9 Chemical Ecology

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Introduction

The ecology of aphids is, like that of most insects, highly dependent upon signals. Signals from host and non-host plants convey information that is vital for selecting feeding, larviposition, and mating sites. Signals from aphids themselves are important in attracting a mate, aggregating with conspecifics, avoiding competition, and sensing, or giving warning of, threats. Chemical signals (semiochemicals) are relatively efficient to produce, specific, easy to disperse into the environment and, not least, easy to detect. Aphid life cycles are characterized by complex interactions, and those species that alternate between hosts and have a sexual phase are faced with considerable challenges such as locating the correct winter (primary) host, finding mates, leaving the winter host in the spring, and successfully colonizing the summer (secondary) host. Therefore, it is no surprise that aphids make extensive use of semiochemicals, both in gathering information from their environment and in signalling to each other. Parasitoids and predators have also evolved responses to some of these semiochemicals. It is has been 15 years since the last major review of aphid chemical ecology (Pickett *et al.*, 1992). In this time, significant advances have been made in studies on systemic production of aphid-induced volatiles by plants, volatiles that sometimes have a composition specific to an aphid species. There have also been advances in respect of the role of aphid sex pheromone-related chemistry beyond parasitoid chemical ecology and into predator behaviour.

There are around 4000 species of aphid. However, for two reasons, this review will necessarily focus on a very small number, principally in the subfamily Aphidinae. First, the great majority of information that exists on aphids as a group has been obtained through the study of species from the temperate regions of the northern hemisphere (Dixon, 1998), and secondly, studies have naturally tended to focus, not exclusively but in large measure, on those species that have attained pest status due to their interference with human activities. This focus has been particularly accentuated in relation to aphid chemical ecology, since the principles and techniques of chemical ecology have so often been directed towards more effective and sustainable management of pests. Arguably the most studied aphids in terms of their chemical ecology have been *Aphis fabae* (black bean aphid) and *Rhopalosiphum padi* (bird cherry–oat aphid), both from the Aphidinae and both host-alternating species that regularly manifest themselves as pests in the northern hemisphere. In the case of *R. padi*, the importance of chemical signals in the complex life cycle

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of a host-alternating aphid is readily apparent, and is summarized in Fig. 9.1. We now also see the first application of molecular biological techniques to aphid chemical ecology and, in particular, in the study of olfactory mechanisms and alarm pheromone biosynthesis (Field *et al.*, 2000). Already, the first aphid gene involved in processing secondary plant metabolites (Jones *et al.*, 2001; Pontoppidan *et al.*, 2001) has been identified (Jones *et al.*, 2002).

In this review, we examine the many varied interactions in aphid ecology in which semiochemicals play important roles. Taking a trophic level approach, we present examples of chemically mediated interactions between aphids, between aphids and plants, and between aphids and their natural enemies.

Methods

Since this is a review of aphid chemical ecology, the 'Methods' section will be brief, but may be useful in highlighting some recent developments that have played an important generic role in the advances described in subsequent sections. It may also be of value to those workers contemplating new studies on aphid chemical ecology.

Bioassay techniques have continued to employ the Pettersson olfactometer (Pettersson, 1970a) and the linear track olfactometer with or without the modification (Hardie *et al.*, 1994a) allowing continuous release of test compounds. Approaches to obtaining volatile samples involve entrainment of air above the natural system by absorption onto porous polymers. Use of the solid-phase microextraction (SPME) technique has become widespread and is extremely convenient. However, SPME does not provide a single consistent sample for multiple analysis, suffers from some other disadvantages, and has been the subject of comparative quantitative investigation, which confirms the value of entrainment systems giving samples as solutions (Agelopoulos and Pickett, 1998).

When studying semiochemical release from intact plants, or plants colonized by particular herbivores such as individual aphid species, any cutting of petioles or damage to roots can alter the volatile profile. Entraining from semi-open vessels placed over the plant material can solve this problem. To avoid pressure or other damage at hermetic seals, a loose seal is established in the enclosing vessel and air is sampled from the positive pressure that can be created within (Dicke *et al.*, 1990; Turlings *et al.*, 1991; Röse *et al.*, 1996; Agelopoulos *et al.*, 1999).

Electrophysiological recordings coupled to capillary gas chromatography (GC) are important approaches for initial identification of volatile semiochemicals and can comprise electroantennogram (EAG) (Birkett *et al.*, 2000) as well as single cell (neuron) recordings (SCR) (Pickett *et al.*, 1992). However, tentative identification by GC coupled mass spectrometry (MS) can remain problematic as the threshold detection level for MS, or at least the level for obtaining useful spectra, can be higher than for GC-SCR, or even the less sensitive technique of GC-EAG. Where GC is directly coupled to behavioural measurement, for example, wing fanning by aphid parasitoids (Nazzi *et al.*, 1996), the response threshold can be even lower than for GC-SCR. Increased sample size can cause loss of GC resolution, but sometimes biological solutions to the problem can be found; for example, if a plant compound is involved, related cultivars or species that may contain more of the active compound can be investigated (L.J. Wadhams, personal communication).

Non-volatile semiochemicals can also mediate important aspects of chemical ecology (Powell and Hardie, 2001; Powell *et al.*, 2006; see also Pettersson *et al.*, Chapter 4 this volume). Techniques for studying these materials have advanced with liquid chromatography (LC) coupled MS using reverse phase columns and electrospray ionization in both positive and negative ion modes (Takemura *et al.*, 2002, 2006). High-performance LC can also be used to produce fractions and pure samples of the putative non-volatile semiochemicals for analysis by

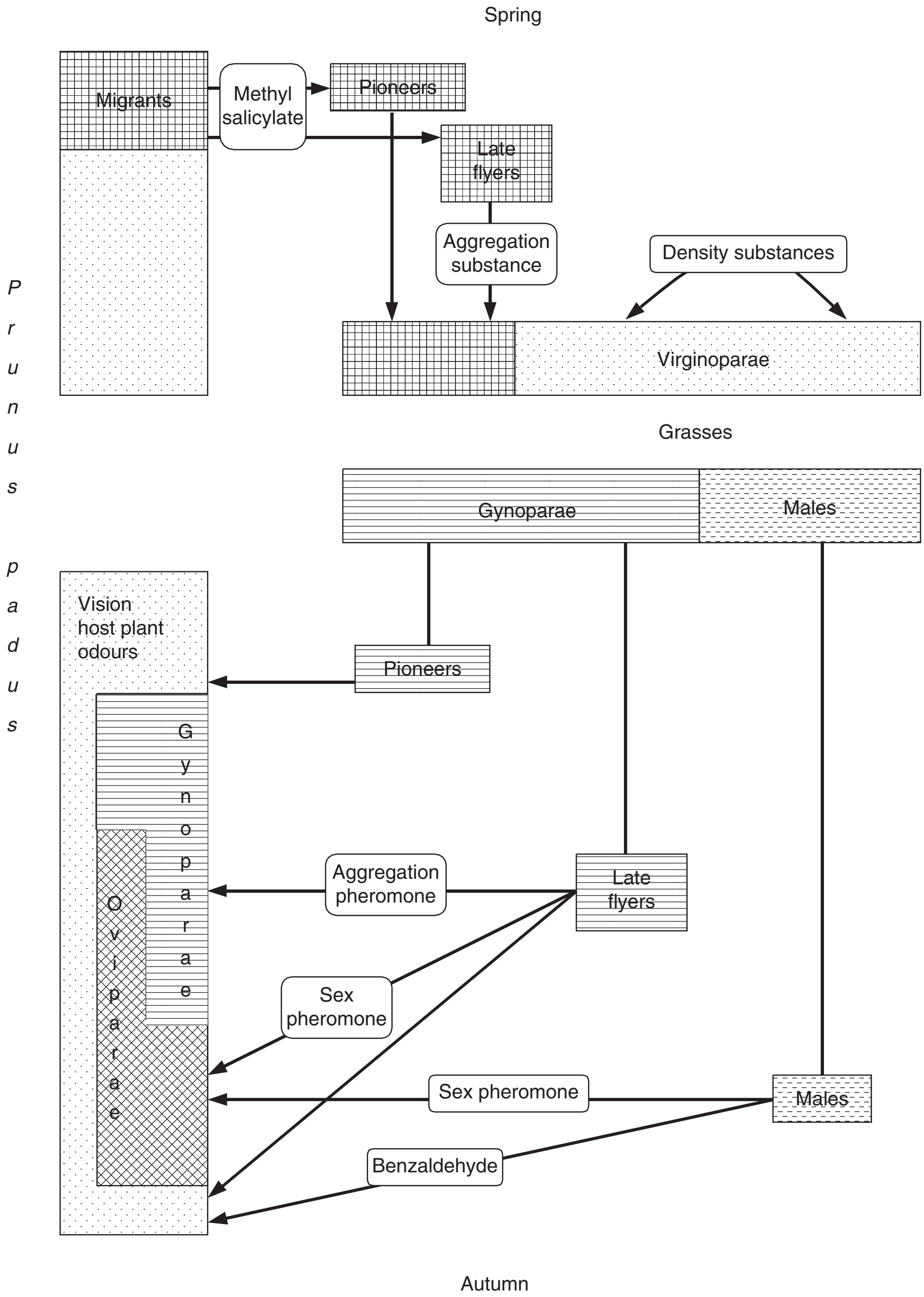


Fig. 9.1. Chemical ecology of host alternation in *Rhopalosiphum padi (*bird cherry–oat aphid). The diagram summarizes the interactions in which chemical signals are important for the aphid as it migrates from its winter host bird cherry *Prunus padus*, to grasses and cereals, and back to *P. padus* in the autumn to undergo the sexual phase (based on Pettersson, 1994; Pettersson *et al*., 1994, 1995; Hardie *et al*., 1996; Glinwood and Pettersson, 2000a; Park *et al*., 2000).

nuclear magnetic resonance spectroscopy using a 500 MHz Fourier Transform instrument (Takemura *et al.*, 2002).

Interactions between Aphids

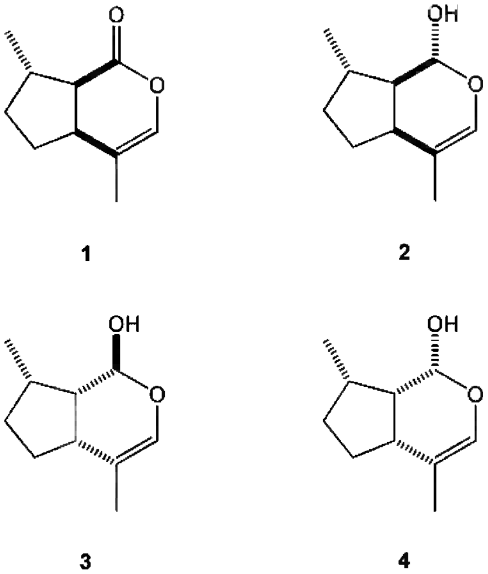
Sex pheromones

Aphid sex pheromones were unequivocally demonstrated by Pettersson (1970b), and the first was chemically characterized in 1987 (Dawson *et al.*, 1987a). The subject was reviewed in 1992 (Pickett *et al.*, 1992) and later by Hardie *et al.* (1999). The sex pheromones, released by the sexual females, excite males and increase mating success (Fig. 9.2). The pheromones are produced in glandular epidermal cells lying beneath scent plaques on the tibiae of the hind legs of the sexual females and released through porous cuticles above the plaques. During pheromone release, the female engages in typical 'calling' behaviour, with the hind legs raised (Fig. 9.3). The olfactory receptors for sex pheromones are placoid sensilla, in the secondary rhinaria on the antennae of male aphids. The secondary rhinaria are mainly on the third, fourth, and sometimes fifth antennal segments, and are highly sensitive to sex pheromone components (see also Pettersson *et al.*, Chapter 4 this volume). There are separate receptor cells for individual pheromone components, which usually have different response amplitudes, both cells being necessarily activated to trigger a behavioural response.

Sex pheromones have been chemically characterized from a number of species, all of which are in the subfamily Aphidinae. GC-SCR has played a major role in these studies, followed by GC-MS and confirmation of structures by synthesis and spectroscopy (Hardie *et al.*, 1999; Boo *et al.*, 2000). The pheromones usually comprise (4a*S*, 7*S*, 7a*R*)-nepetalactone (**1**, Fig. 9.4) and (1*R*, 4a*S*, 7*S*, 7a*R*)-nepetalactol (**2**, Fig. 9.4), which are monoterpenoids in the cyclopentanoid or iridoid series. However, *Phorodon humuli* (damson–hop aphid) employs neither compound **1** nor **2**, but a mixture of the two diastereoisomeric (1S)- and (1*R*,4a*R*,7*S*,

Fig. 9.2. Mating in the aphid *Megoura viciae*. The darker, slightly thinner male (top) is attracted by sex pheromone released by the ovipara, the sexual female aphid (original colour photo courtesy Rothamsted Research).

Fig. 9.3. The typical 'calling' position of a sexual female (ovipara) of *Megoura viciae*, with the hind legs raised. Sex pheromone components are produced in glandular epidermal cells lying beneath scent plaques on the tibiae of the hind legs (original colour photo courtesy Rothamsted Research).



1 2 3 4

Fig. 9.4. Chemical structures for aphid sex pheromone components (4a*S*,7*S*,7a*R*)-nepetalactone (**1**), (1*R*,4a*S*,7*S*,7a*R*)-nepetalactol (**2**), (1*S*,4a*R*,7*S*,7a*S*)-nepetalactol (**3**) and (1*R*,4a*R*,7*S*,7a*S*)-nepetalactol (**4**).

7a*S*)-nepetalactols (**3** and **4**, Fig. 9.4) (Campbell *et al.*, 2003), although EAG responses to **1** have been recorded in males of this species (Pope *et al.*, 2004).

The nepetalactone **1** has long been familiar as a compound from the catmint plant *Nepeta cataria* (Lamiaceae), and can be chemically reduced selectively to the nepetalactol **2**. This now provides a commercial (Botanix Ltd) route to these compounds in which specific cultivars of *N. cataria* are grown as arable crops, harvested wet, and then directly subjected to steam distillation to yield the essential oil with a very high proportion of **1**. Further conversion by hydride reduction to the nepetalactol **2** can also be achieved on a commercial scale. Formulation (AgriSense-BCS Ltd) is as a polymeric rope where the length determines the overall release rate or field dose. The nepetalactone giving the nepetalactols **3** and **4** is also found in the related *Nepeta racemosa* (Birkett and Pickett, 2003).

The biosynthesis of nepetalactones in plants has received considerable attention and may be achieved by a similar route in aphids. The isoprenoid precursor is geraniol and/or the (*Z*)-isomer nerol, or possibly citronellol, and a detailed analytical study of the aphid sex pheromone from various species has revealed that citronellol (with undetermined stereochemistry) is a common, but minor, component of volatiles associated with sex pheromone release. It has shown no electrophysiological or behavioural activity, at least in connection with mate location, and may therefore be the precursor for the cyclopentanoid biosynthesis of aphid sex pheromones (Dawson *et al.*, 1996).

Most aphids examined so far employ a limited range of pheromone components, but there are differences in relative and absolute compositions. Only *P. humuli* produces **3** and **4**, while the other species produce blends of **1** and **2** or the individual components (Hardie *et al.*, 1999; Boo *et al.*, 2000; Goldansaz *et al.*, 2004). However, there does not seem to be sufficient variability to allow species-specificity even merely within the Aphidinae. Indeed, a number of species use the same single component **1** alone, for example, *Sitobion avenae* (grain aphid), *Sitobion fragariae* (blackberry–cereal aphid), and *Brevicoryne brassicae* (cabbage aphid) (Hardie *et al.*, 1999). Laboratory males are more responsive to the pheromone blend closest to that released by the conspecific sexual females than to other blends. Addition of **2** inhibited the olfactometric responses of males of *S. avenae* (Lilley *et al.*, 1995) and *S. fragariae* (Lilley and Hardie, 1996) to their sex pheromone component **1**. Field trap-catch data show a remarkable specificity. Water traps releasing only **1**, recorded males of 21 aphid species, including mainly *S. fragariae* (Hardie *et al.*, 1992). Even the addition of minimal quantities of **1** reduced catches of *R. padi*, which employs only **2** (Hardie *et al.*, 1997), to levels similar to unbaited traps. In traps releasing **1**, **2**, or a 1 : 1 blend of **1** and **2**, 66% of male *Aphis* spp. and 85% of male *Dysaphis* spp., including *Dysaphis plantaginea* (rosy apple aphid), were captured by the blend, while 88% of *Rhopalomyzus lonicerae* were found in traps releasing **1**. *Myzus cerasi* (cherry blackfly) was also captured in traps releasing only **1** (Hardie *et al.*, 1994b; J. Hardie, R. Harrington and L.J. Wadhams, unpublished results). The sex pheromone for *D. plantaginea* comprises compounds **1** and **2**, with the latter the most abundant (S.Y. Dewhirst, personal communication). Trap catches appear to be associated with pheromonally mediated anemotaxis, although this was, before the availability of synthetic pheromone, considered to be an unlikely possibility. Indeed, it has been shown that *P. humuli* can fly towards a source of its pheromone against wind speeds of up to 0.7 m/s, and can detect pheromone 3–4 m downwind (Hardie *et al.*, 1996). The sex pheromones have potential for use in population monitoring and control of pest aphids, and there are a number of practical development programmes under way, but the main agricultural use under development is in the attraction of parasitoids (see 'Natural enemies' below). One particular opportunity for direct aphid control is the use of pheromone in traps for disseminating an entomopathogen from the trap into the aphid population (Hartfield *et al.*, 2001).

There is now clear evidence for an interaction between sex pheromone and host-plant volatiles in some aphid species (Hardie *et al.*, 1999; Powell and Hardie, 2001). More *P. humuli* males were caught in traps releasing volatiles extracted from winter host plants, *Prunus* spp., with **3/4** than with **3/4** alone. Similar results were found with *R. padi* when combining release of volatile leaf extracts of bird cherry (*Prunus padus*), with **2**, which was at least partially accounted for by the presence of benzaldehyde in the leaf volatiles. Placing pheromone traps in the winter host rather than the non-host tree *Malus sylvestris* also enhanced catches of male *R. padi* (J.R. Storer and J. Hardie, unpublished results). However, this area of aphid chemical ecology requires further investigation (Hardie *et al.*, 1999). It should also be mentioned that the various semiochemicals can interact with visual cues, the latter also affecting the behaviour of male aphids. For example, surface colour is crucial and catches of males of three species in water traps were dramatically affected by trap colour (Hardie *et al.*, 1996).

Alarm pheromones

The asexual forms, and most often the wingless females, of many aphids release an alarm pheromone when disturbed. Nearby aphids exhibit a variety of behaviours, ranging from removal of mouthparts from the plant and moving away, to running, dropping off the plant, and even attacking the predator. This aspect of aphid chemical ecology has been reviewed in depth (Hardie *et al*., 1999), and so will be dealt with briefly for completeness, highlighting work subsequent to that review.

Variation in response to alarm pheromone is seen both intra- and interspecifically and relates to the relative risks of predation and costs of escape. A lack of response from the early instars may arise from the risk of predation to these larvae being lower than the risk involved in ceasing to feed. Winged adults, on the other hand, are more responsive to alarm pheromone, perhaps because they move more readily off the natal host. However, with *Ceratovacuna lanigera* (sugar cane woolly aphid), it is the first-instar nymphs that show attack behaviour in response to alarm pheromone, while older nymphs and adults merely disperse (Arakaki, 1989). In this species, however, the first-instar nymphs have long frontal horns and are able to mount an effective defence against predators. Exposure to alarm pheromone can lead to an increase in the production of winged morphs in an aphid colony (Kunert *et al*., 2005). Since this effect was seen when groups of aphids, but not isolated individuals, were exposed to the pheromone, it seems likely that the exposure causes a 'pseudo crowding' effect, with increased physical contacts triggering the shift in morph production.

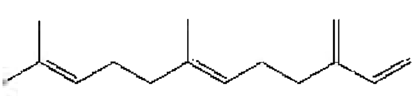
Considerable variation exists among aphid species in their sensitivity to alarm pheromones in both the speed and the form of the response, variation that often can be related to the ecology of the species. For example, some aphids, particularly those tended by ants, respond by walking or 'waggling' their abdomens rather than falling off the plant (H.F. van Emden, personal communication). Response to alarm pheromone is affected by many additional factors, and in some cases may be dependent upon physical cues, or other semiochemicals, as discussed below (Hardie *et al*., 1999).

In terms of variation within species, susceptibility to insecticides has also been found to correlate with alarm pheromone responses, discovered originally in 1983 (Dawson *et al*., 1983). Insecticide-resistant *Myzus persicae* (peach–potato aphid) responded to alarm pheromone more slowly and in lower numbers than insecticide-susceptible forms (Foster *et al*., 1999). This was true for a wide range of aphid clones carrying different combinations of metabolic (carboxylesterase) and target site (kdr) resistance mechanisms (Foster *et al*., 2005; Foster *et al*., Chapter 10 this volume). This may be due to pleiotropic physiological effects associated with resistance affecting mobility, or to impairment of nerve function (Foster *et al*., 1999).

Aphids that have dropped from a plant may either recolonize it or search for another host plant, but in both cases face a greatly increased risk of predation (Griffiths *et al*., 1985; Sunderland *et al*., 1986) or starvation. Fallen aphids exposed to alarm pheromone often appear behaviourally reluctant to regain their host plant (Klingauf, 1976).

Droplets secreted from the siphunculi, on attack or mechanical interference, contain the minor, rapidly vaporizing fraction that is the alarm pheromone. A waxy fraction, consisting mainly of triglycerides, that crystallizes on contact with foreign particles outside of the aphid's body is also produced. The waxy component appears to function as a sticky irritant to predators and parasitoids.

The main component of the alarm pheromone of many aphids is the sesquiterpene hydrocarbon (*E*)-β-farnesene (**5**, Fig. 9.5) (Bowers *et al*., 1972; Edwards *et al*., 1973; Wientjens *et al*., 1973; Pickett and Griffiths, 1980). Other components may also be present. For example, the alarm pheromone of *Megoura viciae* (vetch aphid) contains the monoterpenes (–)-α-pinene, (–)-β-pinene, (*Z*,*E*)-α-farnesene, and (*E*,*E*)-α-farnesene, in addition to **5**, and these can synergize the activity of **5**. There is a high degree of cross-activity of both natural alarm pheromone and **5** among species within the Aphidinae and Chaitophorinae, typical of insect alarm pheromones in general. The main component of the alarm pheromone of *Therioaphis trifolii maculata* (spotted alfalfa aphid) and *Therioaphis riehmi* (sweet clover aphid) (Calaphidinae), however, is the cyclic sesquiterpene (–)-(10*S*)germacrene-A (**6**, Fig. 9.5). Both **5** and **6** are volatile, highly labile compounds and produce a short-lived, though for some species dramatic, alarm signal.



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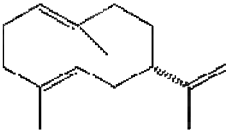

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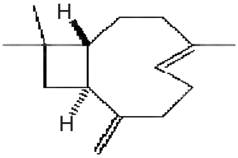

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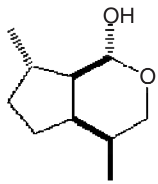

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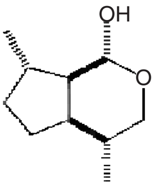

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Fig. 9.5. Chemical structures for other aphid related semiochemicals (*E*)-β-farnesene (**5**), (–)-(10*S*)germacrene-A (**6**), (–)-β-caryophyllene (**7**), (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydro nepetalactol (**8**), and (1*R*,4*R*,4a*R*,7*S*,7a*R*)-dihydr onepetalactol (**9**).

The alarm pheromone is detected by the primary rhinaria on the fifth and sixth antennal segments, which can be seen from recordings from single olfactory cells, for example with *M. persicae* (Dawson *et al.*, 1987b).

Although the biosynthesis of (*E*)-β-farnesene has not been studied in aphids, the gene for an enzyme from peppermint (*Mentha* × *piperita*) that effects conversion of farnesyl pyrophosphate to this sesquiterpene has been cloned and functionally expressed in *Escherichia coli* (Crock *et al.*, 1997). However, many other sesquiterpenes are also produced that could cause inhibition of the alarm response (see below).

Sesquiterpene hydrocarbons, including **5**, are commonly produced by plants. Indeed, compound **5** in the volatiles produced by leaf hairs of the aphid-resistant wild potato *Solanum berthaultii* caused alarm behaviour in wingless adult *M. persicae* (Gibson and Pickett, 1983). However, the sesquiterpene (–)-β-caryophyllene (**7**, Fig 9.5), together with other sesquiterpenes commonly accompanying **5** in plants, was found to inhibit the alarm activity (Dawson *et al.*, 1984). This enables the aphid to distinguish whether **5** emanates from alarm pheromone or from a plant source. Olfactory cells in aphids have been found that are sensitive to either **5** or **7**, but not to both, and the two cell types are located in rhinaria on different antennal segments (Campbell *et al.*, 1993). For *Lipaphis pseudobrassicae* (mustard aphid), there is synergy between **5** and the generally toxic defence compounds such as organic isothiocyanates, which are produced from glucosinolates apparently sequestered from host plants in the Brassicaceae (Dawson *et al.*, 1986). The aphid enzyme responsible for the initial hydrolytic elimination of glucose from the glucosinolate has been characterized (Jones *et al.*, 2001; Pontoppiddan *et al.*, 2001), the associated gene cloned, and the sequence, although similar, shown to confer a structural difference from the corresponding plant thioglucosidase or myrosinase (Jones *et al.*, 2002). Hydrolysis of glucosinolates by the aphid myrosinase proceeds by general acid base catalysis using two glutamate residues as proton donor and nucleophile. Plant myrosinases differ from other members of this family in that one of the glutamate residues has been replaced by a glutamine (Burmeister *et al.*, 1997) and ascorbic acid substitutes for the second proton donor (Burmeister *et al.*, 2000). Thus, unexpectedly, aphid myrosinase acts mechanistically similarly to the *O*-glucosidases in having two glutamate residues; the absence of a proton donor, as in plant myrosinases, is not a prerequisite for the hydrolysis of glucosinolates, as was once thought (Burmeister *et al.*, 1997). The crystal structure at 1.1 Å resolution of the myrosinase of *B. brassicae* has been obtained from milligram amounts of the pure recombinant myrosinase obtained by over-expression in *E. coli*. However, the only residue specific for the aphid myrosinase in proximity of the glycosidic linkage is Tyr180, which may have a catalytic role. The aglycone binding site differs strongly from plant myrosinase, whereas due to the presence of Trp424 in the glucose binding site, this part of the active site is more similar to plant β-*O*-glucosidases, as plant myrosinases carry a phenylalanine residue at this position (Husebye *et al.*, 2005).

An antibody has been raised to the aphid myrosinase and its localization in the insect determined by immunocytochemistry and electron microscopy (Bridges *et al.*, 2002). The enzyme was found to be located in muscle of the head and the thorax and is present as regular crystal-like structures. This represents an apparently unique organizational arrangement, where myrosinase is held in the sarcoplasm of the muscle, while glucosinolates are most likely present in the haemolymph. Remarkably, this arrangement is similar to the brassicaceous plants that the insect feeds on, where myrosinase is compartmentalized into special myrosin cells and glucosinolates appear to be held in separate cells. In both cases, the spatial arrangement suggests a defence mechanism, with the enzyme and substrate remaining separated until tissue damage brings them together.

The use of (*E*)-β-farnesene in aphid control strategies has been hindered by its instability. However, there is renewed interest in exploiting plant essential oils rich in the substance, such as that of *Hemizygia petiolata* (Lamiaceae), which contains > 70% (*E*)-β-farnesene. Although laboratory studies suggest that the presence of minor components (+)-bicyclogermacrene and (–)-germacrene D may inhibit the alarm response of certain aphid species, a slow release formulation reduced *Acyrthosiphon pisum* (pea aphid) numbers in the field, giving a promising route for the deployment of (*E*)-β-farnesene in pest aphid management (Bruce *et al.*, 2005).

Social interactions: aggregation, density regulation, and avoidance

Aphids live in colonies that often reach a high density of individuals. A number of factors explain the existence of this habit and the selective forces that maintain it, and the capacity for rapid reproduction combined with low mobility can be seen as either reasons for, or adaptations to, colonial living. One benefit of aggregation is increased protection from natural enemies (Turchin and Kareiva, 1989), although aphids forming heterospecific aggregations could satisfy this. Therefore, it is possible that conspecific aggregation improves the food quality of the host plant (Way and Cammell, 1970; Way, 1973; see Douglas and van Emden, Chapter 5 this volume). Aphid colonies can grow to such a size that their sustainability on the plant is in no way assured (Way and Banks, 1967), and consequently there should be a point at which the advantage switches from recruitment of further individuals to the plant to their deterrence from it. Aphids of different species can have deleterious effects on each other when feeding on the same plant (Chongrattanmeteekul *et al.*, 1991). Therefore, control over social interactions both within and between species is important for aphid survival, and it is no surprise that semiochemicals play a role in maintaining such interactions. Current knowledge is biased towards aphid species that are considered to be economically important, and aggregation and avoidance interactions may be especially important in host-alternating species.

Aggregation

Gynoparae of *R. padi* show an aggregated distribution of settling on the winter host *P. padus* that cannot be explained by differences in microclimate or leaf quality (Pettersson, 1993). Gynoparae were attracted to the odour of other gynoparae in an olfactometer (Pettersson, 1993) and to water traps baited with gynoparae (Pettersson, 1994). Gynoparae were also attracted to the odour of *P. padus* leaves, but there was no increased response to leaves infested with gynoparae. Thus, aggregation of colonizing aphids on the winter host is mediated by an aggregation pheromone. Although it is unclear whether gynoparae of *R. padi* feed on the winter host, it is likely that one benefit of aggregation is the subsequent aggregated distribution of oviparae, which should maximize the attraction of males to the sex pheromone.

Aggregation of *R. padi* gynoparae is further enhanced by attraction of late flyers to the sex pheromone nepetalactol (**2**, Fig. 9.4), produced by oviparae already on the winter host (Fig. 9.1). Nepetalactol-baited traps captured gynoparae in the field (Hardie *et al.*, 1996), but since the traps caught only a relatively small proportion of the aerial population, it was suggested that the response of gynoparae to nepetalactol is weaker than that of males. This was later confirmed by EAG and behavioural studies (Park *et al.*, 2000). Since gynoparae of *S. fragariae* and *P. humuli* are also attracted to sex pheromones, this aggregation mechanism may be common among host-alternating species (Hardie *et al.*, 1996; Lösel *et al.*, 1996).

Semiochemicals are also involved in causing aphids to aggregate to their summer host plants. In olfactometer tests, spring migrants of *P. humuli* were more attracted by spring migrants feeding on a leaf of their summer host, hop (*Humulus lupulus*), than to an uninfested leaf (Campbell *et al.*, 1993). Entrainment of hops, followed by GC-MS, identified three compounds released by the plant on feeding: (*E*)-2-hexenal, β-caryophyllene, and methyl salicylate, all of which were shown by GC-SCR to elicit responses from separate olfactory receptors on the aphid antenna. Interestingly, addition of methyl salicylate caused the other two compounds to lose attractiveness in the olfactometer, and it was suggested that methyl salicylate might be produced by the plant in response to high densities of feeding aphids, effectively preventing recruitment of further migrants to the plant. Analagous to the above results for *P. humuli*, *R. padi* spring migrants are more attracted to odour produced by spring migrants feeding on oat leaves than to uninfested leaves (Pettersson, 1994), again suggesting the presence of an aggregation substance for the migrating morph. In this case, the origin of the substance or substances has been difficult to identify. The migrant–oat complex is highly attractive to the migrants in the olfactometer. Migrants alone, however, are not attractive when removed from the leaf and presented immediately in the olfactometer, and when a previously attractive migrant–oat complex was separated and the odours of both the aphids and the previously infested leaf presented simultaneously, they did not elicit attraction (R. Glinwood, unpublished results). The rapid loss of attractiveness suggests that aggregation is mediated by an aphid pheromone, production of which is disrupted by removing the aphid from the plant, rather than by an aphid-induced plant volatile.

Aggregation pheromones in non-migratory morphs have also been reported. Alatae of two brassica-feeding aphids, *B. brassicae* and *L. pseudobrassicae*, emit an odour that attracts conspecific alatae, but not conspecific apterae (Pettersson and Stephansson, 1991). Apterae of *Aphis craccivora* (cowpea aphid) were attracted to the odours emitted by both apterae and alatae in small groups (≤ 10 individuals), and alatae were also attracted to small groups of apterae (Pettersson *et al.*, 1998). In both these studies, it appears that the aggregation signals are also involved in density regulation and avoidance interactions (see following section). Arrestment of alatae by odour from settled alatae was shown in *A. fabae* (Kay, 1976). In the examples reviewed here, early colonizers can be considered as pioneers that act as 'beacons' for later arriving individuals (Pettersson, 1994).

Density regulation and avoidance

Based on observations of the increase in walking activity of *R. padi* apterae when colony density passes a critical threshold, Pettersson *et al.* (1995) investigated the presence of a semiochemical-based, density-regulating mechanism. In olfactometer tests, colonies of *R. padi* apterae on oat leaves became repellent to other apterae when the density threshold was exceeded. Air entrainment, followed by GC-MS, identified three compounds associated with high, but not lower, densities of *R. padi* apterae feeding on wheat; 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, and 2-tridecanone, with the enantiomeric composition of 6-methyl-5-hepten-2-ol determined as a 1 : 3 ratio of (+) and (–) (Quiroz *et al.*, 1997). In olfactometer tests, all the compounds were repellent, the strongest effect being obtained with the ratio of compounds present in air entrainment samples. However, when the enantiomers of 6-methyl-5-hepten-2-ol were presented either individually or in the incorrect ratio, they were behaviourally inactive (Quiroz and Niemeyer, 1998). Chemicals that influence aphid behaviour, especially those that are repellent, are of great interest as components of integrated pest management strategies. Trials are under way using the *R. padi* density related substances applied to cereal crops in the form of slow release wax pellets, and initial results are encouraging, especially when used in combination with other behaviour modifying chemicals such as methyl salicylate (Ninkovic *et al.*, 2003).

A similar density-regulating mechanism to that of *R. padi* may operate in *A. craccivora*. Odours from groups of apterae repelled both apterae and alatae in an olfactometer when a critical group size of 20 or more individuals was surpassed (Pettersson *et al.*, 1998). The aggregation pheromones emitted by alatae of the brassica-feeding aphids *B. brassicae* and *L. pseudobrassicae*, described earlier, also appear to facilitate interspecies avoidance, with alatae of both species repelled by the odour of alatae of the other species (Pettersson and Stephansson, 1991).

Although, in some cases, semiochemicals that influence aphid social interactions are pheromonal, in other cases their origins are unclear. In functional terms, it makes little difference whether the aphid of the plant produces the substance; the message is the same. Indeed, induction of plant chemicals by aphids and their ability to detect them has certain advantages, such as avoidance of the costs of pheromone production and the conveyance of additional information about the condition of the plant. It is interesting that species that share a common host plant have the ability to detect each other using odour alone (Pettersson and Stephansson, 1991; Johansson *et al.*, 1997).

Interaction with Plants

Host-plant semiochemicals

Where host-plant semiochemicals have been identified, their perception has been found generally to be associated with highly specific cells, usually in the primary rhinaria of the fifth and sixth antennal segments.

Semiochemicals of the primary host of host-alternating aphids

The use of chemicals by return migrants searching for their primary hosts in the autumn has been reviewed recently (Powell and Hardie, 2001), providing evidence of semiochemical use by *A. fabae*, *P. humuli*, *R. padi*, and *S. fragariae*, among others.

In the autumn, the orientation of *R. padi* to its primary host *P. padus* is largely dependent upon aphid-produced signals, as described earlier, although the role of the host chemical, benzaldehyde, has also been demonstrated (Pettersson, 1970a). In the spring, the challenge facing the migrating morphs is to leave *P. padus* and, rather than recolonizing it, to successfully locate a suitable summer host. In the search for substances that could be used for control of the summer generations, Pettersson *et al.* (1994) identified methyl salicylate as a volatile chemical occurring in the primary, but not the summer, host. The substance was repellent to *R. padi* spring migrants in olfactometer tests, and also switched off the response to their aggregation cue (the odour of migrant-infested cereals). Pettersson *et al.* (1994) hypothesized that methyl salicylate could be used by aphids as a host discrimination cue, and also pointed out the potential role of the substance in plant defence signalling and as a promoter for induced resistance (Shulaev *et al.*, 1997).

Methyl salicylate may also function as a host-leaving stimulus. Using air entrainments *in situ* on intact *P. padus* trees in the field, Glinwood and Pettersson (2000a) showed that volatiles from foliage infested with developing spring populations of *R. padi* were repellent to spring migrants in olfactometer tests, whereas volatiles from uninfested foliage were behaviourally neutral. Subsequent collection and identification of volatiles from intact uninfested and infested foliage on *P. padus* saplings revealed that methyl salicylate is released in response to aphid infestation (Fig. 9.6). Thus, it appears that methyl salicylate is a damage-induced plant signal that is used as a behavioural cue by the aphid that induces its production. Methyl salicylate has been deployed successfully in integrated control of cereal aphids (Pettersson *et al.*, 1994), although there is evidence that the response of *R. padi* to this substance is dynamic and varies with the physiological state and/or age of the aphid (Glinwood and Pettersson, 2000b). It may be used most successfully in combination with other behaviour modifying chemicals such as the density-regulating substances described earlier (Ninkovic *et al.*, 2003).

Aphis fabae on spindle does not seem to parallel the *R. padi–P. padus* system in

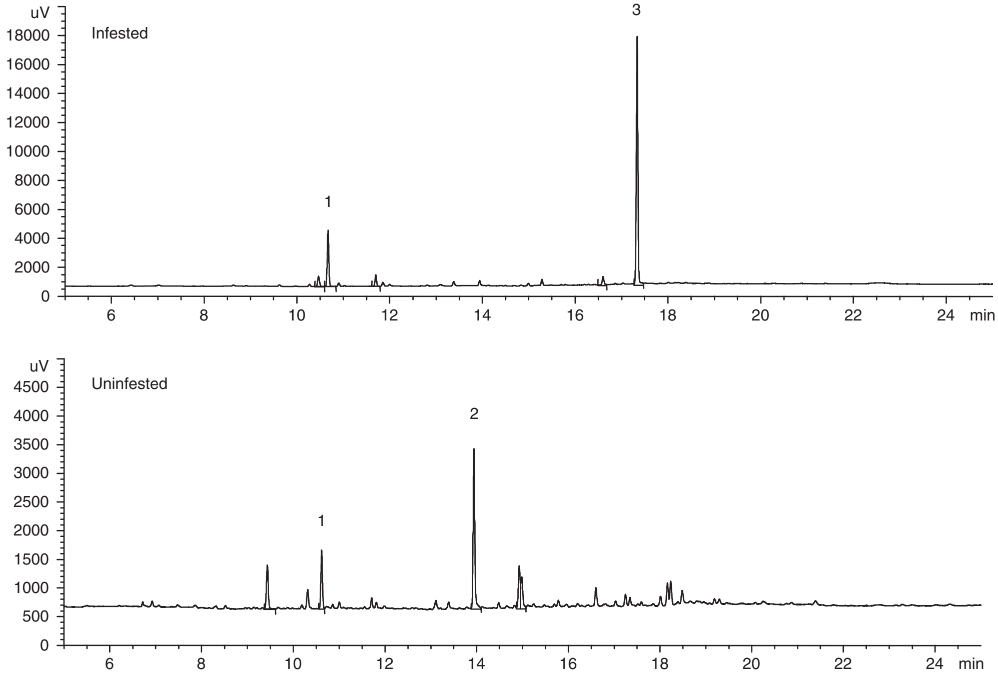

Fig. 9.6. GC of volatiles collected from *Prunus padus* that was either infested or uninfested with spring generations of *Rhopalosiphum padi* (bird cherry–oat aphid). Major peaks identified by GC-MS as (1) (*Z*)-3-hexenyl-acetate, (2) benzaldehyde, (3) methyl salicylate.

terms of the importance of chemical signals. Evidence of attraction of gynoparae to spindle volatiles is contradictory (Powell and Hardie, 2001), and there is no evidence of induction of volatiles by feeding spring generations analogous to the induction of methyl salicylate in the *R. padi*–*P. padus* system (R. Glinwood, unpublished results). These two aphid–plant systems therefore provide an interesting arena for comparative studies.

Semiochemicals of the secondary host of host-alternating aphids

A substantial part of the existing knowledge in this area was reviewed extensively by Pickett *et al.* (1992) and therefore will not be dealt with here. While the ability of aphids to perceive chemical cues (Visser and Piron, 1995; Park and Hardie, 2003, 2004) and the importance of these cues in plant finding is undoubted, eventual host acceptance is expected to depend upon the interaction of chemical, visual, and nutritional cues available to the aphid (Hori, 1999; Powell *et al.*, 2006; see also Pettersson *et al.*, Chapter 4 this volume). It should be noted that volatiles from host plants can also have negative effects on aphids, for example, by directly reducing fecundity (Hildebrand *et al.*, 1993) or by inducing responses in neighbouring plants (Ninkovic *et al.*, 2002; Glinwood *et al.*, 2003, 2004).

Non-host-plant semiochemicals

The study of semiochemicals used by aphids to avoid unsuitable hosts, although also reviewed in 1992 (Pickett *et al.*, 1992), has since provided important new discoveries. In 1994, there was evidence that, besides taxonomically unsuitable hosts being avoided by aphid migrants (Nottingham and Hardie, 1993; Hardie *et al.*, 1994a), in the spring, the primary host became a non-host (Pettersson *et al.*, 1994) and that the associated repellency was perceived by specific olfactory cells, again in the primary rhinaria, that responded to non-host semiochemicals. Two of these, (1*R*,5*S*)-myrtenal from the *A. fabae* non-host Lamiaceae and methyl salicylate from *P. padus*, the primary host for *R. padi*, not only caused repellency but at lower levels also interfered with attraction to host plants (Hardie *et al.*, 1994a; Pettersson *et al.*, 1994). Fieldwork with methyl salicylate showed that the compound reduced populations of several species of cereal aphids in addition to *R. padi*, including *S. avenae* (Pettersson *et al.*, 1994; Ninkovic *et al.*, 2003). The effect on aphid species other than *R. padi* can be seen as a result of this substance acting as a promoter for plant-induced resistance. Plants under stress from herbivory also produced methyl salicylate. The compound presumably is derived from salicylate, which was considered a product of the inducible phenylalanine ammonia lyase pathway, although an alternative involvement of isochorismate synthase has been suggested (Wildermuth *et al.*, 2001).

During studies on the semiochemical basis for host alternation by *Nasonovia ribisnigri* (currant–lettuce aphid), a number of compounds from the primary host *Ribes nigrum* (Saxifragaceae) were identified that interfered with the attractancy of the secondary host lettuce (*Lactuca sativa*) (Asteraceae). One, *cis*-jasmone, was found to repel other aphids and also to attract *Coccinella septempunctata* (7-spot ladybird) and the parasitoid *Aphidius ervi* (Birkett *et al.*, 2000). This positive effect on the higher trophic level was also observed for methyl salicylate. Since methyl salicylate had, furthermore, been shown directly to induce defence against pathogens by intact plants (Shulaev *et al.*, 1997), the possible role of *cis*-jasmone as a plant signal was investigated. *cis*-Jasmone, although commonly induced during herbivory, was widely considered and originally expected by ourselves to be semiochemically inactive, except when released from flowers as a pollination attractant. However, surprisingly, when released into air above intact broad bean (*Vicia faba*) plants, *cis*-jasmone caused induction of a defence response resulting in the attraction of parasitoids, which persisted long after the *cis*-jasmone could no longer be detected (Birkett *et al.*, 2000). Although *cis*-jasmone is considered to be a biosynthetic product from jasmonic acid (Koch *et al*., 1997), this compound and its volatile methyl ester do not give such a persistent effect in comparative studies also using *V. faba* (Birkett *et al*., 2000). Since then, an effect of *cis*-jasmone on cereals, which causes repellency of aphids, has been exploited in the field (Bruce *et al*., 2003). Because of the availability of microarrays for searching gene expression, studies were directed to *Arabidopsis thaliana*, and genes possibly associated with aphid defence have been identified that are specifically induced by *cis*-jasmone (Matthes *et al*., 2003). However, in terms of plant–plant interactions, the plant produced insufficient *cis*-jasmone during aphid feeding to account solely for induction of plant defence against aphids. For crop protection, though, the persistence of the *cis*-jasmone induction effect, with the initial success in the field and the prospects of exploiting the developing molecular mechanism by which *cis*-jasmone acts, is promising for the future (Birkett *et al*., 2001; Chamberlain *et al*., 2001).

Aphid effects on the plant

The physical and chemical association between a feeding aphid and its host plant is relatively intimate, and aphids cause changes in the chemistry of the plants on which they feed. These changes are reviewed thoroughly in Petterson *et al*., Chapter 4 this volume and Quisenberry and Ni, Chapter 13 this volume. There is evidence that barley plants infested with *R. padi* produce volatile signals that induce neighbouring plants to become less acceptable to the aphid (Pettersson *et al*., 1996). The study and identification of such aphid-induced signals is likely to be an area of great interest to chemical ecologists.

Interaction with Natural Enemies

A disadvantage of employing semiochemicals in intraspecific communication is that they can betray the often-sophisticated crypsis that an insect has attained. An individual may be coloured to blend into the foliage, or concealed on a lower leaf surface or inside a rolled leaf, but when it releases chemicals into the environment it will reveal its presence to any natural enemy that has evolved the ability to detect those signals. Of course, insects leave further clues from which searching predators and parasitoids can elicit information, such as waste products and other excretions. Given the importance of chemical signals to aphids, it is no surprise that there are several examples of their exploitation by natural enemies. Recently, major developments have been made in the study of the use by aphid parasitoids of damage-induced chemicals produced by aphid-attacked plants. These will be dealt with separately to chemicals of direct aphid origin.

Responses of natural enemies to aphid-produced chemicals

Parasitoids

Initial studies on the use of kairomones as host location cues by aphid parasitoids focused on aphid honeydew. It is an obvious target for interest since it is relatively apparent and is produced as a necessity by all aphids. Indeed, it is strongly indicative of the presence of aphids, and parasitoids respond to it with intensified searching behaviours (Gardner and Dixon, 1985; Cloutier and Bauduin, 1990) and by increasing their residence time in honeydew-contaminated patches (Hågvar and Hofsvang, 1989; Budenberg *et al*., 1992). These experimental studies all strongly suggest that honeydew is detected on contact by the parasitoid, although there are reports of parasitoid attraction to honeydew odour in an olfactometer. Such attraction was shown by Bouchard and Cloutier (1985) for *Aphidius nigripes* and by Wickremasinghe and van Emden (1992) for *A. ervi*, *Aphidius rhopalosiphi*, *Aphelinus flavus*, *Lysiphlebus fabarum*, *Trioxys* sp. and *Praon* sp. It has also been shown that *A. rhopalosophi* has olfactory attraction to indole-3-acetaldehyde, a volatile breakdown product of tryptophan in the honeydew of aphids (van Emden and Hagen, 1976).

The first evidence that parasitoids were attracted to aphid sex pheromones came during field experiments designed to observe the response of field-flying male aphids to synthetic sex pheromone (Hardie *et al.*, 1991). Besides capturing male aphids, large numbers of parasitoids of the genus *Praon* were recovered from water traps baited with nepetalactone (**1**, Fig. 9.4). All the wasps were female, and 98.5% were found in pheromone-baited traps, as opposed to unbaited controls. Since clear traps and synthetic pheromone were used, all other visual and olfactory cues were ruled out, clearly demonstrating that parasitoids were attracted by the pheromone.

When the experiments were repeated during the following autumn in winter cereal fields, attraction to nepetalactone was again demonstrated (Powell *et al.*, 1993; Hardie *et al.*, 1994c). This time, 89% of the captured wasps were *Praon volucre*, with over 99% occurring in the pheromone-baited traps. The trials were replicated at sites in southwestern, central and northern England, and in northern Germany, and the response to sex pheromone was apparent at all sites that had parasitoid populations. In several of the trials, nepetalactol-baited traps were included, but caught significantly fewer parasitoids than those baited with nepetalactone.

Attraction to aphid sex pheromones in laboratory assays has been demonstrated for *P. volucre* (Lilley *et al.*, 1994), *Aphidius eadyi* and *A. ervi* (Glinwood *et al.*, 1999a), *Aphidius matricariae* (Isaacs, 1994), *A. rhopalosiphi* and *Ephedrus plagiator* (Glinwood, 1998). *Diaeretiella rapae* was attracted to traps baited with nepetalactone in the field (Gabrys *et al.*, 1997). Electroantennogram responses have been shown in *Lysiphlebia japonica* (Hou and Yan, 1995).

There are opportunities for applied use of aphid sex pheromones in integrated management of aphid pests because aphid parasitoids are important members of the aphid natural enemy complex, yet their impact is often diminished by poor synchrony between their arrival in crops and aphid population development (Carter *et al.*, 1980; Wratten and Powell, 1991). Aphid sex pheromone deployed in field margins in the autumn (Powell *et al.*, 1993) or in the crop in the spring (Glinwood, 1998) might reduce this asynchrony. Attempts to increase parasitization in aphid colonies in the field using sex pheromones have been successful. When potted cereal plants were artificially infested with *S. avenae* and exposed in the field, the presence of nepetalactone significantly increased parasitism of *S. avenae* by *Praon* spp. (Lilley *et al.*, 1994; Glinwood *et al.*, 1998) and *A. rhopalosiphi* (Glinwood *et al.*, 1998). In a field trial (Glinwood, 1998), the deployment of nepetalactone lures in spring wheat significantly increased the numbers of parasitized aphids in treated plots (Fig. 9.7). Although the impact of parasitization was not sufficient to control the aphid population, levels of parasitization in pheromone plots were clearly shifted to earlier in aphid population development, indicating that this strategy may indeed enhance the synchrony discussed previously.

Initially, it was concluded that the parasitoid response to aphid sex pheromones was either induced by environmental stimuli (i.e. the changes in temperature and photoperiod which occur in the autumn) or developed in response to parasitoids learning to associate suitable hosts with the presence of pheromone by attacking sexual aphids when they appeared in the autumn (Hardie *et al.*, 1994c). However, parasitoids have since been shown to respond to sex pheromones in laboratory bioassays at temperatures and photoperiods that do not match those which exist in the field in autumn, thus ruling out the environmental induction theory. Furthermore, since test insects were prevented from having contact with either sexual aphids or sex pheromones, the response does not appear to be learned *via* association. Therefore, the parasitoid response to aphid sex pheromones appears to be innate.

Both *P. volucre* and *A. ervi* were attracted equally by plant-extracted and fully synthetic (4a*S*,7*S*,7a*R*)-nepetalactone (**1**, Fig. 9.4) in wind tunnel bioassays (Glinwood *et al.*, 1999b).

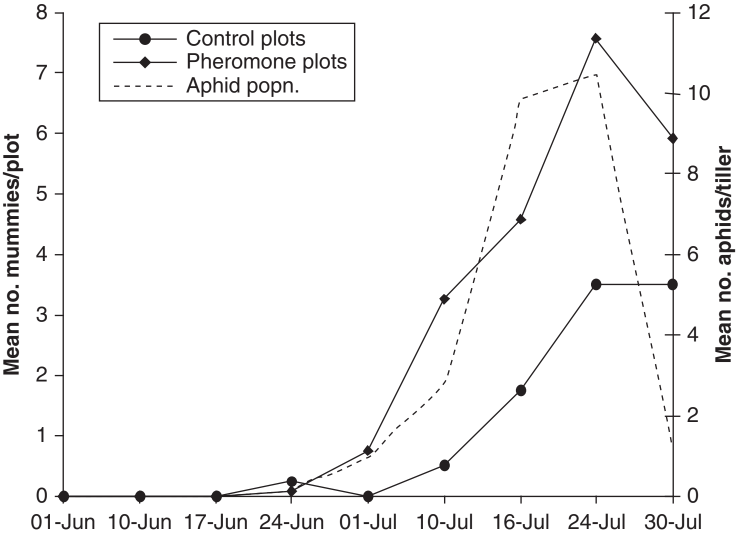

Fig. 9.7. Effect of aphid sex pheromone on parasitization levels of cereal aphids in plots of spring wheat. Parasitization increased earlier in plots treated with pheromone, and in greater synchrony with the increase in the aphid population (from Glinwood, 1998).

However, in field tests with baited aphid-infested plants, the synthetic 7*R* isomer was unattractive, and even rendered the 7*S* isomer unattractive when combined in a 50% blend with it (Glinwood *et al.*, 1999b). This indicated that enantiomeric purity of the pheromone is more important for parasitoid response than is the presence of plant-derived contaminants. This is in contrast to the response of male aphids (Hardie *et al.*, 1997), which raises interesting questions about the evolution of the response of the two different insects to this signal.

The parasitoid *Aphidius uzbekistanicus* was attracted to the aphid alarm pheromone (*E*)-β-farnesene (**5**, Fig. 9.5) in a simple Y-tube olfactometer bioassay (Micha and Wyss, 1996). Detailed measurements of the foraging response of the parasitoid *D. rapae* to **5** showed increased movement and tighter turning angles, behaviours likely to increase the probability of host encounters (Foster *et al.*, 2005).

The searching behaviour of aphid parasitoids seems to be influenced by cues present in the aphid mummy that are experienced by the adult wasp upon emergence. Several reports have shown that this experience can prime parasitoids to preferentially search for and oviposit in a particular aphid species (van Emden *et al.*, 1996; Storeck *et al.*, 2000; Blande *et al.*, 2004). This underlines the subtlety of chemical interactions between parasitoids and their aphid hosts, about which more surely remains to be discovered.

Predators

Predators generally have been considered as less sophisticated users of host location kairomones than parasitoids. One reason may be that a parasitoid's reproductive success is more critically dependent upon finding suitable hosts for oviposition, and therefore suitable for its offspring, than that of a predator whose search is usually directed towards a food resource for that individual. However, in the case of predators such as coccinellids, syrphids, and chrysopids, the location of aphid colonies by adults is also very important for the survival of their offspring. Thus, they may be considered as similar to parasitoids in this respect.

The responses of several ladybird species to aphid siphunculus secretions and alarm pheromone ((*E*)-β-farnesene, **5**, Fig. 9.5) have been investigated, and there appears to be some variation in response between species and between larval and adult stages. In olfactometer tests, *Adalia bipunctata* (2-spot ladybird) larvae were attracted to the odour of aphids under attack by conspecific larvae, whereas there was no response to larvae or aphids alone (Hemptinne *et al.*, 2000). The hypothesis was that the larval response is adaptive since it allows them to share the aphid prey of larvae that have hunted successfully. Adults of *A. bipunctata* have also been shown to respond to **5** in an olfactometer (Francis *et al.*, 2004). Adults of *Harmonia axyridis* (harlequin ladybird) did not show any response to siphunculus droplets produced by *A. pisum* (Mondor and Roitberg, 2000). Responses of larvae were not tested in this study. Electroantennograms in response to **5** have been recorded from *Coleomegilla maculata* (12-spot ladybird) (Zhu *et al.*, 1999). Behavioural studies were not reported, but the authors claimed to have obtained behavioural responses from two further species, *Hippodamia convergens* and *H. axyridis*, in preliminary studies subsequently confirmed to involve **5** for attraction of *H. convergens* (Acar *et al.*, 2001).

The most comprehensive study of ladybird response to **5** (Al Abassi *et al.*, 2000) demonstrates both electrophysiological and behavioural responses in *C. septempunctata* (7-spot ladybird). Not only was attraction to **5** demonstrated, but it was also shown that increasing concentrations of the plant-derived alarm pheromone inhibitor (−)-β-caryophyllene (**7**, Fig. 9.5), analogous to the system that operates inhibition of response in aphids (Dawson *et al.*, 1984), inhibited ladybird response. The receptor cells for **5** and **7** in *C. septempunctata* are paired and are frequently found in close proximity, with larger amplitude consistently recorded for the cell specifically responding to **5**. The co-location of these cells may provide the mechanism by which different behavioural responses are exhibited when the inhibitor is present together with the attractant (Al Abassi *et al.*, 2000). Although **5** did not influence the fine-scale searching behaviours of *C. septempunctata* such as turning rate and 'meander' (Nakamuta, 1991), it is likely that it plays some role as a host location kairomone in this and other ladybird species.

The behavioural responses of ladybirds to aphid sex pheromones either have not been studied or studies have not been reported due to a negative outcome. Electroantennogram responses to the nepetalactone and nepetalactol sex pheromone components have, however, been shown in *C. maculata* (Zhu *et al.*, 1999). The same authors also obtained EAG responses to both of these sex pheromone components from *Chrysoperla carnea* (green lacewing). Another lacewing, *Chrysopa cognata*, showed EAG responses to both nepetalactone and nepetalactol, as well as positive behavioural responses in an olfactometer and attraction to pheromone-baited traps in the field (Boo *et al.*, 1998). *Chrysopa oculata* showed EAG responses to sex pheromone components, and the (1*R*, 4a*S*,7*S*, 7a*R*)-nepetalactol attracted adults to field traps (Zhu *et al.*, 2005). Interestingly, the same study also demonstrated field attraction of *C. carnea* females to 2-phenylethanol, a component of its prey aphid's host plant. *Chrysoperla carnea* showed an EAG response to **5** (Zhu *et al.*, 1999), whereas *C. cognata* did not (Boo *et al.*, 1998).

Earlier literature revealed that the lacewing *Chrysopa pallens* was attracted to the vine *Actinidia polygama*, a plant that generates two dihydronepetalactols previously named neomatatabiol and isoneomatatabiol (Hyeon *et al.*, 1968), structurally related to nepetalactone and nepetalactol but incompletely characterized. The enantiomerically pure diastereoisomers (1*R*,4 S,4a*R*,7*S*,7a*R*)-(**8**, Fig. 9.5) and (1*R*,4*R*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**9**, Fig. 9.5) were synthesized (Hooper *et al.*, 2002) diastereoselectively from (4a*S*,7*S*,7a*R*)-nepetalactone (**1**). The stereochemistry of the compounds was determined by NMR spectroscopy and X-ray crystallography, and the compounds were shown to be identical to those previously referenced as neomatatabiol and isoneomatatabiol, respectively. (1*R*,4*S*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**8**) was found to catch significant numbers of three species of lacewing in the field: in Korea, *C. cognata* and, in the UK, *Nineta vittata* and most notably, *Peyerimhoffina gracilis*. All species caught in significant numbers were found more frequently in traps releasing (1*R*,4*S*,4a*R*,7*S*, 7a*R*)-(**8**) rather than (1*R*,4*R*,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**9**), while more *C. cognata*, *Chrysopa formosa*, and *Chrysopa phyllochroma* were found in traps releasing the nepetalactol (Hooper *et al.*, 2002; Boo *et al.*, 2003). The capture of *P. gracilis* with (1*R*,4 S,4a*R*,7*S*,7a*R*)-dihydronepetalactol (**8**) was of particular interest as this lacewing has been recorded only recently in the UK; indeed, its discovery was made possible by attraction to the aphid sex pheromone components (Donato *et al.*, 2001).

The efficacy of certain sprayed yeast hydrolysates in attracting lacewings into lucerne crops in California was ascribed to the fact that they contain tryptophan, an amino acid present in aphid honeydew (Hagen *et al.*, 1971). van Emden and Hagen (1976) found that, for *C. carnea*, this attraction stemmed from indole acetaldehyde, a volatile breakdown product of tryptophan (see analogous results with parasitoids above). Interestingly, later work (Hagen, 1986) showed that the response of the lacewing was not triggered in the absence of a synomone from the lucerne plant.

Hover fly responses to aphid semiochemicals are notably absent from the literature, and again it may be that they have been sought but not found. Non-volatile kairomones present in extracts of the siphunculus secretion of *A. fabae* influenced the searching behaviour of adult females of *Eupeodes (Metasyrphus) corollae* (Shonouda *et al.*, 1998). Effects included stimulation of oviposition and arrestment on bean plants treated with the extract. Olfactory responses to aphid honeydew have been shown in the aphidophagous gall midge, *Aphidoletes aphidimyza* (Choi *et al.*, 2004).

There is one report of attraction of non-aphid specialist predators to an aphid semiochemical. In olfactometer tests, adults of the ground beetles *Pterostichus melanarius* and *Harpalus rufipes* (Coleoptera: Carabidae) responded positively to (*E*)-β-farnesene (**5**) (Kielty *et al.*, 1996).

Responses of natural enemies to aphid-induced plant signals

Herbivore-induced chemicals from infested plants provide natural enemies with information that is both easily detectable and a highly reliable indicator of the presence of hosts or prey (Vet and Dicke, 1992). These signals are often considered to be plant SOS signals, i.e. synomones that enhance recruitment of the attacking herbivores' enemies. However, in most cases, the consequences of production of these signals for plant fitness are unknown.

Parasitoids

In wind tunnel bioassays, *A. ervi* was attracted equally to the plant–host complex (*V. faba–A. pisum*) and to aphid-damaged plants from which aphids had been removed (Du *et al.*, 1996). The parasitoid was able to discriminate between bean plants previously infested with *A. pisum* and a non-host aphid, *A. fabae*, even after the plants had been washed to remove traces of aphid honeydew and exuviae. Thus, the plant produces aphid species-specific volatile cues that can be used by female parasitoids in host location. The plant response that leads to the production of these volatiles is now known to be systemic, the upper leaves becoming attractive to parasitoids 48–72 h after aphid infestation of basal leaves (Guerrieri *et al.*, 1999). Air entrainment of the bean plants, followed by GC-MS, identified several volatiles that were produced at higher levels by plants infested with *A. pisum*, including 6-methyl-5-hepten-2-one, (*E*)-β-ocimene, (*Z*)-3-hexen-1-ol, (*Z*)-3-hexenyl acetate, linalool, and (*E*)-β-farnesene from the plant (Du *et al.*, 1998). All the chemicals were behaviourally active, with 6-methyl-5-hepten-2-one eliciting significantly more upwind flights by *A. ervi* in a wind tunnel than the other substances. Recent work on the transfer of damage-induced messenger substances between plants has demonstrated that root exudates of *A. pisum*-infested bean plants contain compounds that are systemically translocated and, when taken up by an intact plant, elicit the release of the same volatiles that are induced by aphid feeding (Chamberlain *et al.*, 2001). These volatiles are also attractive to the parasitoid *A. ervi*.

Predators

Studies on predator attraction to aphid-induced plant signals are in their infancy compared with those on parasitoids. Adult female ladybirds (*A. bipunctata*) were more attracted to the odour of *V. faba* infested with *A. pisum* than to uninfested *V. faba* (Raymond *et al*., 2000). However, when aphids and aphid products (exuviae and honeydew) were removed from the plant, the attraction was lost. Therefore, attraction of ladybirds to honeydew and alarm pheromone cannot be ruled out in this study. There is stronger evidence for attraction to aphid-induced signals in *C. septempunctata* (Ninkovic *et al*., 2001). Olfactometer responses to solvent extracts from air entrainments of barley and the aphid *R. padi* showed that attraction to a host-damaged plant from which aphids had been removed was as strong as attraction to the plant–host complex. There was no response to the undamaged plant or to aphids alone, and it is likely that the aphids in this case were undisturbed and thus not producing alarm pheromone (J. Pettersson, personal communication).

It is clear from laboratory experiments that a broad range of aphid parasitoids and predators has evolved behavioural responses to both aphid kairomones and aphid-induced plant signals. What is not as clear, however, is the importance of such responses for the success of natural enemies in their natural habitats and, in the case of plant signals, whether there are genuine fitness benefits for the emitting plants. It is also unclear how useful these substances will prove as components of integrated aphid control strategies. Nevertheless, the few attempts to use aphid kairomones to concentrate natural enemies on plants have been encouraging (Lilley *et al*., 1994; Glinwood *et al*., 1998; Shonouda *et al*., 1998).

Conclusions

The intimate relationship that aphids have with their host plants has resulted in the evolution of fascinating chemical ecological interactions. The range of molecular structures of the semiochemicals involved is restricted by the limited array of plant secondary metabolites with appropriate physicochemical characteristics, and has given rise to diverse activities from relatively simple compounds such as methyl salicylate, 6-methyl-5-hepten-2-one and nepetalactone, which appear in various roles in this review. This parsimony in usage of semiochemicals in aphid chemical ecology bodes well for their applied use in pest management through restricting the number of compounds required for registration. However, it should be borne in mind that there are still areas, for example, involving the chemical ecology of aphid parasitoids and predators, where new chemistries will be identified.

It appears from recent work that the main problem in developing semiochemicals for aphid pest management, which at least for arable crops is cheap delivery, is now being solved. Thus, the discovery that genes relating to production of aphid semiochemicals by plants can be ‘switched on’ by benign chemical signals means that the plants themselves can generate target semiochemicals. Already, volatile signals from damaged plants that have this potential have been identified, but further work is needed, both in this area and on signals active in the rhizosphere. Signalling by undamaged plants is also an area of increasing interest (Pettersson *et al*., Chapter 4 this volume). These studies will be enhanced greatly by a deeper understanding of the molecular biology of aphid chemosensory receptors, and of the genetics underpinning plant secondary metabolism, particularly those pathways induced by external signals. Currently, the latter studies rely heavily on *A. thaliana*, but as genomic information from other plants more relevant to pest aphid chemical ecology becomes available, more rapid advances will be made.

Finally, it must be emphasized that determination of a complete aphid genome is now seen to be essential for a full understanding and exploitation of aphid chemical ecology.

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