How predatory arthropods learn to use herbivore-induced plant volatiles. Evidence from behavioural experiments and the field

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GENERAL DISCUSSION – BEHAVIOURAL RESPONSES OF PREDATORY AND HERBIVOROUS ARTHROPODS TO INDUCED PLANT VOLATILES: FROM EVOLUTIONARY ECOLOGY TO AGRICULTURAL APPLICATIONS

Herbivory is known to induce the production of volatiles in plants. These signals are thought to betray herbivores to their predators, which are then attracted or arrested near the plant under attack. Evidence for involvement of herbivore-induced plant volatiles in predator recruitment is largely based on experiments with olfactometers designed to demonstrate a response to odours, not to elucidate the behavioural mechanisms used to locate the source. Since the mechanisms underlying orientation may well operate at a spatial scale beyond that considered in the lab, experiments are required to unravel the tactic and kinetic responses in carefully designed laboratory experiments at a larger scale and to assess the responses under more realistic (greenhouse, field) conditions. Experiments are discussed showing the role of odour-conditioned anemotaxis, tactic/kinetic responses to odour gradients, odour-conditioned landing and take-off responses, as well as the role of hunger, associative learning and innate responses in predatory arthropods.

By producing volatiles, plants also betray their presence to arthropods that do not confer a direct benefit to the plant, such as other herbivores, hyperpredators and omnivores. Recent results on responses of such non-beneficial arthropods to odours from plants under herbivore attack are reviewed. Finally, conditions are discussed under which herbivore-induced synomones have an overall positive effect on plant fitness, and possibilities for the practical application of herbivore-induced synomones in plant protection are highlighted.


Plants defend themselves against herbivorous arthropods, not only directly (toxins, digestion inhibitors, glandular hairs, tough cuticle, etc.), but also indirectly by promoting the effectiveness of the herbivores’ predators (Price et al. 1980, Fig. 1a). Indirect plant defense may be achieved by creating protective structures, providing food (pollen, nectar)
and by releasing chemical cues which betray the presence of herbivores (Dicke and Sabelis 1988a, Sabelis et al. 1999ab). These plant-provided facilities can be utilized by predatory arthropods that help the plant to get rid of herbivorous arthropods, but they are also open to other organisms that are not beneficial to the plant. Indeed, mutualistic interactions are never foolproof (Bronstein 1994a) and plant-predator mutualisms are no exception. Here, the role of herbivore-induced plant volatiles (HIPV) is discussed from a food web perspective.

There are two problems to be addressed before we can conclude that attracting predators is indeed one of the functions of HIPV. Firstly, most studies showing attraction of predators by these volatiles are done under idealized laboratory conditions and it remains to be shown that attraction also occurs under natural conditions. Secondly, if plants do attract predators by producing HIPV, it is inevitable that other organisms that are not beneficial to the plant also use these volatiles to their own benefit. Here, advances in knowledge on how these volatiles influence the behaviour of (1) predatory arthropods and (2) other (possibly non-beneficial) arthropods are reviewed. By considering the responses to HIPV in the food web of plant-inhabiting arthropods a more realistic view on the overall effect of producing “alarm calls” on plant fitness may be obtained.

![Figure 1](image-url) Diagrams of systems with one, two and three trophic levels (top) or four trophic level and variants thereof (bottom). The spheres represent the biomass at each trophic level, assuming 10-20% conversion efficiency for the herbivores and 70-80% for the (hyper-)predators. Drawn arrows stand for the trophic interactions, whereas dashed arrows stand for avoidance of (herbivore-herbivore, herbivore-predator, predator-predator) interactions. Defensive interactions are left out to keep the diagram simple.
A food-web perspective on the effects of HIPV is crucial to understand the evolution of plant-predator mutualisms. Hairston et al. (1960) hypothesized that predators control herbivore densities, resulting in the plants being relieved from herbivore damage. This implicitly assumes that direct plant defenses are ineffective. Although these defenses are successful against many herbivores, they are not effective against all (Strong et al. 1984), and this leaves room for the evolution of indirect defenses (plant-predator mutualisms). While the "World is Green" hypothesis (Hairston et al. 1960) may hold for linear tritrophic food chains, one may wonder what happens in more complex food webs (Polis and Strong 1996). When hyperpredation and intraguild predation are prevalent, plant alarm calls may not only benefit the herbivores' predators, but also the predators' predators and competitors. These effects on the fourth trophic level will cascade down the food chain (Fig. 1b): the herbivores' enemies become less abundant, the herbivores increase in number and the plant will suffer more from herbivory. This cascading effect may reduce the plant's benefit in sending out alarm calls to the point that it does not outweigh production costs and risks of alerting other herbivores. Thus, it is crucial to determine whether communities of arthropods on plants behave like linear tritrophic food chains or not (such as a four trophic level system or a web of interactions between linear food chains). In the first case, plant investment in indirect defenses is more likely to be favored by selection, since predators will suppress herbivore populations to a greater extent and plants will suffer less from herbivory (Hairston et al. 1960, Strong et al. 1984, Sabelis et al. 1999ab). Consequently, to explain why plants usually retain a green appearance under natural conditions, one should identify the mechanisms that make complex food webs behave much like linear tritrophic food chains (Fig. 1b). The key mechanisms probably lie in the adaptive behaviour of members of the food web. Odour-mediated attraction and avoidance may lead to niche partitioning, thereby decreasing the strength of food web interactions. This is why the focus is on behavioural responses to HIPV and, where possible on responses to other volatiles conveying information on niche quality (Janssen et al. 1998).

Food webs of plant-inhabiting arthropods vary in space and time with respect to their structure and the abundance of their members. Herbivores and their natural enemies make foraging decisions that not only depend on the presence and quality of food but also on the risk of being eaten and the intensity of competition. Thus, these decisions depend on the current and local state of the food web. Hence, although the ultimate evaluation of effects of HIPV on plant fitness should be done under natural conditions, there is a danger in evaluating plant fitness effects exclusively in the field, because the temporal and spatial scale of field experiments is bound to be such that only a subset of food web states play a role. Lab experiments allow a free choice of the composition of the food web, whereas in the field, species composition may vary in space and time. Hence, it seems wiser to develop insight by considering a chain of experiments, starting from controlled conditions in the laboratory to real, complex conditions in the field.

There is a rich literature on indirect plant defense against herbivores via the attraction of natural enemies by HIPV (Dicke and Sabelis 1988b, 1989, 1992, Dicke et al. 1990b, Dicke 1994, Turlings et al. 1995, Takabayashi and Dicke 1996). In this chapter, literature on the role of HIPV in attracting predatory arthropods is reviewed. The focus is on true predators because the experimental work presented in this thesis is carried out on true predators. Predators – unlike many parasitoids (Van der Meijden and Klinkhammer 2000, Vinson 1999) – have a direct impact on plant fitness: they kill the herbivores immediately,
thereby preventing further feeding damage to the plant. Attraction of true predators may therefore more readily result in an advantage to the individual plants. After reviewing the responses of predators to HIPV, the net effect of HIPV production on plant fitness is evaluated. Finally, the practical perspectives of HIPV for crop protection are discussed.

**BEHAVIOURAL RESPONSES OF PREDATORY ARTHROPODS TO HIPV**

The first indication of olfactory responses of arthropods to odours emanating from herbivore-infested plants came from Y-tube olfactometer tests with predatory mites and spider mites on detached bean and apple leaves (Sabelis and Van de Baan 1983). Subsequent experiments showed that the odours do not emanate from the spider mites alone nor from its silk secretions, but that it comes to some degree from the faeces, and principally from the mite-infested leaves (i.e. after removal of spider mites and their products, Sabelis *et al.* 1984a). Since leaves did not elicit a response until being fed upon by spider mites and infested leaves gradually lost their attractiveness after the removal of spider mites, the hypothesis was formulated that the odours are of plant origin and induced by spider mite feeding (Sabelis and Dicke 1985). Two lines of evidence for the active involvement of plants have been presented. First, headspace analysis revealed that the most abundant chemicals in the blend released upon feeding by two-spotted spider mites, *Tetranychus urticae*, on Lima bean (*e.g.* methyl salicylate, linalool, *(E)-β*-ocimene, 4,8-dimethyl-1,3(E),7-nonatriene, 4,8,12-trimethyl-1,3(E),7(E),11-tridecatetraene) are known as plant compounds (Dicke 1988, Dicke and Sabelis 1988ab, 1989, 1992, Dicke *et al.* 1990ab). Second, not only the infested leaves release the volatiles, but also uninfested leaves of the same plant (Dicke *et al.* 1990b, 1993; see also Turling and Tumlinson 1992). Thus, there is a local, as well as systemic induction of volatiles upon spider-mite attack. Since these results became available, a quest for the elicitor involved in local transduction from the herbivores' oral secretions to the plant and systemic signal transduction from leaf to leaf has started (Paré *et al.* 1998). The ability to produce herbivore-induced alarm signals is probably widespread in the plant kingdom, as there is supporting evidence that herbivores elicit such responses across 23 plant species in 13 plant families (Dicke 1994, Dicke *et al.* 1998). This ability is not limited to the interaction of plants with herbivorous mites. Apart from extensive work on the response of parasitoids to plant volatiles induced by caterpillars (Turling *et al.* 1995) and aphids (Guerrieri *et al.* 1997, Du *et al.* 1998), there is also evidence for responses of anthocorid bugs to odours from bean plants infested by spider mites (Dwumfour 1992), to pear leaves infested by psyllids (Chapter 4, this Thesis; Drukker *et al.* 1995) and to components identified in the odour blends of *Psylla*-infested pear leaves, such as *(E,E)-α*-farnesene and methyl salicylate (Chapter 2, this Thesis; Scutareanu *et al.* 1996, 1997).

There is evidence that the behavioural responses of predatory arthropods to HIPV are based on olfaction. For example, when released in realistic concentrations, single components of the herbivore-induced odour blend (methyl salicylate, linalool, *(E)-β*-ocimene) elicit a positive response of predatory mites in a Y-tube olfactometer (Dicke *et al.* 1990a). Furthermore, the behaviour of chemoreceptor-bearing extremities in response to HIPV has been described for predatory mites (Dicke *et al.* 1991), chemosensors on tarsi of first legs have been described morphologically (Jagers Op Akkerhuis *et al.* 1985) and
electrophysiological recordings have been obtained from the neurons in these sensors when exposed to one of the blend components (linalool) (De Bruyne et al. 1991). We know little, however, of the role of blend composition, even if it concerns only the major blend components. Can mixes of the major components alone explain all behavioural responses? Or are minor blend components or hitherto undetected volatiles essential? Campbell et al. (1993) compared single-cell recordings of odour-receptor cells with gas chromatograms from odours from hop leaves and found that some spikes in the action potential of the receptor cells did not coincide with detected volatiles, suggesting that other volatiles may also play a role. The only test of the response of a parasitoid to a synthetic mixture of the (eleven) major volatiles from corn fed upon by *Spodoptera exigua* caterpillars failed to reproduce the response to a natural blend of HIPV (Turling et al. 1991). Moreover, it is well documented that herbivorous mites of different species on the same species of host plant induce the production of blends that differ only in relative amounts of the (major) components. Yet, predatory mites can readily discriminate between leaves infested by either of the spider mite species (Sabelis and Van de Baan 1988, Sabelis and Dicke 1985, Dicke and Groeneveld 1986, Takabayashi et al. 1991). Apparently, predatory mites can discriminate between quantitative differences in the composition of major blend constituents, a phenomenon recently also reported for parasitoids (De Moraes et al. 1998). Alternatively, the components that enable the mites to discriminate are present in minor quantities and/or are still to be identified. These volatiles are not necessarily of plant origin as they may well be produced by the herbivores themselves (pheromones that are only produced in minute quantities) or even by associated micro-organisms.

**COPING WITH VARIABILITY IN HIPV**

Another salient point arises from the observation that the composition of induced volatiles varies with the species and cultivar of the host plant, even when attacked by the same species of herbivore (Takabayashi et al. 1994). Also, age, tissue and condition of a host plant influence the composition of plant odour blends (Takabayashi et al. 1991 1994, Scutareanu et al. 1997; Chapter 2, this Thesis). Plants even show diurnal cycles in the production of some of the induced volatiles (Loughrin et al. 1994). One may wonder how predators cope with this bewildering variety of signals.

Margolies et al. (1997) showed that it is possible to select for strength of the response to one of the spider-mite-induced volatiles (linalool) in a culture of the predatory mite, *Phytoseiulus persimilis*. A genetic basis may be expected when there is a cost to flexibility (e.g. the capacity of the sensory and central nervous system to perceive and integrate signals and alter behavioural response) and/or a cost to dietary change (e.g. due to differences in nutritional quality and secondary plant compounds). Given a sufficiently constant prey availability and signal environment, there will be selection to avoid paying the cost of flexibility, which will then pave the way for genetically fixed preferences. In fact, genetic polymorphisms for prey preference have been found in a predatory mite species associated with roots and bulbs of lilies (Lesna and Sabelis 1999). Thus, there may well be a (partially) genetic determinant of the olfactory response, but this does not imply a rigid prey/plant preference, because the genetic influence may be overruled by plastic responses to environmental stimuli. Indeed, there are indications of effects of experience
due to rearing on different host plants attacked by the same herbivore (Dicke et al. 1990c, Takabayashi et al. 1994, Krips et al. 1999). Selection, however, cannot be completely ruled out as a factor modifying the response, because (1) mortality during the 7-day rearing period may alter the genetic composition and (2) 8 hours of rearing on cucumber was not sufficient to alter preference. Experiments in this Thesis (Chapter 6; Drukker et al. 2000a), however, have shown that one-day starvation in the presence of HIPV caused a reversal from attraction to avoidance of these odour components. These studies indicate that predatory mites can learn the association between these odours and their state of starvation. Much the same results were obtained with Anthocoris females starved in the presence of single HIPV components from pear leaves (methyl salicylate, (E,E)-α-farnesene) (Chapter 5, this Thesis; Drukker et al. 2000b). Because there was no mortality in these experiments, this is strong evidence that associative learning is implicated in strategies of predatory arthropods to locate herbivorous arthropods. Positive associations may be reversed when volatiles are paired with a hunger stimulus, which leads to dispersal away from the original site. We hypothesize that avoidance responses wane when pairing of the odours and the hunger stimulus is discontinued, which in turn may reinstall the innate responses. When innate responses or coincidental encounters happen to bring the predator in contact with a potential prey on a new host plant, then positive associations with the HIPV in that setting may result (Fig. 2). Learning of positive associations between food and odours has been amply demonstrated in studies on parasitoids of herbivorous insects (Turlings et al. 1993a).

**HIPV-ELICITED MECHANISMS TO LOCATE THE ODOUR SOURCE: RELEVANCE OF SPATIAL SCALE**

Y-tube olfactometers are suitable to assess whether HIPV elicit a response of the predatory arthropod, but not to analyse the orientation mechanisms. The latter requires a more analytical approach, where each mechanism is tested in a specially designed experimental set up. Such an approach has so far only been followed in studies on the predatory mite *P. persimilis* (Sabelis and Dicke 1985). This predator showed an ability to orient to odour gradients in an olfactometer with a vertical airflow passing a horizontal fine-mesh gauze which served as a substrate for the predator (Sabelis et al. 1984b). When the odour source was positioned underneath the center of the gauze, the predatory mites responded to passing the sharp HIPV gradients by performing right-about turns. These turns show that, after passing the gradient, the predator perceived the absence of HIPV and increased the chance of moving back into the area with HIPV. When the odour source was moved in the same direction as the predator’s direction of movement, but faster, the predators also responded to the passing gradient by making about turns. Clearly, this behaviour would not help them to keep track of a moving odour plume. Hence, orientation to odour gradients is probably only relevant where odour gradients are sufficiently static at the time scale of mite movement. This is probably the case very close to the odour source, *i.e.* near a spider-mite colony on a leaf, where wind has relatively little impact on the position of the gradient.
The presence of HIPV also suppresses the tendency to disperse aerially in response to herbivore-induced plant odours, as shown by Sabelis and Afman (1994) using a wind tube designed to observe take-off behaviour of a single predatory mite on a leaf. Predatory mites are thought to disperse passively in air currents (Sabelis and Dicke 1985), but before becoming airborne, they appear to make decisions on whether to take off or not. These decisions are influenced by their state of food deprivation and dehydration, but also by the presence of HIPV. Even when the predatory mites were severely starved and dehydrated (in the absence of HIPV), the presence of HIPV stimulated them to stay. This response leads to arrestment in the area where HIPV signals the presence of prey to the predatory mites. However, the same response is also relevant to attraction towards the odour source. To understand this, one should realize that airborne predatory mites cannot decide when and where to land, but after landing they would gain by finding clusters of spider mite colonies located not too far from the site of landing. This is done...
by ambulatory search and the perception of HIPV probably stimulates the predator to continue searching until the source is found.

The ambulatory search to find clusters of spider-mite colonies on plants would be much more effective if HIPV elicits not only take-off suppression but also movement against the wind guided by orientation on the wind direction (positive anemotaxis). This was studied in a wind tunnel with a homogenous airflow and a uniform odour concentration (thus no odour gradients) (Sabelis and Van der Weel 1993). The results showed that starved predators walk upwind, but this positive anemotaxis is also manifested in the absence of HIPV, though in a somewhat less pronounced form. The most surprising result was that in the absence of HIPV well-fed predators move downwind, whereas in the presence of HIPV this negative anemotaxis disappears or is reversed to a positive anemotaxis depending on the odour concentration. At first sight this result was puzzling: why should satiated predators walk with the wind (= downwind) in absence and against the wind (= upwind) in presence of HIPV? The solution is simple once one realises that predatory mites foraging in clusters of prey colonies are probably satiated and, while moving from one infested leaf to another, may move out of the plant area occupied by the colony cluster. Should the predators happen to move out at the upwind (= windward) side of the cluster, then this takes them into HIPV-free air and negative anemotaxis would bring them back into the area with the colony cluster. Should they move out at the downwind side of the colony cluster, then positive anemotaxis will increase the chance of re-entering the cluster area. The consequence of this behaviour is anemotactic arrestment in the prey cluster area.

Whether the above mechanisms are sufficient to explain the orientation responses to HIPV in the field is an open question for future research. So far, effective location of spider mite-infested plants has been demonstrated in wind tunnels (Sabelis and Schippers, unpublished data), in the laboratory (Sabelis and Van der Weel 1993) and under greenhouse conditions (Janssen 1999). Moreover, there is indirect evidence that arrestment of predatory mites in clusters of spider-mite infested leaves plays a decisive role in the rate at which the local population of spider mites are exploited and ultimately eliminated (Sabelis and Van der Meer 1986, Sabelis 1992, Van Baalen and Sabelis 1995, Pels and Sabelis 1999). Simulation models of the interaction between *T. urticae* and *P. persimilis* only provided a good fit to data on local predator-prey dynamics when it was assumed that the predatory mites do not emigrate until after all prey are eliminated. Even small rates of predator emigration from the cluster led to drastic effects on the simulated local predator-prey dynamics (much higher prey population peaks, longer periods of predator-prey interaction, much higher overall predator yield). Since predatory mites have to move between spider-mite infested leaves, they have to pass leaf surface, stems and petioles that are devoid of prey. The cloud of HIPV surrounding the cluster of infested leaves may well be instrumental for the predatory mites to stay arrested within the cluster.

**THE IMPACT OF HIPV IN THE FIELD**

The first demonstration of the impact of HIPV in the field came from research on biocontrol of psyllids in pear orchards in the Netherlands (Chapter 4, this Thesis; Drukker et al. 1995). Pear orchards suffer from only a few plant pests, the most important
of which are leaf suckers, *Psylla pyri* and *P. pyricola*. These psyllids are absent in spring (March-May), begin to show up in June and may become numerous from July to September. In spring and early summer the predators thought to be most effective against the psyllids, *(i.e.,* heteropteran bugs such as *Anthocoris nemorum* (L.), *A. nemoralis* (Fabricius) and various *Orius* spp.) feed on aphids on other trees *(e.g.,* alder, hawthorn). By the time the psyllid populations start to increase in July predators begin to immigrate, show up first as adults in June-July, then later in July-September in all developmental stages, and building up strong cross-correlations with the population sizes of psyllids (Chapter 3, this Thesis; Scutareanu et al. 1999). It was hypothesized that the immigration of adult predators was triggered by HIPV from *Psylla*-infested pear trees. This was assessed in the field by sampling trees next to cages with trees harboring *Psylla* populations of various sizes. Densities of predatory bugs increased with the density of psyllids in the cages. Since covering the cage with an infested pear tree by a plastic sheet led to a sudden drop in the density of predatory bugs and removal of the sheet was quickly followed by a build-up in predator numbers, there was support for HIPV-triggered immigration of predators (Chapter 4, this Thesis; Drukker et al. 1995). This was further substantiated by identification of headspace volatiles from *Psylla*-infested leaves *(methyl salicylate and *(E, E)-α-farnesene)* and by testing the behavioural response to *Psylla*-infested vs. clean leaves and to single HIPV-compounds in a Y-tube olfactometer (Chapter 2, this Thesis; Scutareanu et al. 1997). Naive predatory bugs showed a weak innate response to HIPV from pear, but once they experienced the association between food and these odours they exhibited a strong positive response (Chapter 5, this Thesis; Drukker et al. 2000b). Pairing HIPV to a hunger stimulus, the predators exhibited a strong negative response. This ability to learn associations between odours and positive or negative stimuli is probably of great importance in the field since the predators are faced with a wealth of volatile infochemicals emanating from various plants and plant-herbivore combinations (Fig. 2). We suspect that predatory bugs leave their original prey resources when hungry *(a negative association with plant volatiles)*, subsequently rely on their innate responses until they contact suitable herbivorous prey, such as pear leaf suckers, and experience the associated HIPV *(a positive association)*. These experienced predators subsequently continue to respond positively to the HIPV and end up close to the cages harbouring large *Psylla* populations in the experimental orchard. This may explain the somewhat puzzling observation that predatory bugs aggregate around the cages without being able to contact the prey on the trees inside these cages.

Other examples of attraction in the field are still scarce. Shimoda *et al.* (1997) studied the response of a predatory thrips to odours produced by bean plants infested with spider mites. In the field, thrips were caught on sticky traps baited with infested bean plants but not on traps baited with clean bean plants. However, only a few predatory thrips were recaptured, and only after a long period, and it therefore remains to be seen whether predators arrived in time to benefit the plant.
LIMITS TO ATTRACTIVENESS: THE EFFECT OF THE PRESENCE OF CONSPECIFIC PREDATORS

There may well be limitations to the number of predators that a plant can attract (Janssen et al. 1997). When predatory mites are offered a choice between odours from spider mite colonies with and without conspecific predators, they prefer odours from the latter. This probably means that predatory mites are initially attracted towards a plant infested by spider mites, but once the infested leaves are discovered and colonized, others will move on to neighboring plants with spider-mite colonies free of predators. Predatory mites make their own foraging decisions and may well be capable of balancing food gains and competition effects, as predicted by ideal-free foraging theory. They not only use information to locate their prey, but also to avoid intraspecific competition. An open question is where this information on competitors is coming from and whether this information allows predators to discriminate between different competitor species. Janssen et al. (1997) show that production of the odours is associated with the presence of adult spider mites, and they suggest that it is an alarm pheromone of the spider mites. Whether this alarm pheromone conveys information that allows the receiver to specify the enemy (competitor, predator) is not known.

RESPONSES OF NON-BENEFICIAL ARTHROPODS TO HIPV

To understand the evolution of HIPV it is necessary to change gears from simple tritrophic food chains to food webs. Volatile signals may also be picked up by other organisms in the food web, such as herbivores and hyperpredators (Fig. 3), and this can have severe consequences for the net fitness effects of the production of volatiles by plants. Consider, for example, a simple food web occurring on greenhouse cucumber in The Netherlands. One tritrophic food chain studied in this system consists of the plant, two-spotted spider mites and their natural enemy, the predatory mite P. persimilis. Another food chain consists of the plant, western flower thrips (Frankliniella occidentalis), and its natural enemies. When combining these two food chains in a food web, many more interactions can occur (Janssen et al. 1998, Pallini 1998, Pallini et al. 1997, 1998).

Herbivores could take advantage of the presence of other herbivores on plants, for instance by using the volatiles induced by the other herbivore to acquire information from a distance on the state of the plant. Attack by the first herbivore may induce plant defenses, including indirect defense through attraction of natural enemies, making infested plants less suitable for colonization. In this case, the volatiles would signal this defended state to other herbivores that may use them to avoid well-defended plants (e.g. Ohsaki and Sato 1994). Herbivores may also use HIPV to avoid competition on plants that have a high probability of being defended by predators attracted by the volatiles. Under all scenarios, production of HIPV would lead to avoidance of the plant by other herbivores, which will have a positive effect on plant fitness. Alternatively, herbivores attacking the plant may deplete plant constitutive defenses, making the plants a more suitable food source (Tallamy 1986). In this case, production of HIPV could lead to an increase of herbivore numbers and species, which is obviously detrimental to the plant.
Production of HIPV may also attract natural enemies that interfere directly, through interference competition or intraguild predation, with natural enemies already present on the plant (see Janssen et al. 1998, for a review). Effects of these interactions on herbivore densities can be positive or negative (Rosenheim et al. 1995, Holt and Polis 1997, Janssen et al. 1998), and hence will affect the plant’s net benefits of HIPV production. Below we review the evidence for the use of HIPV by other members of the food web (see Turlings and Benrey 1998 for a review), as well as the evolutionary consequences of this for volatile production.

**Figure 3** Herbivory (e.g. by spider mites) induces plant-wide production of volatiles in their host plant (e.g. cucumber), thereby providing free information for other herbivores (e.g. thrips), predators (e.g. predatory mites), hyper- and intraguild predators (e.g. predatory bugs), and possibly others (e.g. fungivorous or pollen-feeding mites and insects).
THE RESPONSE OF HERBIVORES TO HIPV

Effects of HIPV on conspecific herbivores

Dicke (1986) found that two-spotted spider mites dispersed when exposed to odours of bean leaves infested with conspecifics. Although other studies indicate that many of the volatiles emitted by such infested leaves (i.e. general green-leaf volatiles) such as hexenol and specific herbivore-induced volatiles such as terpenoids and the ester methyl salicylate) are indeed produced by the plant, it cannot be ruled out that spider mites themselves also produce volatiles that play a role in eliciting dispersal. Another study of the response of these mites showed a slight attraction to cucumber plants with conspecifics (Pallini et al. 1997). Apart from differences in the plant species, the differences between the two series of experiments may be explained by differences in the spatial scale at which the response occurs; from a distance, spider mites are slightly attracted to plants with conspecifics, but once on the plant, they prefer leaf areas that are unoccupied (see Pallini et al. 1997 for details). This matches with further experiments by Dicke (1986), in which odours from a mixture of clean and infested leaves did not induce dispersal. Hence, from a distance, spider mites perceive a mix of odours from clean and infested leaves to which they are attracted. Once on a plant, they preferentially settle on clean leaf areas.

Landolt (1993) studied the response of adult female cabbage looper moths (Trichoplusia ni) to odours of cotton and cabbage plants that were uninfested, mechanically damaged or damaged by conspecifics in a laboratory wind tunnel. In choice experiments, artificially damaged or herbivore-damaged cotton attracted more moths than clean plants, but moths oviposited more on clean plants. In contrast, cabbage with herbivores attracted significantly fewer moths than clean plants did, and there was no difference in attractiveness between clean and artificially damaged cabbage.

Harari et al. (1994) investigated the volatile stimuli that cause aggregation in a polyphagous beetle (the scarabaeid Maladera matrida) with a Y-tube olfactometer. Beetles were not attracted to odours from conspecifics only, but were attracted when beetles were feeding on Duranta repens leaves. Bolter et al. (1997) studied the response of the Colorado potato beetle (Leptinotarsa decemlineata), to clean plants, artificially damaged plants and plants damaged by conspecifics. More beetles were found to walk upwind when mechanically damaged plants, rather than clean plants, were offered as the odour source, but plants lost their attractiveness soon after being damaged. Plants with older beetle infestations were attractive for a longer period. The volatiles produced were a mix of green-leaf volatiles (fatty acid derivatives) and induced volatiles (terpenoids such as linalool). The responses found in these two studies (Harari et al. 1994, Bolter et al. 1997) may have been elicited by a combination of plant-produced volatiles and beetle odours.

In laboratory olfactometer experiments, Campbell et al. (1998) showed that spring morphs of damson-hop aphids (Phorodon humuli) have a significant preference for odours from hop leaves with conspecifics over clean hop leaves. Single-cell recordings of olfactory receptors combined with gas chromatography showed that this preference is likely to be caused by volatiles produced by the plants upon induction by the aphids (hexenal, β-caryophyllene, methyl salicylate and (E)—farnesene). As mentioned before, some spikes in the action potential of the receptor cells did not coincide with detected
volatiles, suggesting that also other volatiles may play a role. The origin of these compounds is unknown, hence, volatiles other than HIPV may play a role. This study also shows some other striking features of arthropod responses to blends of volatiles. First, responses depend on the relative concentration of various components; two components in a natural ratio ((E)-2-hexenal and β-caryophyllene) were attractive whereas a 1:1 ratio was unattractive. Second, responses to mixtures differ from responses to individual components; for example, the full blend of HIPV is attractive despite the presence of one component that, by itself functions as a repelling alarm pheromone ((E)-farnesene). Moreover, the attractive mix of (E)-2-hexenal and β-caryophyllene in natural ratios became unattractive when a third compound (methyl salicylate) was added in the natural ratio.

In all of the above examples, it is still possible that minor or even undetected components are responsible for eliciting the responses. A more conclusive example of increased attraction of herbivores by HIPV comes from the work of Loughrin and colleagues on Japanese beetles (Popillia japonica: Scarabaeidae). These beetles were shown to induce production of volatiles in crab apple and grapevines (*i.e.* terpenoids such as ocimene, linalool and farnesene, aliphatic compounds such as hexenyl acetate, and aromatics such as phenylacetonitrile and hexenyl benzoate; Loughrin *et al.* 1995, 1996). In the field, vines producing these volatiles after overnight feeding by beetles attracted 15 to 30 times as many beetles as plants with non-feeding beetles (Loughrin *et al.* 1996). Trapping experiments in the field showed that single compounds of the induced volatiles are attractive and that the attractiveness of mixtures of synthetic volatiles increased with the number of compounds added (Loughrin *et al.* 1998). Some of the most prevalent volatiles produced by plants induced by herbivores (acyclic terpene hydrocarbons such as (E)-ocimene and farnesene) were not tested however, because they were not commercially available. It is possible that these single compounds would elicit even stronger responses from the herbivores. In conclusion, there are several examples of conspecific herbivores that respond to the odours emanating from plant-herbivore complexes, and there is every reason to assume that heterospecific herbivores use the same volatiles to their own benefit.

**Effects of HIPV on heterospecific herbivores**

Responses to plant volatiles induced by heterospecific herbivores are less well studied. Some of the already cited studies compare plants infested with heterospecifics to plants with conspecifics. Harari *et al.* (1994), for example, found that leaves with the desert locust Schistocerca gregaria were as attractive to the scarabaeid beetles as leaves with conspecifics, suggesting that common damage-induced volatiles are responsible for the aggregation. The authors further state that in the field, aggregations of beetles were seen close to feeding larvae of Spodoptera littoralis. Bolter *et al.* (1997) found that feeding by beet armyworm larvae (*Spodoptera exigua*) led to similar responses in the Colorado potato beetle as conspecific feeding. Pallini *et al.* (1997) found that two-spotted spider mites avoided plants infested with western flower thrips under greenhouse conditions. In contrast, western flower thrips did not show preference when offered clean plants and plants infested with spider mites (Pallini *et al.* 1999). In all of these studies, it is possible that herbivores responded to odours that were not produced by the plants, such as faeces or body odours of the heterospecific herbivores.
The only study that unambiguously shows that herbivores use plant volatiles induced by heterospecific herbivores when searching for plants is that of Bernasconi et al. (1998). They studied plant selection by the corn leaf aphid (Rhopalosiphum maidis). Corn plants were induced to produce volatiles by treating artificially damaged plants with caterpillar (S. littoralis) regurgitate. Plants treated in this way are known to produce many typical herbivore-induced volatiles (Turlings et al. 1993b, 1998), amongst others (E)—farnesene which is an alarm pheromone of aphids. In a Y-tube olfactometer in the lab and in release experiments in the field, it was found that aphids prefer clean plants over induced plants. By using plants that were artificially induced (without herbivores), effects of the presence of odours of herbivores themselves or their faeces were ruled out as a possible cue for avoidance.

**Functional explanations**

The above shows that there is evidence that herbivores can use volatiles emitted by plant-herbivore complexes to avoid or find plants infested with con- and heterospecific herbivores. Little is known about the adaptive value for such avoidance or attraction. All animals for which odour-mediated attraction towards plants with conspecifics was shown are known to form aggregations in the field, but it is unclear why they do this, although some reasons have been suggested (Loughrin et al. 1995, 1998, Harari et al. 1994). Bernasconi et al. (1998) suggest that their aphids avoid plants because the volatiles indicate (1) that the plant has started to produce toxic compounds in response to damage, (2) that potential competitors are present on the plant, or (3) that the plant is attractive to natural enemies. Moreover, they suggest that aphids may avoid damaged plants because they produce an aphid alarm pheromone. This last explanation hinges on the biological function of one isolated compound in aphid ecology, and the avoidance is seen as an inevitable by-product of this. However, farnesene was also present in the headspace of infested hops (Campbell et al. 1998) and this did not lead to the whole mixture of volatiles being unattractive to aphids. This last study provides further evidence that mixtures of volatiles may contain unattractive compounds without losing their overall attractiveness (Campbell et al. 1999). It therefore seems necessary to study and explain responses to blends of volatiles rather than to isolated compounds.

Another study that speculates on the functional explanation for the response of herbivores to HIPV is that of Pallini et al. (1997), who state that two-spotted spider mites avoid plants with thrips to avoid interspecific competition and intraguild predation. Indeed, greenhouse experiments show that the population growth rate of spider mites is lower in the presence than in the absence of thrips (Brodsgaard and Enkegaard 1995).

From the plant's perspective, it is clear that production of induced volatiles may keep other herbivores away in some cases but can have devastating effects in others. Aggregation of Japanese beetles on certain plants, for example, causes complete defoliation while other hosts in the vicinity are hardly attacked (Loughrin et al. 1996). Hence, in this case the production of volatiles by the plants seems to have only negative effects. Since Japanese beetles are an imported pest at the study site, plants may not have adapted to their occurrence. It is expected that plants that have coevolved with this pest would have been selected not to produce volatiles.
ATTRACTION OF HYPERPREDATORS AND INTRAGUILD PREDATORS BY HIPV

Hyperpredators and intraguild predators may also be attracted by HIPV. There are no examples of this in the literature. Recently, the response of an omnivorous predatory bug, *Orius laevigatus*, to odours of plants attacked by two herbivores was investigated: the western flower thrips, the target pest of the predator in greenhouses in The Netherlands, and the two-spotted spider mite, a non-target pest (Venzon *et al.* 1999). The bug was attracted to plants infested with either herbivore. Predatory bugs not only attack herbivores, but can also prey on other predators such as predatory mites, the natural enemies of spider mites (Cloutier and Johnson 1993, Brodsgaard and Enkegaard 1995). For this intraguild predation to occur, it is not sufficient that predators are attracted to plants with the same prey, but also that they do not avoid plants with the other predators present (Janssen *et al.* 1995ab, 1997). When offered a choice between plants with spider mite prey plus spider mite predators and plants with spider mites only, the predatory bug showed no preference for either of the two. This indicates that it does not avoid plants with the other predator present (Venzon 2000). Likewise, the natural enemy of spider mites did not avoid plants with prey and the predatory bug (Janssen *et al.* 1999). This indicates that predators of both species may interact on plants with spider mites. That this can have negative effects on the control of spider mites is indicated by experiments by Brodsgaard and Enkegaard (1995). They compared the dynamics of two-spotted spider mites and *P. persimilis* in the presence or absence of another *Orius* species, *O. majusculus* (Reuter), on gerbera. Their results clearly show that the number of spider mites remained higher in presence of *O. majusculus*. Unfortunately, no data were given on the densities of *P. persimilis*, but it suggests that intraguild predation of *Orius* on *P. persimilis* initially has a positive effect on spider mite densities. Hence, attraction of a generalist predator by odours produced by the plant has positive effects on herbivore density, and may therefore have negative effects on plant fitness. The effects of HIPV on the attraction of hyper- and intraguild predators clearly need further study.

SYNTHESIS: OVERALL IMPACT OF HIPV ON PLANT FITNESS

Most studies reviewed above do not enable distinction between volatiles emanating from the herbivores and plant-produced volatiles. Moreover, volatiles may also be produced by microorganisms associated with the plant or with herbivores. The distinction between odours of these various sources and their effects on other herbivores is essential from an evolutionary perspective since plants may be unable to affect production of volatiles by herbivores or microorganisms. We therefore stress that further identification of the volatiles and determination of their source is essential for determining costs and benefits of HIPV-production by plants.

The concept of plants producing volatiles to attract members of the third trophic level hinges on the assumption that hyperpredators and intraguild predators are unimportant in determining herbivore density and plant fitness. In other words, the effects of hyperpredators should not cascade down to the first trophic level. This is in agreement with the once widely accepted view that herbivore densities are mainly determined by
predators, and predator densities are, determined by competition rather than hyperpredation (the "World is green" hypothesis, Hairston et al. 1960). This idea however, has lost some of its credibility since it became clear that competition among herbivores occurs frequently (Sih et al. 1985, Denno et al. 1995), and that intraguild predation is widespread and can have important effects on herbivore dynamics (Rosenheim et al. 1995, Holt and Polis 1997). Moreover, the idea that species can be assigned to one trophic level is also the subject of discussion (Polis and Strong 1996, Janssens et al. 1998). Many species are omnivorous and attack both plant and herbivore, or herbivore and other predators. A remarkable example of this is the western flower thrips. It attacks both plants and spider mites, but is mainly viewed as a pest. However, in cotton in California and Texas it is regarded as a predator of spider mites and it is mostly not controlled, although it may occasionally cause substantial damage (Trichilo and Leigh 1986). The thrips can also act as hyperpredator; it kills eggs of predatory mites that prey on spider mites or on the thrips itself (F. Faraji, pers. obs.; A. Pallini, pers. obs.).

Viewed from the perspective of such food web complexities, one is inclined to think that conditions favouring the evolution of production of HIPV can only occur under a restrictive set of conditions, i.e. when the overall effect of hyper-, intraguild-, and simple predators on herbivore densities is negative, or when HIPV-production does not lead to attraction of other herbivores that cause more damage. Moreover, when benefits of volatile production are relatively small, as will be the case when a plant is attacked by a minor pest, the costs may well outweigh the benefits. Hence, assuming that plants can distinguish between herbivore species, we hypothesize that attack by herbivores that cause small negative effects on plant fitness will not lead to induction of volatile production. Yet, we are aware of only one example of a herbivore-damaged plant species that did not produce any volatiles: maize plants infested with aphids (Rhopalosiphum maidis) did not show any production of induced volatiles (Turlings et al. 1998). This species can be a pest in the US and China but is less important than stem-borers that do elicit HIPV production (T.C.J. Turlings, pers. comm.) Hence, it is possible that volatile production would be detrimental to the plant because it attracts more severe pests. Alternatively, it is possible that volatiles were produced, but were not detectable by the techniques used. Study of the response of natural enemies of aphids, as well as stem-borers, to plants with aphids will give more insight.

A reason for the apparent generality of HIPV production among plants may be that negative results, i.e. the absence of attraction of natural enemies or production of volatiles, may not be published as readily as positive results. Negative results, however, would enhance our understanding of the evolution of induced volatile production. Another reason might be that most herbivores studied so far are serious pests of crops, hence have enormous effects on plant fitness. In these cases, any defense is better than patiently awaiting death or defoliation. This topic raises further questions on the specificity of volatile signals produced; besides being able to produce specific signals for each herbivore (De Moraes et al. 1998), are plants also capable of shutting down this line of defense when they are attacked by a minor pest?

A third reason for the generality of production of herbivore-induced volatiles may be that complex food webs behave as simple tritrophic food chains (Fig. 4). In other words, the negative effects of hyperpredators and generalist predators on plant fitness are minor compared to the positive effects of predators, and it therefore always pays for a plant to advertise the presence of herbivores. This seems to contradict the notion that interactions
such as intraguild predation are widespread (Rosenheim et al. 1995, Holt and Polis 1997). However, many of the empirical studies underpinning this conclusion used confines to study food web interactions and this leads to inevitable interactions among species (Janssen et al. 1998). Antipredator behaviour (Lima and Dill 1990, Lima 1998, Kats and Dill 1998, Pallini et al. 1998) and avoidance of other adverse interactions (Janssen et al. 1995ab, 1997) may substantially reduce the direct interactions between predators, hyperpredators and generalist intraguild predators (Fig. 4). Still, such avoidance always comes with a cost, so even if predators escape from their adversaries, the act of escaping may reduce their efficiency at reducing herbivore populations.

A last important point to make is that costs and benefits of indirect plant defenses are likely to vary greatly in space and time, as is generally the case in mutualistic interactions (Bronstein 1994ab). For example, the number of predatory arthropods, but also the number of herbivores and hyperpredators in the environment of a plant may vary, thereby causing differential net effects of indirect plant defenses.

Figure 4 Complex interactions in an artificial food web on cucumber plants consisting of spider mites (the two-spotted spider mite, *Tetranychus urticae*) and their enemies (the predatory mite *Phytoseiulus persimilis*), as well as thrips (the western flower thrips, *Frankliniella occidentalis*) and its enemies (the predatory mites, *Amblyseius cucumeris* and *Iphiseius degenerans*, the predatory bug, *Orius laevigatus*) (left). The complexities involve herbivore-herbivore, herbivore-predator and predator-predator interactions, as well as the avoidance of these interactions. When the avoidance responses are of overriding importance, the food web behaves like two linear food chains (right).
In conclusion, we hope to have convinced the reader that to assess the impact of HIPV on plant fitness it is essential to consider (1) interactions with other members of the food web; (2) a spatial scale that enables manifestation of the full behavioural repertoire of all organisms in the food web, such as antipredator and avoidance behaviour; (3) a temporal scale that allows the expression of the full range of possible outcomes of interspecific interactions (Bronstein 1994b).

FUTURE DIRECTIONS: PERSPECTIVES OF HIPV FOR CROP PROTECTION

At first sight, the active role of plants in promoting efficiency of the third trophic level seems an appealing idea for improving crop protection, but it is essential to realize that these plant traits have evolved under natural conditions. One should be cautious when using these traits to improve pest control under agricultural conditions. Clearly, plant fitness and crop yield are not synonymous, although they may overlap in many cases. Moreover, food web structure of arthropods on agricultural crops may differ drastically from those under natural conditions due to, for example, pesticide use, the introduction of (alien) natural enemies and reduced plant diversity. Nevertheless, bearing these restrictions in mind, one may speculate on the possibilities for manipulating HIPV production to improve crop protection (Bottrell et al. 1998). Below, we give five applications and discuss their advantages and disadvantages.

Development of predator lures and herbivore deterrents

Components or mixtures of several compounds of HIPV can be used to monitor and/or lure naturally occurring predators to crop fields, or to arrest released predators in the crop where they have been released. Attractive as this might seem, predators may be confused within the crop by the omnipresence of cues signaling prey. This may eventually alter their responsiveness to the compounds either via selection or via learning to avoid them. In fact, when the compounds are offered dissociated from the prey, the application of lures may even become counterproductive because these signals then become associated with hunger (Drukker et al. 2000b). Also, the supposed predator lures may additionally attract herbivores. This is nicely illustrated by the field experiments of Molleman et al. (1997). They attempted to attract anthocorid predators to pear orchards by placing delta and funnel traps releasing methyl salicylate, one of the HIPV components from *Psylla*-infested pear leaves that was shown to be attractive in Y-tube experiments. Rather than attraction of these predators, they found significant attraction of herbivores, such as silver-Y and apple clearwing moths.

Natural enemies were also attracted – in greater numbers than herbivores – but these consisted mainly of hoverflies and lacewings and to a much lesser extent of predatory bugs (*Orius* spp.). These were caught either very early (March) or very late (October) in the season. In an experiment with methyl salicylate lures where no traps were used, but where samples were taken with beating nets (see Chapter 4 for methodology), it was found that within 24 h after suspending the lures, anthocorid predators were found in greater numbers on trees with lures than on trees without lures (Drukker, unpublished data). When sticky traps were used later on, fewer predators were caught (but still
significantly more on trees with lures). Whether this decrease was due to the method of collection or indeed due to negative inforcement is impossible to tell, but given the finding that anthocorids learn by association (Chapter 5), it would be interesting to see what will happen if some of the lures are accompanied by a positive reinforcement such as pollen or a sugar solution. A subsequent increase in predator numbers near all lures would demonstrate associative learning in the field, but it would still not be final proof for a role as HIPV for methyl salicylate.

**Conditioning of mass-reared predators on the appropriate cues**

It is a widely recognised problem that mass-reared natural enemies do not end up on the target crop after release. One possible cause may be that the food/prey used for mass-rearing lacks the association with cues relevant to searching in the field. The simple solution may be that before release the predators are conditioned by offering food associated with appropriate HIPV. For example, the number of *Orius laevigatus* recovered on thrips-infested and spider mite-infested cucumber plants in the greenhouse increased by 30% when the predators had experienced the association of odours and spider mites on cucumber prior to the release experiment (Venzon and Janssen, unpublished data). Another example of such a conditioning before mass-release, is the release of tomato-conditioned *P. persimilis*. Before mass-release in a tomato greenhouse infested with spider mites, predators were kept overnight on a spider-mite-infested tomato leaf. Compared to predators without such an experience, they performed significantly better (Bruin and Drukker, unpublished data).

**Plant breeding for improved HIPV production**

Plant species and cultivars differ in the composition and amounts of green leaf volatiles and HIPV, as shown for example in cotton (Elzen *et al*. 1985), apple (Takabayashi *et al*. 1991), gerbera (Krips 2000), and pear (Drukker, Bruin and Posthumus, unpublished data). The responsiveness of natural enemies is not necessarily related to the total amounts of volatiles, but rather to the extent to which the blends stand out in the context of the environment. Conspicuousness may well prove a selectable trait, but the impact on biocontrol is probably context-dependent. In addition, dose-response curves usually exhibit a plateau above which an increase in stimulus is not matched by an increased response. Nevertheless, selecting more attractive varieties due to HIPV release upon herbivore attack has the major advantage that target and signal coincide. Plant varieties attractive due to green leaf volatiles may attract more predators from the environment surrounding the crop, but share the disadvantage with predator lures that the volatiles are not associated with prey and predators may be confused by the omnipresence of cues within the crop.

**Elicitors to induce HIPV production**

Exogenous application of jasmonic acid and release of volatile methyl jasmonate activates the octadecanoid pathway in plants which in turn triggers the production of proteinase inhibitors, oxidative enzymes (Farmer and Ryan 1990, Thaler *et al*. 1996, Adviushko *et al*. 1997, McConn *et al*. 1997), but also of plant volatiles known to attract natural enemies of herbivores (Hopke *et al*. 1994). Thus, these treatments have multiple effects involving both direct and indirect defenses against herbivores (Thaler in Agrawal *et al*. 1999).
Treating plants with an exogenous elicitor (jasmonic acid) is the most elegant way of producing predator lures; the plant itself produces most of the right ingredients! Whether such application to crops can yield less herbivore damage remains to be seen. For one thing herbivory also triggers these defenses on site and, for another, application away from the site of herbivore attack may cause the plant to invest in defenses when herbivore densities are low, thus reducing crop yield. Again, there is the danger of attracting predators to places without herbivores, in fact, the bouquet produced by plants consists of major and minor compounds and is thus a realistic mimic of HIPV. This is an extra reason for caution since predators may have difficulty learning to discriminate between JA-induced plant volatiles and plant volatiles induced by herbivory.

**Intercropping with plants attractive to predatory arthropods**

The use of this technique has recently been illustrated by Khan et al. (1997). They intercropped maize in Africa with molasses grass, a plant that was not colonized by stem-borers, a major pest of maize in this area. When undamaged, this grass normally produces several volatiles that are otherwise known as HIPV, such as β-caryophyllene, humulene and 4,8-dimethyl-1,3(Δ)7-nonatriene. The entire grass plant, as well as one of these volatiles in pure form (nonatriene), was attractive to stem-borer parasitoids, whereas plant volatiles extracted by hydrodistillation deterred oviposition by the stem-borers. Indeed, intercropping resulted in an increase in rate of parasitism and a decrease of stem-borer attacks. This study suggests that undamaged plants releasing volatiles that are otherwise only produced upon herbivore attack may improve parasitoid efficiency, but the role of the volatiles is by no means proven. Moreover, the use of such plants as natural lures faces some of the same problems as described in the previous section.

However, if the alternate crop produces apart from HIPV also additional cohorts of predators, this objection does not apply. In this thesis such an example was already mentioned in several chapters. Pear orchards are frequently bordered by trees and shrubs producing predatory bugs, and without any doubt also HIPV. In the Netherlands, alder and hawthorn are inhabited by a rich predatory fauna. Some evidence is available for the production of HIPV by alder (Drukker, Bruin and Posthumus, unpublished data). Both alder and hawthorn are traditionally planted in hedges surrounding orchards as windbrakes. Both shrubs are ideal for two reasons: (1) they produce *Psylla*-predators, while their inhabiting psyllid species do not feed on fruit trees, and (2) they form refuges for predators when orchards are sprayed with insecticides. In France and Switzerland hedge-planting strategies are being developed to optimise this role for predator *reservoirs* (Staubli et al. 1992; R. Rieux, pers. comm.). Shrubs were selected for the amounts of anthocorids they harbour. It should be interesting to select shrubs producing blends of HIPV, similar to those produced by *Psylla*-infested pear trees in order to provide the predators with the right conditioning.

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**GENERAL DISCUSSION**


