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Effect of seasons on the decomposition by necrophagous insects of pig (*Sus scrofa domesticus* L.) carcasses exposed to the open air in the Sudano-guinean and Sub-sudanese zones of Côte d'Ivoire

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Abstract

Determining the post-mortem interval (PMI) of a corpse discovered 72 hours after death is becoming complex and imprecise in forensic medicine. It is necessary to use other investigative methods to determine the date of death. The aim of this research was to determine the impact of the seasons on the decomposition of pig corpses exposed to the open air by necrophagous insects. The research was carried out in the North (at the Botanical Garden of the Peleforo Gon Coulibaly University in Korhogo) and in the Centre (in the Bouaké region). Insects were collected directly from decomposing carcasses using a mowing net and flexible forceps for larvae and Coleoptera. Pitfall-traps were also used for collection. Diptera and Coleoptera were collected during the dry and rainy seasons in the Sudano-guinean and Sub-Sudanese zones. *Chrysomya albiceps*, belonging to the Calliphoridae, was the majority species. Because of its plethora of species in the two study areas, *C. albiceps* could be one of the main species for determining the minimum MPI in Côte d'Ivoire. Five stages of decomposition were observed in the two study zones, although a sixth stage (dry putrefaction) was observed in the Sub-sudanese zone during the harmattan period. Stratiomyidae and Piophilidae contributed to the total decomposition of carcasses during the rainy season. The Histeridae family was the richest in species. *Dermestes maculatus* was the richest in individuals. Carcasses in the rainy and dry seasons were respectively decomposed by an average of 97.18% in the rainy season and 84.32% in the dry season in the two study areas.

Keywords: Decomposition; Corpses; Insects; Seasons; Sudano-guinean; Sub-sudanese; Côte d'Ivoire

1. Introduction

Determining the post-mortem interval (PMI) of a corpse discovered 72 hours after death is becoming complex and imprecise in forensic medicine [1, 2]. For this reason, it is necessary to use other investigative methods to determine the date of death. Forensic entomology, which uses insects present on the cadaver to establish the date of death, is a relevant solution. The corpse is a source of food and reproduction for insects [3]. Studies on necrophagous insects have been carried out in developed countries [4]. However, in Africa, there is little research into the use of necrophagous insects in criminal investigations. In Côte d'Ivoire, some work has been carried out on the activity of these insects. However, there are no studies in the literature on the decomposition of a corpse exposed to the open air during the different seasons in the Sudano-Guinean and Sub-sudanese areas of Côte d'Ivoire. The aim of this research is to determine the impact of the seasons on the decomposition of pig carcasses exposed to the open air by necrophagous insects.

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2. Materials and methods

2.1. Study site and weather conditions

The research was carried out at the Botanical Garden of the Peleforo Gon Coulibaly University in Korhogo, in the north of Côte d'Ivoire, located at geographical coordinates 9°26' N - 5°38' W, at an altitude of 381 m. The site is characterized by its wooded savannah and gallery forest vegetation. The open area is adjacent to a river and has a grassy cover dotted with bushes. Most of the trees lose their leaves during the dry season. The traps were placed in an open environment, allowing insects easy access to the corpses. The Sub-Sudanese region is characterized by two seasons: the rainy season and the dry season. Temperature and relative humidity data were collected using a thermos-hygrometer and a rain gauge at the site where the pig carcasses were exposed to the open air and subjected to various environmental factors (temperature, humidity, rainfall, sunshine). Temperatures varied between 24 and 35.8°C in the dry season, with an average of 29.75°C, and between 24.5 and 32.8°C in the rainy season, with an average of 27.84°C. Relative humidity ranged from 12.5 to 62% in the dry season (average 46.64%) and from 67.8 to 86.2% in the wet season (average 76.78%). Rainfall was low in the dry season, with an average of 33.67 mm, compared with 45 to 345 mm in the wet season.

2.2. Experimental set-up

The experimental set-up used consisted of two types of cages, Gt and Ge [5]. Gt was the conditioning cage for the control cadaver and Ge was the conditioning cage for the test cadaver.

The G cages had the shape of a straight block with dimensions of L = 1.5 m, l = 0.80 m and h = 0.70 m [5]. The cages for the control cadavers were entirely covered with a stainless-steel grid with a very fine mesh of 1 mm, preventing any insect access to the cadavers. The test cages were also covered in stainless steel, but with 3 cm mesh. The cages were cemented to the ground at the experiment site to prevent scavengers from gaining access to the corpses (Figure 1). The domestic pig was chosen as the biological material because it is considered an excellent model of human cadaver decomposition [6, 7].

Figure 1 Experimental set-up

A: Control corpse (the corpse is isolated from insects by a very fine mesh screen); B: Test corpse (the corpse is accessible to insects thanks to the large mesh screen which protects it from scavengers).

2.3. Collecting and identifying necrophagous insects

Necrophagous insects were collected directly from decomposing carcasses using a mowing net and flexible forceps for larvae and Coleoptera. Pitfall-traps were also used for collection. Larvae and pupae were collected from or near decomposing corpses [8]. The larvae and pupae were transported to the laboratory and reared, enabling us to confirm the period of activity of the various insects. After emergence, imagoes obtained were killed with ether and then sorted by family [8]. Identification was carried out using an "Optika LAB20" version 4.0 binocular magnifier and various identification keys [9-14].

2.4. Weekly weighing of pig carcasses

Twelve (12) pigs weighing 50 kg each were used as baits, divided into six pigs per study area. The pigs were transported live to the study sites to avoid colonization by insects prior to exposure [7,8]. They were anaesthetized with a sedative and then euthanized [7,8]. In wire cages, each cadaver was placed on a 2 cm mesh net and weighed weekly. The contribution of necrophagous insects was assessed on the basis of the initial body mass loss observed during the decomposition process [7,8]. The cumulative rate of body mass loss (Tc) for a given postmortem interval (PMI) was calculated using the following formula [5, 7]:

$$\mathbf{Tc} = \frac{\mathrm{Mi} - \mathrm{Mf}}{\mathrm{Mi}} \times 100$$

- Tc (Df): represents the cumulative rate of body mass loss (%) for a given post-mortem interval (MPI) Df; - Mi (in Kg): represents the initial body mass of the pig corpse just before slaughter - Mf (Df) (in Kg): represents the final mass corresponding to a given post-mortem interval (IPM).

The Tcc corrected rate of initial body mass loss was obtained using the Abbott formula [7- 9, 15]:

$$\mathrm{Tcc}(\%) = \frac{\mathrm{Tce} - \mathrm{Tct}}{100 - \mathrm{Tct}} \times 100$$

- Tcc: Corrected rate of body mass loss; - Tct: Rate of body mass lost by the control corpse; - Tce: Rate of body mass lost by the cadaver test.

2.5. Data processing

Total wealth

The total richness of a biocenosis corresponds to the total number of species observed during N surveys [16].

S = Sp1+Sp2+...+Spn

- S: total number of species observed during N surveys;

- Sp1, Sp2, Spn: species observed.

Shannon-Weaver index and equitability index

Shannon-Weaver index (H')

This index makes it possible to quantify the heterogeneity of biodiversity over the different study seasons, providing an overview of temporal changes [8, 17]. Recognized and widely used by specialists, it is independent of sample size and takes into account the relative abundance of each species [17]. H' is minimal (= 0) when all the individuals belong to a single species or when each species is represented by a single individual, except for one species that dominates the stand. Conversely, the index reaches its maximum when all the individuals are equally distributed between all the species [18].

$H' = -\sum Pi \times \log_2 Pi$

Pi = ni/N; ni: number of individuals of a given species i, ranging from 1 to S (total number of species); N: total number of individuals.

Equitability index (E)

Equitability (E) was used to study the regularity of species distribution in each study area [19]. This index can vary from 0 to 1, and is highest when species have identical abundances in the stand and lowest when a single species dominates the entire stand. Unaffected by species richness, it is very useful for comparing potential dominance between stations or sampling dates.

$E = H' / \log_2 (S)$

H': Shannon index

S: Total richness

Jaccard similarity index

Jaccard's similarity or community index [20] measures the similarity between two communities. This index was used to establish a qualitative study based on the presence or absence of species in the different samples by comparing the number of different species (genus, family or order) in the different zones in pairs. It is calculated using the following formula:

$$\mathbf{Ji} = \frac{\mathrm{Sc}}{(\mathrm{Sx} + \mathrm{Sy}) - \mathrm{Sc}} \times 100$$

Sx: number of species in sample x ; Sy: number of species in the sample y ; Sc: number of species common to samples x and y ; Ji: Similarity index

2.6. Statistical processing

Statistical analyses were performed using R 3.0.3. The ANOVA test followed by the Newman-Keuls test with a probability threshold of 5% was used to determine the homogeneity of the different groups. Daily climatic data were used to calculate weekly averages of temperature, humidity and rainfall, corresponding to the weekly periods when pig carcasses were weighed [7, 8].

3. Results

3.1. Abundance of insects collected in the two study areas

A total of 78 956 individuals were collected in the two study zones, 43567 individuals (55.18%) in the Sudano-guinean zone and 35389 individuals (44.82%) in the Sub-sudanese zone. Two main orders, Coleoptera and Diptera, were obtained from the identification. In the Sudano-guinean zone, the number of Coleoptera in the zone amounted to 10709 individuals (24.58%), while Diptera comprised 32858 individuals (75.42%). In the Sub-sudanese zone, Coleoptera numbered 5783 individuals (16.35%) and Diptera 29606 individuals (83.65%).

3.2. Specific richness and abundance of necrophagous insects collected in the different seasons and the two study areas

In the rainy season, 20452 individuals of Diptera were collected while only 12406 individuals were collected in the dry season in the Sudano-Guinean zone. Among Coleoptera, 8793 individuals were collected in the dry season, while 1916 individuals were collected in the rainy season in the same zone. In the sub-Sudanese zone, 18791 Diptera were collected in the rainy season and 10815 in the dry season. For Coleoptera, 4327 individuals were collected in the dry season and 1456 in the rainy season. The identification of Diptera collected in the Sub-Sudanese zone enabled them to be divided into 18 species belonging to seven (7) families. There were nine (9) species in the Calliphoridae family: *Chrysomya marginalis, Chrysomya albiceps*, *Chrysomya megacephala*, *Calliphora vomitoria, Lucilia sericata, Lucilia caesar, Chrysomya putoria, Protophormia terraenovae,* and *Calliphora vicina*. Of these species, *C. albiceps* was in the majority in both seasons (F = 31.545; ddl = 17; P = 0.0001). The least abundant species in the Calliphoridae family was *Calliphora vomitoria* (F = 31.545; ddl = 17; P = 0.0001). *Calliphora vicina*, also belonging to the family Calliphoridae, was only found on corpses during the rainy season. Among the Muscidae, only *Musca domestica* was collected during both seasons. Among the Sarcophagidae, four (4) species were collected from cadavers: *Sarcophaga carnaria*, *S. africa*, *S. haerrhomoidalis* and *Wohlfahrtia nuba*. These were all observed and collected from cadavers in both seasons. Of these species, *Sarcophaga carnaria* was the most abundant (F = 23.087; ddl = 7; P = 0.0001). *Wohlfahrtia nuba* was the least abundant species in the Sarcophagidae family (F = 23.087; ddl = 7; P = 0.0001). In the Piophilidae, Stratiomyidae and Fanniidae, only one species (*Piophila casei*, *Hermetia illucens* and *Fannia canicularis*) was obtained from each family respectively. These species were only found on cadavers during the rainy season. In the Sudano-Guinean zone, 20 species of Diptera were identified. These species were identical to those found in the Sub-Sudanese zone, with numbers greater than or equal to those found in the Sub-Sudanese zone. However, two (2) species belonging to the Muscidae family (*Musca* sp. and *Stomoxys calcitrans*) were specific to the Sudano-Guinean zone, while *Wohlfahrtia nuba* belonging to the Sarcophagidae family was specific to the Sub-Sudanese zone.

3.3. Seasonal richness and abundance of necrophagous Coleoptera species in the two

3.3.1. study areas

In the Sub-Sudanese zone, the identification of Coleoptera revealed five (5) families: Dermestidae, Cleridae, Histeridae, Tenebrionidae and Scarabaeidae. The Histeridae was the richest family with four (4) species: *Hister cadaverinus*, *Margarinotus brunneus*, *Hister quadrinotatus* and *Pachylister inaequalis*. Among the Histeridae, *M. brunneus* was the most abundant (F = 34.5412; ddl = 7; P = 0.0001). The Dermestidae, Tenebrionidae, Scarabaeidae and Cleridae all represented a single species, respectively *Necrobia rufipes*, *Tenebrio molitor*, *Onthophagus taurus* and *Dermestes maculatus*. All these species were collected during both seasons in the Sub-sudanese zone, with the exception of *Onthophagus taurus*, which was only found on cadavers during the rainy season. In the Sudano-Guinean zone, the same Coleoptera species were collected.

3.4. Richness and diversity of species

Total species richness in the Sudano-Guinean zone was 28, while in the Sub-Sudanese zone it was 26. In the Sudano-guinean zone, the Shannon index (H' = 1.85) was lower than in the Sub-Sudanese zone (H' = 1.98). Jaccard's similarity index for the two communities (Sudano-guinean and Sub-sudanese zones) was 86.21%.

Table 1 Ecological indices for the two study areas

	Sudano-Guinean zone	**Sub-Sudanese zone**
Total number of Diptera species	20	18
Total number of Coleoptera species	8	8
Totale richness (S)	28	26
Shannon index (H’)	1,85	1,98
Equitability (E)	0,55	0,61
Species common to both zones	25	
Similarity index (J)	86,21%	

3.5. Stage of decomposition of corpses

The stages of cadaver decomposition during the first week of exposure were fresh cadaver (D0-J1), swelling (D1-J2) and active decomposition (D3-J7). During the swelling stage of the cadavers, only Diptera eggs were observed on the hidden faces and in the natural orifices. At the active decomposition stage, the corpses were colonized by Diptera larvae from their eggs. At this stage, adult Diptera and Cleridae beetles were found in large numbers on the corpses. The active decomposition stage continued until 15^{e} days post mortem in the rainy season and 10^{e} days in the dry season in both study areas. The advanced decomposition stage began with the entry into pupation of the first larvae of Diptera Calliphoridae, Sarcophagidae and Muscidae. At this stage, the number of individuals and species of Coleoptera increased, with a preponderance of Histeridae, Cleridae and Dermestidae. These observations were made in both study areas. Other Diptera such as Stratiomyidae and Piophilidae appeared between 30 and 49^{e} days post mortem only in the rainy season in the Sub-sudanese zone and in both seasons in the Sudano-guinean zone. Species of these two families of Diptera are responsible for the appearance of the skeletonization stage in the rainy season, whereas the Dermestidae play this role in the dry season in the Sub-sudanese zone.

3.6. Action of necrophagous insects on the corpses’ mass loss

In the Sudano-guinean zone, the cumulative mass loss rates of corpses exposed during the rainy and dry seasons increased very rapidly during the first week post-mortem, reaching 73.64 and 51.26% respectively. The control corpses, to which the insects had no access during the same periods, lost 5.23 and 3.57% of their initial body mass respectively. In the Sub-sudanese zone, the cumulative rates of body mass loss for corpses exposed to the open air during the rainy and dry seasons increased rapidly during the first week after death, reaching 65.64 and 35.06% respectively. Body mass loss rates for corpses from both seasons continued to increase until the third week post-mortem (21^{e} day post-mortem), reaching 78.15 and 67.43% respectively in the Sudano-guinean zone, while in the Sub-sudanese zone they reached 70.15 and 47.63% respectively. Control cadavers lost an average of just 11.43% of their body mass. From the third week onwards, the cumulative rates of body mass loss gradually increased, reaching a peak (97.18% on average) at 15e weeks in the rainy season and at 18 weeks in the dry season (84.32% on average).

4. Discussion

Two orders of necrophagous insects, Diptera and Coleoptera, were identified during the rainy and dry seasons in the Sudano-Guinean and Sub-Sudanese zones. The main families of Diptera were: Calliphoridae, Muscidae, Sarcophagidae, Piophilidae, and Stratiomyidae. Histeridae, Cleridae and Dermestidae were the main families of Coleoptera. These results are similar to those obtained by Tabor et al [21] and Dao et al [8]. The early appearance of these insects on corpses shows their importance in forensic investigations [6, 8]. Several other studies, such as those by Faria et al [22] and Koffi et al [7, 23] confirm the presence of these insects on decomposing corpses. The Calliphoridae family was the most numerous in terms of individuals and species during the rainy season, with *C. albiceps* the dominant species, present during both seasons. This species is very abundant in the rainy season, probably because of its ability to colonize and recolonize cadavers in favorable conditions, and the competitiveness of its larvae [8]. These observations are in agreement with studies by Carvalho and Linhares [24] and Carvalho and Godoy [25]. Among the Sarcophagidae, *S. carnaria* was the most abundant species, particularly during the rainy season, due to the favorable conditions for the wet decomposition of corpses [26]. The Piophilidae and Stratiomyidae families were mainly present during the rainy season, probably because the high humidity favored their colonization [3, 5]. Among the Coleoptera, the Histeridae family was the richest in species. *Necrobia rufipes* (Cleridae) was also observed at this time, probably because of its similar feeding requirements to those of the Histeridae. Coleoptera appeared from the third post-mortem day, often as predators of Diptera eggs or larvae, a phenomenon also described by Tabor et al [21] and Dao et al [8]. *D. maculatus* (Dermestidae) was particularly abundant during the harmattan in the Sub-Sudanese zone, a dry period when corpses dried out under the effect of low humidity levels [5]. The Shannon index and equitability were higher during the rainy season, indicating a more balanced distribution of species during this period [18]. Corpse mass loss was more rapid in the wet season, probably due to the moistening of the corpses, which allowed the larvae of colonizing insects to decompose the flesh more efficiently. In the dry season, decomposition was slowed by the drying out of the cadavers and predation of the larvae by Histeridae and Cleridae [3, 5]. The latent phase in mass loss observed in the dry season could be due to the inactivity of the insects because of the dried-out corpses, or to the incubation of the eggs of certain Coleoptera such as the Dermestidae [5]. On the other hand, in the rainy season, advanced decomposition was favored by the action of Stratiomyidae and Piophilidae larvae, already observed at the end of decomposition by Pujol-Luz et al [27] in Brazil.

5. Conclusion

The work carried out in the Sudano-Guinean and Sub-Sudanese zones during the dry and rainy seasons enabled us to collect mainly two orders of necrophagous insects: Diptera and Coleoptera. Diptera were more numerous in the rainy season and Coleoptera in the dry season.

Among the Diptera, the Calliphoridae family was the richest in species. All the species in this family were collected in both seasons and in both study areas. They are *Chrysomya albiceps, Chrysomya marginalis*, *Chrysomya megacephala*, *Chrysomya putoria, Lucilia sericata, Lucilia caesar, Protophormia terraenovae, Calliphora vomitoria*, and *Calliphora vicina*. *C. albiceps* was the majority species. Because of its plethoric numbers in the two study areas, *C. albiceps* could be one of the main species in determining the minimum MPI in Côte d'Ivoire. It was more abundant in the rainy season than in the dry season. Stratiomyidae and Piophilidae contributed to the total decomposition of carcasses in the rainy season. The Histeridae family was the richest in species (*Margarinotus brunneus*, *Hister cadaverinus*, *Pachylister inaequalis* and *Hister quadrinotatus*). *D. maculatus* was richest in individuals. Rainy and dry season carcasses were respectively degraded to an average of 97.18% in the rainy season and 84.32% in the dry season in the two study areas. As part of an entomological expertise to date a death, at the time of the discovery of a corpse, the forensic entomologist expert will already be able from this study to have an idea of the evolution of the decomposition of the corpse in this zone.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest with respect to this article.

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