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TIME OF DEATH ESTIMATION: THE ROLE OF *HERMETIA ILLUCENS* IN BONES IN THE SAVANNAH OF GOIÁS

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Abstract: This study highlights the importance of determining the Minimum Post-Death Interval (MPI) in forensic investigations, with a particular focus on the role of forensic entomology and the specific characteristics of the savannah biome. Using *Hermetia illucens* as a keystone species, we investigated its colonization behavior and life cycle to improve IPMm estimates. Observations and controlled experiments led to a preliminary estimate of the IPMm situated on January 5, 2023, although we highlighted the influence of environmental and biological variables and the possibility of successive generations of insects occurring.

The study reiterates the need to consider the complexity of the activity of scavenging insects, as well as the specific conditions of each decomposition scenario. We highlight the importance of interdisciplinary approaches, combining entomology, climatology and other forensic sciences to produce more accurate and reliable estimates of IPMm. The research not only contributes to the knowledge base in forensic entomology, but also provides practical perspectives for resolving criminal cases, underlining the relevance of adapting forensic techniques to the specific environment and circumstances of each case investigated.

INTRODUCTION

Accurately determining the minimum postmortem interval (MPI) is a crucial element in forensic investigations, serving as a key tool for narrowing the timeline of suspicious activity or identifying potential suspects. Within this field, forensic entomology emerges as a prominent discipline, utilizing insects, specifically scavenging flies, to provide estimates of time since death [1].

The savannah, with its distinct flora and fauna, presents a complex scenario for decomposition and subsequent colonization by insects. The dynamics of ephemeral communities in this biome are of particular interest to forensic entomology, as the corpse represents a valuable resource, attracting a wealth of local colonizers. The composition and abundance of these scavenger communities vary seasonally, directly influencing the IPMm estimate [2]. Understanding the interaction between these colonizers, the savannah environment and decomposing bodies is vital to improving forensic estimates of the post-mortem interval and providing more accurate and reliable results in legal investigations.

Efforts to explore forensic fauna in the savannah have been notable. Lira Júnior [3] focused on beetles and their role in the forensic ecology of the savannah; Rosa et al. [4] examined dipterans in different savanna vegetation profiles in Uberlândia, expanding understanding of forensic insect communities; and Da Silva et al. [5] conducted a detailed survey of dipterans in an area of the savannah in Brasília, expanding the corpus of knowledge on the biodiversity of forensically relevant insects and their implications for calculating the MPI.

Predominantly found in tropical regions and hot climates, *Hermetia illucens* colonizes decomposing matter, both plant and animal. The life cycle of the species varies from 38 to 42 days from oviposition to the adult stage, but these periods can be significantly extended under adverse environmental conditions, such as variations in temperature [6] [7]. In extreme scenarios, larval development can last up to five months, expanding the scope of analysis for IPMm, a crucial consideration in biomes with different climatic conditions such as the savannah. This ecosystem, characterized by its specific temperatures and humidity, provides a unique environment where corpses become ephemeral resources highly desired by several scavenger species.

The specific contribution of *Hermetia*

illucens in this field is marked by a distinct life cycle, divided into egg, larva, pupa and adult phases, each with characteristics and durations that respond to environmental conditions [7]. The larvae, passing through six instars, change color and size until they reach maturity [8]. The work of Barros-Cordeiro and collaborators [8] detailed the entire intra-puparial development of H. illucens, revealing the critical morphological transformations over time. These insights are vital not only for understanding the basic biology of this species, but also provide morphological parameters that are fundamental for estimating mIPM in forensic contexts.

This study aims, therefore, to determine the minimum post-mortem interval of a bone found in the Savannah of Goiás, through an interdisciplinary approach that considers both the entomological and environmental aspects specific to this biome. Using the data and methods accumulated in the literature and previous studies, together with field investigations, we will seek to establish more accurate and reliable estimates of the IPMm, thus contributing to the field of forensic entomology and the solution of criminal cases in the region.

MATERIALS AND METHODS

COLLECTION AND PACKAGING OF LARVAE OF *HERMETIA ILLUCENS*

On January 31, 2023, fly larvae were collected from the cadaver in a skeletal state and immediately packaged for later analysis (Figure 01). The larvae, belonging to a single morphotype identified as larval instar L6, were carefully collected and transferred to an airtight margarine-type container containing fresh ground meat. This approach ensured the preservation of the larvae and avoided any contamination during transport [9].

Figure 01: Fly larvae in bones for forensic analysis, carried out on January 31, 2023.

DIET PREPARATION FOR LIVE LARVAE OF *HERMETIA ILLUCENS*

In the laboratory, live larvae were isolated and placed in a cylindrical container. A specific diet was prepared to feed the larvae during their development. The diet was formulated by combining 5 measures of wheat bran, 1 measure of powdered milk, 1 measure of brewer's yeast and a pinch of nipagin, with the addition of distilled water until the mixture reached the consistency of solid porridge. It should be noted that the diet was positioned in one of the corners of the container, while the vermiculite was placed in the remaining area to provide adequate humidity conditions [9].

LABORATORY CONDITIONS FOR LARVAE OF *HERMETIA ILLUCENS*

The container containing the larvae and the diet was sealed with a filo fabric secured by an elastic band, allowing adequate ventilation and preventing the larvae from escaping. This set was then placed in a room designated as an "insectarium" with controlled temperature and humidity [9].

MONITORING THE DEVELOPMENT OF LARVAE *HERMETIA ILLUCENS*

During the follow-up period, we meticulously recorded the time from emergence of larvae to adults. The larvae were raised at a temperature of 27°C. As soon as the larvae completed their development cycle, the adult insects were carefully removed from the container and fixed in 70% alcohol for later analysis [9].

IDENTIFICATION OF ADULT FLIES *HERMETIA ILLUCENS*

The adult flies obtained were identified as belonging to the species: *Hermetia illucens* (L.), (*Diptera, Stratiomyidae*), known as the black soldier fly. Identification was carried out based on identification keys [10] [11] [12] and specific morphological observations (Figure 02).

Figure 02: Adult fly larvae obtained were identified as belonging to the species: *Hermetia illucens* (L.) (*Diptera*, *Stratiomyidae*), known as the black soldier fly.

CALCULATION OF THE MINIMUM POSTMORTEM INTERVAL (MPI) FOR THE FLY *HERMETIA ILLUCENS*

The minimum post-mortem interval was calculated based on the Insect Activity Pattern (PAI), considering the development time of the *Hermetia illucens* species, as reported in previous studies [11] [12] [13] [14].

DISCUSSION

The calculation of the Minimum Post-Death Interval (IPMm) is one of the crucial aspects of Forensic Entomology, and its accuracy fundamentally depends on detailed knowledge of the developmental biology of each insect species involved in the colonization of corpses. In case of *Hermetia illucens* (L.) (*Diptera, Stratiomyidae*), This species stands out as a significant object of study due to its wide distribution and adaptation to urban and rural environments in Brazil [15] [16].

Extensive research conducted in the laboratory has contributed to a deep understanding of the life cycle of this black soldier fly, which has proven to be a species of great use in Forensic Entomology, especially in estimating mIPM [11] [12] [16] [17]. Being a generalist detritivore, *Hermetia illucens* colonizes different types of decomposing organic matter, which makes it relevant in forensic scenarios with different substrates [18]. Furthermore, its occurrence in both natural habitats and urban environments in Brazil makes it a common and frequently found species [15].

One of the notable results of research into the development of *Hermetia illucens* is the estimate that the life cycle of this species, from egg to adult, occurs in approximately 40 days at an average temperature of 27°C [19] [20]. However, it is worth noting that this estimate is based on experiments carried out under controlled laboratory conditions, which may differ from the conditions found in real crime scenarios.

A valuable contribution to Forensic Entomology is the current emphasis on the use of the Insect Activity Interval (I.A.I.) which portrays a preferred alternative to estimate the minimum IPM. This marks a significant shift away from calculations based solely on temperature and larval length, recognizing the complexity of the developmental biology

of scavenging insect species [11] [12] [15] [16] [17] [18] [19].

In the present study, based on the observations made and the results of previous experiments, it was estimated that an oviposition of *Hermetia illucens* on the cadaver occurred on January 5th, resulting in an estimated minimum Post-Death Interval (PMI). The timeline (Figure 03) clearly presents the events from the detection of larvae to the emergence of black soldier fly adults.

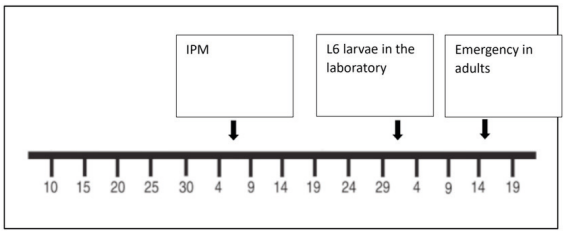

Figure 03: Chronology indicating the main events: corpse found (January 31); larva arrived at the laboratory (February 1); adult fly emerged in the laboratory (February 15); Estimated minimum PMI (January 5).

It is crucial to recognize that, due to the behavioral pattern of *Hermetia illucens* in establishing itself in bodies in advanced decomposition, the initial estimate of the Minimum Postmortem Interval (MPI) may indicate that death occurred weeks before the first oviposition record observed. However, it is essential to consider that several elements, including variations in climate and environment, in addition to the particular conditions of the place where the body was found, can influence the accuracy of these IPMm estimates.

In summary, the study on the life cycle of *Hermetia illucens* highlights the need for an inclusive methodology in Forensic Entomology, highlighting the relevance of the Insect Activity Index (A.I.) as an essential resource to determine the minimum IPM. The adoption of certain fly species as biological indicators can offer valuable insights for criminal investigations, significantly contributing to solving intricate forensic cases.

CONCLUSION

We have determined, based on collected and examined entomological data, that the Minimum Postmortem Interval (MPI) for this specific incident is likely and preliminarily placed at January 5, 2023. However, it is important to point out that this milestone does not precisely define the moment of death, given the possible involvement of successive generations of insects. The late colonization behavior of the fly *Hermetia illucens* suggests that death may have occurred weeks before the initial detection of oviposition, considering the tendency of this species to associate with bodies in more advanced stages of decomposition.

The inference based on the Insect Activity Index (A.I.) and the concentration on primary colonization by *Hermetia illucens* demonstrate the need to consider specific biological and environmental variations in each forensic analysis. We emphasize the importance of multidisciplinary strategies, involving forensic entomology, climatology and other scientific areas, to ensure more accurate assessments of IPMm in future investigations. While we provide detailed and contextualized estimates for the case under study, we highlight the uniqueness of each forensic event, requiring a meticulous and individualized assessment of all pertinent evidence and factors.

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