

# **Influence of the Conditions of Exposure of Pigs Carcasses (*Sus scrofa domesticus* L.) on the Egg-Laying Delay of Necrophagous Diptera in the Guinean Zone of Ivory Coast**

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**Abstract:** A decaying cadaver is particularly attractive to necrophagous insects, more specifically Diptera. These are the first to lay their eggs on corpses. For the post mortem interval determination, the entomologist needs to know the precise time of the first egg-laying. The objective of this study is to determine the egg laying delay of these insects on a cadaver exposed in different conditions in the Guinean zone of Côte d'Ivoire. To do this, our work was carried out in a natural environment at the National Agronomic Research Center. The experimental setup consisted of four types of wire mesh cages corresponding to the following cases: cadavers exposed to the open air or control cadavers, semi-immersed cadavers, cadavers wrapped in a shroud and suspended cadavers. Work on the site has been made from 29 October to 5 November 2019. The spawning period in species of Calliphoridae, was shorter on control and suspended cadavers. In Sarcophagidae, we noted a larviposition late on the suspended cadavers. Muscidae and Fanniidae, which intervened later on the decomposing corpses, were not observed on the suspended cadavers. These carcasses quickly dried out, no longer being able to provide nutrients essential for the proper development of larvae of species of these Diptera families. Depending on the accessibility of the corpses to necrophagous insects, the first egg-laying of Diptera were observed after 6 hours of exposure and the last after 174 hours. The exposure conditions of the corpses significantly influenced the time taken to lay the main necrophagous Diptera. As the first egg-laying of the flies occurs in the first moments after death, as long as the corpses is accessible, the results obtained during these experiments should be taken into account by the expert entomologist, in the estimation of the interval post-mortem upon discovery of a corpse.

**Keywords:** Eggs-Laying Delay, Necrophagous Diptera, Corpses, Guinean Zone, Ivory Coast

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## **1. Introduction**

A decomposing corpse attracts a large and diverse fauna. This is particularly composed of necrophagous insects, in particular Diptera, whose activities allow the post-mortem interval to be estimated. The spawning involved in the first moments after death [1]. The eggs are laid in or in the immediate vicinity of the natural orifices of the cadaver [2,

3]. Knowledge of the data relating to the lengths of the development cycles of fly species and to the various environmental conditions will enable investigators to determine the dates of the first flies laying eggs [4]. Nevertheless, the triggering of egg laying depends on the state of the corpses and the pre-existing egg laying on the

cadaver [5]. The determination of the egg-laying delay can be influenced by bad climatic conditions, or human action [6]. The degradation of a corpse and its colonization by insects are two closely related phenomena and are influenced by many factors intrinsic and extrinsic to the cadaver [5, 7]. The post-mortem interval (PMI) is the time elapsed between the moment of death and the discovery of the corpse [4]. The investigations carried out for the determination of the PMI through several studies in Asia, America, Europe and South Africa, made it possible to establish databases. In Côte d'Ivoire, the work carried out by [8-12] made it possible to establish a local database of necrophagous Diptera, but the literature does not mention any data on necrophagous dipterans' egg laying delay on corpses. This study is part of a perspective of enriching this database of necrophagous Diptera. The objective of this study is to determine the egg-laying delay of these insects on a cadaver exposed in different conditions in the Guinean zone of Ivory Coast.

## 2. Material and Methods

### 2.1. Study Site

The work was carried out at the National Agronomic Research Center (NARC), located west of the city of Abidjan, precisely in Adiopodoumé at km 17. The experimental site (5° 19'40.13"N - 4° 07 '54, 80"W - Altitude 17 m) is limited to the south and east by the Ebrié lagoon, to the west by the road leading to the Institut Pasteur in Adiopodoumé and to the north by the road which leads to Dabou. It covers an area of approximately 230 hectares. There is vegetation essentially made up of a relic of primary lagoon forest covering 4/5 of the site. In its western part, a fringe of this forest relic has undergone severe degradation under the action of man to make way for vegetation made up of grasses and cassava plantations. This site houses many buildings for administration and accommodation for the agents who work there.



Figure 1. Satellite view of the study site [13].

### 2.2. Experimental Climatic Conditions

On the study site, a thermo-hygrometer recorder of the "IHM - 172SI" type was installed to record the daily temperatures and relative humidity. The Meteorological Service of the Airport, Aeronautical and Meteorological Exploitation and Development Company (SODEXAM - Abidjan) provided rainfall data. The daily climatic data recorded were used to calculate the monthly averages of temperature and relative humidity as well as the monthly total rainfall. Temperatures were between 25 and 29°C, and relative humidity between 77 and 85%. The rainfall of the study period ranged from 10.2 to 292.61 mm of rain. However, here is no rain was recorded in the first seven post-mortem days.

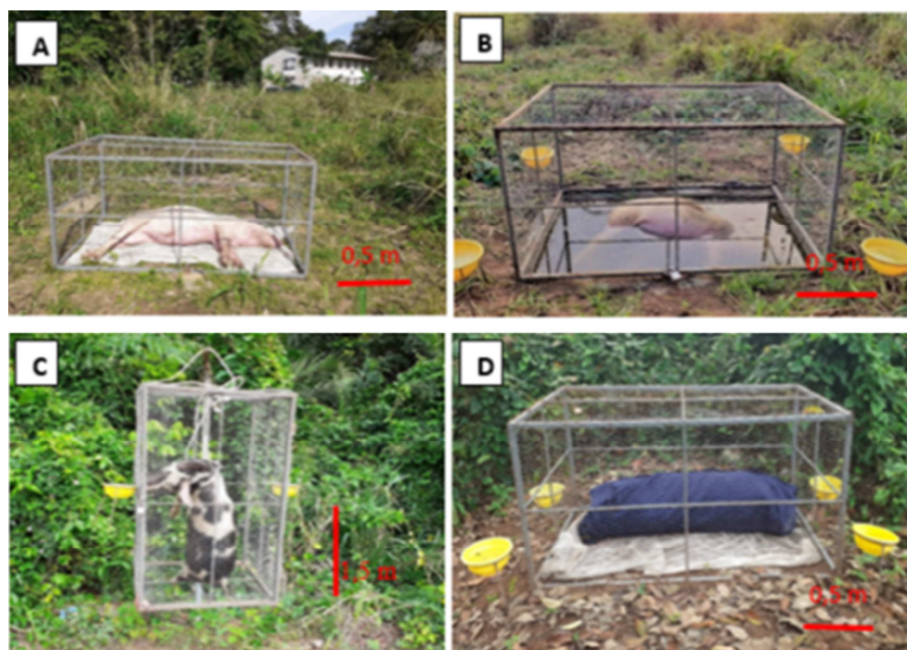
### 2.3. Methods

#### 2.3.1. Experimental Apparatus

The experimental device consisted of four types of wire mesh cages corresponding to the following cases: corpses exposed to the open air or Control Cadavers (CC), Semi-Immersed Cadavers (SIC), Cadavers Wrapped in a Shroud (CWS) and Suspended Cadavers (SPC). A freshly slaughtered pig was introduced into each cage. The dimensions of the cages of the first three cases were L=1.5 m, l=1 m and h=0.8 m. Those of the cage housing the suspended corpse were L=0.9 m, l=0.9 m and h=1.5 m.

The dimensions of the basin were L=1.7 m, l=1.2 m and the pool depth 0.7 m. Three 60 kg pigs corresponding to three repetitions were used in each case, ie a total of 12 pigs.

Twelve (12) technicians were recruited for data collection, one per repeat, or one data collector per exposed cadavers.



**Figure 2.** Exposure of the corpses in the various protection cages.

A: Exposure of the control cadavers, B: Exposure of the semi-immersed cadavers  
C: Exposure of the suspended cadavers, D: Exhibition of the wrapped cadavers.

### 2.3.2. Egg-laying Delay on Corpses Exposed in Different Types of Cages

#### 1) Exhibition of pig carcasses

After the slaughter on October 29, 2019, noted day 0 (D0), the pigs were exposed in different situations and separated from each other by a distance of 300 m in an area comprising three repetitions. The zones were separated from each other by a distance of 800 m on the same site. The aim of this distancing was to avoid a possible phenomenon of competition in the process of colonization of the various corpses by the necrophagous Diptera.

#### 2) Collection of eggs laid by the necrophagous Diptera

Observations were made every 30 minutes. It was a question of recording, using the technical sheets, the time of the first laying. Pictures were taken with a digital camera from the exposure of the pigs to the laying of the female Diptera. The collection of data relating to the laying of eggs and their samples was carried out from October 29, 2019 to November 05, 2019.

#### 3) Breeding in the laboratory

The clusters of eggs laid by female necrophagous Diptera on pig carcasses were removed five times using a knife and flexible forceps. They were then incubated on a substrate, in plastic tubs containing sand sterilized in an autoclave at 121°C and at a pressure of 1.5 bar. The sand was lightly moistened and covered with muslin. After the eggs hatched, these boxes were placed in breeding tanks and sent to the laboratory. The larvae which came out were fed with 300 g of pork liver. These larvae were followed until pupae were obtained. After sorting, these pupae were grouped together by resemblance and then introduced into

different cages and monitoring was done until the emergence of adult flies. Twenty-four hours after emergence, the flies obtained were killed by asphyxiation using carbon dioxide gas (CO<sub>2</sub>) then sorted, stung and identified. Then, the egg-laying delay of the species was determined.

#### 4) Preparation and identification of emerged Diptera

The preparation of the emerged Diptera consisted in pricking them carefully mounted on an emalene flake fixed by an entomological pin. All the prepared specimens were kept in a sorting box.

The identification was made using a binocular magnifier of the brand "Optika LAB20 ". Identification keys were used to determine the various emerged species [3, 14-24].

### 2.4. Data Processing

#### 1) Specific wealth (S)

The specific richness or total richness of a Biocenosis corresponds to the total number of all the species observed during N surveys [25].

$$S = Sp_1 + Sp_2 + \dots + Sp_n \quad (1)$$

Where S: Total number of species observed during N surveys; Sp<sub>1</sub>, Sp<sub>2</sub>, Sp<sub>n</sub> are the species collected.

#### 2) Frequency of occurrence

The frequency of occurrence (C) represents the ratio between the number of records where the species is found (P<sub>i</sub>) and the total number of records (P) [26].

$$C (\%) = P_i / P \times 100 \quad (2)$$

There are 5 classes of frequency of occurrence:

- a) Ubiquitous species ( $C=100\%$ )
- b) Constant species ( $50\% \leq C < 100\%$ )
- c) Common species ( $25\% \leq C < 50\%$ )
- d) Accessory species ( $5\% \leq C < 25\%$ )
- e) Rare species ( $C < 5\%$ )

### 3) Statistical analyzes

The software Statistica version 7.1 was used to perform ANOVA variance analyzes. The Newman-Keuls test, at the 5% probability threshold, allowed to separate the different average of egg-laying delay of different species on the different cadavers.

## 3. Results and Discussion

### 3.1. Results

#### 3.1.1. Ecological Indices

##### 1) Specific wealth

The rearing carried out from the eggs collected made it possible to identify thirteen (13) species of Diptera from all the corpses exposed. These species have been grouped into four (4) families: the Calliphoridae (*Calliphora vomitoria*, *Calliphora vicina*, *Chrysomya albiceps*, *Chrysomya marginalis*, *Chrysomya megacephala*, *Chrysomya putoria*,

*Lucilia caesar*, *Lucilia sericata* and *Protophormia terraenovae*), the Muscidae (*Musca domestica*) the Sarcophagidae (*Sarcophaga haerromoidalis* and *Sarcophaga carnaria*) and the Fanniidae (*Fannia canicularis*). Of these, the Calliphoridae family has been the most diverse.

##### 2) Frequency of occurrence

The frequency of occurrence in the case of pork witness showed that of the five (5) egg samples taken, the species: *C. albiceps*, *C. marginalis*, *L. caesar*, have been ubiquitous; or a frequency of occurrence of 100%. That of *C. vomitoria*, *C. putoria*, *L. sericata*, *P. terraenovae* and *S. carnaria* was 40%. The species *C. vicina*, *C. megacephala*, *M. domestica*, *S. haerromoidalis*, *F. canicularis*) were respectively present in 60%, 80%, and 20% of cases (Table 1). On the corpses hanging cadaver, 100% of sampled eggs were those of *C. albiceps* and *L. caesar*. While species such as *C. vomitoria*, *C. putoria*, *Lucilia sericata* and *S. haerromoidalis* had a frequency of occurrence of 20%. The species *Calliphora vicina*, *C. megacephala*, *P. terraenovae* and *S. carnaria* presented an occurrence rate of 40% while that of *C. marginalis* was 80%. *Musca domestica*, *F. canicularis*, were rare on hanging cadavers ( $C=0\%$ ) (Table 1).

Table 1. Frequency of occurrence of the different species at the level of each case.

| Families      | Species                          | Control Cadavers (CC) |       | Suspended Cadavers (SPC) |       | Wrapped Cadavers in a Shroud (WCS) |       | Semi-Immersed Cadavers (SIC) |       | Numbers of eggs sampling |
|---------------|----------------------------------|-----------------------|-------|--------------------------|-------|------------------------------------|-------|------------------------------|-------|--------------------------|
|               |                                  | Nber of app           | C (%) | Nber of app              | C (%) | Nber of app                        | C (%) | Nber of app                  | C (%) |                          |
| Calliphoridae | <i>Calliphora vomitoria</i>      | 2                     | 40    | 1                        | 20    | 2                                  | 40    | 1                            | 20    | 5                        |
|               | <i>Calliphora vicina</i>         | 3                     | 60    | 2                        | 40    | 1                                  | 20    | 2                            | 40    | 5                        |
|               | <i>Chrysomya albiceps</i>        | 5                     | 100   | 5                        | 100   | 5                                  | 100   | 5                            | 100   | 5                        |
|               | <i>Chrysomya marginalis</i>      | 5                     | 100   | 4                        | 80    | 5                                  | 100   | 2                            | 40    | 5                        |
|               | <i>Chrysomya megacephala</i>     | 4                     | 80    | 2                        | 40    | 3                                  | 60    | 1                            | 20    | 5                        |
|               | <i>Chrysomya putoria</i>         | 2                     | 40    | 1                        | 20    | 1                                  | 20    | 1                            | 20    | 5                        |
|               | <i>Lucilia caesar</i>            | 5                     | 100   | 5                        | 100   | 5                                  | 100   | 4                            | 80    | 5                        |
|               | <i>Lucilia sericata</i>          | 2                     | 40    | 1                        | 20    | 1                                  | 20    | 1                            | 20    | 5                        |
|               | <i>Protophormia terraenovae</i>  | 2                     | 40    | 2                        | 40    | 3                                  | 60    | 2                            | 40    | 5                        |
| Muscidae      | <i>Musca domestica</i>           | 4                     | 80    | 0                        | 0     | 4                                  | 80    | 1                            | 20    | 5                        |
| Sarcophagidae | <i>Sarcophaga carnaria</i>       | 2                     | 40    | 2                        | 40    | 2                                  | 40    | 1                            | 20    | 5                        |
|               | <i>Sarcophaga haerromoidalis</i> | 1                     | 20    | 1                        | 20    | 2                                  | 40    | 1                            | 20    | 5                        |
| Fanniidae     | <i>Fannia canicularis</i>        | 1                     | 20    | 0                        | 0     | 2                                  | 40    | 1                            | 20    | 5                        |

Nber of app: Number of apparition.

Concerning the wrapped cadavers, the frequency of occurrence was 100% for species such as *C. albiceps*, *C. marginalis*, *L. caesar*. It was 20% for species such as *C. vicina*, *C. putoria* and *L. sericata*. While some species such as *C. vomitoria*, *S. carnaria* and *S. haerromoidalis* showed a frequency of occurrence of 40%, other species such as *C. megacephala*, *P. terraenovae*, and *Musca domestica* respectively exhibited a frequency of 60% occurrence, 80% (Table 1).

The frequency of occurrence in the case of the semi-submerged pig was 100% for the species *C. albiceps*, while it was 40% for the species such as *C. vicina*, *C. marginalis* and *P. terraenovae*. The species *C. vomitoria*, *C. megacephala*, *C. putoria*, *L. sericata*, *M. domestica*, *S. carnaria*, *S.*

*haerromoidalis*, *F. canicularis* were common in 20% of cases (Table 1).

#### 3.1.2. Time Taken by Necrophagous Diptera to Lay Eggs After Slaughter and Exposure of Corpses

The egg-laying delay of *C. vomitoria* on the control cadavers, suspended cadavers, wrapped cadavers and semi-immersed have respectively was  $8.66 \pm 1.76$ ,  $12.00 \pm 0.57$ ,  $11.00 \pm 0.58$  and  $11.66 \pm 1.45$  hours. The ANOVA test followed by that of Newman Keuls at the 5% level did not reveal any significant difference between the egg-laying delay of this species at the level of the different situations ( $F=1.5346$ ,  $df=3$ ,  $P=0.278801$ ) (Table 2).

The egg-laying delay of *C. vicina* on control cadavers,



hanging cadavers, wrapped cadavers and semi-immersed cadavers have respectively was  $7.00 \pm 0.57$ ,  $8.66 \pm 0.88$ ;  $10.66 \pm 1.45$  and  $12.33 \pm 0.88$  hours ( $F=5.4074$ ,  $df=3$ ,  $P=0.025101$ ) (Table 2).

At *Chrysomya albiceps*, the egg-laying delay were  $10.33 \pm 1.20$  hours on the control cadavers,  $11.66 \pm 1.21$  hours on suspended cadavers,  $18.66 \pm 1.45$  hours on semi-immersed cadavers and  $28.33 \pm 2.18$  hours on wrapped cadavers. The

ANOVA test followed by that of Newman Keuls at the 5% threshold showed a significant difference between the egg-laying delay of this species at the level of the different situations ( $F=27.7992$ ,  $df=3$ ,  $P=0.000139$ ) (Table 2).

The egg-laying delay of *C. megacephala* on control, suspended, wrapped and semi-immersed cadavers were  $11.00 \pm 1.52$  respectively,  $11.00 \pm 1.15$ ,  $11.33 \pm 0.88$  and  $9.33 \pm 1.45$  hours ( $F=0.4972$ ,  $df=3$ ,  $P=0.694381$ ) (Table 2).

**Table 2.** Egg-laying delay of the Families of the first necrophagous Diptera on corpses exposed under different conditions.

| Families      | Species                          | Necrophagous Diptera egg-laying delay (hours) |                      |                              |                        | df | F       | P        |
|---------------|----------------------------------|---|----------------------|------------------------------|------------------------|----|---------|----------|
|               |                                  | Control Cadavers                              | Suspended Cadavers   | Wrapped Cadavers in a Shroud | Semi-Immersed Cadavers |    |         |          |
| Calliphoridae | <i>Calliphora vomitoria</i>      | $8.66 \pm 1.76^a$                             | $12.00 \pm 0.57^a$   | $11.00 \pm 0.58^a$           | $11.66 \pm 1.45^a$     | 3  | 1.5346  | 0.278801 |
|               | <i>Calliphora vicina</i>         | $7.00 \pm 0.57^b$                             | $12.00 \pm 1.73^b$   | $10.66 \pm 1.45^{ab}$        | $12.33 \pm 0.88^a$     | 3  | 5.4074  | 0.025101 |
|               | <i>Chrysomya albiceps</i>        | $10.33 \pm 1.20^c$                            | $11.66 \pm 1.21^c$   | $28.33 \pm 2.18^a$           | $18.66 \pm 1.45^b$     | 3  | 27.7992 | 0.000139 |
|               | <i>Chrysomya marginalis</i>      | $8.33 \pm 0.88^b$                             | $8.66 \pm 0.88^{ab}$ | $21.66 \pm 2.40^a$           | $12.00 \pm 0.57^b$     | 3  | 13.1985 | 0.001827 |
|               | <i>Chrysomya megacephala</i>     | $11.00 \pm 1.52^a$                            | $11.00 \pm 1.15^a$   | $11.33 \pm 0.88^a$           | $9.33 \pm 1.45^a$      | 3  | 0.4972  | 0.694381 |
|               | <i>Chrysomya putoria</i>         | $16.33 \pm 2.60^c$                            | $18.66 \pm 4.81^c$   | $48.00 \pm 2.01^b$           | $32.33 \pm 4.09^a$     | 3  | 16.8216 | 0.000814 |
|               | <i>Lucilia caesar</i>            | $6.66 \pm 0.88^c$                             | $10.33 \pm 0.88^b$   | $12.33 \pm 0.87^a$           | $8.00 \pm 0.57^{bc}$   | 3  | 9.4444  | 0.005249 |
|               | <i>Lucilia sericata</i>          | $9.33 \pm 1.76^a$                             | $9.33 \pm 1.20^a$    | $10.00 \pm 1.52^a$           | $12.66 \pm 0.88^a$     | 3  | 1.3140  | 0.335465 |
|               | <i>Protophormia terraenovae</i>  | $26.00 \pm 1.57^a$                            | $12.66 \pm 4.35^a$   | $30.66 \pm 3.52^a$           | $22.66 \pm 4.09^a$     | 3  | 3.2346  | 0.081864 |
| Muscidae      | <i>Musca domestica</i>           | $12.26 \pm 1.25^b$                            | -                    | $20.33 \pm 1.85^a$           | $18.66 \pm 2.60^a$     | 3  | 28.9037 | 0.000121 |
| Sarcophagidae | <i>Sarcophaga carnaria</i>       | $12.66 \pm 1.76^c$                            | $19.00 \pm 2.88^b$   | $11.00 \pm 0.57^c$           | $32.33 \pm 0.88^a$     | 3  | 29.9086 | 0.000107 |
|               | <i>Sarcophaga haerromoidalis</i> | $9.00 \pm 1.52^b$                             | $30.66 \pm 2.96^a$   | $11.33 \pm 0.88^b$           | $12.33 \pm 0.87^b$     | 3  | 31.4971 | 0.000088 |
| Fanniidae     | <i>Fannia canicularis</i>        | $170.66 \pm 2.18^a$                           | -                    | $174.00 \pm 3.61^a$          | $136.00 \pm 8.32^b$    | 3  | 308.250 | 0.000000 |

The numbers followed by the same letter, in the same line, are not significantly different according to the Newman-Keuls test at the 5% level.

*Chrysomya marginalis* laid its first eggs on the control cadavers,  $8.33 \pm 0.88$  hours after exposure, on the suspended cadavers, after  $12.00 \pm 1.73$  hours post-mortem, at the level of the wrapped cadavers,  $21.66 \pm 2.40$  hours after exposure and on semi-submerged cadavers, after  $12.00 \pm 0.57$  post-mortem. The ANOVA test followed by that of Newman Keuls at a threshold of 5% showed a significant difference between the times to lay eggs of this species at the level of the different situations ( $F=13.1985$ ,  $df=3$ ,  $P=0.001827$ ) (Table 2).

The egg-laying delay of *C. putoria* on control cadavers, suspended, packaged and semi-immersed have respectively been  $16.33 \pm 2.60$ ,  $18.66 \pm 4.81$ ,  $48.00 \pm 2.01$  and  $32.33 \pm 4.09$  hours ( $F=16.8216$ ,  $df=3$ ,  $P=0.000814$ ) (Table 2).

The egg-laying delay for *L. caesar* were  $6.66 \pm 0.88$  hours on the control cadavers,  $10.33 \pm 0.88$  hours on suspended cadavers,  $12.33 \pm 0.87$  hours on wrapped cadavers and  $8.00 \pm 0.57$  hours on semi-immersed cadavers ( $F=9.4444$ ,  $df=3$ ,  $P=0.005249$ ) (Table 2).

In *L. sericata*, the egg-laying delay were  $9.33 \pm 1.76$  hours, on the control cadavers,  $9.33 \pm 1.20$  hours on suspended cadavers,  $10.00 \pm 1.52$  hours for wrapped cadavers and  $12.66 \pm 0.88$  hours for semi-immersed cadavers. The ANOVA test followed by that of Newman Keuls at the 5% threshold showed a significant difference between the egg-laying delay of this species at the level of the different situations ( $F=1.3140$ ,  $df=3$ ,  $P=0.335465$ ) (Table 2). *Protophormia. terraenovae* laid its first eggs on the control cadavers  $26.00 \pm 1.57$  hours after exposure, on the suspended corpses, after  $12.66 \pm 4.35$  hours post mortem, at the level of the wrapped cadavers,  $30.66 \pm 3.52$  hours after exposure and  $22.66 \pm 4.09$  hours post mortem on semi-immersed cadavers ( $F=3.2346$ ,  $df=3$ ,  $P=0.081864$ ) (Table

2).

The egg-laying delay of *M. domestica* were respectively was  $12.26 \pm 1.25$  hours,  $20.33 \pm 1.85$  hours and  $18.66 \pm 2.60$  hours on control cadavers, packaged and semi-immersed. On the other hand, no spawning of *M. domestica* was observed on the suspended cadavers ( $F=28.9037$ ,  $df=3$ ,  $P=0.000121$ ) (Table 2).

In *Sarcophaga carnaria*, the egg-laying delay were  $12.66 \pm 1.76$  hours on the control cadavers,  $19.00 \pm 2.88$  hours on the suspended cadavers,  $11.00 \pm 0.57$  hours on the wrapped cadavers and  $32.33 \pm 0.88$  hours on the semi-immersed cadavers. The ANOVA test followed by that of Newman Keuls at the 5% threshold showed a significant difference between the delay to lay eggs for this species at the level of the different situations ( $F=29.9086$ ,  $df=3$ ,  $P=0.000107$ ) (Table 2).

The delay of egg-laying of *S. haerromoidalis* on control, suspended, wrapped and semi-immersed corpses were  $9.00 \pm 1.52$  respectively,  $30.66 \pm 2.96$ ,  $11.33 \pm 0.88$  and  $12.33 \pm 0.87$  hours ( $F=31.4971$ ,  $df=3$ ,  $P=0.000088$ ) (Table 2). The egg-laying delay of *Fannia canicularis* have respectively been  $170.66 \pm 2.18$  hours,  $174.00 \pm 3.61$  hours and  $8.32$  hours on the control cadavers, wrapped cadavers and semi-immersed cadavers. On the other hand, no eggs of this species were taken from the suspended cadavers ( $F=308.250$ ,  $df=3$ ,  $P=0.00001$ ) (Table 2).

### 3.2. Discussion

The pig cadavers used in our experiments were exposed under different experimental conditions (control cadavers exposed to the open air, cadavers suspended in the open air,

semi-immersed cadavers and wrapped cadavers). These were exposed at specific sites separated by at least 300 m from each other, so that colonization of carcasses by necrophagous Diptera was not influenced by any phenomenon of intra-specific competition. The 13 species of necrophagous Diptera identified each exhibited an egg-laying delay that varied from one species to another and from one type of cadaver to another. The rapid drying out of the suspended corpses and their position did not allow Diptera belonging to the Muscidae (*M. domestica*) and Fanniidae (*F. canicularis*) families to carry out their first eggs. These desiccated corpses were no longer attractive to the rest of the necrophagous Diptera seeking to lay eggs [11].

A few hours after the slaughter and exposure of the pig corpses according to each situation, many adults of Diptera belonging to the families of Calliphoridae, Sarcophagidae and Muscidae were attracted by the first odors emitted by the onset of decomposition. The early appearance of these Diptera families on exposed corpses could be explained by their particularly developed chemotactile system (olfactory system), allowing them to detect very weak odors several tens of meters away [8, 12, 25-29] have also observed this early colonization of corpses by species from these families. Many species are associated with the cadaver ecosystem and in particular, adult females of necrophagous Diptera, in search of protein inputs necessary for vitellogenesis [30]. The early arrival of species from these Diptera families could also be due to favorable climatic conditions. Among these pioneer species, we find mostly Calliphoridae Diptera [9, 12]. During our observations, the egg-laying took place in very hidden areas. In the control corpses, the necrophagous Diptera laid their eggs on the back facing the ground. Concerning the suspended corpses, the laying was carried out in the mouth, the snout and the hollow of the eyes. In the case of wrapped corpses, the eggs laid were observed on the tissue covering the head and in semi-immersed corpses, the first eggs were laid in the hollows of the hind legs and the ear. This behavior, which had also been described by [31] during his work on Pig97 and Pig99, would be at the origin of the choice of place of egg-laying of females. These would act in this way to protect their eggs against the light intensity and heat of the sun [9, 12].

In the species of Diptera Calliphoridae recorded on corpses exposed in different situations, an order of appearance was observed. When we consider the *C. marginalis* egg-laying time, we notice that it was  $8.33 \pm 0.88$  hours on the control corpses,  $8.66 \pm 0.88$  hours on the suspended corpses,  $12.00 \pm 0.57$  on semi-immersed corpses and  $21.66 \pm 2.40$  hours on wrapped corpses, at an ambient temperature varying between 25.6 and 29.8°C and at a relative humidity ranging from 78.7 to 89%. Although the corpses were subjected to identical environmental and climatic conditions, a significant difference was observed in the laying time of *C. marginalis*. This could be explained by the variation in accessibility to corpses. Indeed, the witness and suspended corpses being exposed to the open air, were the first to be colonized by necrophagous insects. The semi-immersed corpses followed in second position with a delay of 3 hours 30 minutes

compared to the time obtained for the control and suspended corpses. This delay is mainly due to the reduction in available laying sites, which delayed the access to the semi-immersed corpses. When the bodies were placed in the water, they have first been immersed in 4/5 of their volume and this is from the 8 hours post mortem they have begun to emerge [32]. The progressive swelling of pig corpses is believed to be the cause of the late emergence of carcasses from the water. This late corpses emergence has favored the emergence of laying sites of necrophagous Diptera. The delay taken to lay eggs for these different Diptera varied from one species to another. This variability in the laying delay could be explained by the fact that these carcasses had only a small part of the laying sites and that the water closed the rest of the body. It was therefore difficult for some Diptera to access the corpses despite their presence. The presence of water around the corpses would influence the accessibility of Diptera to the substrate. Indeed, water was an obstacle to the activity of flies because they looked for sites favorable to the development of their offspring.

On the control cadavers, suspended cadavers, wrapped cadavers and semi-immersed cadavers, the first eggs of *S. carnaria* respectively were observed at  $12.66 \pm 1.76$ ;  $19.00 \pm 2.88$ ;  $11.00 \pm 0.57$  and  $32.33 \pm 0.88$  hours after exposure of the carcasses. The late laying in semi-immersed corpses could be explained by the same causes. The slight difference observed in the egg-laying time of this species (larviparous) on suspended corpses is due to the search for a favorable site to deposit their larvae, in order to ensure their development.

Other species of Diptera such as *M. domestica* (Muscidae) and *F. canicularis* (Fanniidae) were observed a little later on the other three types of pig carcasses with the exception of the suspended corpses which where the latter carried out no laying. The late arrival of these species is said to be due to the characteristic odors emitted by the decomposing corpses. Indeed, these same species were also collected late on decaying corpses exposed to the open air by [9, 12]. The absence of activity by Muscidae and Fanniidae in the suspended corpses could be explained by the drying out of these. Indeed, when the corpses become dry, the insects no longer have nutrients favorable to the proper development of the larvae of these species. These observations join those of [12] who carried out work on corpses exposed to the open air in the Sub-Saharan zone of Côte d'Ivoire during the dry and rainy seasons.

As decomposition is strongly linked to the characteristics of the cadaver ecosystem, insect succession is therefore highly variable [7]. It depends on the environmental conditions and the initial state of the corpse, but other factors such as the action of plants, fungi or carnivorous mammals may intervene [5].

## 4. Conclusion

Thirteen (13) identified species of necrophagous Diptera which laid eggs on exposed corpses were grouped into four families: the Calliphoridae (*C. vomitoria*, *C. vicina*, *C.*

*albiceps* *C. marginalis*, *C. megacephala*, *C. putoria*, *L. caesar*, *L. sericata* and *P. terraenovae*), Muscidae (*M. domestica*), Sarcophagidae (*S. haerromoidalis*, *S. carnaria*) and Fanniidae (*F. canicularis*). Of these, Calliphoridae family has been the most diverse.

Regarding the egg-laying delay in species of Calliphoridae (*L. caesar* and *C. vicina*) was shorter on control and suspended corpses or respectively  $6.66 \pm 0.88$  and  $8.66 \pm 0.88$  hours after slaughter and exposure of the corpses.

On semi-immersed and wrapped cadavers, the first egg-laying respectively observed after  $8.00 \pm 0.57$  and  $10.66 \pm 1.45$  hours post mortem, were those of *Lucilia caesar* and *Calliphora vicina*.

The Muscidae and the Fanniidae, which appeared later on the decomposing corpses, were not observed on the suspended corpses which very quickly dried up and therefore could no longer provide the nutrients essential for the proper development of the larvae of the species of these families of Diptera. The species laid eggs on the hidden parts of the exposed corpses to allow protection of the eggs from solar radiation and the intensity of light.

At the end of this study, we can retain that according to the accessibility of the insects to the corpses to the necrophagous insects, the first Diptera egg-laying was observed at least six hours (6 h) and at most 174 hours after exposure of the carcasses [33]. Thus, it clearly appears that the conditions of exposure of pig cadavers significantly influenced the egg-laying delay of the main necrophagous Diptera [34]. As part of an entomological expertise to date deaths in the Guinean zone of Côte d'Ivoire, these variable of egg-laying delay for the first Diptera should be taken into account in estimating the minimum post-mortem interval upon discovery of a corpse.

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## Declaration

The authors declare that they have no conflict of interest regarding this article.

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