Chapter 11
Exploiting Insect Olfaction in Forensic Entomology

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11.1 Introduction

Insects, specifically blowflies (Diptera: Calliphoridae), are often the first to arrive at the scene of a crime and provide crucial information including post mortem interval and whether the body has been moved from its original location, amongst other useful information. History tells us that insects' association with death was recognised as early as documentation of events could be made (Greenberg and Kunich 2005; Benecke 2001). As we continue to understand this link dramatic advances, such as those mentioned throughout this book, are continually being made in the field of Forensic Entomology in relation to different situations, environments, as well as the incorporation of new approaches. While the methods used to determine the post-mortem interval (PMI), such as larval age determination and arthropod succession, are continually being used and further investigated the mechanism which attracts the flies to the body has not been fully explored. It is well documented that female flies will lay eggs near wounds or natural orifices soon after death so that the larvae may develop in a moist area (Smith 1986; Anderson 2001). However, determining exactly what attracts insects to a decomposing body and cause behavioural responses such as mating and laying eggs (oviposition), has still not been identified.

As humans, we primarily sense our world using vision, sound and touch (Cadré and Millar 2004). It is therefore understandable that we, at times, underestimate the importance of olfaction, the sense of smell. Insects perceive the world differently to humans and their ecology relies, sometimes almost exclusively, on chemicals they detect from their environment. Carrion insects are no exception to this.

It has been widely accepted that female carrion flies are attracted to volatile chemical cues emitted by a decomposing body (their host) in order to establish a

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suitable site for oviposition (Ignell and Hansson 2005). Although we know that body-derived odours are likely to attract the carrion insect to the body, we have not yet identified the chemicals responsible for the attraction and whether the chemicals covey additional information to the insects. The aim of this chapter is to describe recent advances in forensic entomology research and state-of-the-art techniques used to investigate insect responses to volatile chemicals from a decomposing body. The identification of such chemicals could aid in the development of new tools to estimate a more accurate PMI.

11.2 Insect Olfaction and Decomposition

11.2.1 Insect Olfaction

Insects must locate food sources in order to sustain life, obtain energy and gain nutrients required for the production of offspring. This is achieved by means of efficient sensory processes and behavioural mechanisms that are mediated by external and internal stimuli (Agelopoulos and Pickett 1998). Insects use chemical signals to navigate through their environment. They are able to quickly process the information within an odour plume coming from a source, such as a decomposing body. Generally, insects use different sensory perceptions to locate food, a mate, an oviposition site, and detect danger (Cragg and Cole 1956; Borror et al. 1989; Castner 2001). The cues can be visual, auditory, olfactory, gustatory, and physical and each is likely to play a role in the induction of a series of behaviours, leading to the successful location of the source of interest. The most dominant cues used by insects is considered to be olfactory stimuli. Most insects have a highly developed olfactory system and use it to detect volatile chemicals.

The capacity to detect and respond to volatile chemicals present in the environment exists in nearly all living creatures; however, this ability is particularly important in insects (Vickers 2000). Insect olfactory organs involved in the response to volatile chemicals are located primarily on the antennae (Borror et al. 1989). Often in nature the morphology and position of chemosensory appendages, such as the antenna, may help determine its importance and efficiency in capturing chemical cues. For example, most insects possess long, movable antennae which provide greater capacity to detect volatiles without requiring the insect to re-position its body frequently to detect an odour (Vickers 2000). These evolutionary features indicate that chemical cues play an important role in insect behaviour and survival.

Insect antennae are covered with a large number of sensillae (Castner 2001; Shields and Hildebrand 2001). Each sensillum houses olfactory receptor neurones (ORN) which detect volatile chemicals (Shields and Hildebrand 2001). The chemicals enter through pores on the sensilla where they are transported across the sensillum lymph by odorant binding proteins to the dendrites of the olfactory neurones (McIver 1982) (Fig. 11.1). The sensory neurones input information directly into the central nervous system and this induces a behavioural response in the insect

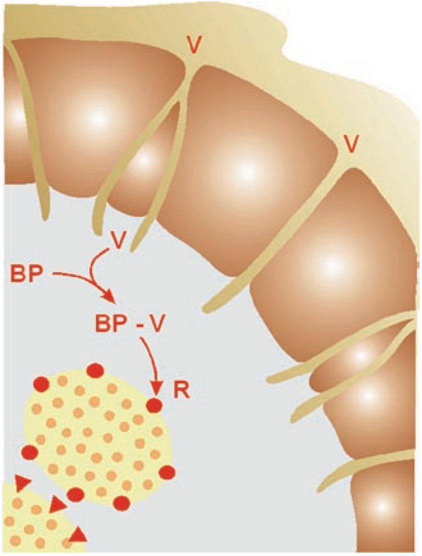

Fig. 11.1 Sensillum. V = volatile compound, BP = binding protein, R = receptor

(Hansson 2002; Zhou et al. 2004). Most insects respond not only to single compounds, but also to mixtures of compounds. With the correct combination of sensory inputs, animals or plants are recognised as hosts (Bruce et al. 2005). Insects also detect chemical gradients, giving them vital information about the location of an odour source (Vickers 2000). They detect volatile chemicals that indicate host suitability and also the presence of potential predators or competitors (Pickett et al 1998; Shields and Hildebrand 2001). As this chapter explains, even the state of decomposition of a body is revealed through the volatiles released. These volatile "signals" are also called semiochemicals.

11.2.2 Semiochemicals

The word "semiochemical" is derived from the Greek word *simeon*, which means 'sign' or 'signal' (Agelopoulos et al. 1999). Semiochemicals are volatile in nature and when airborne, they can be detected from long distances and potentially perceived by a number of other organisms of the same or different species (Agelopoulos and Pickett 1998; Selby 2003). Semiochemicals convey information between organisms and can be classified into two groups, pheromones and allelochemicals, according to the effect produced on the receiver or emitter (Nordlund and Lewis 1976; Blight 1990). Pheromones are chemicals which cause interactions between individuals of the same species (intra-specific) such as those that initiate behaviours

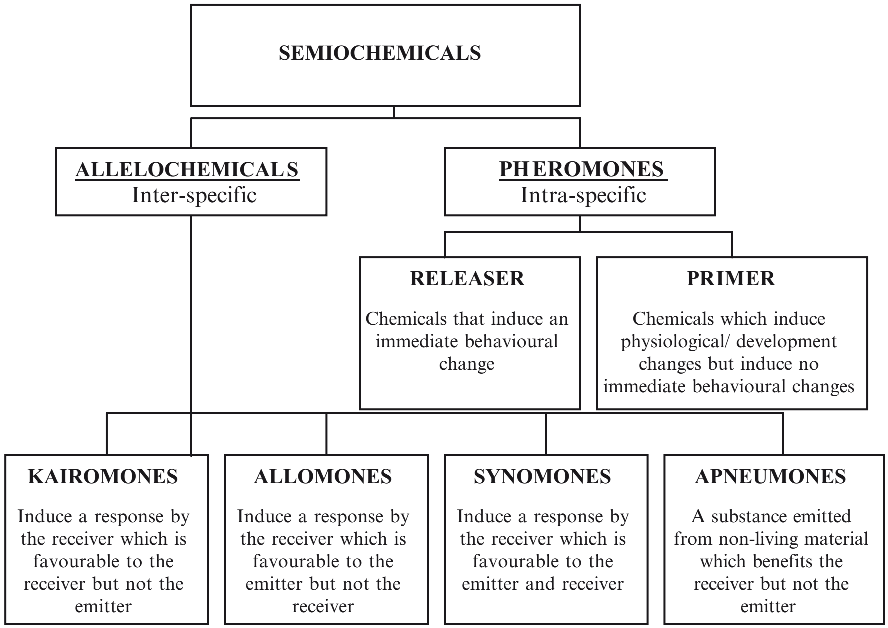

Fig. 11.2 Classification of semiochemicals (Nordlund and Lewis 1976; Howse et al. 1998)

such as mating; while allelochemicals create interactions between different species (inter-specific) (Agelopoulos et al. 1999) (Fig. 11.2). Semiochemicals are often perceived by the receiver beyond its visual range (Gikonyo et al. 2003) and a behavioural response can be triggered with only very small quantities of chemicals (Cork et al. 1990). Some volatiles are released in such small quantities, in fact, that they are barely detectable by the most advanced analytical techniques (Zumwalt et al. 1982); however, these can still be detected by insects.

The successful location of a plant or animal host by an insect is reliant on its ability to detect semiochemicals that give information about host suitability or physiological state (Pickett et al. 1998). For example, female mosquitoes (Diptera: Culicidae) detect odours such as carbon dioxide, ammonia and lactic acid from their animal or human hosts and these chemicals are of major importance in the successful location of an appropriate host in order to obtain a blood meal (Blackwell et al. 1992; Takken and Knols 1999). Semiochemicals may also be used to locate a suitable oviposition site or mate. For example, volatiles released during decomposition of a body allow blowflies to find the carcass, thereby increasing its chance of finding a suitable oviposition site, a mate, and food for their offspring (Smith 1986). Additionally, olfactory stimuli may function in combination with other stimuli, for example, oriental fruit moths, *Grapholita molesta* (Lepidoptera: Tortricidae), are unable to remain orientated when placed in a visually diminished "blank" environment containing a chemical attractant, implying that a visual cue is vital for site location (Vickers 2000). Similarly, the blackfly, *Simulium arcticum* (Diptera: Simuliidae), relies heavily on visual cues, such as shape and colour, to locate a host at close range and, therefore, responds not only to the CO_2 being released by the living host (Sutcliffe et al. 1991).

11.2.3 The Decomposition Process

Decomposition commences almost immediately after death and it is believed that the semiochemicals utilised by carrion insects are produced at the onset of decomposition (Vass et al. 1992; Dix and Graham 2000; Vass et al. 2002; Dent et al. 2004). The cells begin to die and enzymes digest the cells from the inside out, a process called autolysis or self-digestion. This action causes the cells to rupture and release nutrient-rich fluid (Dix and Graham 2000; Vass 2001). Tissues containing more digestive enzymes, such as the liver, are digested at a faster rate than those containing fewer enzymes. Putrefaction occurs as the bacteria, already present in the large intestine, destroy the soft tissues resulting in the production of liquids and various gasses (hydrogen sulfide, carbon dioxide, methane, ammonia, sulfur dioxide, and hydrogen) (Vass 2001). These bacteria gain access to the vascular system and spread throughout the body. There is often a green discolouration associated with these changes (Williams et al. 2001). As the blood begins to break down within the blood vessels and the skin loses pigmentation, the dark stained blood vessels can be observed through the skin producing an effect called marbling. The outer layers of skin begin to slip off the body while fluid under the slipping skin form blisters. Trapped gasses cause the body to become 'bloated'. The body swells, primarily within the abdomen, and decomposed blood or faecal matter may be 'purged' from the lungs, airways, or rectum. Once the trapped gasses have escaped, a more active stage of decomposition can be observed. Volatile compounds derived from the decomposition of materials, such as proteins and fats, are subsequently produced (Vass et al. 2002; Dent et al. 2004). The greatest physical changes to the cadaver occur at this time. The organs degrade and become unrecognisable forming a grey exudate within and beneath the body.

The odour associated with putrefaction is mainly caused by the release of sulfur-containing compounds and various inorganic gases that are produced in the bowel. However, bacteria, fungi, protozoa and even insects aid in the breakdown of soft tissues of the body during putrefaction and this results in the production of various gases including CO_2, H_2S, CH_4, NH_3,SO_2, H_2 and a variety of volatile organic compounds (Statheropoulos et al. 2005). Following this active decomposition the body may become skeletonised leaving behind just dry leathery skin and bones, depending on the environment in which the body has been resting (Clark et al. 1997; Dix and Graham 2000). The body can go through several phases and rates of decomposition and these are highly dependent on weather, temperature, humidity, and the environment, i.e. indoors, outdoors, buried, under water, wrapped or concealed (Clark et al. 1997; Dix and Graham 2000).

In the initial stages of decomposition, there are no visual or odour effects obvious to humans at this time, however, some insects are able to detect the decomposition immediately (Anderson 2001). Blowflies are most often the first insects to oviposit on a carcass and it is likely that a number of factors initially attract these insects to the body, including volatile semiochemicals. Throughout decomposition, bodies constantly change and emit hundreds of chemicals (Vass et al. 2002). It is currently unclear which semiochemicals are detected by the different carrion insects that are found on a decomposing body at the different stages of decomposition. However, chemical ecology research is unravelling this complex interaction between insects and decomposing corpses. This could potentially be exploited to develop a more accurate time of death, alongside the insect identifications relating to succession.

For the purposes of forensic entomology, the process of decomposition is divided into five visually distinct stages. These are the fresh, bloated, active decay, advanced decay, and dry stages, originally described by Payne (1965) and Anderson and VanLaerhoven (1996). The descriptions of these stages are based on physical condition, odour, and at times varying insect activity (Payne 1965). The "fresh" stage refers to the period immediately after death and continues until the body is bloated. Chemical breakdown occurs during this stage; however, few morphological changes are observed. There is no obvious odour to humans. The "bloated" stage becomes evident when an accumulation of gasses from the activity of anaerobic bacteria produce a swollen, bloated appearance. There is an obvious odour present at this time. The "active decay" stage is recognisable by the deflation of the carcass due to the gases escaping from the body, often due to the insect activity occurring on the body. There is a very strong putrid odour that can be detected – this is when the strongest odours are detected. During the "advanced decay" stage, a large amount of the flesh has been removed; however, there is still some moist tissue present. The odour is less obtrusive than in the previous stage, but it is still quite noticeable. The "dry" stage, also at times refered to as "skeletonisation", has been reached when the carcass has been reduced to bones, cartilage, and dry skin. At this stage, only a slight odour is present.

11.2.4 Carrion Insects and Semiochemicals

Different species of carrion insect have developed different seasonal life-cycle development times, variations in habitat, and preferences in carcass size, host species, and decomposition stage due to the high levels of interspecific competition (Fisher et al. 1998). Some, like the blowfly *Lucilia sericata* (Diptera: Calliphoridae), are able to develop on live sheep and other warm-blooded vertebrates as well as in carrion (Fisher et al. 1998). Such strategies greatly increase the chance of survival of their offspring.

Carrion odour contains a wide range of chemicals. Volatile molecules appear almost immediately after death but human olfactory perception is too insensitive to detect such short-term degradation. However, carrion insects have evolved to detect olfactory stimuli from the corpse, even at the early stages of decomposition. Those semiochemicals emitted from decomposing bodies are classified as 'apneumones'. These are chemicals which are emitted by non-living material, such as a corpse, and evoke a behavioural reaction from the receiver (see Fig. 11.2). The word apneumone was derived from the Greek word *ā-pneum*, meaning breathless or lifeless. This category was originally described by Nordlund and Lewis (1976) and has since become increasingly important when discussing carrion insects.

It is likely that chemicals emitted from a decomposing body may provide information about its location and suitability as a host and may even provide signals to their predators. It is well documented that insect succession occurs on a decomposing body and different species of insect are attracted at different stages of decomposition. While the production of volatile chemicals is caused by the decomposition of bodies due to bacterial and enzymatic activity, many of the volatiles released are as a result of the action of carrion insects on the corpse. While this is still in the early stages of investigation, other areas of research have revealed important findings that could be relevant to forensic entomology. For example, there are many examples in plant-insect interactions where mechanical damage caused by insect colonisation or feeding can alter the semiochemical profile of the host plant (Dewhirst and Pickett 2009). When plants are attacked by aphids (Hemiptera: Aphididae) the volatile profile of the plant is altered, and this profile can even be specific to a particular aphid species (Du et al. 1998). In such cases, plants release herbivore-induced signals that can alert and attract predators (e.g. parasitoids) to the plant, which indirectly protect plants against herbivory. An example of this can be seen when the pea aphid, *Aphis pisum*, attacks the broad bean, *Vicia faba*. Levels of attractive volatile chemicals (including 6-methyl-5-hepten-2-one, linalool, (*E*)-β-farnesene and *E*)-ocimene) increase following aphid feeding (Du et al. 1998). Similarly, lima bean plants infested with spider-mites *Tetranychus urticae* (Prostigmate: Tetranychidae), release an odour which attracts the spider-mites' natural predator, the predatory mite *Phytoseiulus persimilis* (Mesostigmata: Phytoseiidae) (Sabelis and van de Baan 1983; Dicke and Sabelis 1988). Similar instances of predation or parasitism are also witnessed on decomposing bodies. For example, the parasitoid wasp, *Alysia manducator* (Hymenoptera: Braconidae), is a common parasitoid of the blowfly, *Calliphora vicina* (Diptera: Calliphoridae), and will lay eggs on the fly larvae during specific stages of their development (Reznik et al 1992). *Nasonia vitripennis* (Hymenoptera: Pteromalidae), is another common parasitic wasp which comes to a decomposing body to lay eggs on larvae of the Muscidae (Order: Diptera). Their evidential value as indicators of time since death has been explored by Grassberger and Frank (2003) with some degree of success. Others, such as the yellow jacket wasp (Hymenoptera: Vespidae), the beetle *Necrodes littoralis* (Coleoptera: Silphidae) are predators of the adults blowflies and many Dipteran larvae, respectively (LeBlanc 2008). However, in these instances, the chemicals being released, attracting the predators and parasites, or their sources have not yet been identified. Whether it is volatiles from the decomposing body or those produced by the insects themselves which are used by the insects is still unknown.

11.3 Chemical Ecology Research

The identification of semiochemicals and their purpose can be achieved by examining the odour source and understanding their role in the biology of both the insect and the host. Various chemical ecology techniques can facilitate this. Volatile semiochemicals can be isolated using air entrainment (also referred to as headspace collection). This method allows the collection of volatile compounds produced by an odour source (e.g. decomposing body) onto a filter, commonly comprising a porous polymer, such as Porapak or Tenax. During this process contamination is kept to a minimum by, isolating the target source, with only the volatile chemicals produced by the source being collected (Agelopoulos et al. 1999). Subsequent analytical chemistry techniques, such as gas chromatography (GC) and GC–mass spectrometry (GC-MS) allow the chemicals to be identified accurately.

Headspace analysis is an established technique already used in forensic science for the collection of volatiles in blood and organ specimens (Statheropoulos et al. 2005; Hoffman et al. 2009). However, it has also been used successfully to investigate volatile odours from dead bodies. For example, Statheropoulos et al. (2005) used air entrainment to collect volatiles from the bodies of two males and identified and quantified over 80 different chemicals. The most prominent compounds were dimethyl disulfide, toluene, hexane, benzene 1,2,4-trimethyl, 2-propanone and 3-pentanone and they found marked differences in concentration between the two bodies. The authors suggest that these differences could reflect different rates of decomposition between the bodies and thus may provide valuable information about time of death (Statheropoulos et al. 2005). Later Statheropoulos et al. (2007) collected air entrainment samples of a body during the early stages of decomposition (4 days since death) during a period of 24 h. This time, over 30 volatile chemicals were identified. Eleven of these compounds were recovered throughout each sample, forming a "common core". The "common core" was made-up of the following: ethanol, 2-propanone, dimethyl disulfide, methyl benzene, octane, *o*-xylene, *m*-xylene, *p*-xylene, 2-butanone, methyl ethyl disulfide and dimethyl trisulfide (Statheropoulos et al. 2007). More recently, a study was conducted to identify volatile compounds from 14 separate tissue samples (Hoffman et al. 2009). In total 33 compounds were identified and could be grouped into seven chemical classes such as alcohols, acid esters, aldehydes, halogens, aromatic hydrocarbons, ketones and sulfides. There were common compounds identified in all of these studies; however, it was found that, at this stage, no unique set of compounds could be used to create a "chemical signature" of the decomposing tissues.

Similarly, volatiles have been collected successfully from dead pigs (*sus scrofa*). In this case the pigs were contained inside a metal container during the time of sampling and the volatiles were extracted through Porapak and Tenax filters (LeBlanc 2008). These samples were taken daily in order to encompass the different stages of decomposition. A large number compounds were recovered, however, in this research the aim was to locate specific compounds which are detected carrion insects, specifically the blowfly *Calliphora vomitoria* (Diptera: Calliphoridae).

Although air entrainment followed by GC and GC-MS analysis can provide information regarding the general profile from an odour source (often hundreds of volatile chemicals), this alone does not indicate which volatiles are detected by the carrion insects antennae (i.e. those that are electrophysiologically-active). Therefore, additional techniques can be used to discriminate between electrophysiologically active and inactive compounds in the complex extract (Wadhams 1990). By combining different analysis methods, the identification of volatile compounds can be refined to those which have a behavioural impact on insects associated with decomposing bodies and thus, those that are characteristic of a particular stage of decomposition.

Electroantennogram (EAG) recordings were originally utilised by Schneider in 1957. Using microelectrodes, he found that it was possible to record depolarisation of the affected sensillum on the antenna stimulated by a volatile compound introduced over the antennal preparation (Fig. 11.3). In the case of Dipterans such as Muscidae or Calliphoridae, the antennae are connected to microelectrodes that record the response of the olfactory receptor neurones in the antennae. An odour stimulus can be delivered through an air stream flowing continuously over the preparation and a response (depolarisation) can be immediately recorded if the compound elicits an electrophysiological response. This is an effective means of initially identifying semiochemicals because EAG responses are recorded without the influence of environmental or neurological factors which could affect behavioural responses (Cork et al. 1990). EAG can be combined with gas chromatography to give GC-EAG which allows the location of active chemicals within a complex extract. This technique allows the location of EAG-active chemicals within complex extracts by taking advantage of the high-resolution of the GC while simultaneously utilising the

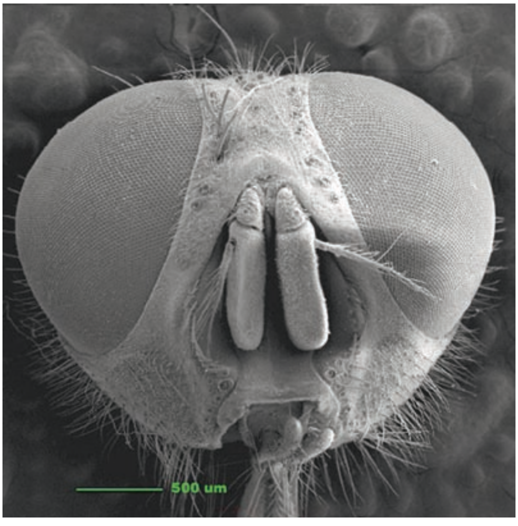

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Fig. 11.3 Scanning electron microscope (SEM) image of Muscidae extreme sensitivity and selectivity of the antennal preparation of an insect. A volatile sample is injected into the GC and the sample is split in two – half of the sample travels to the flame ionisation detector (FID) and half is simultaneously passed over the insect preparation (Fig. 11.4). The compound is recorded and displayed within a chromatogram and the reaction from the fly, if any, is recorded as a depolarisation indicated along with the compound in the chromatogram (Fig. 11.5). GC-EAG was first reported in 1969 (Moorhouse et al. 1969) and was subsequently advanced to

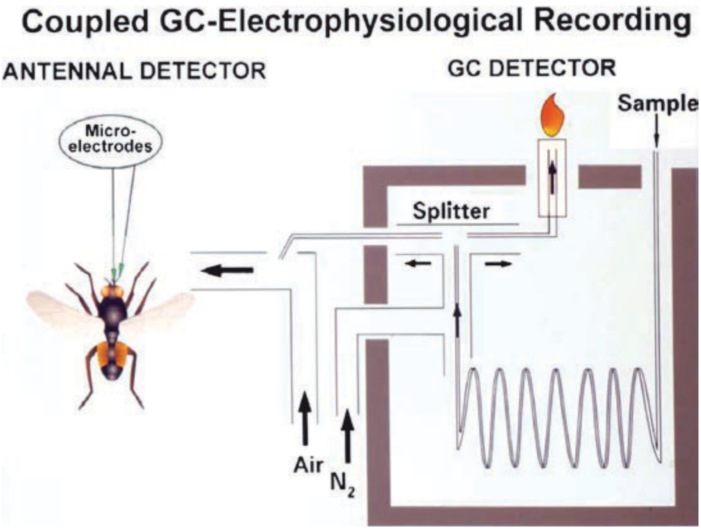

Fig. 11.4 System designed used for coupled gas chromatography and electroantennogram experiments

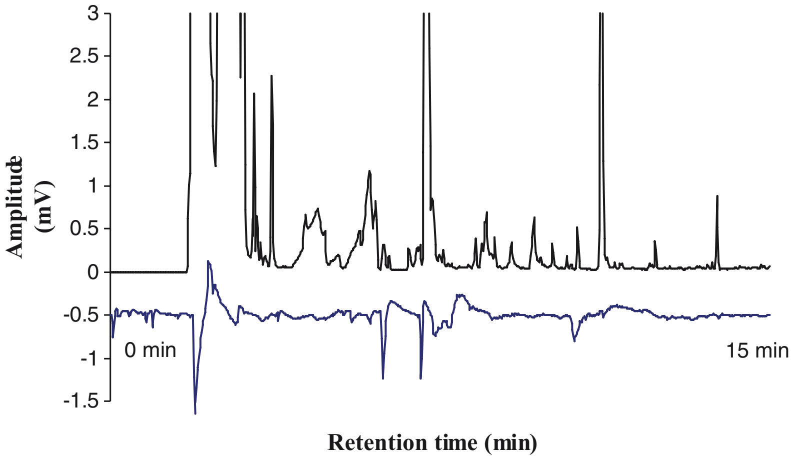

Fig. 11.5 Coupled GC-EAG trace. The upper trace shows the chromatogram of volatiles from the sample extract. The lower trace shows the corresponding EAG response of the insect preparation

possess the capabilities of also detecting responses of single olfactory cells (GC-SCR) (Wadhams et al. 1982). Although behavioural studies, have previously shown that *C. vomitoria* respond odours from bodies, to liver (Woolbridge et al. 2007) and to a compound, dimethyl trisulfide (Nilssen et al. 1996), no study had identified specific compounds associated with dead bodies which elicit a response from blowflies. However, recently the above chemical ecology methods have been used, for the first time in forensic entomology research, to identify semiochemicals from decomposing bodies that could be involved in the attraction of carrion insects (LeBlanc 2008). The identifications of chemicals from this study will be subsequently published.

While there are five recognisable stages in the physical decomposition of the carcass (Anderson and VanLaerhoven 1996), there is often no clearly defined beginning or end to a decomposition stage, especially during the later stages, making the decision of stage change somewhat subjective (Bornemissza 1957; Early and Goff 1986; Shoenly and Reed 1987; Archer 2004; Grassberger and Frank 2004). However, if the chemicals that are characteristic of specific stages of decomposition (i.e. "chemical fingerprinting") can be identified, this could aid confirmation of decomposition stage.

LeBlanc (2008) describes the daily collection of volatiles from decomposing pigs using an air entrainment method and the analysis of these compounds using GC-EAG with blowfly *C. vomitoria*. In these studies it was found that specific compounds, which included mainly sulphur compounds, within the volatile collections triggered an electrophysiological reaction from the blowfly. Most importantly differences were found between the different stages of decomposition. While the five stages of decomposition were determined through physical characteristics, the chemical composition and concentration of the volatiles sampled changed in a manner that closely followed these five stages. The Fresh and Dry stages exhibited the lowest concentrations of volatiles, however, the composition of volatile were different between these two stages. The EAG-active compounds, or semiochemicals, were at their highest concentration during the active decays stage. Visual observations supported these findings as the greatest number of Calliphoridae, adults and larvae, were recorded during this stage of decomposition.

Semiochemicals play a considerable role in mediating insect behaviour (Birkett et al. 2004; Pickett et al. 1998). Identifying these specific compounds and investigating the responses they elicit could provide a better understanding of the insects and, in some cases, allow manipulation of their behaviour. The period between death and the arrival of the first ovipositing blowflies is of great interest to the forensic entomologist, yet not fully understood.

Although some semiochemicals are likely to be attractants, others could have a negative effect on the insects behaviour. For example, Birkett et al. (2004) and Logan et al. (2008, 2009) found that at times when vertebrate hosts are not attractive to pests, there are elevated levels of certain semiochemicals which may act as active repellents or more passively by 'masking' attractants, thereby reducing the sensitivity of flies to these attractants. This could be relevant to decomposing bodies. Semiochemicals could be present at certain stages and may explain why certain carrion insects, such as *Calliphora* spp, are prevalent during the early stages of decomposition while others only appear later during decomposition. Competition and availability of food play an important role in insect succession on a corpse and therefore, repellents or ‘masking’ compounds could be used to prevent attraction of certain insects at particular times. For example, late colonizers could be repelled by early decomposition volatiles or by those produced by the immature stages (larvae) of the early colonizers to help avoid competition. However, the alternative is that the flies are simply not attracted to the odours present on a body at a particular time.

11.3.1 Decomposing Body Odour Mimicry

Olfactory stimuli associated with decomposing bodies is even exploited by plants. Certain plants of the Araceae family use mimicry and deception by releasing compounds similar to those associated with decomposing bodies to lure carrion insects such as blowflies and carrion beetles for pollination (Kite and Hetterschieid1997; Stensmyr et al. 2002). Semiochemicals associated with decomposing bodies are produced by the Mediterranean flower dead-horse arum, *Helicodiceros muscivorus* (Araceae: Aroideae) to trick the carrion flies in favour of the plant. This flower lures blowflies to act as pollinators by emitting dimethyl sulfide, dimethyl disulfide, dimethyl trisulfide, and dimethyl trisulfide derivatives (confirmed through GC-EAG experiments), which are also found in decomposing meat. The flies are thus enticed and trapped into a floral chamber that surrounds the female florets (Stensmyr et al. 2002). Other factors such as the appearance of the plant – the flower is said to resemble the anal section of a dead mammal – and the pseudo-thermogenic properties of the plant are also key factors in the fly’s attraction to the plant (Stensmyr et al. 2003). Circadian activity may also play a role in fly attraction as the odours are only emitted between sunrise and noon. GC-EAG tests also showed that female *C. vicina*, *L. caesar*, and other Calliphoridae species were attracted to these compounds and were not able to discriminate between decomposing meat and the flower through olfaction alone (Stensmyr et al. 2002).

11.4 Future Prospects

11.4.1 Time of Death

To date, entomology still remains the most reliable method of determining the post mortem interval, however, it is anticipated that in the future volatiles associated with a decomposing body, a “chemical fingerprint”, could be used to determine time of death and provide the pathologist with important forensic details (Vass et al. 2002; Statheropoulos et al. 2007). As described earlier, decomposition-related volatiles are greatly influenced by a variety of factors such as enzymatic and bacterial activity, temperature, humidity, body size, soil composition, the presence of clothing, as well as stomach content (Vass et al. 2002; Dent et al. 2004). Further investigations are required to account for such variations. Although insect development can also be affected by factors such as s temperature, humidity, and geographical location, these external influences have been studied in great detail in certain species and are thus highly predictable. Despite this, an accurate estimation of the post-mortem interval still remains a difficult task (Amendt et al. 2004).

Although there are many parallels, in terms of chemical ecology-related mechanisms, between carrion flies that interact with decomposing bodies, and other insects that interact with plants, the production and release of volatile chemicals are not the same. Nevertheless, it is certain that decomposing bodies release different volatiles at different stages of decomposition. LeBlanc (2008) has found that the change in composition and concentration of volatiles released change in sync with the physical changes noted on the pig often termed Fresh, Bloated, Active Decay, Advanced Decay, and Dry stages of decomposition; therefore, giving information on the physical state of the body. Portable methods of collecting volatiles from a body have already been developed (LeBlanc 2008; Logan et al. 2009). This means that volatile samples from a decomposing body could be collected from the crime scene and analysed in order to gain added information about the stage of decay and the behaviour of the insects on the carcass. While estimating a more accurate PMI is the ultimate goal, it may also be possible to determine volatile compounds which insects have evolved to avoid, providing useful repellents (Pickett et al 1998).

11.4.2 Body Recovery

The methods of detection and analysis mentioned in this chapter have a wide application. Efforts are already being made to use the identified volatiles which are associated with decomposition to make portable detection systems which would locate decomposing bodies (Smedts 2004; Hoffman et al. 2009). It is hoped that this technique would compliment canine lead victim recovery searches and that the volatiles identified could be used to help train the canines (Hoffman et al. 2009).

11.4.3 Pest-Control

Another function of semiochemical studies could have great agricultural applications. Already baited traps are being tested and used to lure myiasis causing blowfly, *Lucilia sericata*, away from livestock in order to reduce the amount of sheep strikes (Ashworth and Wall 1994; Smith and Wall 1998). These often include coloured adhesive boards with a bait such as sodium sulphate and liver mixture or the chemical attractant Swormlure-4, developed to more efficiently trap the New World screwworm fly, *Cochliomyia hominivorax* (Diptera: Calliphoridae). (Freney 1937;

Mackley and Brown 1984; Hall 1995; Smith and Wall 1998; Fisher et al. 1998; Hall et al. 2003; Woolbridge et al. 2007). A similar technique could be used, and possibly improved, for use in slaughterhouses and butcher shops where meat must remain at a high grade and therefore free of any insect larvae.

As research continues in the area of decomposition volatiles, new groundbreaking findings will be made. However, insects remain the most accurate method of determining the post-mortem interval and, therefore, it is important that the two, in conjunction, are studied further in order to determine their specific link to decomposition and time since death.

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