OSAC 2022-N-0039 Collecting and Preserving Entomological Evidence from a Terrestrial Environment

Crime Scene Investigation & Reconstruction Subcommittee
Scene Examination Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

Draft OSAC Proposed Standard

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Prepared by
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Foreword

This document was developed to provide best practices for collecting and preserving entomological evidence from terrestrial scenes. Entomological evidence may be collected by either a properly trained entomologist, medicolegal death investigator, crime scene investigator, or law enforcement officer. This document is intended to assist individuals engaging in collecting and preserving entomological evidence from a scene or victim. Portions of this document are adapted from Sanford et al. (2019). Appreciation is expressed to Taylor and Francis Publishing for allowing the use of these materials in this document.

This document has been drafted by the Crime Scene Investigation and Reconstruction Subcommittee of the Organization of Scientific Area Committees (OSAC) for Forensic Science through a consensus process.

Keywords: forensic entomology, insect evidence collecting, evidence preservation

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1. Scope

Arthropods typically colonize vertebrates (i.e., humans, pets, or other animals) while alive or soon after death. A common objective of forensic entomology is to estimate the time of insect colonization. Information determined from an assessment of forensic entomology evidence could generate investigative leads in cases involving death, abuse, neglect, or contamination where insects have colonized or become associated with humans or other vertebrates.

This document provides best practice recommendations for collecting and preserving entomological evidence within the course of an investigation.

2. Normative References

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Haskell, N.H., and R.E. Williams. *Entomology & Death: A Procedural Guide.* Clemson, Joyce's Print Shop, Inc., 2008.

Sanford, Michelle R., et al. "Entomological Evidence Collections Methods: American Board of Forensic Entomology Approved Protocols." *Forensic Entomology: The Utility of Arthropods in Legal Investigations,* edited by Jason Byrd and Jeffrey Tomberlin. CRC Press, 2019. 63-85.

Organization of Scientific Area Committees (OSAC) for Forensic Science, Scene Investigation Subcommittee. OSAC 2021-N-0015. *Guiding Principles for Scene Investigation and Reconstruction* [OSAC Proposed Standard], March 2020.

Organization of Scientific Area Committees (OSAC) for Forensic Science, Scene Investigation Subcommittee. OSAC 2021-N-0018. *Standard for On-Scene Collection and Preservation of Physical Evidence* [OSAC Proposed Standard], March 2021.

Organization of Scientific Area Committee (OSAC) for Video/Imaging Technology (VITAL) Subcommittee. OSAC 2021-S-0013. *Standard Guide for Post Mortem Examination Photography* [OSAC Proposed Standard], March 2022.

3. Terms and Definitions

For the purposes of this document, the following definitions and acronyms apply.

3.1 exuvium (plural: exuvia)
The cast skin of immature insects as they complete immature stages (Nichols 1989).

3.2 larva (plural: larvae)
An immature stage of an insect (Nichols 1989) that undergoes complete metamorphosis.

3.3 minimum postmortem interval (minPMI)
The minimum estimated amount of time that has elapsed since death, i.e., death could have been earlier.

3.4 myiasis
The colonization or infestation of healthy or decaying tissues of living humans, pets, or other animals by fly (Diptera) immatures (eggs, larvae) (Amendt et al., 2011).

3.5 nymph
An immature stage of an insect with incomplete metamorphosis (Nichols 1989).

3.6 postmortem interval (PMI)
The time between death and discovery of the decedent or remains; also known as time since death (OSAC Lexicon).

3.7 pupa (plural: pupae)
The insect life stage during which complete metamorphosis from larva to adult occurs (Nichols 1989).

3.8 puparium (plural: puparia)
The sclerotized exuvium of the third instar of higher Diptera in which the pupa is formed (Nichols 1989).

3.9 scene
A place or object that is subject to and/or requires forensic examination (ISO/FDIS 21043-1:2018[E]).

NOTE A crime scene is a common description of a scene where a presumed crime has been committed. The scene can be a person or an animal.

3.10 time of colonization (TOC)
The time when insect offspring (i.e., eggs or larvae) were deposited on a human, pet, or other animal (Amendt et al., 2011) or other decomposing organic matter (e.g., food, feces).

4. Recommendations

For the purposes of this document, the following information serves as a guide for a properly trained entomologist, medicolegal death investigator, scene investigator, or law enforcement officer encountering entomological evidence associated with an investigation. Sanford et al. (2019) serve as the foundation for the methods described below for collecting and preserving entomological evidence. These methods should be integrated with the OSAC Proposed Standard *Guiding Principles for Scene Investigation and Reconstruction* (OSAC 2021-N-0015),.

4.1 General Considerations

If responding scene investigators do not have adequate training in the collection of forensic entomological samples, a certified forensic entomologist should be contacted for additional guidance. Should a forensic entomologist be unable to be contacted, the recommendations in this document should be adhered to, to ensure proper collection and preservation of entomological evidence. Annex A provides a list of suggested collection and preservation materials that should be utilized on scene and compiled prior to a scene response involving entomological evidence.

4.2 Documentation

4.2.1 In addition to the general scene photography standards and recommendations of the OSAC VITAL subcommittee (OSAC 2021-S-0013), photographs specific to entomological evidence should be taken of the following, with and without a scale:

a) if present, the body *in situ*
b) the scene after the removal of the body
c) close-up images of the body and wounds
d) insects on and off the body

4.2.2 In addition to the documentation standards and recommendations provided in the documentation standards and recommendations of the OSAC Forensic Science, Scene Investigation Subcommittee (OSAC 2021-N-15, OSAC 2021-N-0018), notes specific to entomological evidence should be taken, including:

a) body diagrams to indicate locations of insect colonization
b) the environmental conditions of the scene (e.g., in sun or shade, indoor or outdoor), including changes made to the environment after the discovery of remains (e.g., the opening of windows, changing of thermostat settings, etc.).
c) time/date the body was discovered
d) any times and details relating to specimen collection and further actions to preserve or kill specimen(s)
e) if specimens are collected at autopsy, the time the body is placed in refrigeration, the time removed for autopsy, and the temperature of both refrigeration unit and morgue rooms should be recorded
f) where possible, provide:

1) hourly air temperature at the scene using calibrated temperature data loggers (Hofer et al., 2020).
2) dependent upon scene/circumstances:
   i) body surface temperature
   ii) maggot mass temperature(s) (if present)
   iii) when specimens are collected from the soil, take the temperature of the soil (i.e., site)

4.3 Specimen Collection and Preservation (based on Sanford et al. 2019)

4.3.1 Collecting Specimens

Representative specimens of all life stages (Annex B) present at a suspected crime scene should be collected if possible (Haskell and Williams 2008). A representative sample includes some (≤ 50 individuals) of the largest and smallest specimens present, as well as specimens from visually different insect species and from different sites of infestation or off-body scene locations. Each sample should be collected separately and placed in appropriate vials with labels. Include date, time, location, and collector, as well as case and exhibit number. Most immature insects (i.e., eggs, larvae/nymphs, pupae) can be collected using forceps or gloved fingers. For collecting adults, a sweep net or sticky trap can be used. Sticky traps can be placed near the victim. Once the scene has been processed, the trap can be labeled, inverted into a cylinder with the sticky side on the inside ensuring not to overlap the sticky portion, and placed in a sealable plastic bag. Avoid crushing the cylinder as it will prevent the removal and identification of the flies collected.

a) Appropriate life stages (Annex B) should be collected from on and around the body, bearing in mind that:

   1) certain stages might not be present
   2) early stages can be very small
   3) later stages (mature larvae, puparia) can be dispersed several meters around the body and, depending on the environment, may have burrowed into soil, under carpets, and so forth
   4) specimens can vary in size and appearance: Examples of the following are presented in Annex B:
      i) eggs
      ii) larvae
      iii) pupae or puparia
      iv) adults
      v) empty puparia and larval skins (exuvia)

b) For larval samples, collect specimens representing a full range of sizes (Amendt et al. 2007).

c) All samples to be preserved should be preserved ideally at the scene (described below).
d) Comparable samples should be collected for rearing (see Standard on Rearing Insects XXX).
e) Use tandem labeling (i.e., place a label in the container - using a pencil or alcohol-resistant archival ink - and a duplicate label on the outside of the container);
f) Labels should include case number, exhibit number, collector name, time and date, geographical location, site of the collection - on or around the victim, ethanol concentration for relevant samples;
g) For preserved samples collected,
    1) To prevent sample decomposition in the early stages of preservation, the sample should be killed by immersion in hot water (at least 80°C / 176°F) for at least 30 seconds. Ideally, a thermos of hot water should be brought to the scene. Alternatively, a car-powered immersion heater should be used to boil water at the scene. If this is not possible, live specimens should be transported back to the lab to be hot water processed. However, the sooner the insects are preserved, the better as this 'stops the biological clock' and subsequent identification of life stages and calculation of TOC will depend on this time. Once hot water killed, the insects should be strained using a tea strainer and then transferred to a container with 70%+ ethanol (see Adams and Hall, 2003). However, if resources are unavailable for a hot water kill, the sample should be placed alive directly into the preservative, but this should be noted.
    2) For samples collected for DNA analysis, see XXX Standard for DNA Analysis of Entomological Evidence.
h) Adult specimens should be placed directly into a container of 70%+ ethanol or should be simply placed alive into a vial with a paper towel to protect them and then sealed before killing them back in the laboratory (e.g., by placing in a freezer for ?? hours).

4.4.2 Other Information Required

Forensic entomology can be used to estimate the period of neglect for a living person or other animal. In such cases, it is usually advised that the collection is made by medical personnel under the direction of a forensic entomologist. Other potential sources of insects near the decedent are also important to note as these might confuse the evidence. These may include other carrion, decomposing food, garbage, or dirty diapers.

Temperature is vital to the calculation of TOC as insect development is temperature-dependent. Temperature data from government weather stations are most commonly utilized.

4.4.3 Indoor Scenes

Bodies discovered indoors involve extra challenges, including questions concerning insect access and potential modification of indoor temperatures.

For insect samples taken indoors, it is important to note:

- if windows/doors are open and with or without insect screens
- was the sample collected from a site in direct sunlight
- thermostat temperature and time settings
- presence of decomposing food or feces
- any existing potential points of entry for insects, such as broken windows, missing doorknobs, gaps in the ceiling, etc.

In indoor scenes, it is important to note when first responders entered and whether they left doors open or opened windows to ventilate the scene, which can then allow both ingress and egress of adult flies. Flies that may have emerged from the scene may leave, whereas outdoor flies may enter to lay eggs, attracted by decomposition fluids and blood. In the latter case, the flies may lay eggs, but these will have no bearing on TOC estimations as such colonization occurred after discovery. Also, if doors and windows are opened, this changes the inside conditions, potentially increasing or decreasing the temperature. As well, the entomologist needs to consider how insects accessed the body so must know the setting of the scene before it is disturbed. First responders and other law enforcement personnel may also alter thermostat settings on property heating systems, so it is important to ask what the settings were prior to any changes.

After a period of time, fly larvae usually leave the remains and conceal themselves to avoid predation during pupariation. In outdoor scenes, a suitable site may be found by burrowing into the nearby soil, but in indoor scenes with hard floor surfaces, the larvae may crawl much further and may be located under rugs, in other rooms, within furniture, and under appliances. Therefore, a more extensive search may be required. Fly puparia may also be confused with rodent feces. When carpet/hide beetles have colonized the decedent, large accumulations of frass or feces and larval exuvia may be present, and the fragile pupae or exuvia may be easy to overlook.

4.4.4 Outdoor Scenes

Outdoor scenes often have many more unknowns than indoor scenes, particularly as regards temperature. However, there is usually no impediment to insect access unless the body is buried or wrapped. Adult flies should be collected via a sticky trap or a traditional insect sweep net as long as it does not interfere with trace evidence. Whether the body is in the sun or shade and for how long should be noted as this can impact temperature. Once the body has been removed, the soil beneath should be searched to a depth of 10 cm and soil samples collected from beneath the body to about two meters distant.

4.4.5 Autopsy

Ideally, insect evidence should be collected at the scene. However, in some cases, it may not be possible to collect at the scene, or insects may not be noticed until the

autopsy. In such cases, the time that the remains entered the morgue refrigerator and the time that they were removed from the refrigerator should be noted as insect development may cease or slow down when refrigerated. The temperature of the refrigerator and the morgue suite should also be recorded. As the ambient temperature of the morgue suite may allow some insect development, to mitigate this issue, insects should be collected as soon as possible after the body is removed from refrigeration. During the autopsy, ticks (larva, nymph, or adult), lice (nymph or adult), or fleas may be noticed and should be collected and placed in 70%+ ethanol. Notes should include whether such specimens were alive when collected as well if any insect artifacts are present (e.g., bee, wasp, or ant stings as well as roach or other insect feeding-sites).

4.46 Burials, Wrapped, Confined

Insects should be collected from buried bodies in the same way as from any other body, but an increased search of the surrounding soil may require the use of coarse sieves. When bodies are wrapped, insects may be found throughout the wrapping. When dealing with confined spaces, methods previously outlined for other locations (e.g., indoor) as specimens may be found in the seams or crevices of the confined area.

Annex A
Suggested equipment and supplies list

A suggested Equipment and Supplies List for Entomology-Based Collection in Forensic Investigations.

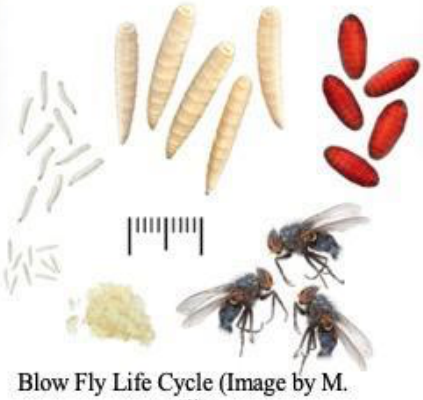
- **Case notebook** including body outline templates (see 4.2.2 and Amendt et al., 2007) for recording information relevant to forensic entomology assessment (e.g., location of insect activity)
- **Camera** for photo-documenting remains at the scene and location and type of entomology evidence
- **Ruler** to be used as a scale of reference for images taken with a camera
- **Container for hot water** kill fixation of soft-bodied specimens (e.g., a thermos of at least approximately 80°C / 176°F water and cup, electric hot water heater that can plug into automobile or other external power source)
- **500 mL of 70%+ ethanol** (avoid isopropyl and denatured alcohol as these interfere with DNA extraction)
- **Forceps** for collecting entomological specimens
- **Positive closure screw cap containers** or other leak-proof containers (e.g., 5 mL containers)
- **Waterproof ink pen and ethanol-proof writing utensils** (e.g., pencil, micron pen)
- **Precut paper or stickers** for preparing labels which are placed inside containers with live or preserved specimens (e.g., containing ethanol) as well as attached to the outside of the container
- **Evidence bags** (include an outer paper bag to protect specimens from direct sun and spectators from viewing contents)
- **Ambient air temperature thermometer** to record the temperature at the time the scene is processed
- **Probe thermometer** for measuring substrate (e.g., water, soil) temperatures or maggot mass temperatures
- **Non-contact thermometer** (e.g., infrared, laser) for measuring maggot mass temperatures or other surfaces where contact will either disturb evidence or the body
- Standard appropriate **personal protective equipment (PPE)** (e.g., one-piece suit, gloves, shoe covers, mask, mosquito repellent)
- **Headlamp flashlight** (facilitates using both hands to collect at the scene) and **extra batteries**
- **Packing material** (e.g., paper towels) to secure glass vials during transport
- **Flat trowel** to scrape and search the soil for insects associated with the decomposing remains
- **Temperature data logger** for scene temperature collection on the hour every hour for five days (
- **Hand-held net** for sampling adult entomological specimens
- **Sticky traps** for the passive collection of adult insects

Annex B

Life Stages of a Blow Fly

Blow Fly Larvae (Image by M. Hall)

Blow Fly Eggs in Cup (Image by G. Anderson)



Blow Fly Life Cycle (Image by M. Hall)

Blow Fly Pupae (Image by G. Anderson)

Blow Fly Adult (Image by M. Hall)

Annex C

Bibliography

There are a number of publications that provide guidance on the collection and handling of entomological evidence and associated parameters to be recorded. The most recent at the time of this writing, and approved by the American Board of Forensic Entomology, is Sanford et al. (2019). The following were cited in this Standard.

[1] Adams, Z.J.O. and M.J.R. Hall. "Methods used for the killing and preservation of blowfly larvae, and their effect on postmortem larval length." *Forensic Science International*, vol. 138, 2003, pp. 50-61.

[2] Hofer, Ines MJ, et al. "Estimating crime scene temperatures from nearby meteorological station data." *Forensic Science International*., vol. 306, 2020, 110028.

[3] Nichols, S.W. Torre-Bueno glossary of entomology. *New York Entomological Society*, 1989.