J Forensic Sci, January 2009, Vol. 54, No. 1
doi: 10.1111/j.1556-4029.2008.00926.x
Available online at: www.blackwell-synergy.com

Helena G. Oliveira,[1] *B.Sc.; Guilherme Gomes,*[1] *M.Sc.; José J. Morlin Jr,*[1] *B.Sc.; Cláudio J. Von Zuben,*[1] *Ph.D.; and Arício X. Linhares,*[2] *Ph.D.*

The Effect of Buscopan® on the Development of the Blow Fly *Chrysomya megacephala* (F.) (Diptera: Calliphoridae)

ABSTRACT: This work investigated the effects of butylscopolamine bromide, a drug present in the pharmaceutical formulation Buscopan®, on the development of *Chrysomya megacephala*, a blow fly species of considerable forensic and medical importance in Brazil. Larvae exposed to the drug showed a decreased rate of development, with higher drug concentrations further retarding the development. Besides, larvae reared on the presence of the drug showed smaller body weight and body length when compared with larvae reared on the absence of Buscopan®. The drug also affected the mortality of the species.

KEYWORDS: forensic science, *Chrysomya megacephala,* entomotoxicology, forensic entomology, butylscopolamine bromide

Flies of the genus *Chrysomya* (Diptera: Calliphoridae) are originally from Africa and Australasia, and three species, *C. megacephala* (Fabricius), *C. albiceps* (Wiedemann), and *C. putoria* (Wiedemann), were probably introduced into the New World by ships (1–3). These flies have great medico-sanitary importance as they carry enteropathogenic organisms such as viruses, bacteria, protozoans, and helminths (4), and may cause myiasis on animals and humans (5,6). They are also of fundamental importance in forensic entomology studies, as they can be indicators of the postmortem interval (PMI) of human cadavers (7,8).

Insects associated with human remains can provide valuable information in criminal investigations. Forensic entomology is the application of studies with insects and other arthropods associated with decomposing human corpses, and may provide valuable information in investigations of the causes of death (8–11). Of vital importance is the PMI, which is calculated based on the capture and age determination of the specimens, mainly insects, found in the corpse when it is first located. The determination of the PMI requires knowledge about the habits and biology of the necrophagous species (11,12).

Several factors may affect the determination of the PMI, making the criminal investigation more difficult and, when not taken into consideration, leading to errors in the PMI estimate. Postfeeding larval dispersion, competition, predation, parasitism, environmental conditions, and the presence of toxins/drugs in the corpse should be analyzed together, so that errors in the PMI estimate are minimized as much as possible (13).

Entomotoxicology is a relatively recent branch of forensic entomology, and it has advanced greatly over the last several decades. This science deals with the application of toxicological analyses of carrion-feeding insects to identify drugs and toxins present in tissues (14). It is based on the principle that insects may serve as a reliable alternative for toxicological analysis of bodies in advanced states of decomposition, where other commonly used sources such as blood, urine, or internal organs are no longer available (14–18). The results obtained from chromatographic analyses are among the positive aspects of using insect larvae instead of corpse tissues. In addition to being easily collected and reared, larvae contain fewer contaminants than the corpse tissues (19).

The presence of drugs in corpse tissues may affect the development rate of the larvae breeding there (20–25). As the developmental rate is the basis for PMI determination, toxicological analysis of larvae is of great importance to correctly determine the time of death.

Drugs such as atropine, scopolamine, and others are competitive antagonists of acetylcholine in autonomous effectors innervated by cholinergic postganglion nerves, as well as in smooth muscle, which does not have cholinergic innervations (26,27). Butylscopolamine bromide is the active ingredient of Buscopan® (Boehringer Ingelheim, Brazil), which acts as a depressant of the central nervous system. It was chosen for the study for its common administration as an antispasmodic drug. The effects produced by scopolamine, such as sedation, calming, and amnesia are useful in many hospital procedures, including obstetrics. It is commonly administrated as butylscopolamine bromide, which is more rapidly absorbed (26,27). Scopolamine intoxication is relatively common, as it is included in some commercial hypnotic solutions (27).

In this study, we conducted experiments with the blow fly, *C. megacephala*, reared in artificial diets containing Buscopan®, to study the pattern of larvae development when exposed to the drug.

Methodology

Adults of *C. megacephala* were collected at the Biosciences Institute of UNESP (Rio Claro City, São Paulo, Brazil). Decaying organic matter, such as bovine and fish meat, was used as bait and the specimens collected were maintained in screen cages in a controlled temperature room at 27 ± 1°C, 60% ± 10% relative humidity, and a 12-h photoperiod. The larvae used in the experiment

[1]Departamento de Zoologia/IB, UNESP—São Paulo State University, Campus de Rio Claro, CP 199, Bela Vista, CEP 13506-900, Rio Claro, SP, Brazil.

[2]Departamento de Parasitologia, Instituto de Biologia, Universidade Estadual de Campinas, CP 6109, CEP 13083-970, Campinas, SP, Brazil.

Received 18 Aug. 2007; and in revised form 17 May 2008; accepted 17 Aug. 2008.

were obtained from colonies established from eggs of these flies. We used a ZEISS® Stemi SV 11 stereomicroscope (ZEISS®, Germany) to count and separate the newly hatched larvae in groups of 400 individuals each, which were kept in glass vials covered with nylon. The vials were maintained in an Eletrolab® climatic chamber (Eletrolab®, São Paulo, Brazil) with temperature, relative humidity, and photoperiod as cited for the adults. The larvae were fed on artificial diet prepared according to Leal et al. (28). After the diet preparation, and while it was still liquid, Buscopan® was added to the diet in four different concentrations, according to a rat lethal dose (RLD) of 1270 mg/kg (29). The concentrations studied were: a quarter of the rat lethal dose (0.25RLD), half of the rat lethal dose (0.5RLD), full rat lethal dose (RLD), and twice the rat lethal dose (2RLD). Four replicates of 400 larvae each were performed for each concentration and for the control group (control), which was performed with artificial diet without the drug. All experiments were performed with 400 larvae in 130 g of diet for each concentration and each replicate. Every 6 h, 10 larvae from each vial were randomly chosen, and individually weighted and measured, as an indicator of the development rate. We measured the wet weights with an OHAUS® digital scale (Ohaus Corporation, Pine Brooke, NJ). The larvae measures were performed with a ZEISS® stereomicroscope. Before the measurements, larvae were placed in small vessels containing water at 60°C, which causes an immediate stretching of the larvae and thus allows a more precise length measurement (30).

Near the end of their development, the larvae were transferred to pots with vermiculite to pupate. Following the emergence of the adults, the vermiculite pots containing the puparia were kept in the growth chambers for 3 days, to watch for any possible additional adult emergence. The puparia were then removed and the vermiculite was checked for remaining unenclosed pupae, which would indicate pupal mortality.

Statistical analyses of the blow fly developmental rate were performed using SAS®—Statistical Analysis System (31). Analysis of variance (one-way) was performed to investigate possible differences between the different treatments and the posteriori Duncan multiple comparisons test was performed to compare the collected data and identify the nonsimilar groups. All tests were performed with a global level of significance of 5% ($p < 0.05$). The response variables of larval weight and larval length were used to compare the different treatments and development intervals (every 6 h). We also compared the response variable total development time with the treatments studied in this experiment.

Results

Effect of Different Buscopan® Concentrations on Larval Development

The immatures that were exposed to 2RLD showed no significant increase in weight or length until their death, which occurred after 54 h of development. As a result, data on 2RLD concentration was not analyzed statistically.

Despite the exclusion of the 2RLD data, the statistical tests indicated a significantly different effect of the groups studied (control, 0.25RLD, 0.5RLD, and RLD) on the three response variables analyzed: developmental time ($F = 100.39$; $p < 0.0001$), larval weight ($F = 99.10$; $p < 0.0001$), and larval length ($F = 41.15$; $p < 0.0001$).

We observed significant differences among development times in larvae reared on different drug concentrations (Table 1). The curves for developmental time versus body weight and length are shown in Figs. 1 and 2, respectively. Those figures show the values of body weight and body length that were obtained every 6 h throughout the whole development period.

TABLE 1—*Results of Duncan's test for mean time of development of* Chrysomya megacephala *larvae reared at different concentrations of the RLD of butylscopolamine bromide.*

Treatment	Mean Time of Development (h)
Control	96 a*
0.25 RLD	96 a
0.5RLD	106 b
RLD	150 c

*Mean in a line followed by the same letters are not significantly different ($p < 0.05$).
RLD, rat lethal dose.

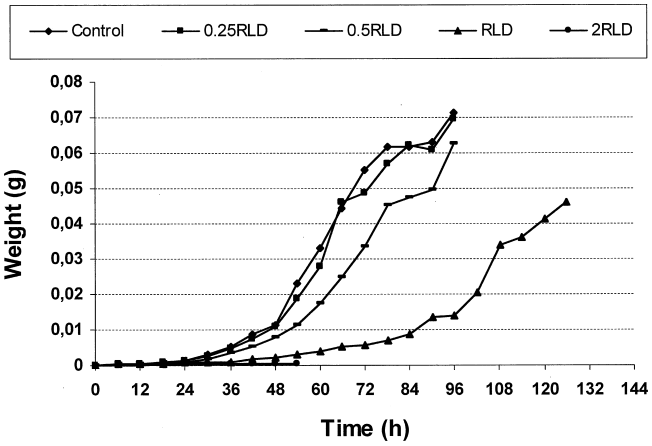

FIG. 1—*Development rates of* Chrysomya megacephala *larvae reared on diet containing different concentrations of the rat lethal doses (RLD) of butylscopolamine bromide, as correlated to body weight.*

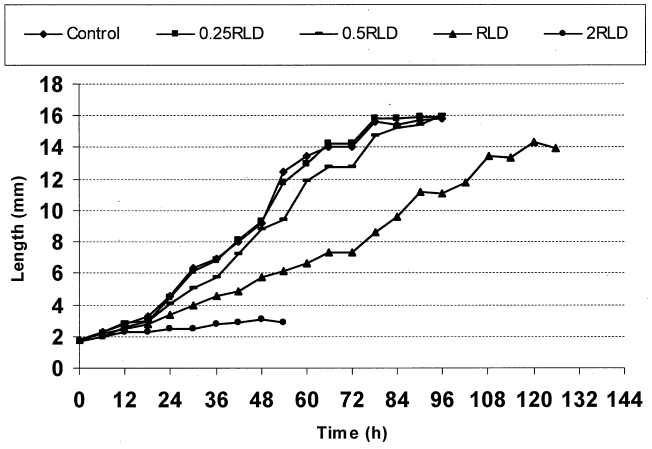

FIG. 2—*Development rates of* Chrysomya megacephala *larvae reared on diet containing different concentrations of the rat lethal doses (RLD) of butylscopolamine bromide, as correlated to body length.*

The mean values and SDs of larval weight are shown in Table 2. The observed differences in larval weight related to drug exposure started at 12 h ($F = 25.34$; $p < 0.0001$). The Duncan test showed that 0.25RLD and control were statistically similar at 12 h, while 0.5RLD differed from both treatments by showing a smaller weight than control and 0.25RLD, while RLD was different from all the others, with the smallest mean weight of all groups. At 18 h, all

TABLE 2—*Mean weights (mg) (±SD) of* Chrysomya megacephala *during development on five different concentrations of the RLD of butylscopolamine bromide.*

Time (h)	Control	0.25RLD	0.5RLD	RLD	2RLD
0	0.11 (0.01)a	0.12 (0.01)a	0.12 (0.01)a	0.12 (0.01)a	0.13 (0.01)a
6	0.26 (0.03)a	0.27 (0.03)a	0.27 (0.04)a	0.24 (0.01)a	0.19 (0.04)b
12	0.53 (0.10)a	0.49 (0.05)a	0.44 (0.05)b	0.33 (0.03)c	0.18 (0.04)d
18	0.93 (0.10)a	0.81 (0.09)b	0.74 (0.14)c	0.42 (0.12)d	0.19 (0.06)e
24	1.64 (0.40)a	1.36 (0.28)b	1.01 (0.22)c	0.71 (0.06)d	0.18 (0.06)e
30	3.26 (0.20)a	2.54 (0.43)b	1.88 (0.36)c	0.84 (0.10)d	0.25 (0.02)e
36	05.5 (0.45)a	4.61 (0.83)b	3.07 (1.01)c	1.05 (0.12)d	0.35 (0.05)e
42	9.04 (0.29)a	7.13 (1.31)b	4.83 (1.74)c	1.66 (0.22)d	0.41 (0.11)e
48	14.12 (3.40)a	10.25 (1.21)b	7.66 (1.52)c	2.33 (0.24)d	0.40 (0.06)e
54	28.67 (8.19)a	16.15 (4.02)b	9.92 (3.44)c	2.94 (0.57)d	0.36 (0.07)e
60	38.73 (7.92)a	23.36 (7.75)b	15.83 (4.58)c	3.70 (0.73)d	
66	50.57 (10.91)a	39.77 (7.83)b	21.10 (10.58)c	4.87 (1.33)d	
72	58.55 (5.95)a	47.63 (6.47)b	30.25 (13.24)c	5.61 (1.13)d	
78	63.32 (4.04)a	55.23 (2.87)b	41.84 (9.64)c	6.85 (2.08)d	
84	63.54 (4.64)a	60.74 (1.83)a	42.71 (11.59)b	8.11 (2.31)c	
90	65.33 (9.42)a	63.21 (3.76)a	48.78 (5.14)b	11.41 (4.43)c	
96	71.28 (3.84)a	70.36 (2.09)a	59.06 (8.46)b	13.61 (5.08)c	
102			54.77 (5.17)a	18.44 (6.94)b	
108			51.6 (6.32)a	33.91 (20.19)b	
114			55.77 (5.87)a	36.00 (13.31)b	
120			60.55 (7.86)a	41.42 (23.41)b	
126			57.45 (6.54)a	42.99 (16.18)b	
132				40.00 (17.77)	
138				37.48 (17.20)	
144				40.58 (18.05)	
150				52.19 (28.62)	

Mean in a line followed by the same letters are not significantly different.

RLD, rat lethal dose.

TABLE 3—*Mean lengths (mm) (±SD) of* Chrysomya megacephala *during development on five different concentrations of the RLD of butylscopolamine bromide.*

Time (h)	Control	0.25RLD	0.5RLD	RLD	2RLD
0	1.74 (0.13)a	1.70 (0.09)a	1.68 (0.19)a	1.73 (0.09)a	1.64 (0.21)a
6	2.30 (0.23)a	1.98 (0.31)b	2.30 (0.38)b	2.21 (0.14)b	2.01 (0.21)c
12	2.72 (0.12)a	2.55 (0.36)ab	2.83 (0.27)a	2.43 (0.19)b	2.31 (0.31)c
18	3.23 (0.14)a	3.00 (0.21)a	2.98 (0.39)a	2.82 (0.26)b	2.23 (0.36)c
24	4.58 (0.49)a	4.50 (0.41)b	4.10 (0.78)c	3.33 (0.13)d	2.44 (0.27)e
30	6.32 (0.32)a	6.18 (0.37)b	5.04 (0.83)c	3.94 (0.50)d	2.48 (0.45)e
36	6.93 (0.35)a	6.82 (0.43)a	5.75 (1.02)b	4.51 (0.30)c	2.79 (0.42)d
42	8.00 (0.42)a	8.07 (0.70)b	7.19 (0.74)c	4.83 (0.43)d	2.89 (0.25)e
48	9.21 (1.48)a	9.34 (0.48)b	8.81 (1.08)c	5.73 (0.26)d	3.01 (0.26)e
54	12.41 (1.26)a	11.79 (0.96)b	9.37 (1.26)c	6.15 (0.43)d	2.83 (0.37)e
60	13.41 (0.56)a	12.95 (0.66)b	11.89 (0.89)c	6.65 (0.47)d	
66	14.05 (1.11)a	14.26 (0.59)b	12.80 (1.37)c	7.34 (0.61)d	
72	15.57 (0.66)a	14.94 (0.77)b	13.09 (1.32)c	7.60 (0.73)d	
78	15.60 (0.59)a	15.82 (0.33)a	14.77 (0.98)b	8.57 (0.13)c	
84	15.42 (0.70)a	15.82 (0.47)a	15.24 (1.04)b	9.55 (0.23)c	
90	15.68 (0.58)a	15.94 (0.32)a	15.43 (0.76)b	11.17 (1.89)c	
96	15.86 (0.80)a	15.96 (0.23)a	15.97 (0.82)a	11.09 (1.91)b	
102			15.06 (0.59)a	11.80 (1.70)b	
108			15.41 (0.72)a	13.46 (3.93)b	
114			15.17 (0.63)a	13.33 (2.80)b	
120			15.58 (0.77)a	14.34 (3.94)b	
126			15.70 (0.72)a	13.98 (2.12)b	
132				13.20 (2.17)	
138				14.01 (2.79)	
144				14.58 (2.57)	
150				15.05 (3.29)	

Mean in a line followed by the same letters are not significantly different.

RLD, rat lethal dose.

groups were different from each other ($F = 53.69$; $p < 0.0001$), forming a gradation of decreasing weight as the drug concentration increased. That difference continued up to 84 h, when treatments 0.25RLD and control were similar. Groups 0.5RLD and RLD remained different up to the end of their development.

A similar pattern of drug effect was observed in relation to the larval body length. The mean values and SDs of larval length are shown in Table 3. The differences among the groups initiated as soon as 6 h ($F = 5.19$; $p = 0.0019$). Duncan tests showed that at 6 h, 0.25RLD, 0.5RLD, and RLD did not differ from each other, but larvae from all of them were shorter than those from the control group. At 12 h, control, 0.25RLD, and 0.5RLD had similar lengths, but RLD was statistically similar to 0.25RLD and different from 0.5RLD and control ($F = 5.30$, $p = 0.0017$). At 18 h, RLD showed smaller values of length than the other treatments ($F = 6.48$; $p = 0.0004$), but there was no difference between control, 0.25RLD and 0.5RLD. At hours 24 and 30, all groups were different from each other.

At 36 h, both 0.5RLD and RLD individually showed lower values of body lengths and they also differed from all other treatments ($F = 128.62$; $p < 0.0001$), but control and 0.25RLD had similar lengths. From 42 to 72 h, all groups were statistically different from each other, and higher concentrations of the drug showed smaller lengths. At 78 h, there still were differences between the groups ($F = 498.20$; $p < 0.0001$), but 0.25RLD was similar to control. This situation continued up to 96 h, when there were significant differences ($F = 248.41$; $p < 0.0001$) between RLD and the other groups (control, 0.25RLD, and 0.5RLD were similar to each other). At 96 h, larvae from the control groups and 0.25RLD left the diet to pupate. The 0.5RLD larvae pupated at 126 h. From 96 up to 126 h, the treatments 0.5RLD and RLD showed statistically different values of lengths.

Larval and Pupal Mortality

Larval and pupal mortality percentages in each experimental group are illustrated in Table 4. The values indicate that higher drug doses are related to a greater mortality, especially during the larval stage. During that period, all treatments showed higher mortality values than control. The mortality percentages of the drug doses 0.25RLD and 0.5RLD were similar and higher than control. The treatment RLD showed an expressive increase on mortality values, and the highest dose tested, 2RLD, caused all the larvae to die within 54 h of development. Hence, there were no pupae originated from the treatment 2RLD. The highest mortality during the pupal stage was observed on treatment RLD.

Discussion

Effect of Different Drug Concentrations on Larval Development

All larvae of the 2RLD treatment died within 54 h. This indicates a strong negative effect of butylscopolamine bromide on the

TABLE 4—*Larval and pupal mortality rates of* Chrysomya megacephala *larvae reared on diet containing different concentrations of the RLD of butylscopolamine bromide.*

Treatment	Larval Mortality Rate (%)	Pupal Mortality Rate (%)
Control	21.75	3.19
0.25RLD	25.75	2.35
0.5RLD	25.25	2.67
RLD	31.25	8.00
2RLD	100	–

RLD, rat lethal dose.

development and survivorship of this species when reared in an artificial medium containing the drug. In addition, a strong negative influence was also caused by the lethal dose RLD, which caused a 54 h delay in the development of the flies. Such influence of butylscopolamine bromide on developmental time required for pupation suggests that this drug may cause significant bias in PMI estimation when large amounts of the drug are ingested prior to death. Similar effects were also determined in other studies (16,24,32) that performed experiments with methamphetamine, cocaine, and morphine. In those studies, morphine also caused a delay in the development, while methamphetamine and cocaine were related to faster development rates. Other studies that were performed with artificial diet (15,33) have also showed the effects of drugs on blow fly larvae. However, attention must be paid to the fact that in this study, the larvae were reared in an artificial diet containing different concentrations of the drug, and thus the drug was not metabolized by a living system, which would have altered the availability of the drug and its metabolites for the larvae.

In our work, there was a decrease in the rate of weight gain on the control group at 72 h. At that time, the control larvae were close to an ideal weight for pupation, and so they slow down their weight gain. The treatment 0.25RLD continues to gain weight up to the end of the larval development (96 h), when the control group is slightly heavier than 0.25RLD. Thus, the control and 0.25RLD pupate at the same time with not much difference on the larval weight.

In general, control larvae developed faster than the ones from all other groups. In addition, larvae exposed to butylscopolamine bromide weighed less than the control larvae. With regards to length gain, the fact that the control group had a higher mean length at 6 h, and then was equal to 0.25RLD and 0.5RLD at 12 and 18 h, suggests that butylscopolamine bromide may have a strong effect in the beginning of development, and then the effects occur at a lower rate, after a prolonged exposure to higher concentrations of the drug.

If weight and length values achieved by the control group are considered the ideal condition for pupation, the deviations on those values caused by butylscopolamine bromide may induce biological alterations on adults, such as small body size and lower fecundity rate. Although there are no studies on the effect of butylscopolamine bromide on insects, the present study and the depressive effect of it in the human nervous system suggest that this drug may alter the biology of *C. megacephala*, causing the larvae to take a longer time to achieve an ideal weight for pupation.

Pupal and Larval Mortality

The larval mortality rate expected for the initial level of larval aggregation established in this experiment is around 30% (34), which probably differs from the data shown in our experiment because the other authors did not remove any larvae from the experimental vials. Thus, in that work, there was no interference from the decreasing number of individuals on the larvae aggregation.

The mortality rates suggest that the larval stage is more susceptible to butylscopolamine bromide, causing a delay in the larval development and an increase in the mortality rate. Besides, the larvae that are able to survive in the presence of the drug seem to have a better chance of completing development, which is suggested by the lower pupal mortality rate in 0.5RLD and 0.25RLD rather than in control. Only when reared at the RLD concentration did *C. megacephala* present higher mortality rates in all development stages. Such a fact is probably due to the lower weight shown by larvae reared at this concentration by the time of pupation.

In terms of differential mortality between larvae and pupae, there is also the possibility of the larvae actually eliminating a lot of the drug before pupation. This may also contribute to higher pupal survival rates.

References

1. Imbiriba AS, Izutani DT, Milhoreto IT, Luz E. Introdução da *Chrysomya chloropyga* (Wiedemann, 1818) na região Neotropical (Diptera: Calliphoridae). Arquivos de Biologia e Tecnologia 1977;20:35–9.
2. Guimarães JH, Prado AP, Linhares AX. Three newly introduced blow fly species in Southern Brazil (Diptera: Calliphoridae). Revista Brasileira de Entomologia 1978;22:53–60.
3. Guimarães JH, Prado AP, Buralli GM. Dispersal and distribution of three newly introduced species of Chrysomya Robineau-Desvoidy in Brazil (Diptera, Calliphoridae). Revista Brasileira de Entomologia 1979;23:245–55.
4. Furlanetto SMP, Campos MLC, Hársi CM, Buralli GM, Ishihata GK. Microrganismos enteropatogênicos em moscas africanas pertencentes ao gênero *Chrysomya* (Diptera: Calliphoridae) no Brasil. Revista de Microbiologia 1984;15:170–4.
5. Zumpt F. Myiasis in man and animals in the Old World. London: Butterworths, 1965.
6. Guimarães JH, Papavero N, Prado AP. As miíases na região neotropical (identificação, biologia, bibliografia). Revista Brasileira de Zoologia 1983;1(4):239–416.
7. Greenberg B. Behavior of postfeeding larvae of some Calliphoridae and a muscid (Diptera). Ann Entomol Soc Am 1990;83:1210–4.
8. Von Zuben CJ, Bassanezi RC, Dos Reis SF, Godoy WAC, Von Zuben FJ. Theoretical approaches to forensic entomology: I. Mathematical model of postfeeding larval dispersal. J Appl Ent 1996;120:379–82.
9. Keh B. Scope and applications of forensic entomology. Annu Rev Entomol 1985;30:137–54.
10. Smith KGV. A manual of forensic entomology. London: British Museum and Cornell University Press, 1986.
11. Von Zuben CJ. Zoologia aplicada: recentes avanços em estudos de entomologia forense. Entomologia y Vectores 2001;8(2):173–83.
12. Oliveira-Costa J. Entomologia forense: quando os insetos são vestígios. Campinas: Millenium, 2003.
13. Amendt J, Krettek R, Zehner R. Forensic entomology. Naturwissenschaften 2004;91:51–65.
14. Introna F, Campobasso CP, Goff ML. Entomotoxicology. Forensic Sci Int 2001;120:42–7.
15. Sadler DW, Robertson L, Brown G, Fuke C, Pounder DJ. Barbiturates and analgesics in *Calliphora vicina* larvae. J Forensic Sci 1997; 42(3):481–5.
16. Hédouin V, Bourel B, Martin-Bouyer L, Bécart A, Tournel G, Deveaux M, et al. Morphine perfuse rabbits: a tool for experiments in forensic entomotoxicology. J Forensic Sci 1999;44(2):347–50.
17. Carvalho LML, Linhares AC, Trigo JR. Determination of drug levels and the effect of diazepam on the growth of necrophagous flies of forensic importance in southeastern Brazil. Forensic Sci Int 2001;120: 140–4.
18. Campobasso CP, Gherardi M. Drug analysis in blow fly larvae and in human tissues: a comparative study. Int J Legal Med 2004;118:210–4.
19. Kintz P, Tracqui A, Mangin P. Toxicology and fly larvae on a putrefied cadaver. J Forensic Sci Soc 1990;30:243–6.
20. Goff ML, Omori AI, Goodbrod JR. Effect of cocaine in tissues on the development rate of *Boetcherisca peregrina* (Diptera: Sarcophagidae). J Med Entomology 1989;26:91–3.
21. Goff ML, Brown WA, Hewadikaram KA, Omori AI. Effects of heroin in decomposing tissues on the development rate of *Boettcherisca peregrina* (Diptera: Sarcophagidae) and implications of this effect on estimation of postmortem intervals using arthropod development patterns. J Forensic Sci 1991;36:537–42.
22. Goff ML, Brown WA, Omori AI, LaPointe DA. Preliminary observations of the effects of amitriptyline in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and implications of this effect to estimation of postmortem interval. J Forensic Sci 1993;38:316–22.
23. Introna F, Lo Dico C, Caplan YH, Smialek JE. Opiate analysis in cadaveric blow fly larvae as an indicator of narcotic intoxication. J Forensic Sci 1990;35:118–22.

24. Nolte KB, Pinder RD, Lord WD. Insect larvae used to detect cocaine poisoning in a decomposed body. J Forensic Sci 1992;37:1179–85.
25. Gagliano-Candela R, Aventaggiato L. The detection of toxic substances in entomological specimens. Int J Legal Med 2001;114:197–203.
26. Brown JH, Taylor P. Muscarinic receptor agonists and antagonists. In: Goodman & Gilman's. The Pharmacological Basis of Therapeutics, 9th edn. International Edition: McGraw-Hill, 1996;141–60.
27. Goth AMD. Grupo atropínico das substâncias bloqueadoras colinérgicas. In: Koogan G, editor. Farmacologia médica, 9th edn. Rio de Janeiro: Guanabara Koogan, 1981;96–106.
28. Leal TTS, Prado AP, Antunes AJ. Rearing the larvae of the blow fly *Chrysomya chloropyga* (Wiedemann) (Diptera: Calliphoridae) on oligidic diets. Revista Bras Zoologia 1982;1(4):1–44.
29. NTP—National Toxicology Program, 1978. http://www.ntp-server.niehs.nih.gov, accessed 12/2004.
30. Tantawi TI, Greenberg B. The effect of killing and preservative solutions on estimates of maggot age in forensic cases. J Forensic Sci 1993;38:702–7.
31. SAS Institute Inc. SAS user's guide: statistics, 6th ed. Cary, NC: SAS Institute Inc., 1986.
32. Goff ML, Brown WA, Omori AI. Preliminary observations of the effects of methamphetamine in decomposing tissues on the development of *Parasarcophaga ruficornis* (Diptera: Sarcophagidae) and implications of this effect to estimation of postmortem interval. J Forensic Sci 1992;38:316–22.
33. Sadler DW, Richartson J, Haigh S, Bruce G, Pounder DJ. Amitriptyline accumulation and elimination in *Calliphora vicina* larvae. Am J Forensic Med Pathol 1997;18:397–403.
34. Godoy WAC, Von Zuben CJ, Reis SF, Von Zuben FJ. The spatial dynamics of native and introduced blow flies (Dipt., Calliphoridae). J Appl Entomol Alemanha 1997;121:305–9.

Additional information and reprint requests:
Helena G. Oliveira, B.Sc.
Departamento de Zoologia/IB
UNESP—São Paulo State University
Campus de Rio Claro
CP 199, Bela Vista
CEP 13506-900
Rio Claro, SP
Brazil
E-mail: helenago@gmail.com