

**Foresight**

Infectious Diseases: preparing for the future

OFFICE OF SCIENCE AND INNOVATION

**S8: State-of-Science Review:  
Interrogation of signals/biomarkers**

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## 1 Introduction

Infectious diseases are a global problem as they are not restricted by borders or boundaries. The global burden of disease in plants and animals through the impact of infectious organisms can be measured using a number of different parameters. For plants and animals, this can be measured directly in terms of product yields arising from plant and livestock-based agriculture, with a reduction in either of these leading to a significant reduction in the amount of food available for consumption (FAO 2005). This can therefore contribute directly to food insecurity and poverty on a global scale. For humans, the global burden of diseases can be measured in disability-adjusted life years (DALYs). Of these, approximately 40% of all DALYs lost can be attributed specifically to acute respiratory infections, HIV/AIDS, tuberculosis (TB), and vector-borne diseases (WHO 2000).

This review aims to assess the current state of research on the identification of natural signals/biomarkers emitted by plants and animals (including humans) as a consequence of interactions with pathogens that cause infectious diseases. In addition, it reviews the current status of the technologies for remotely assessing these signals arising from the host response to pathogen infection, and for measuring their presence, density and distribution. Finally, it explores the mid- to long-term future directions of the science, along with an assessment of the technologies' timing and likelihood of occurrence.

In many cases, infectious diseases of plants and animals that are caused by pathogens elicit changes in the volatile profile emitted by the host. These changes may be detected and identified remotely by means of interrogating the volatile natural signals that are generated and emitted as a consequence of infection. For both plants and animals, these signals can comprise small-molecular-weight, lipophilic compounds. The onset of infectious disease can also be determined prior to the manifestation of physical symptoms by detecting such signals associated with the very early, and often otherwise undetectable, stages of infection. Furthermore, these signals provide potential for the development of extremely sensitive and specific remote sensing systems for disease detection and diagnosis, thereby enabling monitoring for the prevention of epidemics.

Plant and animal pathogens that cause infectious diseases can be transmitted via either abiotic or biotic agents. Although pathogens can be transmitted without biotic agents, e.g. physical contact, for many, biotic agents such as animal vectors are often involved. These predominantly belong to the insect class of arthropods. With these vectors, pheromones, including sex pheromones, although not always directly related to the vectoring component of the lifecycle, represent a potentially extremely potent means of disease detection, through the deployment of pheromone-baited trapping systems. These monitoring systems, which are highly specific for the target insect and work at low population densities, allow for the mapping of vector populations that have the potential to transmit diseases. Furthermore, they allow for the detection of diseases through subsequent screening of trapped individuals for

pathogen presence, using diagnostic techniques such as real-time polymerase chain reaction (RT-PCR). Where appropriate pheromones are not available for monitoring purposes, other related chemical signals, generically known as semiochemicals (derived from the Greek *σημειον* semeion, meaning 'sign' or 'signal'), can be identified and utilised. These are typically host-derived (plant or animal) chemicals that act as attractants for the insect during the host-seeking process, where the insect is attempting to obtain nutritional components from host secretions for full development, e.g. egg maturation in gravid (pregnant) female mosquitoes. Detection of infectious diseases can also be achieved remotely through Earth observation via satellite, surface, airborne or submarine observations (see Foresight project state of science review (S10) Earth Observation). These can be used assess to climatic conditions, which play an important role in the spread of many diseases.

For many plant pathogens of the world's main broad-acre crops, aphids and leafhoppers are the major insect pathogen vectors. For animals and human beings, mosquitoes and other biting and blood-sucking (haematophagous) flies are the major vectors responsible for the transmission of pathogens that cause major global diseases such as malaria, dengue and filariasis. Although the role and identity of pheromones and host-derived semiochemicals is well understood for the major crop pests such as aphids, there is, by comparison, a paucity of information relating to their role and identity for haematophagous arthropods. This is in spite of the obvious potential that such compounds have in facilitating the development of specific vector monitoring and control, and hence disease control, programmes.

## **2 Disease Detection: Literature Review**

### **2.1 Chemical markers for diseases in plants**

The infection of plants with pathogenic oomycetes, bacteria and fungi leads to the production, by the host, of volatile chemical markers, typically isoprenoid oxidation products, but which can also be derived via other biosynthetic pathways. There are a number of examples of this phenomenon in the literature, and these are mentioned here.

For a number of plant–pathogen systems, ethylene, salicylic acid and jasmonic acid are known to play an important role in plant defence in response to pathogen attack (Kunkel and Brooks 2002). Ethylene has been identified as an important signalling component in plant–pathogen interactions, and increased production of this compound from infected plant tissue has been reported for numerous pathogens (Chaudry et al. 1998). An increase in the production and emission of methyl salicylate by tobacco plants *Nicotiana tabacum* following infection has been observed (Shulaev et al. 1997). Furthermore, inoculation of plants with *Pseudomonas syringae* leads to the release of volatile products. For beans *Phaseolus vulgaris*, these comprise 'green leaf' volatiles (GLVs), such as (*Z*)-3-hexenal, (*E*)-2-hexenal, (*Z*)-3-hexen-1-ol, (*E*)-2-hexen-1-ol and hexenyl esters, generated via the lipoxygenase pathway (Croft et al. 1993), and for *N. tabacum*, these comprise (*E*)-ocimene, linalool, methyl salicylate, indole, caryophyllene,  $\beta$ -elemene,  $\alpha$ -

farnesene and two unidentified sesquiterpenes (Huang et al. 2005). Other plants, e.g., peanut plants *Arachis hypogaea*, infected with white mould fungus *Sclerotium rolfsii*, emit methyl salicylate (Cardoza et al. 2002), while barley seedlings *Hordeum vulgare* elicit altered volatile profiles when inoculated with leaf scald, *Rhynchosporium secalis* (Lucas 2003). Volatile production by fungally infected and uninfected potatoes, onions and apples has also been investigated (Lui et al. 2005; Vikram et al. 2004a, 2004b, 2005), as has production by industrial, non-food crops such as oilseed rape (Doughty et al. 1996). For potatoes, volatiles released by tubers on infection with *Phytophthora infestans* have been identified (Schultz et al. 1996). Infection of potato plants with potato leafroll virus (PLRV) has been shown to change the volatile profile of plants, such that differences are observed in the behavioural response of the peach-potato aphid *Myzus persicae* (Eigenbrode et al. 2002), whereby the infected plant is more attractive to the aphid than the uninfected plant. Thus, the influence of viral disease symptoms on the behaviour of the virus vector has implications for disease epidemiology.

The early onset of arthropod infestation on plants is characterised by induced production of volatile chemical markers. Detection of these markers can indicate the presence of arthropod populations at low densities, and where pathogen vectors are concerned, can give early indications of possible pathogen presence. However, it cannot be assumed that the pathogen is present in the vector, and a trapping system as described below (see Section 2.2) is also required to allow individuals to be caught and screened for pathogen presence (e.g. Fabre et al. 2003 and references therein). The major arthropod species responsible for transmitting plant diseases are sucking pests such as aphids and leafhoppers. However, species belonging to other insect orders are also able to act as vectors, such as Coleoptera (beetles) and Thysanoptera, as are non-insect arthropods such as members of the Acarina, specifically mites.

Volatile markers that are generated by plants as a consequence of arthropod damage typically comprise isoprenoid oxidation products, but they can also be compounds derived via other induced biosynthetic pathways, e.g. the production of phenolic compounds via the phenylalanine–ammonia lyase pathway (Dicke 1999; Wadhams et al. 1999; Tumlinson et al. 1999; Turlings and Fritzsche 1999; Boland et al. 1999). Between plant species, the composition of blends of herbivore-induced volatiles can vary qualitatively, but many shared components exist, e.g. GLVs as defined above, the homosesquiterpene compounds (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (*E,E*)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), and indole. For plants in the same species, blends usually vary quantitatively. Despite these variations, the induced volatiles are usually highly species-specific, depending on the herbivore species, and such specificity leads to either the recruitment, by infested plants, of beneficial insects, or to an antixenotic effect, that is pest repulsion, leading to a reduction in colonisation. The latter has been demonstrated for a number of aphid species. For the interaction of cereals and *Rhopalosiphum padi*, four compounds, 6-methyl-5-hepten-2-one, (–) and (+)-6-methyl-5-hepten-2-ol and 2-tridecanone, were shown to be present in volatiles from aphid-infested wheat seedlings but not from intact plants. A

mixture of the four compounds counteracted the attraction of intact seedlings (Quiroz et al. 1997). Methyl salicylate has also been shown to be released by plants when under attack (e.g. maize following aphid attack), and to repel *R. maidis* (Bernasconi et al. 1998). Production of the same compound has also been reported for soybean *Glycine max* when infested with soybean aphids *Aphis glycines* and this compound appears to be responsible for the recruitment of seven-spot ladybirds *Coccinella septempunctata* (Zhu and Park 2005). Similar examples of beneficial insect recruitment have been reported elsewhere. For the aphid parasitoid *Aphidius ervi*, which uses the pea aphid *Acyrtosiphum pisum*, as one of its hosts, but not the black bean aphid *Aphis fabae* or the vetch aphid *Megoura viciae*, coupled gas chromatography (GC)-electrophysiology showed that compounds such as (*E*)-ocimene, 6-methyl-5-hepten-2-one, linalool, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate and (*E*)- $\beta$ -farnesene were produced by infested bean plants *Vicia faba* and detected by the parasitoid (Du et al. 1998). Some of these compounds were found in intact plants, but were released at higher levels from infested plants. 6-Methyl-5-hepten-2-one was found to be produced specifically by *V. faba* upon feeding by *A. pisum*, but not by plants infested with other aphid species. A range of volatile compounds emitted by *P. vulgaris* plants that are associated with infestations of whiteflies *Trialeurodes vaporariorum* have been reported (Birkett et al. 2003). Of the 20 compounds identified, four were emitted at higher levels than in undamaged plants, including (*Z*)-3-hexen-1-ol, DMNT, 3-octanone and another unidentified compound. The three identified cues all induced flight responses in the whitefly parasitoid *Encarsia formosa*. Plant volatile production is also induced by thrips infestations. Comparison of infested and uninfested host-plant chrysanthemums showed that levels of some components, particularly sesquiterpenes including  $\beta$ -caryophyllene, (*E*)- $\beta$ -farnesene, germacrene D and (*E,E*)- $\alpha$ -farnesene, were higher in infested buds (Pow et al. 1998). Similar types of compounds are released with specificity by plants infested by spider mites, *Tetranychus urticae*. (*Z*)-3-Hexenyl acetate, (*Z*)-3-hexen-1-ol, DMNT and (*E*)-ocimene are released by lima beans *Phaseolus lunatus* in response to mite feeding (Dicke 1999). Furthermore, different herbivores on the same plant lead to emission of different volatile blends. This is exemplified by apple foliage, which, when infested with two different mite herbivore species, results in quantitative differences in the volatile blends. These differences are detected by predatory mites that prey specifically on particular species, thereby allowing them to discriminate between prey and non-prey species at a distance (Dicke 1999). Plants can also emit different volatiles depending on whether they are responding to pathogens or herbivores. This is exemplified by *A. hypogaea*, where although infection by *S. rolfisii* leads to methyl salicylate production (see above), feeding damage by the beet armyworm *Spodoptera exigua* leads to release of GLVs, terpenoids and indole (Cardoza et al. 2002).

## **2.2 Pheromone and other semiochemical tools for monitoring plant pathogen vector populations**

The infection of plants by disease pathogens often involves animal vectors which predominantly belong to the insect class of arthropods. Where the presence of pathogens in plants can be monitored directly by detecting

changes in the production of volatile chemical markers (see section 2.1), the presence of pathogens in vector populations can also be assessed by capturing insects using traps and screening those caught insects for the presence of pathogens using rapid diagnostic techniques, some of which are already established, e.g. RT-PCR (Fabre et al. 2003 and references therein). This not only provides information on the presence of a vector, but also whether the vector carries the pathogen. Furthermore, such trapping systems can be used to map vector populations which have the potential for transmission. A more sophisticated approach is to utilise insect pheromones, which, although not always directly related to the vectoring component of the lifecycle, represent a potentially extremely powerful means of vector detection through the deployment of pheromone baits in trapping systems. Thus, the pheromone monitoring systems can be highly specific for the target insect. Such monitoring systems, which incorporate traps for capturing insects, are already utilised on a wide scale, and are effective at very low population densities. Where appropriate pheromones are not available for monitoring purposes, other related chemical signals, generically known as semiochemicals, typically plant-derived kairomones (attractants), can be used in traps to check for vector presence. When used in conjunction with pheromones, such traps can be used to assess the vector capacity.

Insect pheromones, which mediate interactions between members of the same species, can be divided into different categories, depending on the type of behaviour that is mediated, e.g. sex, aggregation, oviposition (egg-laying) and invitation behaviour. Each class of pheromone has the potential to be utilised in traps for vector detection, and examples of identified pheromones in each class are described below. The function of sex pheromones, which are usually released by only one of the sexes, is to initiate either attraction or sexual behaviour in the opposite sex. Aggregation pheromones promote aggregation of both sexes, while oviposition pheromones promote egg-laying behaviour by gravid (egg-laying) females. Examples of invitation pheromones are highlighted in Section 2.4. In terms of suitability for disease detection, this can be viewed in the following order: sex < aggregation < oviposition. Sex pheromones are usually detected by the uninfected sex, e.g. male aphids are attracted to aphid sex pheromones (see below), whereas aggregation pheromones are detected by both sexes, e.g. for Coleopteran insects (beetles). Oviposition pheromones are used by the infected sex, e.g. the *Culex* spp. oviposition pheromone is utilised by gravid female *Culex* spp. mosquitoes, after they have obtained a blood meal from a human or animal host.

Aphids (Homoptera, Aphididae) are the main insect pests of temperate agriculture in many parts of the developed and developing world (Minks and Harrewijn 1987). In addition to the considerable direct damage that these sucking pests can cause to crops through feeding, a number of species are also major vectors of luteoviruses, e.g. Barley yellow dwarf virus, which can severely affect crop yield (Reavy and Mayo 2002). The sex pheromone components for aphids in the sub-family Aphidinae have been identified as cyclopentanoid monoterpenoids. The first identification was from the vetch aphid *M. viciae*, identified as (4aS,7S,7aR)-nepetalactone and

(1*R*,4*aS*,7*S*,7*aR*)-nepetalactol. Since this initial discovery, the sex pheromones for a number of other aphid species in the Aphidinae have been identified, and principally comprise these two compounds (Hardie et al. 1999; Birkett and Pickett 2003). However, an investigation of the damson-hop aphid *Phorodon humuli* showed that the pheromone comprised two diastereoisomers (1*S*)- and (1*R*,4*aR*,7*S*,7*aS*)-nepetalactol. In-depth behavioural studies conducted in the laboratory have shown long-range attraction of male aphids, even at low population densities in the field (e.g. Hardie et al. 1992; Gabrys et al. 1997), thereby demonstrating the very high sensitivity that pheromone-baited systems have, compared to conventional traps e.g. suction traps, which rely on just physical trapping. However, the disadvantage of the pheromone-baited system is that the males do not carry the pathogen, and therefore this approach can only be used to check the presence of the vector, and not the pathogen.

Sex pheromone components have been identified for a number of phytophagous Coleoptera (beetles) in the Chrysomelidae, Curculionidae and Coccinellidae that are also able to vector plant pathogens (Gergerich 2002). For *Diabrotica* spp., *D. undecimpunctata* and *D. balteata*, female-produced sex pheromones have been identified as (10*R*)-10-methyltridecan-2-one and (*R,R*)-6,12-dimethylpentadecan-2-one respectively (Guss et al. 1983; McLaughlin et al. 1991). *D. barberi* and *D. virgifera* respond to 8-methyl-2-decylpropionate (Guss et al. 1984, 1985), and *D. lemniscata* and *D. longicornis* respond to (2*S*,8*R*)-8-methyl-10-propionate (Krysan et al. 1986).

There are few reports on the existence or identity of sex pheromones for other plant disease vectors, which could be applied to disease detection and eradication through monitoring and control systems. Apart from aphids, the other major group of insect vectors of plant viruses are the leafhoppers, planthoppers and treehoppers. To date, nearly 60 plant viruses are known to be transmitted by hoppers in the Cicadellidae, Membracidae, Cixiidae and the Delphacidae (Ammar and Nault 2002). However, reports of pheromone identification are limited to a single leafhopper species. A recent study reported the isolation of a compound, mevalonolactone, from the headspace of *Psammotettix alienus* (Alla et al. 2002).

A large number of aggregation pheromones have been identified for disease vectors, but mainly for Coleoptera. For the cereal leaf beetle *Oulema melanopus*, an aggregation pheromone was identified as (*E*)-8-hydroxy-6-methyl-6-octen-3-one (Rao et al. 2003). For *Acalymma vittatum*, an aggregation pheromone was identified as (3*R*,4*R*)-3-Methyl-4-(1,3,5,7-tetramethyloctyl)-oxetan-2-one (Vittatalactone) (Morris et al. 2005). For weevils, these typically comprise 8, 9 or 10 carbon, methyl-branched secondary alcohols (Bartelt 1999). A number of thrips (Thysanoptera) species, e.g. the Western Flower Thrips *Frankliniella occidentalis* (Thysanoptera) possess the capacity to vector plant Tospoviruses, especially within vegetables grown under glass conditions (Ullman et al. 2002). A male-produced aggregation pheromone for *F. occidentalis* was identified as a mixture of (*R*)-lavandulyl acetate and neryl (*S*)-2-methylbutanoate (Hamilton et al. 2005).

Host-plant volatiles have been shown to enhance pheromone attractiveness. This was first demonstrated for the pea-and-bean weevil *Sitona lineatus* (Curculionidae), for which the aggregation pheromone has been identified as 4-methyl-3-5-heptanedione (Blight et al. 1984), and for which a monitoring system has been developed, which also incorporates host-plant volatile compounds that synergise pheromone activity (Smart et al. 1994).

Interactions between plant disease vectors and their host plants are mediated through the perception of a complex mixture of olfactory, visual and gustatory cues. In some cases, physical cues such as temperature are also thought to influence host location. Host location is mediated primarily by detection of volatile chemicals (kairomones) emitted by host plants. The importance of host blends is discussed in more detail in Section 2.5.

For aphids, in spite of numerous studies by several research groups, few examples exist of the detection of taxonomically characteristic compounds that are unique to particular plant–aphid interactions. Of these, isothiocyanates, volatile catabolites of the glucosinolates characteristic of brassicaceous plants, have been most studied. The specialist cabbage aphid *Brevicoryne brassicae* and the turnip aphid *Lipaphis erysimi* are attracted to 3-butenyl and 4-butenyl isothiocyanate (Nottingham et al. 1991). For ubiquitous plant volatiles, a number of studies have shown olfactory activity for aphids. Fatty acid derivatives, phenyl propanoids (benzaldehyde and phenylacetaldehyde) and isoprenoids have been shown to be perceived by a number of aphid species (Visser and Fan 1995; Visser et al. 1996; Park and Hardie 2003; Quiroz et al. 1998). Coupled GC-SCR studies identified host cues for the black-bean aphid *Aphis fabae* from sugar beet (Wadhams 1990). For the damson-hop aphid *Phorodon humuli*, attraction to host odours, and a blend comprising host odour components (*E*)-2-hexenal and  $\beta$ -caryophyllene was shown in the laboratory (Campbell et al. 1993). A few field studies have examined the role of plant volatiles in host location. An increase in trap catch of the bird-cherry-oat aphid *Rhopalosiphum padi* was reported for benzaldehyde, as was *B. brassicae* when sinigrin was used, with the attraction being presumed to be towards the hydrolysis product (Pettersson 1979). The willow-carrot aphid *Cavariella aegopodii* was attracted to traps baited with (+) or (–)-carvone (Chapman et al. 1981). For *P. humuli*, optimum catches were obtained through a combination of host kairomones, sex pheromones and visual cues (Losel et al. 1996a, 1996b). A similar effect was noted for *R. padi* with volatiles of the winter host *Prunus padus* and with the major winter host component, benzaldehyde (Hardie et al. 1994).

Whiteflies (Homoptera, Aleyrodidae) are recognised as major vectors of plant viruses, in particular *Bemisia tabaci*, *Trialeurodes vaporariorum* and *T. abutilonea* (Brown and Czosnek 2002). Of these, *B. tabaci* is the most important, being associated with more than 100 plant viral diseases found mainly in the tropics and subtropics, such as cassava mosaic virus. Despite the considerable damage that these pests can do to crops, little or nothing is known of the role of volatile semiochemicals in host location. There is a similar situation with hoppers, where little or nothing is known of the role of plant volatiles in host location. Potato leafhoppers *Empoasca fabae* are attracted to

volatiles from alfalfa plants (Ranger et al. 2005), and plant compounds with olfactory activity have been identified for the rice brown leafhopper (Masri 1995; Youn 2002).

A number of host kairomones have been identified for Coleoptera. For Chrysomelidae, *D. balteata* and *D. undecimpunctata* are attracted to traps baited with plant kairomones (Jackson et al. 2005), while *D. barberi* and *D. virgifera* are attracted to maize volatiles (Hammack et al. 1999; Hammack, 2001; Hibbard et al. 1997a). *Diabrotica* spp. were attracted in the field to a blend of blossom volatiles (Lampman and Metcalf 1987), as was *Alacymma vittatum* (Metcalf et al. 1995). A number of olfactory and behaviourally active compounds were identified for *Diabrotica* spp. from powdered roots of buffalo gourd (Hibbard et al. 1997b; Cosse and Baker 1999). *D. speciosa* were attracted to traps baited with host-plant compounds (Ventura et al. 2000). For Coccinellidae, the Mexican bean beetle *Epilachnia varivestis* is the only known disease vector, but little is known of its host-plant semiochemistry. For weevils (Curculionidae), a number of species are pathogen vectors. *Sitona* spp. e.g. the pea-and-bean weevil *S. lineatus* responds at the olfactory and behavioural level to host-plant compounds (Blight et al. 1991). For the West Indian sugarcane weevil *Metamasius hemipterus*, sugarcane volatiles were identified (Perez et al. 1997). Host attractants were identified for the red weevil *Rhynchophorus ferrugineus* (Gunawardena et al. 1998), and the American palm weevil *R. palmurum* (Rochat et al. 2000). In each case for the weevils concerned, studies showed clearly that host-produced attractants synergise the activity of pheromones in the field.

For thrips, attraction of *F. occidentalis* to volatiles from the host-plant chrysanthemums, in particular (*E*)- $\beta$ -farnesene, was demonstrated in the laboratory and in glasshouse trials (Pow et al. 1998). Attraction of *F. occidentalis* to a number of plant-derived compounds has also been demonstrated (Koschier et al. 2000).

### **2.3 Chemical markers for disease in animals and human beings**

For many infectious diseases in animals and humans, early onset is characterised by the generation of small-molecular-weight compounds, similar to the situation for plants. These chemical markers can potentially be used to detect and diagnose diseases through the analysis of breath and biological fluids, i.e. urine, sputum and blood.

Although there has been a tremendous worldwide effort to develop diagnostic tools for detecting infectious diseases in animals and humans, there are few examples where volatile chemicals emitted have been studied specifically for the purpose of detecting infections. TB, one of the major respiratory diseases in animals and humans, was considered to be under control in the latter half of the 20th century, but is now re-emerging as a major global threat. Chemical markers for diagnosis of TB infection were first identified in the 1970s, of which tuberculostearic acid (TBSA) was identified as a key marker (see Stopforth et al. 2005 and references therein). However, these markers are not volatile, and originate from the pathogen. Volatile compounds have been identified in the breath of rats infected with pneumonia, specifically 2,6-

dimethyloctane, 2-propanoic acid and 2-methyl-2H-tetrazole (Phillips et al. 1994).

For other major respiratory diseases, e.g. influenza and SARS, there are no reports on the identity of chemical markers. This is also the case for the major vector-borne infectious diseases in humans, such as malaria, dengue, West Nile virus and filariasis, and in animals, for diseases such as bluetongue and African horse sickness. Recently, however, it was suggested that infection of humans with the malarial parasite *Plasmodium falciparum* increases their attractiveness to mosquitoes, thus suggesting a change in volatile chemical emission following infection (Lacroix et al. 2005).

A number of studies have reported on the identification of chemical markers in animals and humans, as a consequence of major illnesses and disorders other than infectious diseases. Although not directly relevant to the remit of this review, these demonstrate the applicability of using volatile markers to confirm disease status.

For humans, the major focus on the use of volatile disease markers has been on the detection of cancer. Analysis of volatile compounds in exhaled breath has been developed as an approach for the detection of lung cancer, breast cancer and hyperlipidemia (Phillips et al. 1999a, 2003a; Di Natale et al. 2003; Phillips et al. 2003b; Rieder et al. 2001), while urine analysis has been developed for detecting bladder and prostate cancer (Spanel et al. 1999). Volatile markers of disease in blood have been studied, including hydrocarbons such as pentane and isoprene, acetone, halogenated compounds such as isoflurane, and thioethers such as dimethyl sulphide (Miekisch et al. 2001). Similar compounds, collected by breath analysis, have been studied as biomarkers for human liver disease. They include carbonyl sulphide, carbon disulphide, dimethyl disulphide, methanethiol and isoprene (Sehnert et al. 2002). Increased exhalation of isoprene has been suggested as a biomarker for end-stage renal failure (Davies et al. 2001).

Studies have also attempted to provide a greater understanding of exhaled air profiles from healthy individuals as reference points for disease detection (Phillips et al. 1997, 1999b; Moser et al. 2005). These studies suggest that there is small variation in the total number of volatile compounds emitted, and there is a 'common core' of volatile compounds emitted by all subjects. However, they also highlight the complexity and variability of volatile profiles of individuals, and also the difficulty in attempting to construct a baseline 'healthy' condition, from which disease status can be resolved.

For other human disorders and conditions, changes in the emission of volatile compounds have also been reported. These include heart disease, schizophrenia, malnutrition, rheumatoid arthritis, inflammatory bowel disease, damage due to exposure to environmental toxins and psychological stress (see Phillips 1997 and references therein; Di Natale et al. 2005; Queiroz et al. 2002). Although not related to disease, increased attractancy for mosquitoes has been reported for women throughout the menstrual cycle (Mordue and Pickett, unpublished data), and for pregnant women (Lindsay et al. 2000), thereby illustrating that volatile emissions from women are dependent on their

reproductive status. Production of malodorous volatile compounds originating from axillary sweat and human waste has also been studied (Natsch et al. 2004; Troccaz et al. 2004; Sato et al. 2001). In dairy cows, ketosis can be detected by the analysis of exhaled breath (Dobbelaar et al. 1996; Elliott-Martin et al. 1997; Mottram et al. 1999).

## **2.4 Pheromone and semiochemical tools for monitoring animal and human disease vectors**

In an analogous situation described for plants in Section 2.1, the infection of animals and human beings (vertebrates) by disease pathogens often involves animal vectors which predominantly belong to the insect class of arthropods. Again, where the presence of pathogens in vertebrates can be monitored directly by detecting the production of chemical markers by the host (see Section 2.3), the presence of pathogens in vector populations can also be assessed by capturing insects using pheromone/semiochemical-baited trapping systems, and screening those caught insects for the presence of pathogens using techniques already established such as RT-PCR (e.g. Ohashi et al. 2004). Furthermore, such trapping systems can be used to map vector populations that have the potential for transmission.

The major orders of insect disease vectors for animals and humans comprise Dipterous flies, including Culicidae (mosquitoes), Muscidae, Ceratopogonidae, Glossinidae and Psychodidae. Non-insect acarine species, such as mites and ticks, also possess disease-vectoring capacity and are also important.

Mosquitoes (Diptera, Culicidae) represent the most significant threat to human health because of their ability to vector pathogens that cause diseases that afflict millions of people worldwide, e.g. malaria, dengue and filariasis (Curtis and Davies 2001). In addition to those areas where mosquito-vectoring diseases are currently endemic, problems are now arising in new areas as a consequence of changes in global climate patterns and increased global travel and trade (Donaldson 2002). Thus, strategies for improved vector surveillance, as well as direct control, are urgently required. These include the use of semiochemicals, particularly those mediating behaviour involved in host and oviposition (egg-laying) site location.

A limited number of pheromones have been identified for animal and human disease vectors. *Culex* spp. mosquitoes are responsible for transmitting a number of pathogens, notably the nematode *Wuchereria bancrofti*, the causative agent for filariasis, and arboviruses, including more recently West Nile virus spread in urbanised areas in the US and Europe (Jonsson and Reid 2000; Turell et al. 2002). *Culex* spp. mosquitoes utilise an oviposition pheromone, (5*R*,6*S*)-6-acetoxy-5-hexadecanolide (Laurence and Pickett 1982). Field trials in several countries in areas where the pathogen is prevalent have demonstrated the efficacy of synthetic pheromone in the field (e.g. Mboera et al. 2000a, 2000b; Olagbemiro et al. 2004), particularly when used in conjunction with site-derived oviposition cues found in grass infusions or soakage pit water. For the development of optimised monitoring systems, the use of individual components identified from organically enriched water would be advantageous, because the erratic responses from variable and

undefined levels of oviposition cues would be eradicated. Thus, the pheromone and the oviposition cue 3-methylindole (skatole) (Mordue et al. 1992; Blackwell et al. 1993) have been used together effectively in the field (Mboera et al. 2000a; Olagbemiro et al. 2004). Furthermore, the oviposition pheromone/site-derived cue combination can also be deployed for the effective control of *Culex* mosquito populations, when used in conjunction with environmentally benign larvicides, such as the insect growth regulator pyriproxyfen, larvae-specific pathogens, such as the fungus *Lagenidium giganteum* Couch (Pickett and Woodcock 1996) or with trapping systems (Mboera et al. 2000a). The blackfly, *Simulium damnosum* (Simuliidae), which vectors onchocerciasis in western Africa, reportedly utilises an oviposition aggregation pheromone (McCall 1995), but the identity of the pheromone has not been reported. An oviposition pheromone has also been identified as dodecanoic acid for the sandfly *Lutzomyia longipalpis* (Diptera, Psychodidae), which is the vector of the protozoan parasite *Leishmania chagasi*, the causative agent of leishmaniasis in the New World (Dougherty et al. 1997).

A number of sex pheromone components have been identified for other Diptera. Male-produced sex pheromones for *L. longipalpis* have been identified from Brazilian populations, and show surprising chemical diversity between geographically separated populations. For individuals found in the Jacobina region, the major component of the sex pheromone was identified as (S)-9-methylgermacrene-B (Hamilton et al. 1996a, 1999), whereas for the population found in the Lapinha region, the principal component was identified as (1S,3S,7R)-3-methyl- $\alpha$ -himachalene (Hamilton et al. 1996b; Mori et al. 2000). Certain *Culicoides* spp. biting midges (Ceratogoponidae) are vectors of a number of viral pathogens of sheep, cattle and horses, including those that cause the diseases bluetongue and African horse sickness. The major component of the female-produced sex pheromone for the farmyard midge *C. nubeculosus* has been identified as *n*-heptadecane (Mordue 2003). Sex pheromones for other Dipterous species have been identified. For the face fly *M. autumnalis* and the stable fly *S. calcitrans*, sex pheromone components were identified as straight-chain monoalkenes (Uebel et al. 1975a) and mono- and dimethyl branched alkanes (Uebel et al. 1975b). A contact sex pheromone was reported for the tsetse fly *Glossina tachinoides* as comprising long-chain dimethylalkanes (Carlson et al. 1998). For the screwworm *C. hominivorax*, a contact sex pheromone has been postulated but not identified (Hammack 1991). For the Scottish biting midge *C. impunctatus*, although this species does not utilise a sex pheromone, parous host-seeking females produce an aggregation (invitation) pheromone which brings many females together to feed on single food resources (Blackwell et al. 1994).

*Triatoma* bugs (Hemiptera) are responsible for the transmission of the protozoan parasite *Trypanosoma cruzi*, the causative agent for American trypanosomiasis, also known as Chagas disease. Evidence of sex pheromones remains equivocal. Acetic and isobutyric acid are released, along with other compounds, by *Rhodnius prolixus* and *T. infestans*, with the former compound being attractive to males in behavioural bioassays (Rojas et al. 2002; Guerenstein and Guerin 2004). Mating pairs of *T. infestans* release a complex mixture of chemicals, including (*R,S*)-2- and 3-methylbutan-1-ol,

short- and long-chain acids, aliphatic aldehydes, benzaldehyde and dipropylsulphide, with the aldehydes being attractive to females (Fontan et al. 2002). *T. infestans* and *T. mazzotti* are attracted to their own faecal matter, but attraction to the major components 2-aminoacetophenone and 4-methylquinazoline was not reported (Cruz-Lopez and Morgan 1995).

Several members of the order Acarina, i.e ticks and mites, are also known to be major disease vectors in animals. Ticks, in particular those species in the Ixodidae, are of medical and veterinary importance in the developed and developing world through their ability to vector pathogens such as the spirochete bacteria from the *Borrelia burgdorferi* species complex, which causes Lyme borreliosis and tick-borne encephalitis and fever in humans and cattle. Pheromonal components have been characterised for mainly the former (Sonenshine 2004). Long-range volatile sex pheromones have been identified for several tick species, and either a single component, 2,6-dichlorophenol, or a mixture of components, including aromatic compounds such as phenol, *p*-cresol, 2,6-dichlorophenol, salicylaldehyde, methyl salicylate, 2-nitrophenol, benzaldehyde and a short-chain carboxylic acid, e.g. the aggregation-attachment pheromone for the tropical bont tick *Amblyomma variegatum* was identified as comprising a mixture of 2-nitrophenol, methyl salicylate and nonanoic acid (Schoni et al. 1984), and as 2-nitrophenol, benzaldehyde and isobutyric acid for *A. hebraeum* (Apps et al. 1988).

Host location by haematophagous insects is mediated primarily by the detection of carbon dioxide (CO<sub>2</sub>) in exhaled breath, along with the detection of kairomones emitted either in the breath, sweat, mucus secretions, urine, faeces or from wound sites (Pickett and Woodcock 1996; Gibson and Torr 1999). Currently, trapping systems baited with CO<sub>2</sub> are the most widely used for monitoring and controlling populations. The development of trapping systems that incorporate volatile chemicals other than CO<sub>2</sub> has been widely acknowledged and studied, and a number of investigations have identified volatile compounds directly emanating from the host. For humans, the majority of human odour compounds are thought to be present in sweat, through microbial or aerial degradation of excreted compounds (Sastry et al. 1980), and sweat is known to be attractive to mosquitoes (Gibson and Torr 1999). Therefore, research on kairomone identification has focused on this source of compounds.

*Anopheline* spp. mosquitoes, e.g. *An. gambiae* s.s. Giles, are responsible for the transmission of the *Plasmodium* spp. pathogens that cause malaria in humans. This disease causes high levels of human suffering and mortality, with several million deaths per year in sub-Saharan Africa alone. Olfactory responses from saturated, mono-unsaturated and methyl-branched aliphatic carboxylic acids in sweat have been reported (Cork and Park 1996). A wide range of compounds (>80) was isolated and identified from sweat, and olfactory responses to a number of the compounds, including 6-methyl-5-hepten-2-one, geranyl acetone and indole, were reported (Meijerink et al. 2000). Incubated but not fresh sweat was shown to be attractive to *An. gambiae* (Braks and Takken 1999), and ammonia has been reported as the kairomone responsible (Braks et al. 2001). Recent studies suggest that host-

seeking behaviour relies on a combination of carboxylic acids, ammonia and lactic acid, the latter being a known sweat component that affects mosquito behaviour (Smallgange et al. 2005). *An. stephensi* were shown to respond to carbon dioxide plus either acetone or 1-octen-3-ol (Takken et al. 1997), and more recently *An. gambiae* to human skin emanations (Qiu et al. 2004). The landing responses of *An. gambiae* in response to sweat and extracts were studied (Healy and Copland 2000), with 40 out of 73 compounds being identified. From this list, 2-oxopentanoic acid was shown to elicit more landings. Concerning the exploitation of host-derived kairomones for monitoring populations in the field, the major breakthrough in this area has come from the discovery that the highly anthropophilic *An. gambiae* responds to three human-specific sweat compounds, 7-octenoic acid, (*E*)-3-methyl-2-hexenoic acid and (*Z*)-3-methyl-2-hexenoic acid. These major sweat compounds were known to be human-specific through their presence in apocrine gland secretions (Zeng et al. 1991). When tested in field experiments as slow-release formulations, these compounds affected the response of natural populations of *An. gambiae* (Costantini et al. 2001). This study was the first demonstration that natural malarial mosquito populations could be significantly influenced by human-specific chemicals, thereby demonstrating that host compounds have the potential to be developed for monitoring and controlling populations.

The yellow fever mosquito, *Aedes aegypti*, has a significant impact on mortality and suffering in many countries worldwide, including regions of North and South America, not only due to its ability to spread yellow fever, but also dengue, including the fatal haemorrhagic form, particularly in urbanised regions in South America (Pinheiro 1997). Despite the fact that *Ae. aegyptii* are known to be attracted to sweat, the only active component to be identified is lactic acid, which was isolated and reported as an attractant over 30 years ago (Acree et al. 1968). More recently, this compound has been shown to be attractive in the laboratory and in the field, when used in combination with acetone and dimethyldisulphide (Bernier et al. 2003; Silva et al. 2005). Similar to that for *An. gambiae*, ammonia was reported as an attractant for *Ae. aegyptii* (Geier et al. 1999), as were short-chain fatty acids, and a combination of these along with lactic acid (Bosch et al. 2000).

Pioneering studies by the Chemical Ecology group at Rothamsted Research in the UK are now demonstrating that vertebrate host and insect vector interactions are more complex than was originally thought. Where it was once assumed that the interaction was mediated by the detection of volatile attractants, research now shows that individual hosts within a single species differ in their attractiveness. It was hypothesised that this phenomenon is mediated by the presence of attractants and repellents/masking agents acting concurrently (Pickett et al. 1995). Evidence for this is being provided by studies on two interactions, human beings and *Ae. aegypti*, and Holstein-Friesian heifers and their associated fly pests (see below). For *Ae. aegypti*, several human-derived compounds with olfactory activity have been identified from isolated human odour using coupled GC-electrophysiology. Studies are now being carried out to test these identified compounds, either as single

components or multi-component blends, for their masking activity (Logan, unpublished data).

For *Culex* spp. mosquitoes, although attraction to human foot skin emanations has been demonstrated (Mboera et al. 1998), no compounds have been reported. For these pests, and other haematophagous insects, the identification of attractant and repellent components/blends will provide semiochemical tools for developing monitoring systems, and a new generation of repellents that are safer alternatives to the synthetic compounds currently used e.g. *N,N*-diethyltoluamide (DEET) and the more recently developed compounds such as Bayrepel and IR3535, for which there are growing safety concerns. There is also growing evidence for a correlation between host mosquito attractiveness, the physiological status of the host, e.g. the reproductive status, such as pregnancy (Lindsay et al. 2000), and the disease status (Lacroix et al. 2005). It is possible that understanding the semiochemical basis of these interactions may also lead to the identification of compounds for use in improving monitoring of mosquito populations.

A considerable number of investigations have studied the interaction between vertebrate hosts and tsetse flies (Diptera: Glossinidae), which vector the *Trypanomyiasis* spp. protozoa responsible for sleeping sickness in animals and human beings in sub-Saharan Africa. A wide range of vertebrates are hosts for this vector, including humans, ox, buffalo and goats. Host-derived kairomones have been identified from ruminants. 1-Octen-3-ol was isolated from ruminant breath (Hall et al. 1984), and multi-component baits including this compound, along with acetone, were shown to be effective in trapping populations in the field (Vale and Hall, 1985). Attractants from cattle urine have also been identified, including the phenolic compounds 3-methylphenol (*m*-cresol) and 4-methylphenol (*p*-cresol), which are released through microbial degradation (Hassanali et al. 1986; Bursell et al. 1988). Mixtures including some of these chemicals, released in a specific ratio, have successfully been used to trap tsetse fly populations in the field (Vale et al. 1986, 1988; Torr et al. 1995). Very recently, host-derived cues emitted by buffalo *Syncerus caffer* were identified using coupled GC-electrophysiology (Gikonyo et al. 2002, 2003). Additionally, volatile cues for the non-host waterbuck *Kobus defassa* were identified in a similar manner. Comparison of the volatile profiles showed that whereas both vertebrates produced a similar range of compounds, the non-host emitted additional compounds that interfered with activity of attractants. This study exploited the natural differential attractiveness of different vertebrate species, to identify semiochemicals that could be used in tsetse fly population monitoring and control.

Differential attractiveness of vertebrate hosts within a single species, as opposed to that observed for tsetse flies between hosts such as humans, buffalo, ox, goats etc., has been observed for cattle flies (Diptera: Muscidae) (Vagn-Jensen et al. 2004). The nuisance behaviour and disease-vectoring capacity of flies such as *Hydrotaea irritans*, *Haematobia irritans*, *Stomoxys calcitrans* and *Musca autumnalis* can lead to significant economic losses through disease incidence, e.g. myiasis, mastitis, pink eye conjunctivitis, and

reduced growth, milk production and fecundity. The role of cattle-derived semiochemicals in differential attractiveness was established through isolation of volatiles from Holstein-Friesian heifers. Twenty-three compounds were identified by coupled GC-electrophysiology (Birkett et al. 2004). Of these, 1-octen-3-ol, a known ruminant-breath compound, along with 6-methyl-5-hepten-2-one and 3-octanol were identified as attractants, whereas naphthalene, propyl butanoate and linalool were identified as repellents. When applied as slow-release formulations in the field, 6-methyl-5-hepten-2-one reduced fly populations on individual animals. The identification of attractants and repellents demonstrated the potential that these compounds have for monitoring and controlling cattle fly populations. For *S. calcitrans*, olfactory and behavioural responses to the known components 1-octen-3-ol and acetone were observed (Schofield et al. 1995; 1997). In field trials with *S. calcitrans*, 1-octen-3-ol has been very successful, with high trap catches (Holloway and Phelps 1991; Mihok et al. 1995). Trap catches of Tabanidae are also enhanced when 1-octen-3-ol, ammonia and carbon dioxide combinations are used (Kristensen and Sommer 2000).

Major host location cues have been defined for *C. nubeculosus* and the Scottish biting midge *C. impunctatus*, with attraction to 1-octen-3-ol, acetone and butanone being demonstrated (Blackwell et al. 1996; Bhasin et al. 2000a). Field studies using *C. impunctatus* showed attraction to 1-octen-3-ol, to acetone and a combination of phenolic compounds found in cow urine, and mixtures of carbon dioxide with either acetone, 1-octen-3-ol or cow urine (Bhasin et al. 2000b, 2001), and to animal extracts (Mands et al. 2004). Field responses to the human sweat compounds 3-methyl-2-hexenoic acid and 7-octenoic acid have been observed (Mordue and Mordue 2003). Recent studies are now showing that, for *C. impunctatus*, humans differ in their attractiveness in a similar manner to that demonstrated for *Ae. aegypti* (Logan, unpublished data).

Host-derived compounds have been studied for other Dipterous species. For myiasis-causing flies in the Oestridae, Calliphoridae and Sarcophagidae, olfactory stimuli have been identified as compounds associated with wounding and putrefaction, e.g. sulphur- and ammonia-rich compounds (Hall 1995). For the New World screwworm *Cochliomyia hominivorax*, 26 compounds were identified from sheep wound extracts by coupled GC-electrophysiology, with the most abundant being straight and methyl-branched C<sub>2</sub>-C<sub>5</sub> aliphatic acids (Cork 1996). Other components were also identified, with 1-octen-3-ol, 3-methylphenol, indole, phenol and dimethyldisulphide showing strong olfactory activity. Traps baited with a synthetic attractant blend, 'Swormlure-4', resulted in attraction of *C. hominivorax* in the field (Green et al. 1993). Host-derived attractants led to increased attraction of the Australian sheep blowfly *Lucilia cuprina* in the laboratory (Eisemann 1995) and increased trapping in the field (Urech et al. 2004).

Although *L. longipalpis* is a major disease vector, the role and identity of host-derived cues has not been fully established. Attraction to human odours in the laboratory has been reported (Hamilton et al. 1994), as has attraction to human odour (Pinto et al. 2001). For *Triatoma* bugs, although they are major

disease vectors, little has been done to identify host odours. Attraction to ammonia, 1-octen-3-ol, and to mixtures of lactic acid and short-chain fatty acids was reported (Taneja and Guerin 1997; Barrozo et al. 2004a, 2004b). Attractive host odour cues, nonanal and isobutyric acid, have been identified from sheep wool, chicken feathers and rabbit odour (Guerenstein and Guerin 2001).

Attraction of the tropical bont tick *Amblyomma variegatum* to human breath and the components 1-octen-3-ol, acetone and nitric oxide, has been reported (McMahan et al. 2001, 2002). Ticks are also attracted to cattle-derived rumen volatile metabolites (Donze et al. 2005). Once on the host, attractive and repellent host odours appear to guide ticks to their respective feeding sites (Wanzala et al. 2004). The red poultry mite *Dermanyssus gallinae* is a major pest of poultry, and is able to vector pathogens such as fowl spirochaetosis, chicken pox virus, Newcastle disease virus, agents of pullorum disease, fowl typhoid, along with salmonella virus (Chauve 1998). However, little is known of volatile semiochemicals involved in host location, although attraction to surface skin lipids has been reported (Zeman 1988).

## **2.5 Detection and identification of volatile chemical markers for infectious diseases and semiochemicals for insect disease vectors**

A number of physical sensors (biosensors) can potentially be deployed to detect infectious diseases. These include electrochemical, mass-sensitive and optical-based sensors (see Foresight project state of science review (S7) Biosensors and Biomarkers). However, these have been applied mainly to the detection of biomarkers other than host-derived volatile chemicals, i.e. proteins and nucleic acids, which originate from the pathogen. An 'electronic nose' system has been developed for the detection of volatile compounds emitted by antibiotic-resistant superbug MRSA in hospitals (Dutta et al. 2005). However, this system is currently of limited practical use as it cannot yet distinguish between resistant and susceptible strains, the latter of which are carried by many people as part of their normal flora.

Where there are examples of biosensors being developed for detecting host-derived volatile chemical markers, these rely on previous knowledge of the appropriate markers. These include a portable hand-held miniaturised GC system (zNose) (Kunert et al. 2002). Biosensors for detection of pathogen infection and insect infestations via detection of induced plant volatiles have been reported (e.g. Schultz et al. 1996). These include a system for detecting fungus-infected plants (Schultz et al. 1999), beetle-damaged plants (Schultz et al. 2000) and stored product pests (Ridgeway et al. 1999). The potential for using mammalian olfactory systems to detect diseases has been demonstrated with the detection of bladder cancer in humans by dogs (Willis et al. 2004).

Mass spectrometers (e.g. magnetic sector, ion trap, quadrupole, time-of-flight) are the natural and logical choice for identifying volatile markers of disease. They are able to generate stable and reproducible physical data at the sub-nanogram level, and have been used in most of the identifications of chemical markers for plant and animal diseases mentioned above. In recent years,

there have been rapid improvements in mass spectrometry instrumentation, which includes the development of *in situ* analysis of trace-level components present in air or water, and, of particular relevance to chemical marker identification, the development of miniaturised lab-scale instruments for rapid, portable use and which may or may not involve pre-concentration of samples. A method developed for rapid screening of potential disease biomarkers uses proton-transfer-reaction mass spectrometry (PTR-MS) (Rieder et al. 2001; Lirk et al. 2004). Another method involves membrane introduction (inlet) mass spectrometry (MIMS), which includes a miniature ion trap mass spectrometer coupled with semi-permeable membranes, e.g. polydimethylsiloxane (PDMS) membranes used in solid-phase microextraction (SPME) (Riter et al. 2003). New ionisation methods are also being developed that have the potential to be applied to portable systems. These include desorption electrospray ionization (DESI) (Takats et al. 2004). Alongside those systems developed specifically for medical research, the current space research programmes are also providing new leads in mass spectrometer miniaturisation. These include the Ptolemy GC-MS instrument developed for the European Space Agency's Rosetta mission, which incorporates a miniature GC coupled to an ion trap mass spectrometer (<http://pssri.open.ac.uk/missions/mis-rosa.htm>), and an ion and neutral mass spectrometer (INMS), which has been used on board the Cassini-Huygens orbiter.

Volatile, host-derived chemical markers of diseases often occur at trace levels, and in complex mixtures. The greatest challenge for detecting these markers is that the detector system has to be employed to 'see' these biomarkers effectively through extraneous material not related directly to the specific disease to be detected. A number of physical devices have been devised in an attempt to satisfy this need, but the most effective are those incorporating GC and MS, e.g. the Ptolemy instrument mentioned above. However, these systems, along with most of the simpler artificial nose systems, suffer from not being able to discriminate very low levels of key biomarkers in the presence of large amounts of irrelevant but related compounds. Portable, user-friendly biosensors need to be developed for this purpose. We at Rothamsted, and others, are studying the olfactory system of insects at the electrophysiological and molecular level to this end. An overview of these approaches forms the rest of this section.

Volatile pheromones emitted by insects, and semiochemicals emitted by host plants and animals, play an important role in enabling insects to locate mates and hosts at a distance (Pickett and Woodcock 1996; Pickett et al. 1998). Insects perceive volatile compounds via olfactory receptor neurons, located primarily on the insect antenna, which act to convert the chemical signal into an electrical impulse that inputs directly to the central nervous system. For plants, although there is considerable knowledge of the identity of these kairomones, the understanding of their role has, until very recently, been limited. Two possible hypotheses have been proposed. One suggests that species-specific odour cues are utilised, the other that location is dependent on the ratios of volatiles distributed generally among plants (Bruce et al. 2005).

Plant and animal volatiles are often complex mixtures, frequently comprising several hundred components (Bruce et al. 2005; Birkett et al. 2004). For collection of volatiles, air entrainment of plants and animals gives an accurate picture of the ratios produced and emitted (Agelopoulos et al. 1998; Birkett et al. 2004). Location of the biologically active components within these mixtures requires the use of sophisticated techniques –electroantennograms (EAGs) – electrophysiological recordings from insect olfactory receptors obtained by implanting electrodes into the insect antenna (Wadhams 1990). By linking the system with high-resolution GC – that is, splitting the effluent from the GC column and presenting it simultaneously to the flame ionisation detector of the GC column and the antennal preparation – it is possible to locate compounds within a complex extract with biological activity (Pickett et al. 1998).

Alternatively, where electrophysiological recordings are not appropriate or where other aspects of biology are more suited, GC can be linked to behavioural systems, for example, in the identification of sex pheromone components (Nazzi et al. 1996) or plant-host compounds for honeybees (Blight et al. 1997; Pickett et al. 1998). The detected compounds are then identified by coupled GC-MS and confirmed by GC co-injection with authentic samples (Pickett 1990) and, where appropriate, by nuclear magnetic resonance (NMR) spectroscopy. Once identified, authentic samples of chemicals, either obtained from commercial sources or by chemical synthesis, are used to confirm both electrophysiological and behavioural activity.

The detection of pheromones and semiochemicals by insects involves proteins that carry the compounds from the porous cuticular surface of the antennal sensilla through the sensillum lymph to the G-protein-coupled receptors and the olfactory neurons which, in turn, activate signalling cascades (Field et al. 2000). Such insect odorant binding proteins (OBPs) participate in the recognition of odorants. Insect OBPs, and the subgroup of pheromone binding proteins (PBPs), share some characteristics with vertebrate OBPs in that they are small (15–20kDa) soluble proteins, concentrated in the sensillum lymph. Genes and cDNAs encoding the OBPs of many insect species have been cloned, and recombinant OBPs generated via a suitable expression system, e.g. *An. gambiae* s.s OBPs (Li et al. 2005) and *Cx. quinquefasciatus* oviposition PBP (Ishida et al. 2002). Similar molecular techniques are now being used to identify genes encoding a subset of OBPs found in aphids, called chemosensory proteins, which will be facilitated by the determination of the full genome sequence for the pea aphid *Acyrtosiphum pisum*, during the course of the next four years.

To determine the link between OBPs and the semiochemicals, a range of experimental approaches, including conventional techniques, such as displacement of fluorescent ligands and kinetic binding assays, can be used to study the interactions of the ligands with the OBPs. New approaches that can determine more specific interactions are also being developed. These include electrospray MS, NMR spectroscopy and saturation transfer differential (STD) NMR. The latter involves a novel approach to searching libraries of potential ligands against particular OBPs. If successful, this will allow the determination of simultaneous ligand/OBP interaction kinetics. Once

the ligand/OBP interactions are established, the interactions with olfactory receptor proteins can be elucidated.

Understanding the fundamental molecular mechanisms – using the approaches outlined above – by which insects detect volatile chemical signals from plants and animals will potentially lead to the development of biosensors for disease detection that are based on detection of low molecular weight, organic compounds by OBPs and genetically engineered analogues. As illustrated by recently emerging breakthroughs on disease detection (see Phillips et al. 1997 etc.), it is likely that infection by disease pathogens leads to subtle changes in odour profiles of plants and animals. In this context, the pioneering studies being conducted at Rothamsted on: (i) differential attractiveness of animals for haematophagous insects being conducted by Rothamsted and its collaborators (Birkett et al. 2004; Logan, unpublished data); and (ii) the ability to ‘train’ insects such as the honeybee *Apis mellifera* to respond to volatiles collected from non-ecologically relevant situations (Pickett et al. 1998) clearly demonstrate that insects are able to detect such subtle changes in odour profiles and therefore demonstrate the potential for using insects to detect subtle changes in relation to disease infection. Also of particular importance is the ability of insects to detect subtle changes in volatile profiles prior to, or at the onset of, deterioration in fresh food or vegetables, e.g. the fruit fly *Drosophila melanogaster* or the blowfly *Calliphora vomitoria*, to detect the early stages of spoilage in food (Pickett 2005). Although these examples are not directly linked to the detection of infectious diseases, it demonstrates the concept that insects can be used to detect subtle changes in volatile profiles at an early, even pre-symptomatic, stage, which is crucial for infectious disease treatment and control.

The identification of pheromones and semiochemicals, often located using coupled GC-EAG described briefly above, requires the use of highly sensitive analytical equipment, as these compounds are produced and collected in vanishingly (<1ng) small amounts, usually within complex volatile mixtures. Mass spectrometers (e.g. magnetic sector, ion trap, quadrupole, time-of-flight) are the natural and logical choice for identifications. They are able to generate stable and reproducible physical data at the nanogram level, and can also be coupled to gas chromatographs, i.e. coupled GC-MS. For many insect disease vectors, GC-MS has been routinely used to identify pheromones as the level of material collected for analysis is usually only dependent on numbers of individual acquired through laboratory rearing or field collection. Collection and identification of host-derived semiochemicals, on the other hand, is a more challenging aspect, as the level of material is often much lower relative to pheromones. This is particularly so for insect disease vectors of vertebrates, where the barrier to semiochemical identification is not only raised by difficulties in obtaining sufficiently robust recordings from olfactory sensilla, but also by the fact that these are more complex mixtures at much lower levels compared to those collected from plants.

However, the pace of development of modern mass spectrometers, where instruments are increasingly sensitive and accurate, are able to detect broad spectra of molecules with diverse chemical and physical properties, and are

generally easier to operate and handle, is now such that identifications should, in theory, become easier, assuming that the underlying ecological aspects are fully understood and consequent semiochemical collection and detection is straightforward. Such instruments are now being widely used in modern metabolomic and metabolite profiling strategies.

### **3 Future prospects and directions**

#### **3.1 Approaches to disease detection**

As stated above, for many infectious diseases in plants and animals, early onset is characterised by increased oxidative metabolism which generates small-molecular-weight lipophilic compounds, for example, oxidation products from the unsaturated fatty acid and isoprenoid pathways. Examples from the literature, cited above, demonstrate the, as yet, largely unrealised, potential of these types of chemical markers in the detection and diagnosis of infectious diseases, especially at the early onset. For the development of rapid detection systems, there are several challenges that need to be addressed. These relate to: (i) the identification of chemical markers which are specific for particular diseases, and which can be clearly resolved from the 'normal' i.e. healthy plant or animal situation; and (ii) the development of advanced physical sensors which can detect the identified markers.

For (i), techniques for rapid collection of airborne and aqueous-derived volatiles from plants and animals are already well established. However, the complex nature and high variability of plant and animal volatile profiles, particularly for the latter, where the majority of components are potentially irrelevant, implies that disease marker detection through direct comparison of profiles from 'healthy' and 'diseased' individuals is unlikely to be a realistic target for rapid disease detection. In the meantime, examples from our own work and in the literature are providing evidence that there already exists the possibility of chemical marker detection, i.e. infection, through the recording of behavioural and electrophysiological responses from ecologically relevant insects, e.g. the use of pathogen-vectoring aphids (Eigenbrode et al. 2002) or mosquitoes (Lacroix et al. 2005) to detect plant or animal disease infection. Where there is no ecologically relevant insect involved, it is already possible use non-ecologically relevant insects, such as honeybees that can be 'trained' to respond to volatile cues generated in a number of different situations (Pickett et al. 1998). These are as yet insufficiently robust for practical use, but can already be used in the laboratory to identify chemical markers.

For (ii), any potential detector system that will be employed will have to be able to 'see' the chemical markers, once they have been identified, effectively through extraneous material not related directly to the specific disease to be detected. A number of physical devices have been devised in an attempt to satisfy this need. However, these systems, in particular the simpler artificial nose systems, can suffer from not being able to discriminate very low levels of key markers in the presence of large amounts of irrelevant but related compounds. Portable, user-friendly biosensors need to be developed for this purpose, and we at Rothamsted, and others, are already studying the

olfactory system of insects at the molecular level to this end. Here, it is envisaged that OBPs, as described above, that specifically recognise chemical markers for disease, will be isolated, characterised and ultimately incorporated into disease detector systems. Alongside these OBP-based biosensors, new, highly tuned physical sensors appear to be highly suited, in particular those miniature systems that utilise MS. Of these, the most effective will be those that incorporate GC and MS, e.g. the Ptolemy instrument mentioned above. The physical devices developed will need to be capable of rapid-throughput screening of airborne and aqueous-derived volatile samples, possibly using multi-location devices, and positioned at points of congregation, e.g. hospitals, homes, surgeries, hotels, and institutions such as prisons, or at points of entry such as airports and ports, and even on board aeroplanes, trains and boats.

As stated above, arthropods, in particular, insects, are often involved in infectious disease transmission in plants and animals. For these vectors, pheromones, including sex pheromones and other semiochemicals, although not always directly related to the vectoring part of the lifecycle, represent a potentially extremely potent means of disease detection, through the deployment of pheromone and semiochemical-baited trapping systems. These monitoring systems, which are highly specific for the target insect and work at low population densities, allow for mapping of populations with the potential to transmit diseases, and the detection of diseases through subsequent screening of trapped individuals for pathogen presence, using rapid, molecular-based diagnostic techniques. As exemplified in Sections 2.2 and 2.4, we and other workers have investigated the role and identity of pheromones and semiochemicals for major global insect pathogen vectors, and a limited number of chemical tools are already available for incorporation into vector trapping systems. Rapid diagnostic techniques for pathogen presence in plant and livestock disease vectors are already being used, e.g. for barley yellow dwarf virus in aphids (Fabre et al. 2003), and bovine arboviruses such as bluetongue in *Culicoides* spp. biting midges (Ohashi et al. 2004). In the next 20 years, it may be possible to use Earth observation in conjunction with trapping systems for vector-borne diseases (see Foresight project state of science review (S10) Earth Observation). Using genetic modification, it may be possible to insert genes into plants that would have a specific response to a disease. Sentinel plants could then react directly to a range of diseases by changing reflectance or fluorescence at particular wavelengths, depending on the disease, which could be detected by aircraft or spacecraft. Alternatively, intelligent *in situ* sensors that are based on global positioning system technology could be developed to sample the environment or plant/animal disease status.

### **3.2 The diseases**

Whereas the underlying principles of infectious disease diagnosis through chemical marker detection are now being elucidated, and the necessary tools and technologies are starting to be developed, the question remains of which infectious diseases should be targeted. For plants, vector-borne plant viruses remain the major contributor to plant diseases. For livestock animals, zoonotic

diseases, e.g. Rift Valley fever, epidemic diseases, e.g. foot-and-mouth disease, and vector-borne diseases, e.g. bluetongue, are major contributors. For humans, according to the World Health Organization (WHO) report on the total global burden of disease lost due to communicable diseases (WHO 2000), major contributors include vector-borne diseases, e.g. malaria, dengue, filariasis and encephalitis, acute and severe respiratory infections, e.g. influenza and TB, and sexually transmitted diseases, e.g. HIV/AIDS and syphilis. For humans, in addition to these current major global diseases, there are also a number of emerging and re-emerging major diseases, including TB, SARS, avian influenza, and vector-borne diseases such as Lyme disease, Leishmaniasis and West Nile virus.

This Foresight project has identified infectious diseases in plants, livestock animals and humans which it considers will be important in the next 15–20 years. In all cases, diseases vectored by insects and other invertebrate arthropods have been rated as the most important, along with acute and chronic respiratory diseases such as influenza and TB. For insect vectors of plant diseases, aphids and leafhoppers are the major class of disease vectors. For livestock animals and humans, biting and blood-sucking arthropods, e.g. mosquitoes, tsetse flies, sandflies, midges, ticks, mites and triatoma bugs all contribute to transmission of infectious diseases.

### **3.3 Vector-borne diseases**

For vector-borne diseases, although the presence and identity of chemical markers emitted by infected plants, livestock animals and humans is starting to be elucidated, the current level of knowledge is poorly defined and needs to be improved using the approaches outlined in Section 3.1. For the development of efficient trapping systems that incorporate pheromones or other semiochemicals, the current level of knowledge is patchy, with some vectors being well understood and others about which there is little or no knowledge at all. For aphids, pioneering studies conducted at Rothamsted have provided a number of pheromone and semiochemicals which are now available for use on a widespread (landscape) area. For other plant disease vectors, such as leafhoppers, the role and identity of pheromones and semiochemicals is poorly understood. For livestock animal and human disease vectors, although the role and identity of pheromones and semiochemicals is starting to be elucidated, by scientists at Rothamsted and elsewhere, these are still poorly understood.

Thus, for the development of vector trapping systems, it is critical that underpinning science is carried out in order to fully elucidate the role and identity of pheromones and semiochemicals, as the incidence of diseases both in the developing world and in the developed world through vector activity is high and rising, the latter as a consequence of increasing global travel and climate change. The underpinning science includes a greater understanding of vector chemical ecology throughout the entire lifecycle, mainly concentrating on host location, but also on reproductive stages such as mating and oviposition where pheromones are involved. This requires expertise in several scientific disciplines, all of which should preferably be linked directly through multidisciplinary groups at research institutes, or

through well-established collaborative links with complementary areas of expertise. The disciplines include insect behaviour, neurophysiology and morphology, analytical and synthetic organic chemistry, insect behaviour in the field, including epidemiology, and insect molecular biology.

Also of importance for understanding vector biology is the development of remote Earth observation technologies which are sufficiently robust and reliable to be applied where trapping systems cannot be deployed.

In addition to the scientific community acquiring a greater understanding of vector chemical ecology and biology, public understanding and awareness of the importance of chemical ecology needs to be substantially increased, compared to other high-profile areas of biological chemistry, e.g. the pharmaceutical and agrochemical industries. The same holds true in the public peer-reviewed arena, where chemical ecology is still considered something of a 'niche' area of science.

In summary, a comprehensive understanding of the identity of chemical markers produced on infection by disease pathogens, and, more specifically, the role and identity of pheromones and semiochemicals for major disease vectors, will allow the development of technologies, either portable physical sensors or trapping systems, for rapid detection of infectious diseases, even at the onset of the infective stage. These are the major challenges for infectious disease control during the next 15–20 years, and beyond.

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