleoptera and Hymenoptera was almost exclusively comprised by Pteromalidae (96.3%); Figitidae had a small frequency (3.7%).

Finally the carcass treatment 2 (T2), with a total of 62 insects collected, had a richness of specimens close to C2. However, the dominance of specimens in each order was more similar to those found in T1: 53.2% of Hymenoptera, 43.6% of Diptera and 3.2% of Coleoptera. The dominance of Diptera was the same obtained in C1, but the dominance of Sarcophagidae was more marked in T2 (70.4%). Calliphoridae had a frequency of 22.2%, similar to those observed in C2 and T1. Coleoptera and Hymenoptera were exclusively represented by Dermestidae and Pteromalidae, respectively.

Significant difference (Yates corrected chi-square test, p > 0,05) between the control and treatment groups was not found for either the total organisms or Coleoptera, but the abundances of Diptera and Hymenoptera were quite distinct (p = 0,0000), with greater frequency of Diptera in control carcasses and Hymenoptera in treatments (Fig. 2).

The total decomposition of the animals lasted 52 days and have been recognized five stages denominated as fresh, bloated, active decay, advanced decay and skeletonized, according to the characteristics described by Bornemissza (1957). The environmental temperature during the experimentation in field varied from 28 to 30º C while the air relative humidity was between 72 to 100%.

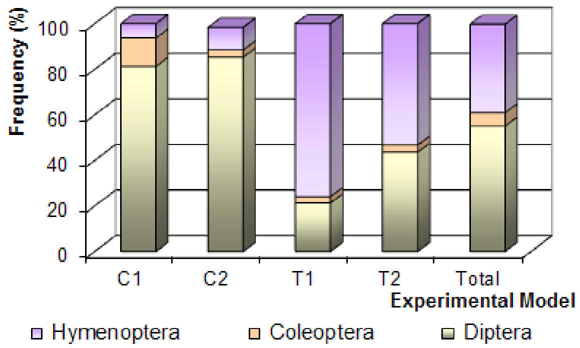

Figure 2. Frequency of insects collected from carcasses exposed in a suburban area of the Atlantic Rainforest Domain from southeastern Brazil, July to August of 2006. C1 = Control 1; C2 = Control 2; T1 = Treatment 1; T2 = Treatment 2.

Discussion

The carcasses that received the insecticide treatment were decomposed more slowly, being found a "pause in decomposition" especially during the bloated stage. The larval activity was comparatively more intense in C1 and C2 than in T1 and T2, revealing the presence of many dead insects (larvae and adults) around the carcasses that received insecticide treatment.

The results showed a direct influence of pyrethrins and pyrethroids in the development of insects in the carcasses, which may have been the cause of lower activity and high mortality of larvae, and may had favored the high level of parasitoids observed.

Gunatilake & Goff (1989) observed a delayed at the oviposition in carcasses by effect of the pesticide malathion; Wolff *et al*. (2004) observed the repellent action effect restricted to the mouth of rabbits treated orally and intracardiac with parathion. Yan-Wei *et al*. (2010) reported altered decomposition rates and species diversity in rabbits treated with malathion, and found increases in the period of larval development sufficient to alter postmortem interval estimates. Abd El-bar & Sawaby (2011) reported an incomplete 40 days post-killing decay of carcasses poisoned by the organophosphate pesticide pirimiphos-methyl, contrasting with control carcasses which reached the skeletal stage in 19 days.

There was no doubt that these compounds can alter the decomposition process, impairing the activity and development of insects present in corpses and modifying the duration of stages of decomposition, which may lead to an error in estimating the postmortem interval, a factor that must be taken into consideration in investigations of such deaths.

The effects of different chemical compounds in the process of decomposition of carcasses and insects of forensic importance should be better studied, since every year are reported hundreds of deaths caused by poisoning, especially in rural areas, where the use of pesticides is more intense.

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REFERENCES

ABD EL-BAR, M.M., SAWABY R.F. 2011. A preliminary investigation of insect colonization and succession on remains of rabbits treated with an organophosphate insecticide in El-Qalyubiya Governorate of Egypt. **Forensic Sci Int 208**: e26-e30

AGENCIA PARA SUSTANCIAS TÓXICAS Y EL REGISTRO DE ENFERMEDADES (ATSDR). 2003. **Reseña Toxicológica de las Piretrinas y los Piretroides**. Atlanta, GA: Departamento de Salud y Servicios Humanos de EE.UU., Servicio de Salud Pública.

ALMEIDA, W.F. 1985. Agrotóxicos. **Cad Saude Publica 1**: 220-249.

BORNEMISSZA, G.F. 1957. An analysis of arthropod succession in carrion and the effect of its decomposition on the soil fauna. ***Aust J Zool 5***: 1-12.

BORROR, D.J., DELONG, D.M. 1969. **Introdução ao estudo dos insetos.** Edgard Blücher/ EDUSP, São Paulo, 653 pp.

CARVALHO, C.J.B., RIBEIRO, P.B. 2000. Chave de identificação das espécies de Calliphoridae (Diptera) do sul do Brasil. ***Rev Bras Parasitol Vet 9***: 169-173.

CEPAGRI – UNICAMP [homepage on the Internet]. Campinas-SP: Centro de Pesquisas Metereológicas e Climáticas Aplicadas à Agricultura. [cited 2006 Nov 06]. Clima dos Municípios Paulistas. São Bernardo do Campo; [about 1 screen]. Available from: <**http://www.cpa.unicamp.br/outras-informacoes/clima_muni_ 547.html**>.

GOFF, M.L., OMORI, A.I., GUNATILAKE, K. 1988. Estimation of postmortem interval by arthropod succession: three cases studies from the Hawaiian Islands. **Am J Forensic Med Pathol 9**: 220-225.

GUNATILAKE, K., GOFF, M.L. 1989. Detection of organophosphate poisoning in a putrefying body by analyzing arthropod larvae. **J Forensic Sci 34**: 714-716.

LEITÃO-FILHO, H.F. 1993. **Ecologia da Mata Atlântica em Cubatão.** UNESP/UNICAMP, Campinas-SP, 184 pp.

MELLO, R.P. 2003. Chave para a identificação das formas adultas das espécies da família Calliphoridae (Diptera, Brachycera, Cyclorrapha) encontradas no Brasil. **Entomologia y Vectores 10**: 255-268.

OLIVEIRA-COSTA, J. 2003. **Entomologia forense – quando os insetos são vestígios.** Millennium, Campinas-SP, 257 pp.

TABARELLI, M., PINTO, L.P., SILVA, J.M.C., HIROTA, M., BEDÊ, L.C. 2005. Desafios e oportunidades para a conservação da biodiversidade na Mata Atlântica brasileira. **Megadiversidade 1**: 132 – 138.

WOLFF, M., BUILES, A.B., 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. **Aggrawal's Internet Journal of Forensic Medicine and Toxicology 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**: 46-51.

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