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REVIEW

Tools for detecting insect semiochemicals: a review

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Abstract

Semiochemicals are chemical compounds that are released by many species as a means of intra- and interspecific communication. Insects have extremely advanced olfactory systems; indeed, they rely on smell when performing many of their main behaviors, such as oviposition, breeding, prey location, and defense. This characteristic of insects implies that semiochemicals could be used for various applications, including in agriculture, where they could be employed along with other tools to control pest insects. The aim of this review is to present the main techniques used and the state of the art in the detection of semiochemicals, focusing on pheromones. In addition to the traditional methods of identifying semiochemicals, such as gas chromatography coupled to a high-resolution detection mode (e.g., flame ionization (FID), electron capture (ECD), photoionization (PID), or mass spectrometry (MS)), other tools are addressed in this review, including sensors and biosensors. While these new technologies may be used under laboratory conditions to improve or complement technologies that are already being used, they are mainly intended for use as new agricultural tools for detecting and controlling pest insects in the field.

Keywords Agriculture · Biosensors · Pest insects · Insect pheromones

Introduction

The chemical language of insects has been the subject of much research in recent decades. For instance, a family of semiochemicals known as pheromones significantly influence the survival, reproduction, and social organization of insects [1]. This also means that pheromones are very useful tools for monitoring and managing pests of agricultural crops [2].

The term "pheromone" was proposed in 1959 by Karlson and Lüscher to define substances secreted by one individual and received by a second individual from the same species. Such intraspecific chemical signals are used for communication. Many important decisions between insects are mediated through pheromones [1, 3]. Animals can receive and respond to numerous information requests by capturing and releasing odors. The mechanisms that detect this olfactory information, process it in the brain, and finally translate it into appropriate behavior are extremely important in neuroscience [4]. Knowledge of the compositions of these semiochemicals and the forms in which they are released into the environment is crucial before we can employ them for various applications.

Pheromones are used in agriculture to control insects and to monitor pest insects; for instance, they can be used to estimate the size of an insect population through field sampling [5, 6]. They have several advantages when employed for pest management. First, pheromones are natural compounds that are generally used in very small quantities (billionth). Second, pheromones break down relatively quickly in the environment (they do not leave residues). Third, they are highly specific—they do not act on non-target organisms. Fourth, they allow the rapid detection of insects in the field, aiding agricultural decision-making [7–10].

Traps containing pheromones have been widely used by various cultures around the world to capture and monitor pest insects in the field, in some cases quite successfully [11, 12]. For example, pheromones have been utilized to trap banana root weevils *Cosmopolites sordidus* (Germ.), cotton boll weevils *Anthonomus grandis* (Boh.), codling moths *Cydia pomonella* (L.), and brown stink bugs *Euschistus heros* (Fabr.) [8]. The development of detection systems based on

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electrochemical sensors and science materials is leading to the emergence of new techniques that employ chemical communication as a way to detect insects in the field.

In this review, we present a brief summary of insect semiochemicals that focuses on sex pheromones. We discuss the extraction and identification of pheromones, the chemical characteristics of these molecules, and recent advances in pheromone-based methods for the real-time detection of pest insects in agriculture.

Insect pheromones

Among all of their senses, insects are most dependent on their sense of smell to perform their main behaviors. They use chemical signals to aid them in various tasks, such as identifying an oviposition site, when breeding, for prey location, and for defense [13].

The chemical substances used by organisms to communicate are called semiochemicals. This term derives from the Greek word *semeon*, meaning signal. Pheromones are a well-known group of semiochemicals used for intraspecific communication (i.e., between individuals belonging to the same species). Another family of semiochemicals are allelochemicals, which are employed in interspecific (i.e., between-species) communication. Allelochemicals are divided into three different types according to their effects on individuals. When the receiving species is favored (and the sending species is disadvantaged) by the communication, the allelochemicals involved are called kairomones; when the reverse is true, the allelochemicals are termed allomones. Finally, allelochemicals that are used in communication that benefits both the receiver and the sender are called synomones (Fig. 1) [14, 15].

Among the different types of pheromones that are known, sex and sex-aggregation pheromones are of particular interest. The former are produced by one gender to attract the other gender; the latter are released to attract both genders [6, 11].

Pheromones can be applied in the field in various ways:

Mass trapping. In this case, pheromones are used in traps to attract a large number of insects and thus reduce the insect population to economically acceptable levels [16].

Confusing. Here, traps containing large amounts of a pheromone are placed in the field to confuse the sexes of the target insect, making it difficult for them to find each other to mate. For this technique to work properly, it is necessary to prevent the insect population to grow. Otherwise the method does not work and they will reproduce anyway. The goal is to disrupt the communication system between individuals, thus sharply decreasing the probability of encounters and mating [2].

Monitoring. Pheromones have been used to determine the population levels of different pests, thus providing guidance for the appropriate application of chemical treatments. In other words, pheromones can provide an overview of the degree of insect infestation in the field; this information can be used to determine the timing of the implementation of insect control measures [17].

Insect pheromones are, in general, mixtures of two or more components, but in some cases only one of the components is needed to both attract and capture insects [11, 18, 19]. The production of sex pheromone molecules varies widely across different insect orders. For instance, in moths, females are generally the pheromone producers. The females release pheromones for short periods (2–5 h) during the scotophase. On the other hand, in species from the family Pentatomidae, males are always the pheromone producers. In Neotropical stink bugs, the male produces a sex pheromone to attract females, whereas male Nearctic stink bugs release a sex-aggregation pheromone that attracts both genders. Among coleopterans, there are examples of males and females acting as sex pheromone producers.

A pheromone-producing insect will release only nanograms of the pheromone. Many pheromone molecules are thermally unstable and/or degrade in the presence of oxygen and UV radiation, making it rather difficult to extract, detect, and apply them in the field. However, this instability of semiochemicals provides a way of protecting the pheromone producer from being found by its natural enemies, and it ensures that pheromones do not contaminate the environment or leave residues in it: the semiochemical only remains in the environment long enough to allow it to reach its main target [20].

The chemistry of pheromone molecules

Insects of the family Pentatomidae (Hemiptera) are well known for their unpleasant odor, which is caused by the release of aldehydes and esters by these stink bugs. The bugs release these compounds when they are stressed. Compounds used for defense are stored in three pairs of dorsal abdominal glands (DAGs) in the nymphs, whereas they are produced in the metathoracic glands (MTGs) and stored in an orange-colored reservoir between these glands in the adults [21, 22].

Several studies in the literature have reported the chemical compositions of the compounds stored in these glands, but only a few studies have considered the roles of these

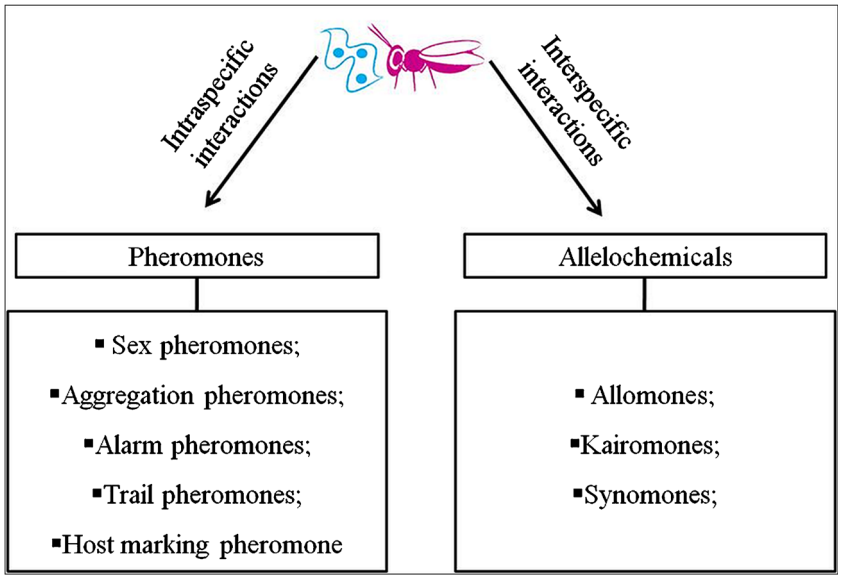

Fig. 1 Semiochemical classification

compounds [11, 23]. The mixture of defensive compounds contains (*E*)-2-alkenals as well as 4-oxo-(*E*)-2-alkenals with C_6, C_8, and C_{10} carbon chain and linear hydrocarbons (mostly C_{11} and C_{13}), including (*E*)-2-hexenyl acetate, (*E*)-2-octenyl acetate, (*E*)-2-decenyl acetate, 4-oxo-(*E*)-2-hexenal, 4-oxo-(*E*)-2-octenal, and 4-oxo-(*E*)-2-decenal (Fig. 2). The (*Z*) isomers of these compounds are sometimes detected in trace quantities. In addition to these major compounds, adult stink bugs also produce some alcohols (C_6–C_8) and their esters, such as (*E*)-2-hexenyl, (*E*)-2-octenyl, and (*E*)-2-decenyl acetates. The chemical structures of the defensive compounds used by several species of stink bugs have been determined, but it is unclear just how these insects use their defensive compounds [24].

Insects present pheromone molecules with a wide variety of chemical structures. Lepidopteran pheromones have been particularly well studied due to their economic importance and abundance, followed by coleopteran pheromones.

A review by Ando et al. [25] highlights the structural diversity of the components of lepidopteran sex pheromones. These pheromones are generally produced by females, and they can be divided into two major structural groups. Type I, the largest group, encompasses sex pheromones with a linear carbon structure (C_{10} to C_{18}) and a terminal functional group (alcohol, acetate, or aldehyde) as well as zero to three double bonds. Type II includes sex pheromones with longer saturated or unsaturated hydrocarbon chains (C_{17} to C_{23}) as well as their oxides. The chemical structures of some type I and type II pheromones are presented in Fig. 3.

While it would be easy to assume that different lepidopteran species have the same sex pheromone composition, this is not the case—the composition is always species-specific. Huge diversity is seen in the degree of unsaturation and the functional groups present on the linear chains of the components included in the blends.

Most type I sex pheromones have an even number of carbons, as they are derived from fatty acids such as palmitic acid (C_{16}: acid) and stearic acid (C_{18}: acid), and the double bonds

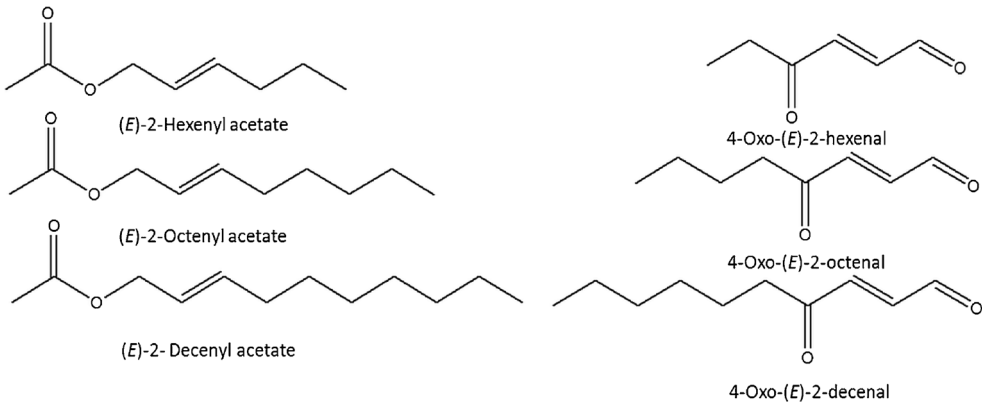

Fig. 2 Chemical structures of the defensive compounds normally found in bed bugs

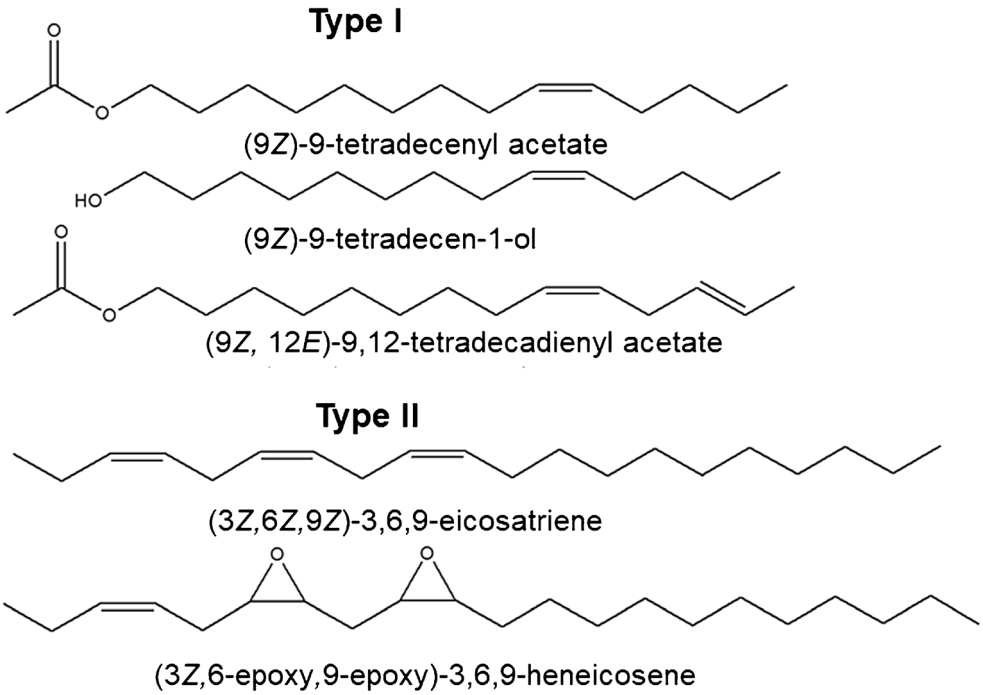

Fig. 3 The structures of some lepidopteran sex pheromone molecules

in type I sex pheromones are mainly located at even-numbered carbons with respect to the functional group at the end of the molecule.

A typical example of a moth that releases type I sex pheromones is the silkworm moth, *Bombyx mori*, which was the first species to have its sex pheromone (bombikol) identified [26]. Bombikol presents a linear chain with 16 carbons, *trans* and *cis* unsaturation at C_{10} and C_{12}, respectively, and an alcohol group at the end of the carbon chain. This compound is also denoted *E*10,*Z*12-16OH in a shortened notation. (*Z*)-7-dodecenyl acetate was identified as the principal component in the sex pheromones of*Trichoplusia ni*, *T. includes*, and 24 other lepidopteran species. To ensure pheromone specificity, *T. ni* also releases another three components: (*Z*)-5-dodecenyl ethyl, (*Z*)-7-tetradecenyl ethyl, and (*Z*)-9-tetradecenyl ethyl, whereas *T. includes* releases two other components: (*Z*)-7-dodecenyl propionate and (*Z*)-7-dodecenyl butyrate.

Type II compounds have been identified in more than 65 moth species, particularly those belonging to the *Geometridae*, *Noctuidae*, *Lymantriidae*, and *Arctiidae*. The structural diversity of heteropteran sex pheromones is greater than that of moth sex pheromones, and there are no established patterns for families or subfamilies. In contrast to lepidopterans, male heteropterans are the sex-pheromone producers. The genera *Acrosternum* and *Nezara* share the same sex-pheromone components: *trans*-(*Z*)-bisabolene epoxide (*trans*-*Z*-BAE; *Z*)-(1′*S*,3′*R*,4′*S*) (−)-2-(3′,4′-epoxy-4′-methylcyclohexyl)-6-methylhepta-2,5-diene) and *cis*-Z-BAE (Fig. 4a) [27–30]. However, the sex pheromones of these two species have different ratios of these two isomers, ensuring that the pheromones are species-specific. Similarly, the sex pheromones of six species of *Nezara viridula* from different regions of the world and six species of *Acrosternum* include the same two bisabolene epoxides, but these epoxides are present in different ratios in the pheromones of these twelve species [30–32].

Three acetates were observed in the sex pheromone of the brown stink bug *Euschistus heros*: methyl 2,6,10-trimethyltridecanoate, methyl (2*E*,4*Z*)-2,4-decadienoate, and methyl 2,6,10-trimethyldodecanoate. Laboratory bioassays and field tests showed that *E. heros* females are mainly attracted by the methyl 2,6,10-trimethyltridecanoate component; indeed, the other two components are not necessary to attract them (Fig. 4b) [7, 33, 34].

Methyl 2,6,10-trimethyltridecanoate presents three chiral centers, leading to eight possible stereoisomers. To determine the absolute configuration of the methyl 2,6,10-trimethyltridecanoate produced by the insect, the eight possible stereoisomers were synthesized and tested in laboratory olfactometer bioassays [33]. Bioassays of individual stereoisomers and the racemic mixture showed that the absolute configuration of the compound produced by *E. heros* males is 2*S*,6*R*,10*S*. The natural isomer produced by males was found to attract significantly more females than the other stereoisomers, but the racemic mixture also captured females in the field [35].

Males of *Thyanta palaeovirus* and *Thyanta custator acerra* produce the sesquiterpenes (7*S*)-(−)-β-sesquiphelandrene, (7*S*)-(−)-zingiberene, and (7*S*)-(−)-α-curcumene for inclusion in their sex pheromones, along with the main component, the

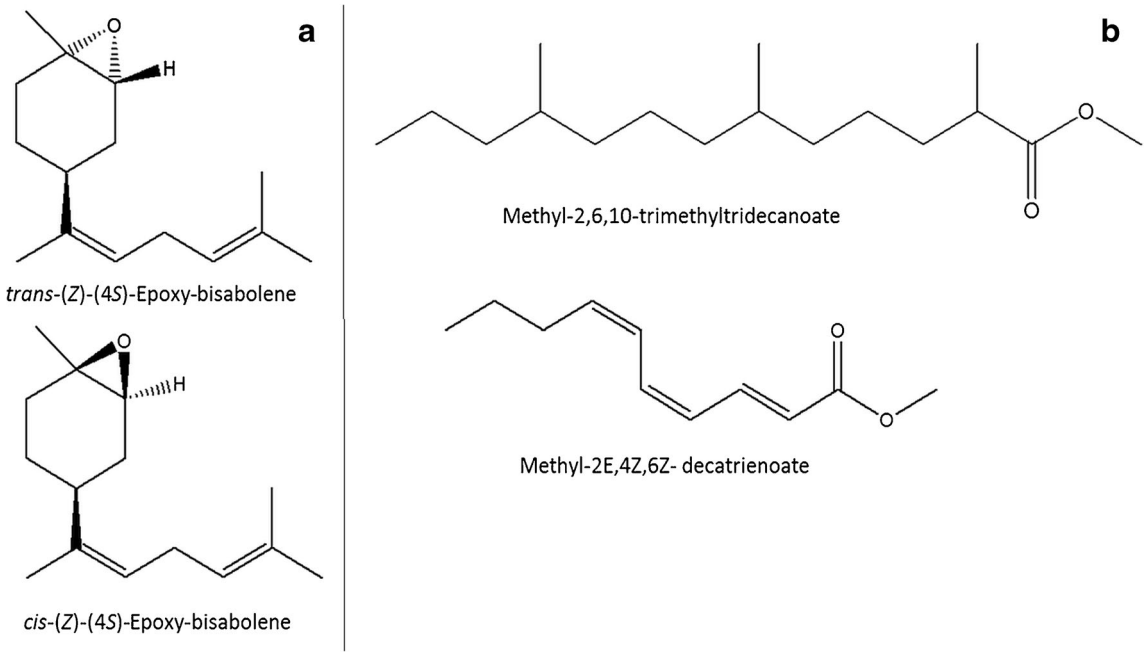

Fig. 4a–b Structures of the components of the sex pheromones of *Acrosternum* and *Nezara* species (**a**) and different species of stink bugs (**b**)

ester methyl (2*E*,4*Z*,6*Z*)-2,4,6-decatrienoate. Air-entrainment extracts from males of the Neotropical stink bug *Thyanta perditor* presented a sex pheromone comprising only one compound—the same ester identified in the sex pheromone of the Nearctic stink bug *Thyanta sp.* [36]. Studies performed to identify the sex pheromones of Pentatomidae suggest that closely related species of stink bugs exhibit the same or similar blends of compounds in their sex pheromones but retain pheromone specificity by using different component ratios or including another unique component.

The Neotropical rice stink bugs *Tibraca limbativentris* and *Oebalus poecilus* produce sesquiterpenes as sex pheromones. Males of *Tibraca limbativentris* produce two isomers of zingiberenol [37] whereas males of *O. poecilus* produce the 3*R*,6*S*,7*R* isomer of zingiberenol [38].

In the Curculionidae, the pheromones are produced by males to attract females to mate with, although they are sex-aggregation pheromones because they actually attract both genders. The majority of these pheromones produced by the Curculionidae can be divided into two categories: those derived from terpene (mainly monoterpenes with four- or six-membered rings or short linear C_7–C_9 chains that include functional groups such as alcohols, aldehydes, and ketones) and those derived from fatty acids.

The cotton boll weevil, *Anthonomus grandis* Boheman, presents four pheromonal components, all of which are derived from the terpene pathway: the alcohol grandisol (+)2-[(1*R*,2*S*)-1-methyl-2-(prop-1-en-2-yl)cyclobutyl]ethanol, (*Z*)-2-(3,3-dimethyl)-cyclohexylidene ethanol, and small amounts of the aldehydes (*Z*)-(3,3-dimethyl)-cyclohexylidene acetaldehyde and (*Z*)-(3,3-dimethyl)-cyclohexylidene acetaldehyde [39]. Another weevil of the same genus, *Anthonomus eugenii*, produces three components: (*Z*)-2-(3,3-dimethyl)-cyclohexylidene ethanol, geraniol, and geranic acid [40].

The palm weevil, *Dynamis borassi* (F.), produces 4-methyl-5-nonanol as an aggregation pheromone. Another pest of coconut crops, *Rhynchophorus palmarum*, produces (2*E*,4*S*)-6-methyl-2-hepten-4-ol as an aggregation pheromone, and two other compounds that were identified as male-specific pheromone compounds: 2,3-epoxy-6-methyl-4-heptanol and 4-methyl-5-nonanol [41, 42].

The isolation and identification of insect pheromones is difficult, largely due to the minute quantities that are released. The development of techniques that can rapidly detect these volatile compounds from insects is therefore of great interest.

Analytical methods employed to obtain pheromone molecules

Methods of extracting and identifying pheromones released and captured by insects are required to facilitate the development of new technologies for detecting these pheromones in the environment and for releasing pheromones into it. Understanding how and when insects release pheromones is crucial if we are to create new technologies to detect these semiochemicals under field conditions. To achieve this understanding, it is necessary to conduct laboratory experiments to extract and identify semiochemicals produced by insects and to discover the roles of these compounds. The particular semiochemical extraction or isolation method that should be

used depends on the insect species studied, the number of insects available, and the type of pheromone system involved [43].

The pheromone extraction method should be tailored to the morphologic, biological, and behavioral characteristics of the insects studied, as well as the chemical composition of the molecules released [44]. The main methodologies that are used to extract pheromones include aeration (volatile collection) and solvent extraction [45].

In air-entrainment volatile collection, all of the volatiles that the insects release over a defined period of time are collected using a small amount (50–200 mg) of a polymeric adsorbent (such as activated carbon, Porapak Q, Tenax GR, or Tenax TA). The volatiles are adsorbed onto the polymers and subsequently desorbed using organic solvents such as ethyl ether, hexane, and dichloromethane [45]. Figure 5 shows a schematic diagram of an aeration system for collecting volatiles from insects. In this system, air is initially pulled through a layer of activated carbon before it passes over the insects of interest. This air, along with volatiles from the insects, is then dragged to the opposite side of the aeration system, which contains the adsorbent polymer.

Male and female insects are placed in separate chambers in the system; the number of insects placed in the chamber depends on the behavior of insect in nature. Solitary insect species can be aerated individually or in small groups (5–10 insects), whereas species that tend to live in groups can be aerated in larger groups. The insects used must also be in good health, sexually mature, and well fed.

One advantage of the aeration technique is that it allows pheromones to be extracted from live insects for a predefined period. This technique is widely used to extract pheromonal compounds that are not stored by insects in compartments or specific glands; for example, they are produced and released continuously by stink bugs. Thus, the insects can be continuously aerated for periods ranging from a few hours to several days, which allows sufficient amounts of the volatile compounds to be released and to accumulate on the adsorbent for chemical analysis. One advantage of this approach is that it yields samples with lower contaminant levels than those obtained using other pheromone collection techniques [46].

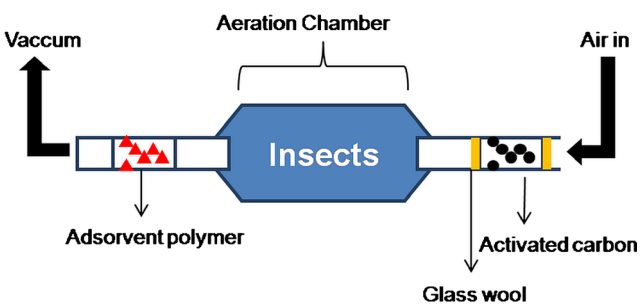

Fig. 5 Schematic diagram of an aeration system used to collect insect volatiles for adsorption on a polymer

Another way to extract pheromones is through liquid extraction with organic solvents, which is a more practical and rapid method of obtaining semiochemicals than the aeration method. However, the samples obtained using liquid extraction can be contaminated with compounds such as fatty acids. This technique is useful for extracting compounds from insects that have storage structures and pheromone production glands. For example, the sex pheromones of female lepidopterans are produced and stored in glands at the end of the abdomen, and the defensive compounds of stink bugs are stored in orange-colored reservoirs between the metathoracic glands.

Knowledge of the body part or structure of the insect that produces the pheromone is beneficial since the liquid extraction can then be made more specific, reducing the potential for contamination. The basic procedure for deriving this information involves submerging the insects into the solvent for a few minutes and then implementing a purification procedure such as filtration of the extracts with celite or a plug of glass wool to remove humidity and solid particles. When the structure that stores the molecule of interest has been identified, it is possible to draw out the secretion from the structure by capillarity using a very thin glass tube. The secretion is then dissolved in the organic solvent, allowing it to be analyzed directly. An alternative is to dissect the insect, obtain the structure, and dip it into the solvent for extraction [24, 47].

Solvents such as pentane, dichloromethane, hexane, and ethyl ether are extensively used in pheromone extraction because of their high volatility—using a volatile solvent allows the extracts to be concentrated at relatively low temperatures [48, 49].

Solid-phase microextraction (SPME) has also been used to extract semiochemicals from insects [50]. SPME involves the adsorption of the analyte on a very thin (7–100 μm) polymeric film coating a silica capillary fiber. The fiber can be placed into the container in which the organism is releasing the pheromone. The air in the chamber must circulate in order to achieve uniform diffusion of the molecules that will be adsorbed onto the fiber. The optimal time to trap the pheromone depends on the pheromone release rate, the chemical characteristics of the compounds, and the fiber used. After adsorption, the fiber is inserted directly into the inlet of a gas chromatograph, where the analyte is desorbed from the fiber due to the high injector temperature (250–300 °C) [51].

There are advantages and disadvantages of using solid-phase microextraction. Advantages include its high sensitivity, its ability to rapidly collect and analyze samples, and its good reproducibility for some compounds. However, its reproducibility and sensitivity vary depending on the affinity of the analyte with the fiber material, so they must be evaluated for each analyte tested. The major disadvantage of this approach is that the material collected can be used only once.

Extract concentration is a very important step in the extraction process. According to Millar and Sims [52], the removal of the solvent is the most critical step in the process because the volatility of the extracted compounds can result in the loss of some of them, which is important given the rather small amount of pheromone extracted in the first place. One of the main ways to minimize this analyte loss is to use solvents with very low boiling points. Conical tubes can also help to minimize any loss of the target compounds. Excess solvent is generally removed from the extract using a gentle nitrogen flow.

Overall, for chemical analysis, the extracts containing the pheromone components are concentrated to volumes of <100 μL. When the amount of pheromone produced and the chemical characteristics of the components are not known, the samples are concentrated to 1 μL per insect.

Analytical techniques for pheromone quantification and identification

As most pheromones are volatile molecules, gas chromatography with flame ionization is the technique most widely used for quantitative pheromone analysis, and gas chromatography coupled to mass spectrometry with electron impact ionization tends to be used for qualitative pheromone analysis [18]. Gas chromatography separates compounds based on their volatilities and polar affinities for the column. Typical chromatograms of aeration samples obtained from males and females of the Neotropical stink bug (*Thyanta perditor*) are shown in Fig. 6. The chromatograms were realized using a nonpolar column (DB-5MS, Agilent, Santa Clara, CA, USA) and a temperature ramp program.

Generally, extracts contain μg/mL to ng/mL quantities of semiochemicals, and the injection modes most commonly used in the analysis of such extracts are the splitless and cool on-column modes.Temperature programs are applied during the analysis to obtain higher compound resolution, and nonpolar columns tend to be used, especially those with dimethylpolysiloxane (DB-1) or 5% diphenyl-dimethyl polysiloxane (DB-5) stationary phases, which are suitable for separating medium- and low-polarity compounds.

Electroantennography coupled to gas chromatography

Electroantennography (EAG) was first described in 1957, when Schneider was attempting to identify a volatile from the silkworm, *Bombyx mori* L. He recorded slight variations in voltage between the tip and the base of the insect antenna when it was stimulated with the pheromone. He also found that the amplitude of the antenna response signal increased with the concentration of the volatile and the air flow rate [53]. Electroantennography can therefore be defined as a technique that uses an insect antenna as a biosensor for identifying physiologically active molecules. The antenna or insect head can be fixed between two electrodes (the working and the reference electrodes). When the antenna receives a chemical stimulus, the recorded electrical potential fluctuates. The apical end of the antenna is generally sensitive to odor molecules, so the tip of the antenna is placed on the working electrode and the base on the reference electrode. Electroantennography is a very useful technique for identifying molecules that exert physiological effects on insects; in other words, it can help to pinpoint compounds with the potential to act as semiochemicals [11, 54].

Although there are different protocols for performing EAG, studies have shown that the main disadvantage of this method is the preparation time required, which is said to vary from 30 min to 2 h [49, 55]. In EAG, the compound of interest is puffed onto the antenna.

When EAG is coupled to gas chromatography (GC), it is possible to evaluate a complex mixture of insect volatiles [56]. In GC-EAD (where EAD refers to electroantennographic detection), the column flow is split using a Y connector. Part of the flow is then directed to a FID detector while the other part is passed to the antenna of the electroantennographic detector [56, 57].

Siciliano et al. [58] isolated the chemicals emitted by (*Ceratitis capitata*) sexually mature flies during the call period (pheromone calling), and evaluated the electrophysiological responses that these compounds caused on the antennas. Fifteen compounds that produced electrophysiological activities in male flies were isolated and identified using EAG coupled to GC, including myrcene, farnesene, tetrahydro-3,4-furandiol, (*E*)-ocimene, and (*R*,*S*)-linalool. Eleven of those compounds induced responses in the antennae of both genders, while the other four prompted responses in female but not male antennae, indicating that these are electrophysiologically active compounds.

Park et al. [59] used GC-EAD for the detection and discrimination of twenty different compounds: *Z*-11-hexadecenal (Z11–16: Ald), *Z*-3-hexenol (Z3–6: OH), hexanoic acid, benzyl acetate 2-methyl-5-nitroaniline, cyclohexanone, α-pinene, *cis*-nerolidol, *trans*-nerolidol, β-caryophyllene, β-ocimene, (*R*)-limonene, methyl jasmonate, 2-diisopropylamino-ethanol, indole, 2,2-thiodiethanol, 1-heptanol, 1-octanol, 1-nonanol, and 1-decanol. The authors evaluated the responses of the antennae of five insect species—*Drosophila melanogaster*, *Heliothis virescens*, *Helicoverpa zea*, *Ostrinia nubilalis*, and *Microplitis croceipes*—to these volatiles, and observed different responses of these species to many of the compounds.

Some studies have also used GC-EAD to correlate active compounds with reactions between insects and/or host plants. Siderhurst and Jang [64] used GC-EAD to evaluate biologically active volatiles of the fruit fly. Olsson et al. [60]

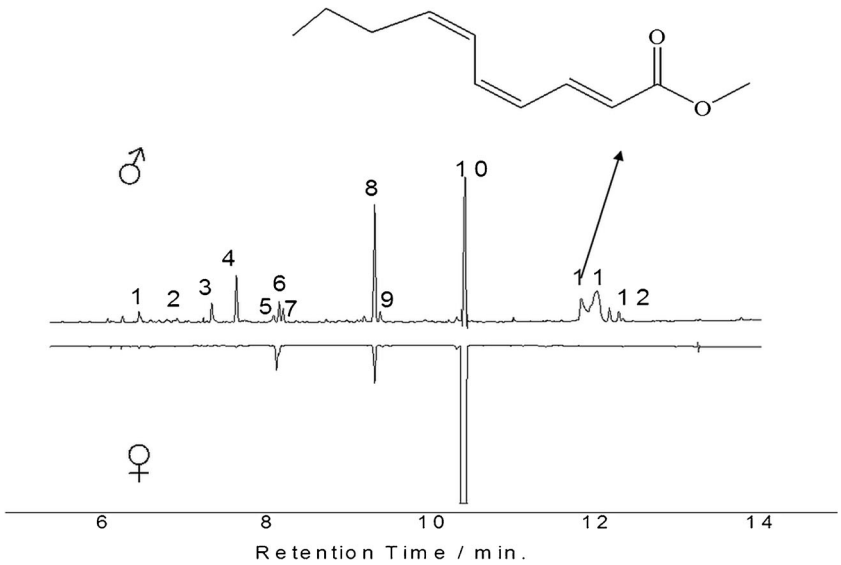

Fig. 6 Chromatographic profiles of the aeration samples obtained from males (*top*) and females (*bottom*) of the Neotropical stink bug, *Thyanta perditor*. The peak (11) is for the male sex pheromone [36] (reprinted with permission from Springer)

evaluated the effects of volatiles from chocolate on the moths *Ephestia cautella* and *Plodia interpunctella*. Zhang et al. [61] used GC-EAD to identify the active compounds involved in chemical communication between beetles, predators of the beetles, and a host plant. Magalhães et al. [54] used EAG to show that males and females of *A. grandis* possess antennal receptors for two homoterpenes emitted by cotton plants, and confirmed the biological roles of these compounds using behavior assays. Thus, the antennal response measured in EAG is an electrophysiological process that can be used to screen compounds released by insects for semiochemical activity.

Sensors to detect semiochemicals

Chemical sensors

The development of sensors to detect volatiles that act as semiochemicals is of great interest in various fields. As discussed above, the detection and identification of volatile compounds can be performed using either gas chromatography or mass spectrometry. However, these methods have some drawbacks: they are time-consuming, involve considerable handling costs, and require high power consumption. An alternative method is to use chemical sensors with partial specificity and an appropriate pattern detection system, which makes it possible to recognize either simple or complex odors.

The miniaturization of chemical sensors have been demonstrated to be highly effective tools for detect specific molecules in small quantity in the environment, in this way, broadly pursued by scientists [1]. Sensors are defined as devices that can transform information derived from a chemical reaction or a physical property of the system into an analytically useful signal [62]. The signal is generated through the chemical or physical interaction of the analyte molecules with a sensitive coating. This interaction triggers fluctuations, usually in electrical and optical properties, that are detected by an appropriate transducer and converted into an electrical signal [2]. The signal is processed using pattern recognition methods, yielding analytical data that can be displayed as a graph or plot.

Sensors can be categorized based on the method of detection into physical sensors, chemical sensors, and biosensors. Sensors vary in terms of the parameter that the sensor monitors for fluctuations (pH, humidity, liquid, or mass), whether the species of interest are chemical or biological, the property measured (resistance, capacitance, reflectance, or fluorescence), the transduction mechanism employed (electrochemical, potentiometric, amperometric, conductimetric, optical, thermal, or piezoelectric), the molecular recognition mechanism employed (microorganisms, enzymes, or immunosensors), and their specificity (specific or semispecific), as well as other characteristics [63, 64]. Desirable properties of sensors include sensitivity, selectivity, reversibility, stability, a fast response time, and a low detection limit. It is, however, difficult to obtain a sensor that fulfills all of these requirements, so it is necessary to prioritize some of those requirements over others [4, 65, 66].

In a pheromone sensor, the material used in the sensitive layer deposited on the sensor surface is responsible for the pheromone detection process. Molecules interact with this sensitive layer in one of two ways: by chemical adsorption, where the volatile molecules chemically bond with the surface, or by physical adsorption, where the volatile molecules interact weakly with the surface through van der Waals forces. In the latter situation, molecules may be adsorbed when they reach the substrate surface, but they do not undergo chemical reactions, so they maintain their original characteristics [67]. There are several review articles that explore the adsorption/

desorption properties of volatile molecules on sensors [68, 69]. Interactions of volatile molecules with the sensitive material on the sensor surface cause property fluctuations that are converted by the sensor into a more easily analyzed signal; in other words, the sensor acts as a transducer. Changes in mass, temperature, and conductivity (among other properties) are transduced by sensors.

Most sensors that are employed to detect volatile compounds are electrochemical (potentiometric, conductimetric, or amperometric) or piezoelectric sensors. These sensors convert the responses of the sensitive sensor layer to interactions with volatile molecules into electrical signals. The sensitive layer can be a metal-oxide semiconductor or a conducting polymer [70]. Semiconductor sensors are simple, robust, and show excellent responses to small concentrations of the analyte. Such devices usually employ a silicon semiconductor layer, a silicon oxide insulator (zinc, tin, titanium, tungsten and iridium oxides for example), and present high sensitivities (5–500 ppm) and fast response times. However, they tend to saturate, even at low volatile concentrations [71].

Sensors based on conducting polymers (polypyrrole, polyaniline, and polythiophene) are advantageous as they are easy to synthesize, inexpensive, versatile, can be used at room temperature, can be manufactured using a variety of methods (depending on the type of dopant and the degree of doping applied), and are highly sensitive to volatile compounds (10–100 ppm) [72]. On the other hand, they show high sensitivity to humidity and low reproducibility in some cases, as it is difficult to control the growth of the polymer film between electrodes [73].

Among the various piezoelectric sensors available, quartz crystal microbalance (QCM) sensors and surface acoustic wave (SAW) sensors are particularly prominent. QCM sensors consist of a quartz disc a few millimeters in diameter that is positioned between two circular metal electrodes. One of these electrodes is coated with the active layer, but the active layer is also connected to the quartz disc. The QCM sensor is characterized by adsorption/desorption: when the quartz disc is connected to an electrical circuit oscillating at a constant frequency, the deposition of a small amount of material on the oscillating crystal leads to a decrease in the frequency [17]. In other words, the QCM sensor acts as a mass balance. Its characteristics can be explained by noting that certain materials generate an electric field when they are subjected to external pressure or deformation, and the electric field generated depends on the change in polarity caused by the mechanical disturbance. These devices have detection limits of below one pictogram, allowing the detection of trace levels of analytes [74]. QCM sensors are used to detect a variety of substances in gas and liquid phases, such as ammonia, acetoin, hydrogen sulfide, sulfur dioxide, hydrochloric acid, nitrogen dioxide, methylamines, mercury vapor, hydrogen, anesthetic gases, organophosphorus compounds, and agrochemicals [17].

SAW sensors are defined as oscillator circuits whose resonance frequencies are controlled by resonator devices. They are used to measure small mass disturbances at the interface with a sensitive layer through acoustoelectrical phenomena. This interaction changes the acoustic wave speed, which in turn modifies the resonance frequency of the sensor. This change in resonance frequency can be related directly to the analyte (e.g., volatile) concentration [75].

Optical sensors, particularly surface plasmon resonance (SPR) sensors [76], are also applied to detect volatiles. SPR sensors utilize the charge density oscillations caused by surface plasmon waves traveling along the interface between a metallic layer and a dielectric medium to generate a signal [77]. Quantification of the species of interest is performed by measuring changes in the refractive index of the surrounding environment. SPR makes use of the change in the wavelength of peak light absorption on a metallic film that occurs if the refractive index of the film changes due to the adsorption of molecules [78]. Replacing the metal sensing layer with nanoparticles makes it possible to expand the range of applications of SPR sensors. Thus, it does not depend on the angle where the light interacts with nanoparticles, but on the collective and local oscillation of the electron cloud, which is called localized surface plasmon resonance (LSPR) [79].

New prospects for device development include the use of chemical sensor arrays, in which many sensor units are operated together in a single transduction system, or higher order sensors, which use multiple sensor units with different transducers. Both of these schemes allow more parameters to be analyzed and increase the information obtained from the sensor [80].

Although they are used for volatile detection, it is worth noting that none of these devices are comparable to the olfactory systems of living organisms in terms of sensitivity, selectivity, and response time: animals, especially insects, utilize highly sophisticated molecular mechanisms that involve the activation of olfactory receptors and permit rapid detection in real time [81].

Biosensors

Biological molecular recognition systems (e.g., olfactory organs) contain sensitive and selective elements that have specific binding affinities for particular molecules, such as semiochemicals [82]. A biosensor utilizes a biological component to detect specific molecules. The biological material may be a whole organism, an isolated organ, tissue, cells, an enzyme, a nucleic acid, an organelle, or microorganisms. It can also be derived from a biological material (such as recombinant antibodies or modified proteins) or it can be a biomimetic system

(such as synthetic receptors, imprinted polymers, or biomimetic catalysts). The signal from the biological component of the biosensor is transduced so that it can be measured optically, electrochemically, thermometrically, piezoelectrically, magnetically, or micromechanically; this transduced signal is proportional to the concentration of the analyte [83].

Biosensors are used in a wide variety of applications: to examine pollution and water contamination, for microbial analysis, for clinical diagnostics and biomedical applications, for fermentation analysis and control, to detect gases (such as toxic gases) and industrial liquids, by the military to detect explosives, and to probe flavors, scents, and pheromones. Their popularity is due to their many advantages: simple operation, inexpensive instrumentation, fast response times, and the need for only minimal sample pretreatment [84].

Odor biosensors based on cells are a very attractive technology as they are highly sensitive and selective, which are prerequisites for practical applications. They have been used to investigate the olfactory systems of many insects, including flies, mosquitoes, moths, and beetles. An olfactory receptor can detect a wide range of odoriferous molecules with different functional groups [85].

Studying how an insect species highly selectively detects and differentiates dozens of semiochemicals present at extremely low concentrations, and is even able to distinguish between isomers with different absolute configurations (*R* and *S* isomers), can provide useful information for researchers attempting to develop sensors that selectively detect very small amounts of volatile substances by imitating the olfactory systems of insects.

Odorant-binding proteins (OBPs), a multigene family of small hydrophilic proteins [1], are the most important part of an insect's olfactory system, implying that they could be useful for developing biosensors intended for biotechnological applications [57]. The first insect OBP to be identified came from the moth *Antheraea polyphemus*. It was a small (14 kDa) protein that was only found on male antenna at levels of approximately 10 mM, and was shown to bind to the sex pheromone of this species [86, 87].

The detection of biological molecules involves phenomena that occur at various length scales. For instance, protein interactions occur at the 1–10 nm scale, and nucleic acid interactions occur at a scale of approximately 20 base pairs (> 6 nm), i.e., both types of interactions occur at the nanoscale. Utilizing OBPs makes it possible to obtain nanoscale devices with excellent sensitivity.

Insects are able to perceive alterations to their environment through chemosensory neurons located in sensory hairs called sensilla (Fig. 7), which are distributed across the insect's body, especially on their antennae [20]. OBPs help to transport odoriferous molecules to those neurons, making OBPs critical to the detection of odors in insect olfactory systems [88]. Insects have a large number of OBPs on their antennae. These OBPs can endure considerable variations in pH and temperature without denaturing or losing their properties, suggesting that they are robust enough to use in biosensors [58].

OBPs are low molecular weight (< 30 kDa) proteins containing six cysteine residues that form three disulfide bonds. Studies show that the presence of these six similarly sized cysteine residues is sufficient to categorize a protein as an OBP [59]. OBPs can be classified into two subfamilies: pheromone-binding proteins (which are species-specific) and general OBPs [89].

By mimicking biological olfaction, Lu et al. [90] developed an olfactory electrochemical impedance biosensor that used interdigitated electrodes coated with immobilized honeybee (*Apis mellifera* L.) OBPs. This system was applied to detect alarm pheromones (isoamyl acetate and methyl *p*-hydroxybenzoate) and floral odors (linalool, geraniol, β-ionone, 4-allylveratrole, phenyl acetaldehyde, and dibutyl phthalate). Photographs and a schematic of the biosensor—an electrode chip containing two groups of eight channels—are presented in Fig. 8. The resistance of the biosensor decreased linearly with the logarithm of the ligand concentration for concentrations of 10^{-6} to 10^{-3} M. Thus the sensor is capable of detecting different concentrations of floral odors and pheromones down to detection limits of micromoles.

An olfactory biosensor that utilizes OBPs from the oriental fruit fly (*Bactrocera dorsalis*) immobilized on interdigitated electrodes to detect semiochemicals (such as isoamyl acetate, β-ionone, and benzaldehyde) emitted from host plants of this insect was developed by Lu et al. [81]. The OBPs (BdorOBP2) were identified and amplified by PCR. The genes of interest were cloned, yielding a recombinant plasmid that was introduced into cells of *Escherichia coli* to initiate the expression of recombinant proteins. Protein immobilization was achieved using self-assembly with specially designed polyethylene glycol on interdigitated electrodes of a glass substrate with a layer of chromium or gold. α-Thio-ω-carboxypoly(ethylene glycol) was then covalently bonded to this layer to give a robust and sensitive membrane. BdorOBP2 was immobilized on the resulting poly(ethylene glycol)-coated electrodes via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide coupling (Fig. 9). The electrochemical biosensor showed high sensitivity and affinity for benzaldehyde, with detection limits for isoamyl acetate, β-ionone, and benzaldehyde of 3.3×10^{-8}, 6.2×10^{-8}, and 8.4×10^{-8} M, respectively. This device could therefore be used in agriculture to detect semiochemicals.

Mitsuno et al. [91] developed a sensor that used ovary cells from the moth *Spodoptera frugiperda*, which express odorant receptors. The selectivity and sensitivity of the biosensor for pheromones were evaluated. Microfluidic devices were constructed on boron silicate glass wrapped in a layer of aluminum. The devices had a semicircular flow channel of radius 100 mm and a space for cell culture. The biosensor presented

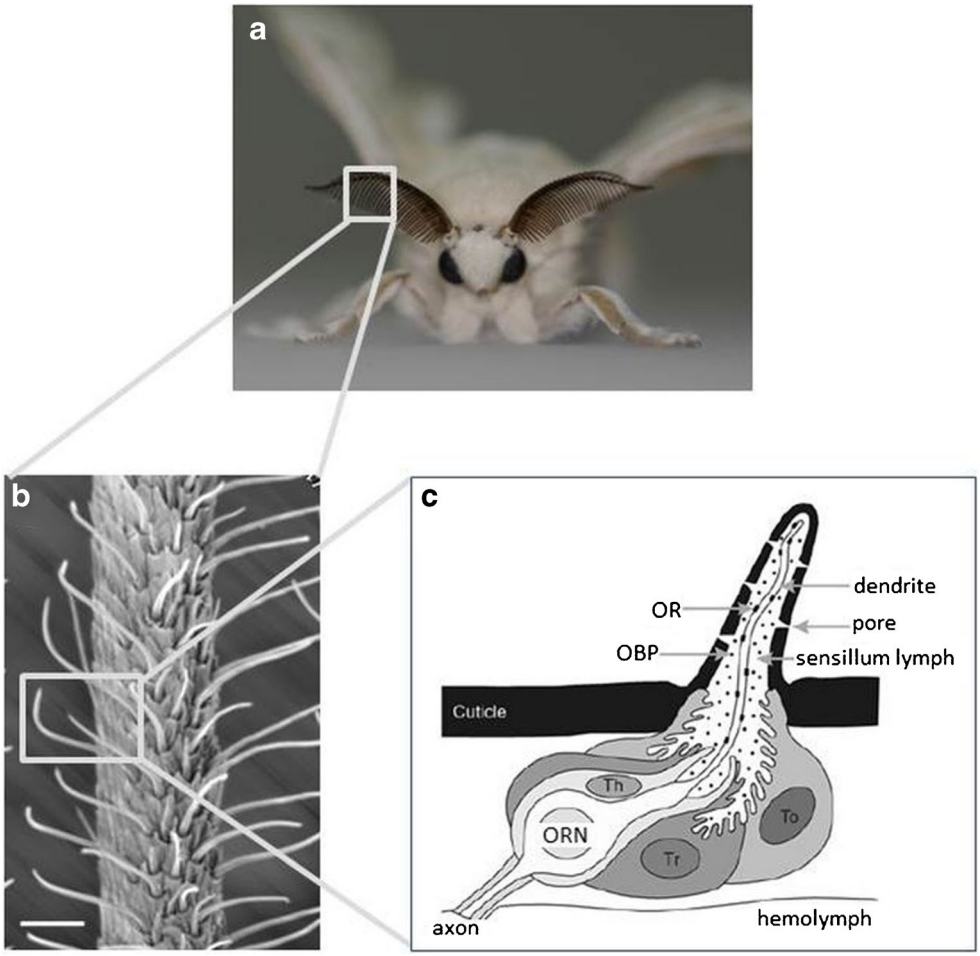

Fig. 7a–c Main olfactory sensory organs of the silk moth, *Bombyx mori*. **a** A male silk moth with its prominent antennae optimized for odorant detection. **b** Scanning electron micrograph of an antenna, displaying the external morphology of sensilla trichodea. **c** Schematic of an olfactory sensillum showing the detailed configuration of the olfactory receptor neurons (*ORN*) and auxiliary cells with respect to the cuticular specializations. The cell bodies of the ORNs are surrounded by three types of auxiliary cells, the tormogen (*To*), trichogen (*Tr*), and thecogen (*Th*) cells, which secrete odorant-binding proteins into the sensillum lymph. Odorants are detected by olfactory receptors (*OR*) expressed on the dendritic membranes of ORNs [20] (reprinted with permission from NCBI)

parts per billion (ppb) sensitivity in solution, and was employed to distinguish between similar olfactory chemical structures. It exhibited a rapid response time (~13 s) and maintained its response intensity for at least two months.

Research is fundamental to improving our understanding of insect communication. For instance, it is necessary to elucidate the synthetic and degradation processes of pheromones, as knowledge of these processes could aid the development of sensors that mimic the insect communication mechanism [1]. Also, it is very important to specify some condition during the execution of an experiment, such as temperature, air flow, rate of evaporation and the kind and amount of solvent used in the application. As well, the chemical affinity of the sensing layer with substrate to interact with pheromone compounds.

An electroantennographic detector (EAD) was applied to measure the pheromone flux in a wind tunnel during the flight of a moth [92]. The antennae of the moth were used as a detector by connecting the extremities of the antennae to two electrodes, yielding a potential difference of nanovolts between the electrodes [93]. Vickers et al. [92] observed that the largest electroantennogram bursts and frequent fluctuations occurred in the central zone of a pheromone plume at low wind speeds.

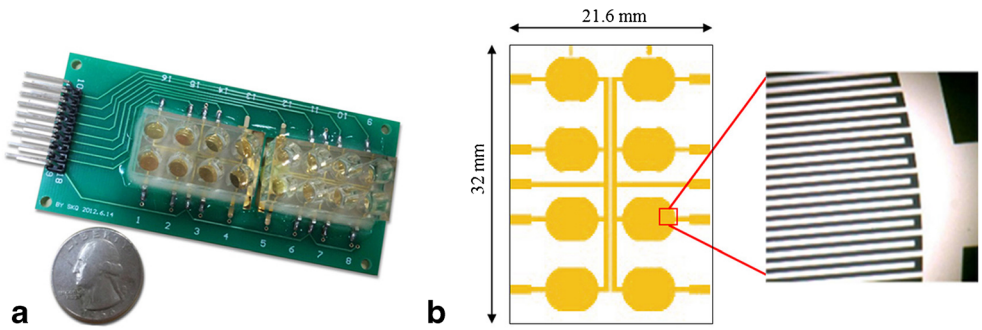

Fig. 8a–b Interdigitated electrodes for impedance detection. **a** The electrode device used in an olfactory electrochemical biosensor system. **b** The structure of the interdigitated electrodes at the bottom of a well [90] (reprinted with permission from Elsevier)

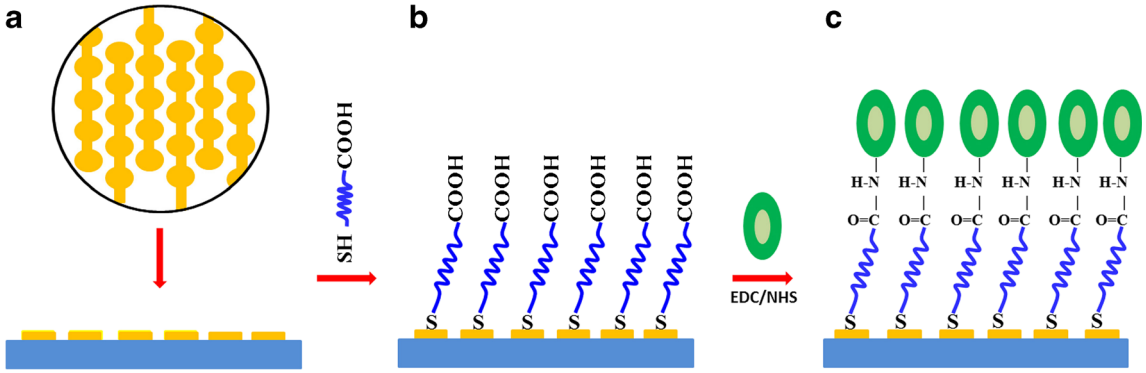

Fig. 9a–c Schematic of the immobilization of OBPs on poly(ethylene glycol)-modified interdigitated electrodes. **a** Interdigitated electrodes; **b** bonding of α-thio-ω-carboxypoly(ethylene glycol) to the surfaces of the gold electrodes; **c** formation of covalent amino bonds between BdorOBP2 and the poly(ethylene glycol)-coated electrodes via 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide coupling [81]. Reprinted with permission from Elsevier

As the wind speed was increased, the plume became more dispersed. This EAD had a response time of milliseconds, but the results given by the EAD were influenced by environmental changes (in temperature and humidity).

Sensors based on a selected enzyme can be used to detect volatile compounds. Enzymes, which catalyze the chemical reactions that occur in living organisms, are capable of driving specific reactions. Some lepidopterans present enzymes in their sex pheromone glands that participate in the biosynthesis of pheromones. One such enzyme is the alcohol acetyl transferase, which has been immobilized in a microreactor that esterifies the alcohol (*Z*,*E*)-9,11-tetradecadienol to (*Z*,*E*)-9,11-tetradecadienyl acetate, allowing the bioreactor to mimic the pheromone production of the Egyptian armyworm *Spodoptera littoralis*. The enzyme acetyl transferase was immobilized by a layer-by-layer technique using polyethyleneimine as the polycation and dextran sulfate as the polyanion. Alternate depositions were conducted, leading to five bilayers on a microreactor connected to a microevaporator—essentially an artificial gland. The performance of the artificial gland was determined by GC-EAD detection using male armyworm antennae, as well as by capturing the pheromone generated by the artificial gland using a polymeric adsorbent and then analyzing it via GC-MS [94]. The pheromone from the artificial gland was 40% as effective as a synthetic pheromone at producing antennal depolarizations, and male armyworms exposed to the pheromone from the artificial gland showed similar behavior to that prompted by the natural pheromone.

Another promising approach to ultrasensitive detection of pheromones was demonstrated by Park et al. [59], who developed an EAD that utilized various insect species (*Helicoverpa zea*, *Heliothis virescens*, *Ostrinia nubilalis*, *Trichoplusia ni*, *Microplitis croceipes*, and *Drosophila melanogaster*) in an array of sensors (i.e., a bioelectronic nose). The array, which was termed a Quadro-probe electroantennogram, was applied to discriminate odorants in plumes in a wind tunnel and in a field. The entire insect antennae (attached to the body) were fixed to thin copper wires that were used to record the signals from the antennae. The sensor array showed that each insect species exhibited a different response profile to the odorants, as well as a response time of just 1 s.

An electronic nose employing an array of nonspecific gas chemical sensors (eight polycrystalline tin-dioxide-based semiconductor sensors) was applied to monitor low codling moth pheromone (coddlemone, E8,E10-dodecadienol) levels in the air of an apple orchard. This pheromone presented a very low vapor pressure (5.0×10^{-8}M), so it was clearly discriminated from other compounds using the electronic nose in real time. Also, the electronic nose was able to continuously and remotely monitor the levels of the pheromone for weeks [95].

Lan et al. [96] used a commercially available portable electronic nose to identify southern green stink bugs, *Nezara viridula*, including their gender. The electronic nose consisted of an array of 32 carbon-polymer-based sensors. The pheromones of male and female southern green stink bugs were discriminated correctly 90% of the time, indicating that this electronic nose has great potential to identify volatiles and odors of stink bugs.

Henderson et al. [97] also used a commercially available electronic nose comprising an array of carbon polymer sensors to detect stink bug pheromones in different situations (under laboratory and field conditions). Stink bugs collected from soybeans and cotton were exposed to a 16 h:8 h light:dark cycle to simulate field conditions. The electronic nose detected whether stink bugs were present with an accuracy of 100% under laboratory conditions, and it was able to identify (*E*)-2-decenal and (*E*)-2-octenal at 0.67, 1.0, 1.33, 2.0, and 4.0 mg/L concentrations.

Another approach is to construct new miniaturized sensors based on microelectromechanical systems (MEMs). In this context, sensors that make use of the deflection or frequency of the cantilever in atomic force microscopy (AFM) have emerged as very sensitive and selective miniature devices (see the next section). Sensitivity can be enhanced through

the functionalization of the AFM tip with conducting polymers, carbon nanotubes, or other organic materials that act as sensitive coatings. Such systems could prove very useful in studies of interspecies communication through pheromones.

Cantilever nanosensors

The cantilever used in atomic force microscopy (AFM) can be used as a physical, chemical, or biological sensor by detecting changes its bending or vibrational frequency. An AFM cantilever is a thin, flexible, suspended beam. Attached to its underside is the AFM tip, which is just a few nanometers wide at its vertex. The materials used to fabricate AFM cantilevers are generally constructed from silicon, silicon nitride, silicon dioxide, and polymers. The length and thickness of a cantilever are typically on the order of microns and/or nanometers that provide specific mass, spring constant, resonant frequencies and sensitivities [98]. Cantilever size is very important because the surface of the cantilever can be coated with a layer that is sensitive to the binding of particular analytes, and the size of the cantilever influences the response time (a key parameter of any sensor) when an analyte is detected.

The main objective when developing cantilever sensors is to construct an inexpensive sensor with a highly stable, selective, sensitive, and reproducible response [99, 100]. It is worth noting at this point that nanosensors such as cantilever sensors use minimal amounts of electricity, making them less expensive to use than conventional industrial sensors [101, 102].

The displacement of the cantilever in a nanomechanical sensor can vary between angstroms and hundreds of nanometers, and it is measured using optical or electrical techniques to a sensitivity of less than 0.1 nm Hz^{-1}. Such a system can be used as a precision mass sensor with a sensitivity (per unit area) in dynamic mode of 0.025 fg/μm^2/Hz, which is superior to those of a quartz crystal microbalance sensor (0.17 fg/μm^2/Hz) and a surface plasmon resonance sensor (1.0 ng/μm^2/Hz). The miniaturized nature of the cantilever sensor results in a relatively low detection limit [103].

Some authors have modified characteristics of the microcantilever such as its geometry, spring constant, and the type of material used, in order to increase its sensitivity to surface stress [104]. This can be achieved, for example, by constructing the cantilever from synthetic materials that mimic the behavior of a natural system. Cantilevers utilize transducers or physical sensors to monitor the weight changes or mobility by the interaction between the volatile and sensor. Although they are less sensitive and specific, biosensors are more stable and reusable, they can overcome the limitations of

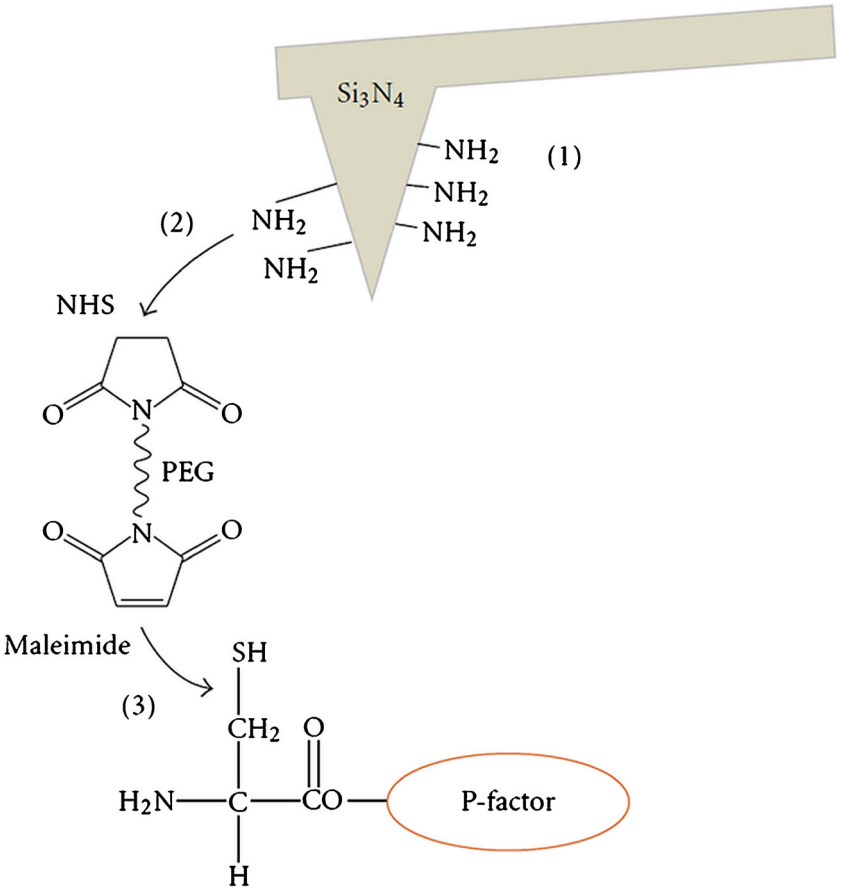

Fig. 10 Illustration of the functionalization of an AFM tip with a crosslinker, amino groups, and a peptide pheromone in preparation for AFM force spectroscopy experiments that determined the interaction force between the pheromone and its receptor [107] (reprinted with permission from NCBI)

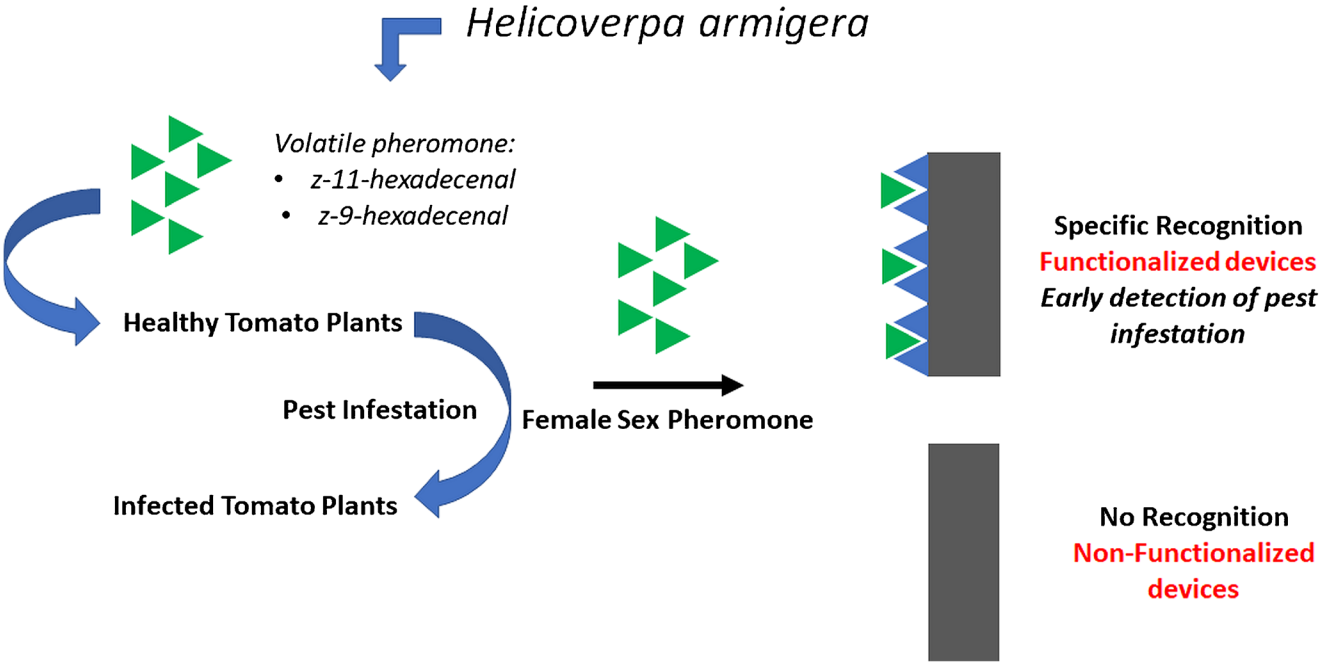

Fig. 11 Photograph and schematic showing the use of a covalently functionalized MEMS device to detect female sex pheromones of *Helicoverpa armigera*, *Scirpophaga incertulas*, and similar pests prior to infestation under simulated field conditions (in the presence of six of tomato plants as well as four male and four female insects) (adapted from [110] and reprinted under a Creative Commons license)

these sensors using preconcentrations, volatile separation and, particularly, the development of sensor array [105].

In this context, Steffens et al. [106] created a bioinspired sensor to detect the pheromone 2-heptanone in air. The sensor employed a cantilever that had been functionalized with a thin film of PANI in the esmeraldine state via spin coating. The resulting miniaturized device showed a linear response to the 2-heptanone concentration at distinct temperatures, and the mechanical behavior, hysteresis, and storage time of the sensor were investigated. The sensor presented a response time of 120 s for a storage time of up to 60 days. The introduction of interfering volatiles such as linalool and orange oil at a concentration of 5% (w/w) did not adversely affect the performance of the cantilever sensor with respect to 2-heptanone. The cantilever deflection method was shown to be very promising for pheromone analysis and sensing.

Pheromone recognition using AFM force spectroscopy was achieved by Sasuga et al. [107], who utilized the interaction force between two molecules, one attached to the AFM tip and the other bound to the surface of interest. The AFM tip was coated with the heterobifunctional crosslinker polyethylene glycol (PEG) and 3-aminopropyl triethoxysilane and then linked to P-factor, a peptide pheromone consisting of 23 amino acid residues (see Fig. 10). The interaction force between the pheromone and its receptor, Mam2, on the cell surface of the fission yeast *Schizosaccharomyces pombe* was measured. During the force spectroscopy measurements, the AFM tip was brought towards the cell surface and then retracted; a z-scan size of 200 nm was used. The results showed an unbinding force of around 120 pN at a probe speed of 1.74 μm/s.

Also, as a future perspective, a insect membrane can be applied as sensitive coating on cantilevers to detect pheromones. The AFM tip forces or cantilever bends can provide results in order of magnitude pN-resolution (interaction forces). Ligand–receptor interactions between protein and membrane immobilized on an AFM tip with a small vertex radius (~10 nm) can be explored by force spectroscopy. This ligand–receptor interaction presents equilibrium dissociation constants ranging from fM to μM [108].

Boshart et al. [109] investigated the intermolecular and intramolecular interactions, unfolding pathways, and energy landscapes of membrane proteins by force spectroscopy. The molecular interactions between L-arginine/agmatine antiporter (AdiC) and its substrates were evaluated, and peak heights of between 210 and 70 pN were observed.

Moitra et al. [110] developed a miniaturized MEMS device that was covalently functionalized for the optical detection of sex pheromones from the pest *Helicoverpa armigera* via laser Doppler vibrometry measurements of the resonance frequency of the MEMS device. The microcantilever sensor was made of silicon and silicon dioxide and functionalized with APTES. Experiments were

performed in the presence of tomato plants and male and female pests, and the device was found to be able to detect femtogram concentrations of pheromones (Fig. 11).

Thus, highly sensitive, selective, label-free, real-time cantilever nanosensors possess great potential for advanced applications of pheromones, and could facilitate the development of sensor arrays to distinguish different molecules in the femtogram range.

Conclusions

The communication between animals with the environment is mediated by chemical interactions through substances released by an individual and received by a second individual. Such chemical-based communication is intimately involved with various animal behaviors, such as prey location, predator avoidance, and intraspecies or interspecies signaling relating to mating or aggregation. The chemical language of insects is particularly sophisticated, and the wide variety of behavioral and physiological responses of insects to chemical messages may be driving the increasing volume of research in this area.

Many of these studies of insect semiochemicals have attempted to improve our understanding of insect olfaction systems and insect communication by developing methodologies to isolate and analyze the semiochemicals, as well as methods to detect pheromones. Such methods have a wide range of applications; for example, in agriculture to control insect infestations, as one of the most difficult tasks in pest management is detecting the presence of pest insects before an actual infestation occurs.

The use of pheromones to monitor pest insects and to tackle infestations is a relatively ecofriendly approach. Indeed, this technique appears to be a promising component of the integrated management of a large number of pest species, which is economically important worldwide. The application of pheromones in conjunction with other control methods leads to new protection strategies for various crops under various conditions.

Sensors or biosensors capable of detecting these semiochemicals, released in small quantities by insects, primarily in controlled environments, testing different methodologies, and thus making up the way development of highly sensitive and selective sensors, as microcantilevers for use in AFM, targeting applications for real-time detection.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

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