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REVIEW

Special feature: predatory ladybirds: individuals, populations, and interactions

Chemical defence, offence and alliance in ants–aphids–ladybirds relationships

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Abstract Chemicals, which mediate the interactions between aphids, ladybirds and ants, are reviewed. Special emphasis is laid on autogenous and plant-derived chemical defence in aphids and ladybirds. Evidences for chemical cues used during foraging and oviposition in ladybirds are assessed. Possible mutualistic interaction between plants and the third trophic level is illustrated by the as yet few reports of indirect plant-defence volatiles induced by aphids or coccids attracting parasitoids or ladybirds. The use of chemical signals allowing aphid parasitoids or ladybirds to squeeze into ant–aphid mutualistic association is briefly described. Questions are raised and hypotheses suggested which could stimulate further research on aphid host-plant influence on ladybird foraging behaviour and fitness, and on the cues used by aphid-web partners for their mutual recognition.

Keywords Chemical communication · Egg laying · Foraging · Indirect plant-defence · Sequestration

Introduction

Thirty years ago, I used a trophic web centred on aphids to illustrate the aim and scope of chemical ecology (Pasteels 1976). The fashion in ecology was to quantify the energy fluxes between trophic levels, whereas chemical ecology planned to investigate the chemicals controlling these fluxes. Chemical mediators appeared to act as negative or positive feedbacks regulating energy flow rates, sometimes passing through the proximate level to act on the next level. They were classified as pheromones, allomones, kairomones or synomones, according to whether or not partners were the same species, and how benefits and losses were distributed between partners (e.g. Dicke and Sabelis 1988). However, chemicals themselves cannot be classified univocally in this way, as the same compound released by the same organism would be labelled differently depending on the partners and the context (Pasteels 1982). It is not the chemical signals that must be classified, but the interactions between the organisms.

Here, I will review some of the recent advances in our understanding of how chemicals regulate the interactions between aphids, ladybirds and ants, illustrating the astonishing refinement of some regulatory mechanisms. This review does not aim at being exhaustive, but lays stress on pending questions that deserve further investigation.

Defence and chemical piracy

A straightforward way to avoid being eaten is to be toxic or at least unpalatable, and chemical defence has been reported in all trophic levels. No further comment is needed here on chemical defence in plants (e.g. Arnonson et al. 2004) and ants (review in Leclercq et al. 2000), and only aphids and ladybirds will be considered. Defensive compounds can be synthesised de novo, but both aphids and ladybirds also sequester toxins from their food, here called chemical piracy.

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Chemical defence and piracy in aphids

Siphuncular wax is for many aphids the most obvious chemical defence against parasitoids or small predators, including ladybird larvae (Dixon 1958; Edwards 1966). Other defences can be plant-derived. All aphids are not equally suitable as food for ladybirds (review in Hodek 1973). It seems most unlikely that this is the result of different nutrient balances in their aphid prey, but is more likely due to the aphid host-plant secondary chemistry. For example, the oleander aphid, *Aphis nerii* Boyer de Fonscolombe, was reported to be toxic for many ladybirds, but not for *Adonia variegata* (Goeze) (Iperti 1965). In Chorefto (Greece), *Adonia variegata* developed normally on a diet of *Aphis nerii* feeding on *Nerium oleander* L. or on the asclepiad, *Cynanchum acutum* L. However, they did not develop normally when their aphid prey was feeding on another asclepiad, *Cionura erecta* (L.) Griseb (Pasteels 1978). In this case, detrimental effects on ladybird fitness were spectacular: high mortality during development, surviving adults without elytra and wings, females with low fecundity and short lifetime. *Aphis nerii* must derive from *Cionura erecta* chemicals, still unknown, which are toxic to the ladybird. These toxins are not cardelonides (cardiac glycosides) frequently present in asclepiads and in oleanders, but not present in *Cionura erecta*.

Sequestration of plant toxins by herbivores for their own defence is widespread and was reported in several specialised aphids (Table 1). Rothschild et al. (1970) first reported sequestration of oleander cardenolides in *Aphis nerii*. Malcolm (1990) confirmed the sequestration of cardenolides by this aphid, and found mean concentrations of about half that observed in monarch butterflies reared on the same host, *Asclepias curassavica* L. The occurrence of cardenolides in *Aphis nerii* explains its toxicity to coccinellids, except the adapted species, *Adonia variegata*. Pyrrolizidine alkaloids from various *Senecio* spp. are sequestered by *Aphis jacobaeae* Schrank (Witte et al. 1990); quinolizidine alkaloids from various legumes, by *Macrosiphum albifrons* Essig, *Aphis cytisorum* Hartig, and *Aphis genistae* Scopoli (Wink et al. 1982; Wink and Römer 1986; Wink and Witte 1991); and glucosinolates from crucifers, by *Brevicoryne brassicae* (L) (Fancis et al. 2001) (see also Hodek and Honek 1996 for possible other examples). Aphids sequestering quinolozidine alkaloids or glucosinolates proved to be toxic or deterrent to predators including ladybirds (Wink and Römer 1986; Francis et al. 2001).

It makes little sense to compare the plant-derived toxin concentrations in the aphids listed in Table 1. Concentrations were measured in different units and, of course, the concentrations of toxins among plants vary a lot. It makes more sense to compare the concentrations of toxins in aphids with those in their food plants (Table 2). Concentrations of cardenolides (Malcolm 1990) and quinolizidine alkaloids (Wink and Römer 1986) in aphids were lower than in plants, although sufficient to efficiently protect the aphids. In contrast, concentrations of pyrrolizidine alkaloids (Witte et al. 1990) and of glucosinolates (Francis et al. 2001) were higher in the aphids than in the plants. In *Brevicoryne brassicae*, bioaccumulation of glucosinolates in aphids reached 15 times the concentrations in plants. Plant toxins are not just present in the aphid gut, but must be stored elsewhere in the body. Plant toxins were in part excreted and found in the honeydew each time it was assayed.

Table 1 Chemical piracy in aphid specialists

Host plants	Aphids	Sequestered toxins	References
Apocynaceae			
Nerium oleander	*Aphis nerii*	Cardenolides 0.4 mg/g DW	Rothschild et al. (1970)
Asclepiadaceae			
Asclepias curassavica	*Aphis nerii*	Cardenolides 10–45 μg/g DW	Malcolm (1990)
Asteraceae			
Senecio jacobaeae, S.inequidens, S. silvaticus	*Aphis jacobaea*	Pyrrolizidine alkaloids 1–3.5 mg/g FW	Witte et al. (1990)
Leguminoseae			
Lupinus albus, and 4 other *Lupinus* species	*Macrosiphum albifrons*	Quinolizidine alkaloids 0.6–1.8 mg/g FW	Wink et al. (1982), Wink and Römer (1986)
Genista tinctoria, Petteria ramentacea, Sophora davidii, Spartium junceum	*Aphis genistae*	Quinolizidine alkaloids 1.4–3.8 mg/g FW	Wink and Witte (1991)
Brassicaceae			
Brassica napus, B. nigra, Sinapis alba	*Brevicoryne brassicae*	Glucosinolates (isothiocyanates) 6–185 μmol/g FW	Francis et al. (2001)

Table 2 Concentration of toxins in plants and aphids

Toxins and plant/aphid associations	Host plants	Change in toxin concentration[a]	Aphids	References
Cardenolides (mg/g DW)				
Nerium oleander/Aphis nerii	2.3–16.4	↓	1.0–4.3	Malcom (1990)
Quinolizidine alkaloids (mg/g FW)				
Lupinus polyphyllus / Macrosiphon albifrons	2.4–3.8	↓	0.6–1.3	Wink and Römer (1986)
Pyrrolizidine alkaloids (mg/g FW)				
Senecio jacobaea/Aphis jacobaeae	0.7–1.8	↑	1.3–3.5	Witte et al. (1990)
S. inaequidens/A. jacobaeae	0.5–1.2	↑	1.1–2.7	
S. silvaticus/A. jacobaeae	0.3–0.9	↑	0.8–2.8	
Glucosinolates (μmol/g FW)				
Brassica napus/Brevicoryne brassicae	1.59±0.04	↑	5.81±1.13	Francis et al. (2001)
B. nigra/B. brassicae	9.54±0.42	⇑	148.20±15.03	
Sinapis alba/B. brassicae	10.93±0.13	⇑	185.16±14.36	

[a] The arrows lay stress on the decrease or increase in toxin concentration from plant to aphid

Generalist aphids tend to avoid highly toxic plants, but not always. Toxic or deterrent compounds, which evolved as protection against herbivory, are widespread in wild herbs. Do generalist aphids derive protection from host-plant chemicals in the same way as specialised aphids? Two studies address this question. Francis et al. (2001) compared the fate of crucifer glucosinolates in the specialist aphid, *Brevicoryne brassicae* (see above), and the generalist, *Myzus persicae* (Sulzer), when fed with either *Brassica napus* L. with low content in glucosinolates, or with two crucifers rich in glucosinolates, *Brassica nigra* L. and *Sinapis alba* L. As expected, the concentrations of glucosinolates in the generalist were much lower when fed on *Brassica napus*, *Brassicanigra* or *Sinapsis alba* than in the specialist (Table 3). Excreted glucosinolates were found in the honeydew of the generalist, but were not analysed for the specialist. Besides, the specialist, but not the generalist, was able to degrade plant-derived glucosinolates into highly toxic isothiocyanates. On all plants, the specialist aphid, but not the generalist, showed toxicity towards *Adalia bipunctata* L. (Francis et al. 2000, 2001).

Generalist aphids or coccids, however, can derive some protection from toxic plants as demonstrated by Mendel et al. (1992). These authors reported that, in Israel, the generalist aphid, *Aphis craccivora* Koch, and the coccids, *Icerya purchasi* Maskell and *Icerya aegyptiaca* (Douglas), are better protected against natural enemies when feeding on alkaloid-rich legumes, *Erythrina corallodendrum* L. and *Spartium junceum* L., than when feeding on *Citrus sinensis* (L.) Osbeck, *Cucurbita moschata* (Duschene ex Lan.) Duschesne ex Poiret, or *Euphorbia tirucalli* L. When preyed on, aphids feeding on *Spartium junceum* induced longer developmental times in *Chrysoperla carnea* (Stephens). A lower parasitic rate was observed on *Spartium junceum* than on *Vicia palaestina* Boiss. and *Melilotus albus* Medicus. The *Icerya* spp. feeding on alkaloid-rich legumes induced higher mortality in *Rodolia cardinalis* (Mulsant) and *Chilochorus bipustulatus* L. Moreover, the honeydew of the polyphagous scale, *Icerya purchasi* feeding on alkaloid-rich legumes reduced the life of the parasitoid, *Encyrtus infelix* (Embleton). Sequestration is not the only way homopterans can use plant-derived chemicals for defence. They also excrete them in their honeydew. Unfortunately, plant alkaloids were not analysed in *Aphis crassivora* or in *Icerya* spp. in Mendel et al.'s study.

Host-derived defence in aphids raises many fascinating questions, both about the proximate mechanisms involved in handling plant secondary chemicals

Table 3 Glucosinolates (μmol/g FW) in a specialist aphid (*Brevicorine brassicae*) and a generalist (*Myzus persicae*) feeding on Brassicaceae. After Francis et al. (2001)

Host plant	*Brevicorine brassicae*	*Myzus persicae*	
	Aphid	Aphid	Honeydew
Brassica napus (1.59 ± 0.04)	5.81 ± 1.13	0.47 ± 0.05	0.34 ± 0.09
Brassica nigra (9.54 ± 0.40)	148.20 ± 15.03	2.33 ± 0.46	1.92 ± 0.17
Sinapis alba (10.93 ± 0.13)	185.16 ± 14.36	1.65 ± 0.09	1.50 ± 0.43

Isothiocyanates are released by *B. brassicae*, but not by *M. persicae* and about its ecological consequence. How do aphids process plant secondary chemicals: detoxification, metabolism, transformation, excretion, transport, storage, etc.? The answer to this question would illuminate specific adaptations of herbivores to their host plant, and also provide for a better perspective on insect–plant coevolution (sensu lato). What is the cost-benefit balance between the metabolic processing of plant secondary chemicals and defence gained by sequestering and/or excreting them? The answer to this question will vary from one aphid–plant association to another, as there is a whole continuum between specialist and generalist aphids and a large qualitative and quantitative diversity of secondary compounds in plants. However, cost-benefit analyses will give a better insight on how plant–aphid communities are structured, and help in designing and implementing integrated pest management programs.

Chemical defence and piracy in ladybirds

Many ladybirds, as part of their defence, display warning colours and odours (i.e. pyrazines, Moore et al. 1990; also used as aggregation pheromone by the seven-spot ladybird, *Coccinella 7-punctata*, Al Abassi et al. 1998). They are protected against ants and birds by autogenous alkaloids which they accumulate in their blood and release by reflex bleeding when attacked (Pasteels et al. 1973; Marples et al. 1989). The diversity of alkaloids synthesised by ladybirds is astonishing: azaphenalenes (monomeric and dimeric), homotropanes, aliphatic and aromatic amines, pyrrolidines, piperidines, azamacrolides, macrocyclic polyamines, quinoline, 3-oxaquinolizidines, etc. (reviewed in Daloze et al. 1995; King and Meinwald 1966; Laurent et al. 2005) (Fig. 1). So far, about 50 different alkaloids have been identified in only 43 species. This census did not count as different the numerous macrocyclic polyamines generated by random combination of three building block units in the secretion of pupal hairs of *Epilachna borealis* (F.) (Schröder et al. 1998). Most species synthesise one or a few alkaloids, which may be different in different life stages in some phytophagous ladybirds. Related species tend to have the same alkaloids. Most if not all these alkaloids follow a common basic biosynthetic pathway, i.e., a hydrocarbon chain to which nitrogen atoms are added from amino acids (Laurent et al. 2003), and thus are “variations on a common theme”. Still, the diversity of ladybird alkaloids used for defence is impressive, and probably unsurpassed in any insect taxon of equivalent size, except perhaps ants.

The selective pressures leading to such diversity are obscure. Possibly, different alkaloids evolved in response to different interguild predation pressures, but there is little evidence supporting this assumption. An arms race between prey and predators could lead to chemical diversity if the prey–predator interactions show some level of specificity as in plant–hebivore interactions. There is only one report of a specific ladybird natural enemy adapting to ladybird alkaloids. The braconid, *Dinocampus coccinellae* Schrank, is attracted by the ladybird alkaloids, precoccinelline and myrrhine (Al Abassi et al. 2001). Actually, the

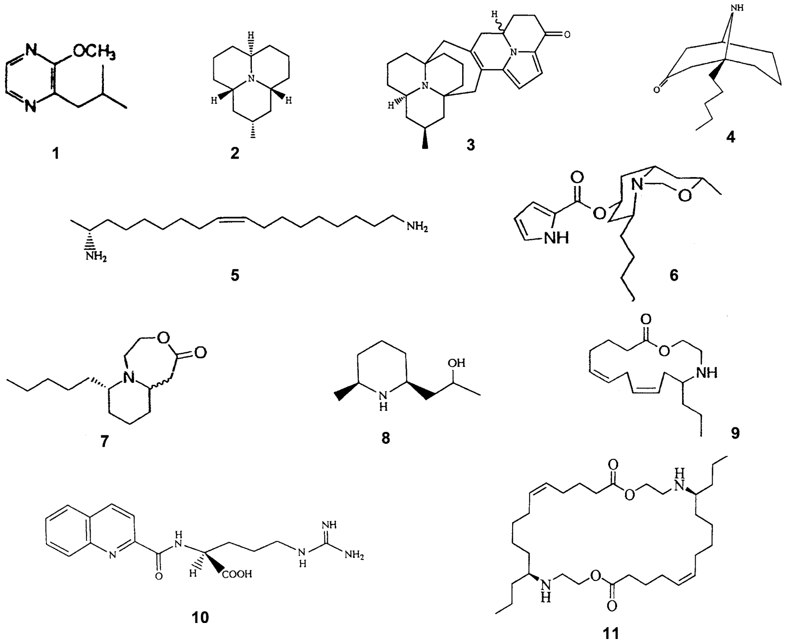

Fig. 1 Examples of ladybird defensive alkaloids. *1* 2-methoxy-3-isobutylpyrazine, *2* precoccinelline, *3* chilocorine A, *4* adaline, *5* harmonine, *6* hyperaspine, *7* calvine (*cis*) and 2-epicalvine (*trans*), *8* piperidine from adult *Epilachna varivestis*, *9* epilachnene from pupal hair secretion of *Epilachna variventis*, *10* N_{α}-quinaldyl-L-arginine from adult *Subcoccinella24-punctata*, *11* polyazamacrolide from from pupal hair secretion of *Subcoccinella* 24-*punctata*. References in Laurent et al. (2005)

similarity of the alkaloids found in different ladybird species appears more as a function of the ladybird taxonomic relatedness than from facing the same potential natural enemies while living in sympatry.

Alkaloids do not protect ladybirds from cannibalism, although they may reduce the risk of intraguild predation. Ladybirds should be immune to their own toxins, but not necessarily to other ladybird alkaloids. Therefore, intraguild predation could be a significant pressure for alkaloid diversity in species living in micro-sympatry. Intraguild predation is frequent among ladybirds, but feeding appears often hesitant and at nutritive cost to the predator (Agarwala and Dixon 1992; Agarwala et al. 1998; Cotrell and Yeargan 1998; Hemptinne et al. 2000a). The possible importance of intraguild predation in the evolution of ladybird chemical defence is further illustrated by the observation that ladybird larvae are far more hesitant to feed on eggs of other species than on their own eggs. Ladybird females endow their eggs with alkaloids. Additionally, alkanes covering the eggs are used as a cue to recognise conspecifics and avoid the eggs of other species (Hemptinne et al. 2000c) (Table 5). Closely related ladybirds possessing identical alkaloids are expected to occupy different niches with little opportunity of encounters. If intraguild predation were a selective pressure for alkaloid diversity in ladybirds, a higher chemical diversity would be expected among sympatric than among allopatric species. This remains to be rigorously tested, but my own experience in collecting ladybirds and analysing their defensive chemistry does not contradict this prediction.

The use of chemical cues in avoidance of intraguild predation was reported for the aphid parasitoid, *Aphidius ervi* Haliday. The braconid avoids alkane trails deposited on leaf surfaces by both larvae and adults of *Coccinella septempunctata* L. (Nakashima et al. 2004) (Table 5).

Chemical piracy was also reported in ladybirds (Table 4). Cardenolides were isolated from *Coccinella undecimpunctata* L. that preyed on *Aphis nerii*, which in turn sequestered the cardenolides from their oleander host-plant (Rothschild et al. 1973). The ladybird collected in Israel was possibly the subspecies *Coccinella* 11-*punctata aegyptiaca* Reiche, for which the oleander aphid is a suitable prey (Hodek 1973). The occurrence of cardenolides was never checked in *Adonia variegata*, a frequent predator of *Aphis nerii* (Iperti 1965). The seven-spot ladybird, not a normal predator of the oleander aphid, did not sequester cardenolides from this aphid (Rothschild et al. 1970). However, it sequesters pyrrrolizidine alkaloids (PAs) from *Aphis jacobaeae*, which in turn obtains them from *Senecio inaequidens* DC (Witte et al. 1990). Concentrations in ladybirds as high as 4.9 mg/g FW were reported. This concentration is higher than in the aphids, itself already higher than in their food plant (see above, Table 2). Bioaccumulation of PAs along trophic levels points to their transport from the gut to storage in the body. PAs were detected in the ladybird blood. Sequestered PAs can increase by nearly 50% the ladybird's average load of autogenous alkaloids. *Hyperaspis trifurcata* Schaeffer sequesters in its blood carminic acid extracted from its prey, the cochineal bug *Dactylopius confusus* (Cockerell). Released by reflex bleeding, the well-known red dye effectively deterred ants (Eisner et al. 1994). It remains unknown if this *Hyperaspis* synthesises alkaloids like *H. campestris* (Herbst) (Lebrun et al. 2001).

Sequestered toxins by insects are ''qualitative toxins'', sensu Feeny 1975. They are found mainly in herbs or ephemeral plant tissues. Polyphagous ladybirds often forage on herbs. How many plant-derived toxins is a polyphagous ladybird, like the seven-spot for example, able to tolerate or sequester from their aphid prey? How do they process them? Do sequestered toxins significantly increase the already high chemical defence diversity of ladybirds? What is the consequence on interguild and intraguild defence? For specialist or generalist ladybirds, what is the balance between the cost of processing plant toxins obtained from aphids and the benefit of accrued defence? Again these questions are relevant for understanding the dynamics of aphid–ladybird communities, and in planning integrated pest management programs.

Table 4 Chemical piracy in ladybirds

Aphids–coccids/host plant	Ladybirds	Sequestered toxins	References
Aphis nerii/*Nerium oleander*	*Coccinella* 11-*punctata*	Cardenolides	Rothschild et al. (1973)
Aphis nerii/*Nerium oleander*	*Coccinella* 7-*punctata*	None	
Aphis jacobaeae/*Senecio jacobaea*	*Coccinella* 7-*punctata*	Pyrrolizidine alkaloids[a]	Witte et al. (1990)
Aphis jacobaeae/*Senecio inequidens*	*Coccinella* 7-*punctata*	Pyrrolizidine alkaloids	
Dactylopius confusus	*Hyperaspis trifucata*	Carminic acid[a]	Eisner et al. (1994)

[a] Present in hemolymph and reflex bleeding

Table 5 Chemical signals regulating oviposition and post-oviposition behaviour in ladybirds and their natural enemies

Chemical signal	Reference
Oviposition stimulants	
Wax filaments covering the ovisacs of soft scales or larvae of mealy bugs (*Cryptolaemus montrouzieri*)	Merlin et al. (1996a)
Oviposition-deterring pheromones	
Wax filaments secreted by conspecific larvae (*Cryptolaemus montrouzieri*)	Merlin et al. (1996b)
Alkanes laid as track by foraging larvae (two-spot and seven-spot ladybirds)	Doumbia et al. (1998), Hemptinne et al. (2001)
Intraguild-predator deterrents	
Alkanes covering the eggs deter other ladybirds (two-spot and seven -spot ladybirds)	Hemptinne et al. (2000c)
Alkanes in tracts left by adults and larvae deter aphid parasitoids (seven-spot ladybirds)	Nakashima et al. (2004)

Offence

Foraging behaviour of coccinellids has been the topic of detailed studies (see Dixon 2000 for an excellent review). The ability to locate and recognise aphids is critical for successful predation. Also, females should lay eggs in numbers, and at times that will optimise larval survival and development by avoiding food depletion, cannibalism and intraguild predation, since patches of prey are ephemeral and limited in size. Foraging and oviposition are partly regulated by chemical cues.

Foraging

It is well known that honeydew induces searching behaviour in many aphidophagous species. However, to my knowledge, there never has been an assessment on how excreted plant-derived toxins in the honeydew (see above) influence this behaviour.

The seven-spot ladybird is attracted by an aphid alarm pheromone (*E*)-*β*-farnesene, and this attraction is inhibited by (–)-*β*-caryophyllene (Al Abassi et al. 2000). Both terpenes are common plant volatiles, and ladybirds would be attracted only when concentration of the farnesene is high relative to that of the caryophyllene, allowing them to detect their prey over the background of common plant odours. Other ladybirds, *Harmonia axyridis* Pallas, *Hippodamia convergens* Guérin, and *Adalia bipunctata*, are also attracted by (*E*)-*β*-farnesene (Zhu et al. 1999; Francis et al. 2004). The aphid alarm pheromone cannot, however, be a primary attractant to aphid patches, as aphids must already be attacked to release their alarm pheromone. It could be a short distance cue during foraging. Young larvae of the two-spot ladybird, *Adalia bipunctata*, attracted by the odour of *β*-farnesene, subdue large aphids by group attack (Hemptinne et al. 2000b). Chemical cues for prey recognition are unknown, but cuticular hydrocarbons seem possible candidates (Dixon 2000).

Egg laying behaviour in ladybirds

Considering the demographic development of an aphid colony, ladybird females should lay few eggs at a time during the beginning of the exponential growth of the colony, the so-called "oviposition window" (Hemptinne and Dixon 1991; Dixon 1997). This will optimise the female fitness by avoiding food depletion for their offspring due to a later decline in the aphid colony. No chemical cues that would allow females to recognise this window are reported. Honeydew of some aphids, but not of others, elicited oviposition by the syrphid *Epysyrphus balteatus* (Deg.), but not by the syrphid *Platycheirus albimanus* (F.) (Budenberg and Powell 1992). Honeydew quality could vary as plant physiology changes during aphid colony development, but honeydew was never reported to elicit oviposition by ladybirds. In the coccidophagous ladybird, *Cryptolaemus montrouzieri* Mulsant, egg laying is stimulated by wax filaments produced by their prey or their ovisac (Merlin et al. 1966a).

Chemicals cues used to limit egg cannibalism are better known (Table 5). Ovipositing female two-spot and seven-spot ladybirds avoid aphid patches already occupied by conspecific larvae, limiting the risks of early patch depletion and cannibalism. The oviposition deterrent pheromone is a mixture of alkanes laid by larvae as tracks on the substrate. These pheromones are species-specific and do not prevent risk of intraguild predation (Doumbia et al. 1998; Hemptinne et al. 2001) (but see above, Hemptinne et al. 2000c). A similar strategy is used by *Cryptolaemus montrouzieri*, where the oviposition deterring pheromone is present in the filament wax of their larvae (Merlin et al. 1996b).

Pheromones, that deter oviposition, are secreted by larvae of other aphidophagous insects, i.e. *Chrysopa* spp, but these pheromones appear less species-specific (Ruzicka 1997a, b). Oviposition deterrents aimed at intraguild predators were mentioned in Chemical defence and piracy in ladybirds.

Alliance

Indirect defence in plants

Plants and natural enemies of herbivores share common interests in defence or offence against herbivores. Alliances between the first and third trophic levels against the second level are thus expected. There is now convincing evidence that plants recruit parasitoids or predators by odours induced by herbivory (e.g. Turlings and Wäckers 2004). Such recruitment is often specific. The specificity in the quality or quantity of volatiles emitted in response to different herbivores, and the specific attraction of natural enemies to these induced volatiles, suggest that this indirect plant-defence has been shaped by coevolutionary processes among the three trophic levels, presently a very active and fascinating topic of research.

Phloem feeders usually induced smaller amounts of plant volatiles than chewing insects (Turlings et al. 1998; W. Boland, personal communication), but recruitment of aphid or mealybug parasitoids by induced plant volatiles has been demonstrated (Table 6). The aphid parasitoid, *Aphidius ervi*, is specifically attracted by 6-methyl-5-hepten-2-one, released by the bean plant, *Vicia faba*, infested with the parasitoid host, *Acyrthosiphon pisum*. The non-host aphid, *Aphis fabae*, does not induce such indirect plant-defence (Du et al. 1998; Powell et al. 1998). Encirtid parasitoids are attracted by cassava plant volatiles, induced by the mealybugs, *Phenacoccus manihoti* and *P. herreni* (Souissi et al. 1998; Souissi and Le Rü 1999; Bertschy et al. 2001).

Conflicting results were obtained with ladybirds. Obata (1986) reported that *Harmonia axyridis* (Pallas) was attracted more by the odour of *Erigeron annuus* (L.) Persoon leaves, infested by *Aphis citricola* van der Goot, than by the odour of healthy leaves. However, aphids alone were not tested in these experiments, and it remains possible that the attractant originated in the aphids and not in the plants. This control was added in more recent studies with the seven-spot ladybird. The ladybird was attracted by plant volatiles induced by the aphids, *Rhopalosiphum padi* (L.) feeding on *Hordeum vulgare* L. (Ninkovic et al. 2001) or *Toxoptera aurentii* (Boyer de Fonscolombe) feeding on tea shoots (Han and Chen 2002). Negative results were reported by Francis et al. (2004) with the two-spot ladybird, which responded to crushed aphids releasing alarm pheromone (see above), but not to intact aphids feeding on various cruciferous plants, possibly because the amounts of volatiles induced in their experimental set up were too low. Possible attraction of ladybirds by induced plant volatiles needs further research. Associative learning by polyphagous ladybirds is an expected requirement for effective recognition of aphid-induced plant volatiles (see Vet and Dicke 1992). Such learned odours could offer useful cues for quickly selecting new, suitable patches when the ladybird has to move from one aphid colony to another.

Ant–aphid mutualism and sneakers

Aphid–ant interactions are usually considered as mutualistic, but see Stadler and Dixon (1999). In truly mutualistic associations, as well as when ants exploit the aphids, alliance is expected between ants and aphids against aphid predators and parasitoids. Indeed it was reported many times that ants actively protect the aphids from their natural enemies (e.g. Way 1963). Parasitoid cuticular hydrocarbons are recognition cues for the ants, releasing aggressive behaviour toward most parasitoids. However, some parasitoids and ladybirds avoid attacks and squeeze themselves into aphid colonies attended by ants. These sneaky parasitoids either mimic recognition cues present in the cuticular lipids of aphids or these lipids lack releasers of aggressive behaviour in ants (Dettner and Liepert 1994). This demonstrates once again the importance of cuticular hydrocarbons as recognition cues at nearly all levels of the food web.

Majerus et al. (this volume) review the relationships between ants and myrmicophilous ladybirds. In the context of the present review, I will mention only that part of the sneaky strategy of the myrmecophilous ladybird, *Coccinella magnifica* Redtenbacher, is to use wood ant trails to reach aphid colonies (Sloggett et al. 1998). Godeau et al. (2003) provided evidence for the ladybird ability to detect the ant trail pheromone. The ants largely ignored the larvae and eggs of the ladybird, but not the adults. Camouflage devices used by ladybird juveniles or ways to prevent ant aggression remain unknown.

Conclusion

Progress in our understanding of how chemical communication regulates the interactions between partners

Table 6 Indirect plant defence against aphids or mealy bugs

Plant/aphids–mealy bugs	Induced volatiles	Recruited parasitoids–predators	References
Vicia faba/*Acyrthosiphum pisum*	6-methyl–5-hepten-2 one	*Aphidius ervi*	Du et al. (1998), Powell et al. (1998)
Vicia faba/ *Aphis fabae*	–	–	
Manihot esculenta-M. glaziovi/*Phenacoccus manihoti*	+	*Apoanagyrus lopezi*	Souissi et al. (1998), Souissi and Le Rü (1999)
Manihot esculenta/*Phenacoccus herreni*	+	*Apoanagyrus diversicornis*; *Aenasius vexans*	Bertschy et al. (2001)
Hordeum vulgare/*Rhopalosiphum padi*	+	*Coccinella 7-punctata*	Ninkovic et al.(2001)
Tea shoots/*Toxoptera aurantii*	+	*Coccinella 7-punctata*	Han and Chen (2002)
Vicia faba, Brassica napus, Sinapis alba/*A. pisum, Brevicoryne brassicae, Myzus persicae*	–	–	Francis et al. (2004)

+/–, positive and negative evidence

in aphid trophic-webs has been spectacular during the past 30 years. No doubt, these interactions will prove in the near future to be even more complex and subtle than presently recognised, as many questions remain open. Some were raised above and will not be repeated here. However, two avenues of research look especially promising to me.

The first avenue concerns the influence of the aphid host–plant on ladybird foraging behaviour and fitness. Aphid–plant interactions could offer cues for ladybirds in their search and recognition of suitable food patches. Plant-volatiles induced by aphids could offer cues, not for primary attraction to aphid patches, but by associative learning for feeding on similar rewarding food sources. Plant secondary compounds excreted in the honeydew could provide additional cues influencing ladybird selection of appropriate resources. To what degree are ladybird responses to these various cues innate or learned? Recent experiments suggest that patch assessment by ladybirds is influenced by experience (Frechette et al. 2004). Besides protecting the aphids in degrees depending on their level of host specialisation, plant secondary-compounds can be sequestered by the ladybirds. Do they incorporate the plant toxins in their eggs, as do other sequestering insects? Does plant-derived defence increase in significant ways the diversity of chemical defence of ladybirds, and does it influence the rate of cannibalism or intraguild predation? Finally, very little is known about how aphids and ladybirds handle plant toxins: excretion, detoxification, transformation, transport and storage.

The second avenue concerns cues used by the aphid-web partners for their mutual recognition. Cuticular hydrocarbons appear prominent in this role. In ladybirds, the same alkanes, but in different proportions, are used for mate recognition, to deter ovipositing females from preoccupied aphid patches and to flag their eggs to intraguild predators (Hemptinne and Dixon 2000). This multiple use of alkanes was considered as an example of semiochemical parsimony. Does it mean that the ladybirds respond to the same signal in a context dependent way, or do they differentiate mixtures of alkanes in different proportions, or both? Where is the signal in a mixture and what is the discriminatory capacity of the ladybirds? Are ladybird olfactory neurones tuned for perceiving peculiar hydrocarbons, in the same way they are for detecting (*E*)-*β*–farnesene and (-)-*β*-cariophyllene (Al Abassi et al. 2000)? Again to what degree are the responses to recognition cues innate or learned, in particular during interspecific interactions?

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