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Experimental study of *Lucilia sericata* (Diptera Calliphoridae) larval development on rat cadavers: Effects of climate and chemical contamination

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ABSTRACT

Household products such as bleach, gasoline or hydrochloric acid have been used to mask the presence of a cadaver or to prevent the colonization of insects. These types of chemicals affect insect development and alter the forensic entomology analysis. This study was designed to test the effects of six household products (bleach, mosquito repellent, perfume, caustic soda, insecticide and unleaded gasoline) on blowfly (*Lucilia sericata*, Diptera: Calliphoridae) larval development. Furthermore, the effects of climate (rain or dry conditions) on larval development were analyzed. For each replication, 100 first instars were placed on a rat cadaver on which one household product was spilled. We observed a decrease in the survival rates of the larvae but no significant effect on their development times or the adult size. The same trends were observed under rainy conditions. However, the rain altered the effects of some tested household products, especially gasoline. These results demonstrate for the first time the successful development of necrophagous larvae on chemically contaminated cadavers, and provide evidence for the range of possible effects to expect.

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1. Introduction

Forensic entomology estimates the time of death by colonization of the corpse with necrophagous insects. However, cadaver accessibility [1], weather conditions [2,3] and chemical contamination [4,5] affect the insect pre-appearance interval (PAI), i.e., the time elapsed between death and the colonization by necrophagous insects [6]. Marchenko [7] showed that gas, paint or a lubricant disrupted the arrival and the larval development of Calliphorid flies. In a 2009 study, Charabidze et al. [4] demonstrated that household products delayed the appearance of flies on a corpse. Using an olfactometer (controlled conditions), the authors showed that an insecticide, unleaded gasoline, hydrochloric acid and patchouli perfume strongly repelled adult flies. Furthermore, field experiments showed that rat cadavers covered with spilled hydrochloric acid, unleaded gasoline or patchouli perfume delayed PAI by up to 60 h. In a recent study, we showed that some household products mixed with beef liver rearing substrate also affected larval development [8]. Patchouli perfume, caustic soda, an insecticide, hydrochloric acid, mosquito repellent and unleaded gasoline increased the larval mortality rate and the larval development time (minimum, maximum and median times) and decreased the size of adults. Additionally, these effects were correlated with the quantities of household products mixed into the meat. Nevertheless, this study was performed under laboratory conditions, and the likely effects of climate was not considered. Using various field experiments, Marchenko [7] demonstrated that weather conditions affected the diffusion or evaporation of contaminants (paint and gasoline) spilled on a corpse and changed the effects on larval development. Moreover, rain increased gasoline diffusion into flesh, and thus altered the effects of this chemical on necrophagous insects.

The present study was performed under laboratory conditions and focused on the effects of rain on the development of necrophagous larvae. The experimental design, based on the study of Aubernon et al. [8], followed larvae development on rat cadavers, treated with various household products, under dry or rain conditions. The survival rate and the development time of the

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larvae and the size of adults were used to assess the effects of rainfall on chemicals and subsequent necrophagous larval development. The relevance of the results for forensic entomology analysis is discussed, particularly for the estimation of the Post Mortem Interval (PMI).

2. Materials and methods

2.1. Insect rearing

The experiments were performed with *Lucilia sericata* (Meigen), (Diptera: Calliphoridae), which were obtained from rearing colonies (Lille, France). The adult flies were raised according to Aubernon et al. [8]. To trigger egg-laying, 25 ± 5 g of minced beef liver was placed in an insectarium. The presence of eggs was checked for hourly, giving an oviposition time known within ±30 min. The eggs were placed on minced beef liver in a climate chamber (SANYO MIR, 554) at 25 ± 0.1 °C.

2.2. Experiment rearing media

The study was performed on rat cadavers (*Rattus norvegicus*, Wistar strain); all the rats were thawed reproductive old males that had been sacrificed with CO_2 by a medicine animal house. Each rat was weighed before the experiment to determine the quantity of product to be spilled on the cadaver. The rats were placed on their right flank in a round plastic bucket (183 × 135 mm) filled with 5 cm of sand. The bucket was placed in a plastic box (420 × 300 × 230 mm) filled with 5 cm of sand and closed with a cover drilled with 20 holes, each 1 cm in diameter. Two hours before the start of the trials, the household products were homogeneously deposited on the left flank of the rat using a p5000 Pipetteman (BIOHIT, Sartorius). According to Charabidze et al. [4], the household product and quantities were as follows: 10 μl g^{-1} for patchouli perfume, 50 μl g^{-1} for mosquito repellent and an insecticide, and 100 μl g^{-1} for the control (water), bleach, caustic soda and unleaded gasoline. Each product had four replicates. For each replication, 100 first instars (age 22 h ± 30 min [9]) were removed from the hatching media and placed in the rat mouth using a thin paintbrush. The boxes were kept at 25 ± 0.1 °C in a climate chamber (SANYO MIR, 554).

Two climatic conditions were tested. The dry condition was maintained with rat cadavers not receiving any additional water. The rainy condition simulated rainy weather by spraying distilled water on the rat cadavers. Based on annual average French pluviometric data, 60 ml was sprayed in 5 min, which was equivalent to a rainfall of 2281 cm^3/m^2 in 5 min (i.e., 832 mm per year, 2013 annual mean Météo France pluviometry). The spray was applied from the start of the experiment until pupation, with the first spray applied 2 h after the larvae were deposited, and the second and the third sprays applied at the same time on the two following days. In the middle of their development (234.5 h ± 30 min [9]), pupae were removed, counted and placed in a plastic box (60 × 85 × 62 mm) filled with 2 cm of sand, and the spraying was discontinued. Although the insects seemed to have different development times, all were subjected to the same protocol with only three sprayings of water.

The emergence of the adults was recorded hourly until emergence ceased after 5 days, manually during the day and with an infrared video camera (KAMATEC, KAM-HWI-SH-7204) during the night. The adult flies were removed, counted, kept in pillboxes for an entire day at 25 °C and then frost-killed (−18 ± 1 °C for 1 h). A total of 5600 larvae were studied. Each seven products was tested 4 times for each dry and rainy condition. That means 28 replicats for each condition, that is to say 56 replicats in total.

2.3. Developmental parameters

Developmental and morphological parameters were used to determine the ability of the larvae to develop on cadavers contaminated with household products. For each replication, the pupae were counted to obtain the pupation rate, and the emerged adults were counted to obtain the survival rate to adult. The time from laying of the egg to emergence was measured with three different approaches: minimum development time (the time from egg-laying to first adult emergence), maximum development time (the time from egg-laying to last adult emergence) and median development time (time to emergence of 50% of the adult population). Adult size was determined according to Ireland and Turner [10]. The flies were sorted by gender, and the left wing of each adult fly was sampled, glued and scanned. The length of each posterior cross vein (dm-cu) was measured three times using Mesurim Pro 3.4 freeware, and a mean length was calculated.

2.4. Statistical analyses

Statistical analyses were conducted using the XLStat 2011.4.02 software by Addinsoft with a significance threshold of $\alpha = 0.05$. The Kruskal–Wallis test with the Dunn procedure was used to compare the development times and the adult sizes of the insects exposed to household products with the control. The z test was used to compare the survival rates of insects exposed to household products with that of the control and to compare the survival rates of larvae in rainy and dry conditions. The Mann–Whitney test was used for the pairwise comparison of the development time and adult size between the two conditions. Finally, the sex ratio was calculated using the ♂/♀ formula, only when the survival rate was above 10%. The χ^2 test was performed on the number of males and females to compare the sex ratio to the expected distribution (♂/♀ = 1).

3. Results

3.1. Dry condition

Under dry condition, gasoline killed all the larvae. Only the larvae exposed to bleach had a survival rate similar to that of the larvae in the control (z test: bleach, $p = 0.144$; other substances, $p < 0.0001$) (Fig. 1). The survival rate for larvae was greater than 60% for bleach and mosquito repellent, but for the other substances, it was less than 20%. The survival rates of the pupae were significantly decreased by perfume, mosquito repellent and insecticide (z test: perfume, $p = 0.011$; mosquito repellent, $p = 0.01$; insecticide, $p < 0.0001$). On the other hand, the bleach and caustic soda did not significantly affect the survival rate of pupae (z test: bleach, $p = 0.350$; caustic soda, $p = 0.345$).

Compared with the control, five of the six substances did not affect the development time of the larvae (Table 1). Only perfume increased the minimum, median and maximum larval development times with delays that exceeded two days (Kruskal–Wallis test: minimum: $K = 15.031$, $p = 0.01$; median: $K = 12.479$, $p = 0.029$; maximum: $K = 10.968$, $p = 0.052$. Dunn test: minimum: $p = 0.004$; median: $p = 0.011$; maximum: $p = 0.009$).

The presence of the substances had no effects on the blowfly sex ratio (χ^2 test: $p > 0.05$), but significantly decrease adult fly size (Kruskal–Wallis test: ♂: $K = 31.405$, $p < 0.0001$; ♀: $K = 22.230$, $p < 0.0005$). Regardless of the substance tested, the females were larger than the males by a factor of approximately 1.05 (Mann–Whitney test: control: $U = 15606$, $p < 0.0001$; bleach: $U = 16522$, $p < 0.0001$; mosquito repellent: $U = 7547.5$, $p < 0.0001$; perfume: $U = 373.5$, $p = 0.015$; caustic soda: $U = 292.5$, $p = 0.004$; insecticide: $U = 224$, $p = 0.018$). For the male flies, bleach and mosquito

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