Tansley review

Indirect defence via tritrophic interactions

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Summary

Many plants interact with carnivores as an indirect defence against herbivores. The release of volatile organic compounds (VOCs) and the secretion of extrafloral nectar (EFN) are induced by insect feeding, a response that is mediated by the plant hormone, jasmonic acid. Although VOCs mainly attract predatory mites and parasitic wasps, while EFN mainly attracts ants, many more animal–plant interactions are influenced by these two traits. Other traits involved in defensive tritrophic interactions are cellular food bodies and domatia, which serve the nutrition and housing of predators. They are not known to respond to herbivory, while food body production can be induced by the presence of the mutualists. Interactions among the different defensive traits, and between them and other biotic and abiotic factors exist on the genetic, physiological, and ecological levels, but so far remain understudied. Indirect defences are increasingly being discussed as an environmentally-friendly crop protection strategy, but much more knowledge on their fitness effects under certain environmental conditions is required before we can understand their ecological and evolutionary relevance, and before tritrophic interactions can serve as a reliable tool in agronomy.

I. Introduction

Some of the most central functions enabling plant survival and reproduction depend on mutualisms. Plants cooperate with animals for pollination and dispersal, and the majority of plants rely on mycorrhizal fungi for the uptake of mineral nutrients. Similarly, many species of higher plants interact with animals of the third trophic level, the carnivores, in order to gain protection from the second trophic level, that is, the herbivores and pathogens.

'Indirect defence' is generally used when plants attract, nourish or house other organisms to reduce enemy pressure. This term was apparently introduced into the literature only some 20 yr ago (Dicke & Sabelis, 1988), but the phenomenon has been under investigation for more than three centuries, as myrmecophytes – plants engaged in obligate mutualisms with ants – were described by some of the earliest European ecologists who worked in the New World (Cobo, 1653; Belt, 1874; Wheeler, 1942). The defensive effect of ants is so conspicuous that there is a long history of using these animals as biocontrol agents. In China, artificial ants' nests have been used for centuries in *Citrus* plantations, and people in various tropical countries traditionally bring ants' nests to cacao and other plantations (Philpott & Foster, 2005; Rico-Gray & Oliveira, 2007).

Ants are, however, not the only group of animals that are engaged in protective interactions with plants. In fact, tritrophic interactions among plants, herbivores and carnivores can generally be influenced by plants as a strategy of defence (Price *et al.*, 1980). Traits expressed in this context are volatile organic compounds (VOCs), extrafloral nectar (EFN), food bodies (FBs), and structures used as refuges or nesting space (domatia; see Fig. 1). Hence, plants may provide information, food or housing to obtain the protective service (Bronstein *et al.*, 2006) from putatively mutualistic carnivores. Many VOCs are induced in response to herbivory and thus represent an active 'cry for help', as does EFN (see Section III. 2).

Several comprehensive overviews have been published on induced plant resistance (Karban & Baldwin, 1997; Agrawal *et al.*, 1999; Tollrian & Harvell, 1999). However, the field lacks communication among disciplines, as VOCs, in particular, have been investigated independently of ant–plant interactions. Although interactions among the different traits are likely, they are usually treated separately (but see Turlings & Wäckers, 2004), and empirical studies on more than one indirect defence trait have mainly concentrated on myrmecophytes (Heil & McKey, 2003).

This review gives an overview on the ecology of indirect defence. Using the book chapter by Turlings & Wäckers
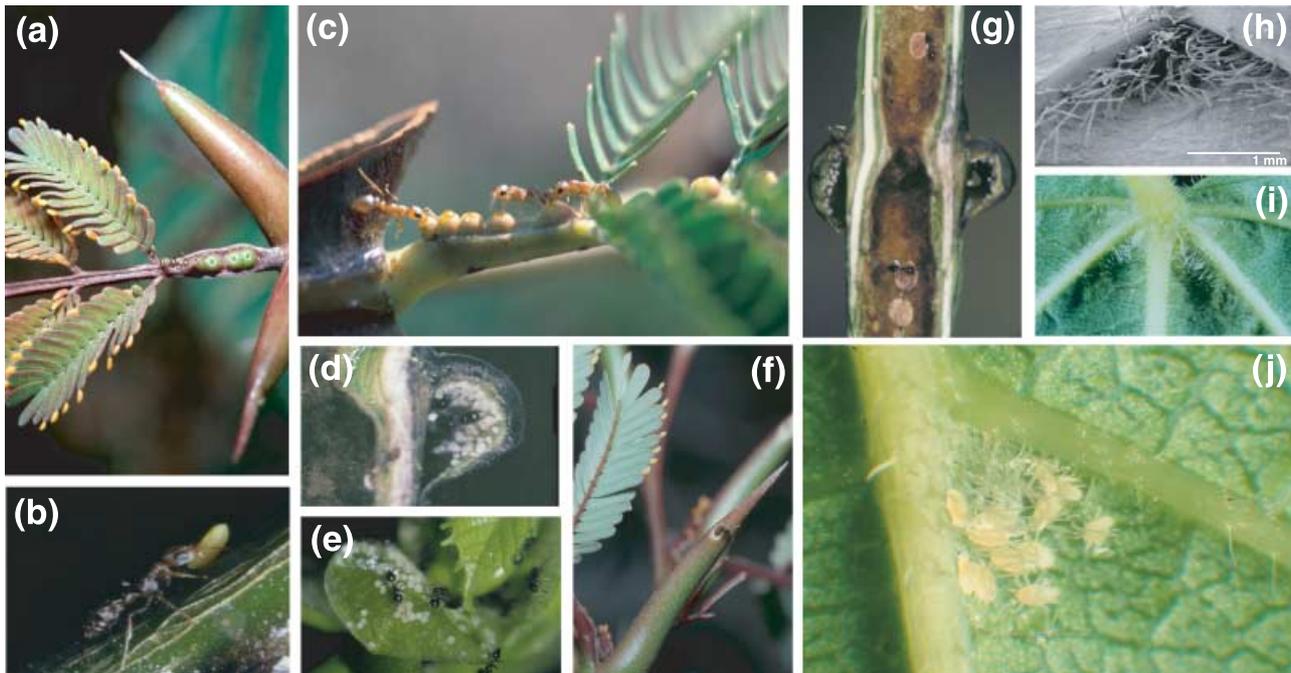
Fig. 1 Anatomical traits that serve indirect defence via tritrophic interactions. Obligate myrmecophytes house and nourish symbiotic ant colonies that function as obligate indirect defence mechanisms, but plants may also provide defenders such as ants and mites with mere nesting space. (a) Young leaf of *Acacia collinsii* with food bodies on the leaflet tips, extrafloral nectaries on the rachis and swollen stipular thorns. (b) *Pseudomyrmex ferrugineus* carrying food body. (c) *Pseudomyrmex peperi* workers consuming extrafloral nectar of *Acacia collinsii*. (d) Food bodies produced under a recurved stipule of *Macaranga bancana*. (e) *Crematogaster* ants collecting food bodies on the surface of *Macaranga hosei* stipule. (f) *Pseudomyrmex* ant leaving domatium in hollow thorn of *Acacia hindsii*. (g) *Crematogaster* ants and scale insects in caulinary domatium of *Macaranga bancana*. (h, i) SEM and light microscopy pictures of mite domatia of cotton. (j) Predatory mites in cotton domatium. (© M. Heil (a–g); © Andrew Norton, Colorado State University (h); © Joe Ogrodnick, Cornell University (i, j)).

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(2004) as a starting point, I will particularly highlight the functional and physiological parallels among the different defensive means. Future studies should consider all defensive traits of a plant, for example by simultaneously investigating both direct and indirect defences, or by making use of those species that exhibit more than an indirect defence trait (Arimura *et al.*, 2005). Only this strategy will allow an understanding of the interactions among different types of defence, and of their manifold interactions with plant metabolism and with the environment.

II. Facultative indirect defences

1. Biology of facultative indirect defences

The number of volatile compounds that are released from plant flowers, vegetative parts or roots, exceeds 1000 (Dudareva *et al.*, 2006; Pichersky *et al.*, 2006). While flower scents are usually released in an ontogenetically programmed way, the quantity and quality of VOCs that are released from vegetative plant parts and roots can change dramatically when plants are damaged (Turlings *et al.*, 1995; Tumlinson *et al.*, 1999; Farmer, 2001). Carnivorous mites were observed to use volatiles released from spider mite-infested lima bean (*Phaseolus lunatus*) plants to localize their prey (Dicke, 1986). After this initial observation, the idea that herbivore-induced VOCs function as an indirect defence was rapidly confirmed (Dicke & Sabelis, 1988; Dicke *et al.*, 1990; Turlings *et al.*, 1990). It is now widely accepted that VOCs can attract predatory arthropods and/or repel herbivores and thus serve as a means of plant resistance (Dicke, 1999; Tumlinson *et al.*, 1999; Dicke *et al.*, 2003b; Turlings & Wäckers, 2004; Arimura *et al.*, 2005) (Fig. 2). However, other functions of VOCs are still being discovered and range from direct roles in protection from microorganisms (Peñuelas & Llusiá, 2004; Kishimoto *et al.*, 2005; Shiojiri *et al.*, 2006) or abiotic stress (Loreto & Velikova, 2001; Velikova *et al.*, 2005; Dudareva *et al.*, 2006; Behnke *et al.*, 2007) to functions as plant 'pheromones' (compounds serving in communication among plants; Baldwin & Schultz, 1983; Rhoades, 1983; Dolch & Tscharntke, 2000; Karban *et al.*, 2000), or as plant 'hormones' (compounds serving in within-plant signalling; Karban *et al.*, 2006; Frost *et al.*, 2007; Heil & Silva Bueno, 2007). Owing to their direct, physiological functions, it has even been suggested that the role of VOCs in tritrophic interactions results from carnivores making use of their unavoidable loss from plants, rather than representing their evolutionary reason of being (Peñuelas & Llusiá, 2004).

Extrafloral nectar is functionally not involved in pollination. Extrafloral nectar secretion has been observed on the shoots, the leaves (Fig. 1c) and the inflorescences of plants belonging to more than 300 genera (Elias, 1983; Koptur, 1992). Since these comprise angiosperms, gymnosperms and even ferns, EFN appears evolutionarily more ancient than floral nectar (Heil, 2007). As for VOCs, a 'physiological theory' (extrafloral nectaries serving in secreting excess carbohydrates) originally competed with an ecological explanation, and some authors regarded the defensive idea as successfully rejected (Schremmer, 1969). An ever-increasing number of studies, however, have since demonstrated convincingly that the attraction of predators to EFN can reduce herbivory rates in nature (reviewed in Bentley, 1977; Koptur, 1992; Heil & McKey, 2003; Rico-Gray & Oliveira, 2007), and there is now general agreement that extrafloral nectaries serve ecological interactions. There are still alternative interpretations in the case of EFN, however, as some authors suggested a function in the distraction of ants from flowers (Wagner & Kay, 2002).

Anatomical structures for which the role in indirect defence was suggested very early are FBs, cellular structures containing mainly carbohydrates, proteins and lipids (O'Dowd, 1982; Webber *et al.*, 2007). Charles Darwin (Darwin, 1877) was apparently the first person to use the term 'food bodies', which he applied to small structures at the leaflet tips of Central American *Acacia* shrubs (Fig. 1a) and on hairy pads located at the leaf bases of *Cecropia peltata*. Food bodies serve as food for ants (Fig. 1b) engaged in both facultative and obligate mutualisms (O'Dowd, 1982; Heil & McKey, 2003; Webber *et al.*, 2007). However, in spite of their wide taxonomic distribution (O'Dowd, 1982), little scientific effort has ever been spent on FBs other than those produced by obligate myrmecophytes.

Plants can increase predator densities also by offering physical structures that serve as nesting or refuge sites. The most prominent example are ant domatia (Fig. 1g), hollow structures inhabited by ants that are engaged in facultative or obligate ant–plant mutualisms (see Section IV. 1). Other domatia types may also house smaller predators such as mites and bugs (Fig. 1h–j). These domatia are generally localized on leaves (O'Dowd & Willson, 1991). While ant domatia appear restricted to the tropics, leaf domatia are also known from temperate regions (Walter, 1996; Romero & Benson, 2005). Removal of leaf domatia reduced the abundance of predatory mites on *Viburnum tinus* (Grostal & O'Dowd, 1994), and their experimental addition to cotton plants significantly increased numbers of predatory thrips and bugs and enhanced plant performance (Agrawal *et al.*, 2000). Defensive effects of mites housed in leaf domatia have also been demonstrated in nature (Romero & Benson, 2004), and mycophagous mites housed in such domatia can even protect plants from fungal infection (English-Loeb & Norton, 2006; Monks *et al.*, 2007).

2. Animals involved in facultative interactions

On the side of carnivorous animals attracted to VOCs, research focused mainly on those organisms for which the phenomenon was first described, that is, predatory mites such as *Phytoseiulus persimilis* (Dicke & Sabelis, 1989; Dicke, 1999) and parasitic wasps such as *Cotesia marginiventris* (Turlings *et al.*, 1990, 1995; Turlings & Tumlinson, 1992). Scattered reports exist on the attraction of predatory nematodes (Rasmann *et al.*, 2005),
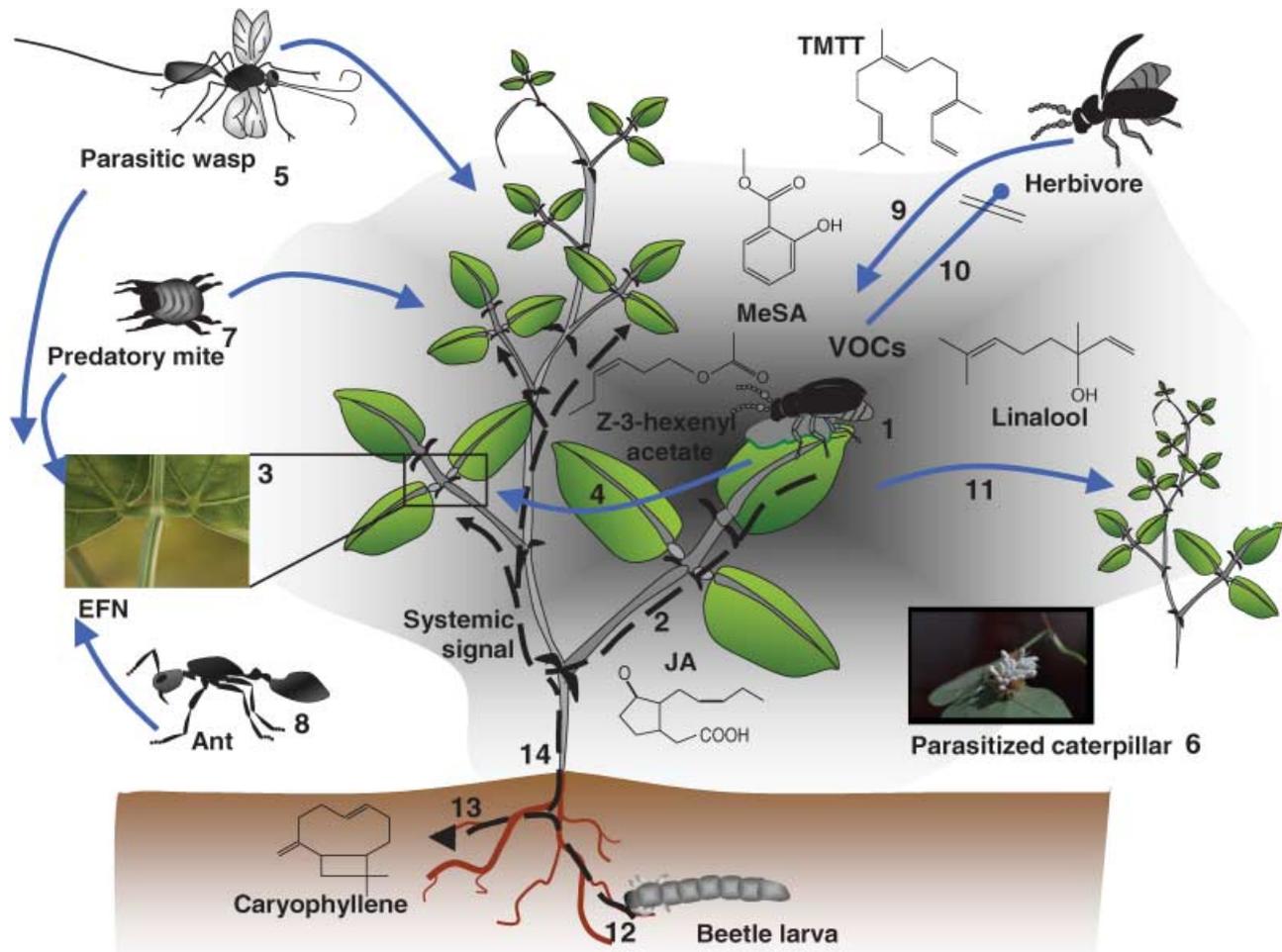
Fig. 2 Biological effects and interactions of volatile organic compounds (VOCs) and extrafloral nectar (EFN). Feeding by a herbivore (1) elicits the octadecanoid cascade (Fig. 3) that leads to the synthesis of jasmonic acid (JA) (2), which induces the release of VOCs and of EFN (3) from both the damaged and intact leaves. Several VOCs, such as Z-3-hexenyl acetate (4), induce indirect defences (EFN, VOCs etc.) in as-yet-undamaged leaves of the attacked plant. The VOCs then attract parasitic wasps (5) that parasitize herbivorous caterpillars (6) or beetles, and they attract predatory mites (7) that feed on smaller herbivores such as spider mites. Both wasps and mites also feed on EFN, as do ants (8), and both ants and mites may be also housed in domatia (Fig. 1). For the response of both con- and heterospecific herbivores, attraction by VOCs (9) as well as repellent effects (10) have been reported. Volatile organic compounds can also be perceived by other plants belonging to the same or a different species (11), which may be primed or directly induced depending on the concentration of VOCs in the headspace. These interactions do not stop above ground, since feeding on leaves can result in the transport of a systemic signal to roots where it elicits the synthesis of defensive compounds, such as nicotine, while feeding on roots by, for example, beetle larvae (12) can induce the release of VOCs, such as (E)-β-Caryophyllene, from roots (13) and also elicit a systemic signal leading to the induced production of EFN or release of VOCs from above-ground parts (14). © C. Kost, Lima bean nectaries (3); © M. Heil, (6). MeSA, Methyl salicylate; TMTT, 4,8,12,-Trimethyl-1,3,7,11,-tridecatetraene.

flies (Hulcr *et al.*, 2005), bugs (Moayeri *et al.*, 2007; Mochizuki & Yano, 2007) and thrips (Shimoda *et al.*, 1997), and VOCs have also been reported to repel herbivores (De Moraes *et al.*, 2001; Kessler & Baldwin, 2001). Some studies used baits with certain VOCs to study the species of parasitoids attracted (James, 2003; James & Price, 2004), but no field studies investigated the role of VOCs in shaping arthropod communities under natural conditions (but see Bernasconi Ockroy *et al.*, 2001 for a study in an agricultural environment).

Volatile organic compounds usually form complex blends that depend on both the genotype of the plant (Loughrin *et al.*, 1995; Halitschke *et al.*, 2000; Fritzsche-Hoballah *et al.*, 2002) and the species and developmental stage of the attacking herbivore (Dicke, 1994, 1999; Takabayashi *et al.*, 1995; Takabayashi & Dicke, 1996; Ozawa *et al.*, 2000). Carnivores can discriminate between damaged and undamaged plants, between plants infested by different herbivore species, and between different plant species infested by the same herbivore (Dicke, 1994). Volatile organic compounds thus have the potential to mediate complex plant–carnivore interactions, which gives the plant an opportunity for fine-tuning its defence according to its actual needs. For example, herbivore-damaged

tobacco plants release different volatile blends during the day and night, to attract parasitoids during the day or repel specific herbivores during the night (De Moraes *et al.*, 2001), and they can also suppress the induction of nicotine in favour of the release of VOCs when attacked by a nicotine-insensitive, specialized herbivore (Kahl *et al.*, 2000). Although positive effects of herbivore parasitation on plant fitness are likely (van Loon *et al.*, 2000), it is strongly dependent on both the type of attacking herbivore and the ecological and developmental situation of the plant whether it is better for the plant to attract parasitoids or predators of the feeding herbivore, predators or parasitoids of herbivore eggs, or to directly repel the herbivores.

Research on EFN consumers has, in general, focused on ants (Bentley, 1977; Heil & McKey, 2003), that is, the quantitatively dominating group of predators in the world. Owing to their effective foraging and recruiting strategies, ants rapidly find and monopolize attractive food sources, and they defend these food sources against putative competitors, including herbivores. Increased ant numbers also inevitably mean an increased predation pressure on herbivores. However, other arthropods belonging to the Araneae, Coleoptera, Dermaptera, Diptera, Hemiptera, Lepidoptera and Orthoptera visit extrafloral nectaries (Koptur, 1992). Some visitors, such as mosquitoes (Foster, 1995) and flies (Heil *et al.*, 2004c), consume EFN, but, because of their life history, are less likely to protect plants, while other nonant EFN consumers may also act as defenders, as has been described for predatory mites and ladybird beetles (Pemberton, 1993; Pemberton & Vandenberg, 1993; van Rijn & Tanigoshi, 1999), ichneumonid and braconid wasps (Bugg *et al.*, 1989; Stapel *et al.*, 1997; Cuautle & Rico-Gray, 2003), lacewing larvae (Limburg & Rosenheim, 2001) and spiders (Ruhren & Handel, 1999; Whitney, 2004). However, while several studies investigated effects of EFN-bearing plants on the ant community (Bentley, 1976; Blüthgen *et al.*, 2000; Díaz-Castelazo *et al.*, 2004; Oliveira & Freitas, 2004; Kost & Heil, 2005), similar work on nonant arthropods is scarce (but see Kost & Heil, 2005).

3. Chemical ecology of facultative indirect defences

Compounds that dominate the headspaces of herbivore-damaged plants are alcohols, esters, aldehydes and various terpenoids (Dudareva *et al.*, 2006; Pichersky *et al.*, 2006). Some substances are immediately released after damage, cause the characteristic odour of freshly mowed pastures, and are therefore called green-leaf volatiles (GLVs). The majority of these substances are isomers of hexenol, hexenal or hexenyl acetate. Some preformed GLVs 'bleed' instantaneously from disrupted tissue (Turlings & Wäckers, 2004), but the rest of these compounds are released rapidly upon damage, since the first intermediate of the octadecanoid cascade, 13-hydroperoxy-linolenic acid, also acts as an intermediate for the synthesis of 6-carbon volatiles (Walling, 2000; Gatehouse, 2002) (Fig. 3).

In contrast, the release of esters such as methyl salicylate and methyl jasmonate, of monoterpenes such as limonene, linalool or ocimene, and of sesquiterpenes such as bergamotene, caryophyllene and farnesene, typically starts 24 h after attack (Paré & Tumlinson, 1997b; Turlings *et al.*, 1998; Dudareva *et al.*, 2006; Pichersky *et al.*, 2006).

The synthesis of these compounds is induced by leaf chewers such as beetles, plant hoppers and caterpillars (Dicke, 1999; Williams *et al.*, 2005) and also in response to feeding by spider mites, herbivorous bugs, aphids and whitefly (Dicke, 1999; Walling, 2000; Colazza *et al.*, 2004b; Moraes *et al.*, 2005; Williams *et al.*, 2005). The type of feeding damage clearly affects the VOCs produced, and a part of the biochemical explanation is that leaf chewers in general induce only jasmonic acid (JA) signalling (Fig. 3), while piercing-sucking herbivores (phloem feeders and single-cell feeders) tend to induce salicylic acid-mediated resistance pathways as well (Walling, 2000; Leitner *et al.*, 2005; Smith & Boyko, 2007). Even mere oviposition on plant surfaces can be enough to affect release of VOCs (Meiners & Hilker, 2000; Hilker & Meiners, 2006) and then usually serves the attraction of egg parasitoids (Meiners & Hilker, 2000; Colazza *et al.*, 2004a).

Using VOCs as the only source of information, carnivores can discriminate among plants infested by different herbivore species (e.g. hosts and nonhosts) and among different plants infested by the same herbivore (Dicke, 1994, 1999; Du *et al.*, 1996; De Moraes *et al.*, 1998; Powell *et al.*, 1998). Volatile blends even vary in response to damage by different instars of the same species (Takabayashi *et al.*, 1995). However, this high specificity is not reflected in the level of the single substances, as surprisingly few compounds are reported as herbivore-induced VOCs, most being released from many different plant species (Dudareva *et al.*, 2006; Pichersky *et al.*, 2006). Plants naturally grow in mixed stands, and carnivores must be able to distinguish their blends from the general background, an aspect overlooked in almost all laboratory studies (Dicke *et al.*, 2003a). In an apparent contrast to the above findings, other studies reported that naturally occurring carnivores responded to isolated compounds (Kessler & Baldwin, 2001; James, 2003; James & Price, 2004; Rasmann *et al.*, 2005). The general pattern appears to be that biochemical investigations find highly characteristic blends with respect to the genotype and developmental stage of both plants and herbivores, and that carnivores are able to distinguish among such blends under laboratory conditions or in simplified agronomic ecosystems, while single VOCs already have a generally attractive function in the complex, natural environment.

To complicate the picture, the majority of herbivore-induced VOCs are also released from flowers (Dicke *et al.*, 1990; Dudareva *et al.*, 2006; Pichersky *et al.*, 2006). In short, the current picture demonstrates a high functional diversity in VOC-mediated communication within and among organisms, but it leaves us with the open question of how misunderstandings in all these communications are avoided (Heil, 2007). How do pollinators
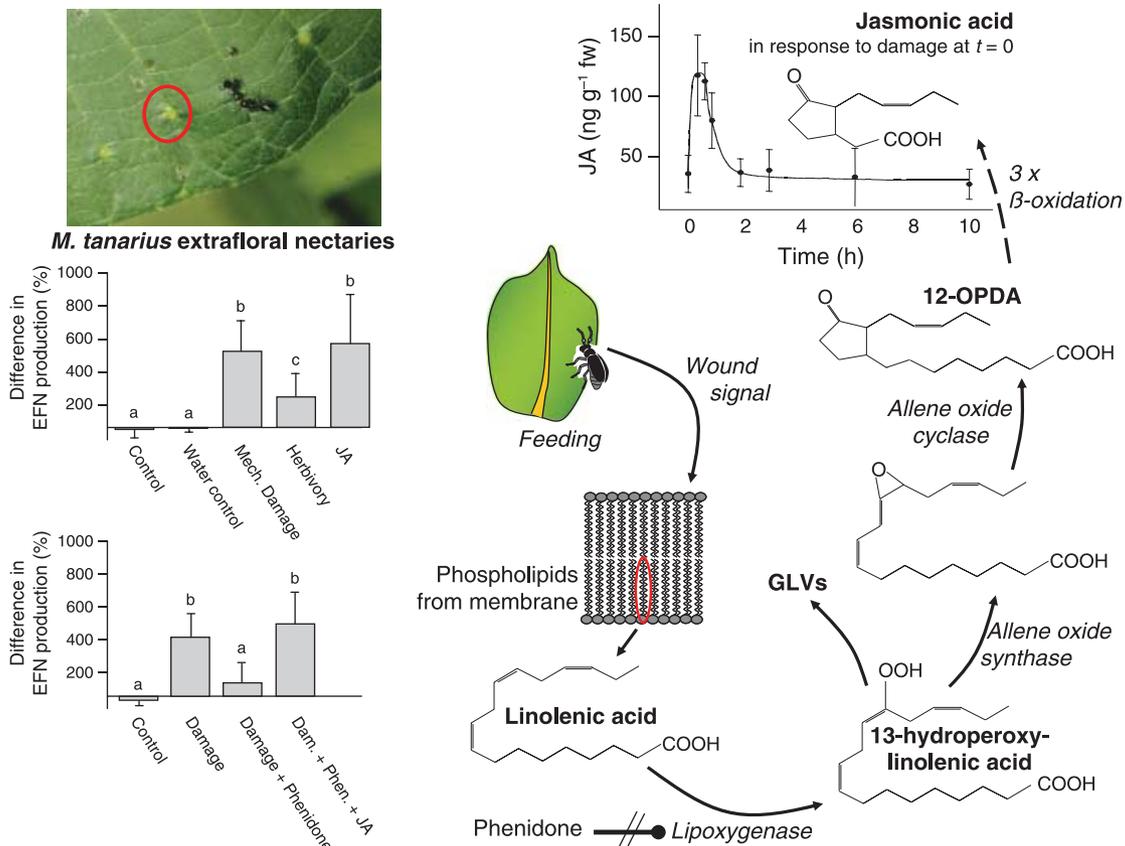
Fig. 3 Role of jasmonic acid (JA) in extrafloral nectar (EFN) induction and the octadecanoid cascade. Extrafloral nectaries on the leaf blade of *Macaranga tanarius* plants are visited by ants. Extrafloral nectar secretion responds positively to mechanical damage, herbivory and exogenous application of an aqueous 1 mM solution of JA, yet not to the application of mere water. The involvement of endogenous JA, whose content increases transiently to up to 120 ng g^{-1} fresh weight in response to damage, was confirmed by the observation that application of phenidone (an inhibitor of lipoxygenase) inhibited the induction of EFN secretion after damage, which could be readily restored when JA was applied additionally to the phenidone treatment. Therefore, the octadecanoid cascade (starting with the release of linolenic acid from biomembranes and leading via several enzymatic steps to the synthesis of JA) is causally involved in the induction of EFN secretion by herbivory or mechanical damage. Bar diagrams and panel on endogenous JA in response to damage at *t* = 0 redrawn from Heil *et al.* (2001b), information on the octadecanoid cascade following Creelman & Mullet (1997a) and Gatehouse (2002). GLVs, green-leaf volatiles; 12-OPDA, 12-oxo-phytodienoic acid.

avoid being attracted by the 'flowerish' odour released from damaged leaves of plants, and how do carnivores searching for herbivores avoid being attracted to flowers? All these questions remain to be studied.

Just as with floral scents and herbivore-induced volatiles, floral and extrafloral nectars may also consist of similar or identical compounds, although their detailed blends can be quite different (Baker *et al.*, 1978; Koptur, 1994). Extrafloral nectar contains mainly mono- and disaccharides (fructose, glucose and sucrose) and free amino acids dissolved in water (Koptur, 1994), but scattered reports exist as to the presence of fatty acids and phospholipids (Stone *et al.*, 1985). Water *per se* can be an important resource (Ruffner & Clark, 1986), but most consumers prefer EFN rich in sugars and amino acids (Baker *et al.*, 1978; Ruffner & Clark, 1986; Smith *et al.*, 1990; Lanza, 1991; Koptur, 1994). Several nonprotein amino acids have been discovered in EFN (Inouye & Inouye, 1980)

and have been discussed as a chemical protection from non-adapted 'nectar thieves'. Even the detailed identity of carbohydrates can be functionally important. As an example, EFN of *Acacia* myrmecophytes has invertase activity to keep it free of sucrose, a disaccharide being generally attractive to hymenopterans. This EFN is thus unattractive to nonsymbiotic ants that might compete with the plants' obligate *Pseudomyrmex* ant partners. The ant inhabitants of these plants, in turn, lack invertase activity in their digestive tracts and thus depend on the 'predigested' EFN of their hosts (Heil *et al.*, 2005).

Other proteins in EFN apparently serve the protection from microbe infection (M. F. Gonzales-Teuber & M. Heil, unpublished), as has been described for floral nectar of tobacco (Carter *et al.*, 1999, 2007; Carter & Thornburg, 2004). From behavioural studies it is now clear that EFN releases odours that facilitate the orientation of carnivores (Röse *et al.*, 2006). These odours, however, remain to be chemically characterized,

and even floral nectar odours have only recently been investigated (Kessler & Baldwin, 2007). In short, the detailed composition of EFN plays a crucial role in its ecological functions, but new substance classes are still being discovered and remain to be chemically and functionally characterized.

4. Evidence for plant–carnivore mutualism

Plant–carnivore interactions that are mediated via EFN or VOCs are generally assumed to be mutualisms. However, positive net effects on plant fitness rather than just reduced herbivory need to be demonstrated before a plant trait can be termed a 'defence' (Karban & Baldwin, 1997), and positive fitness effects on the attracted arthropods must additionally be demonstrated before these interactions can be termed 'mutualisms' between the first and third trophic levels. How good is the evidence for EFN and VOCs?

Higher parasitation rates of herbivores have been observed in nectary-bearing than in nectary-free trees (Pemberton & Lee, 1996; Mathews *et al.*, 2007), and when sugars were applied as an EFN mimic (Jacob & Evans, 1998), but it is not known how this feeds back to plant fitness. By contrast, hundreds of studies have excluded ants from EFN-producing plants and found higher rates of herbivory in ant-free than in ant-tended plants (Bentley, 1977; Koptur, 1992; Heil & McKey, 2003). The drawback of this attempt is that it also excludes crawling herbivores (Freitas *et al.*, 2000; Kost & Heil, 2005). Some researchers applied chemical elicitors or herbivores to induce EFN secretion and reported defensive effects of the attracted ants (Heil *et al.*, 2001b; Ness, 2003), a result that may, however, be influenced by other defensive plant traits also responding to the inducing agent. The most straightforward approach is the experimental application of EFN. While Tempel (1983) did not find a protective effect of sugars externally applied to bracken fern, other studies did indeed find that ants attracted to experimentally applied sugars can significantly reduce herbivory rates (Bentley, 1976; Kost & Heil, 2005).

The outcome of such mutualisms depends on abiotic conditions, identity of the visiting predators, and the type and amounts of herbivores present. Several studies therefore failed to find a clear defensive effect of ants attracted to EFN (O'Dowd & Catchpole, 1983; Tempel, 1983; Rashbrook *et al.*, 1992; Freitas *et al.*, 2000), and even a higher protection by ants and a resulting higher initial fruit set does not necessarily translate to a higher fitness, when factors such as soil nutrients limit fruit production (Oliveira, 1997). However, in spite of these environment-driven uncertainties, many studies found positive effects of ant attraction to EFN on fitness-relevant plant traits (Bentley, 1977; Horvitz & Schemske, 1984; delClaro *et al.*, 1996; Oliveira *et al.*, 1999; Sobrinho *et al.*, 2002; Kost & Heil, 2005).

Much less is known about the importance of EFN for the consumers. As pointed out by Turlings & Wäckers (2004),

'extrafloral nectar by itself falls short from providing a well-balanced diet'. Behaviour and survival of adult parasitoids are energy-limited, and access to carbohydrate sources thus usually has a positive effect on their survival rates, the time they stay on a particular plant, and even on parasitation rates (Stapel *et al.*, 1997; Jacob & Evans, 1998; van Rijn & Tanigoshi, 1999; Gnanvossou *et al.*, 2005; Röse *et al.*, 2006; Olson & Wäckers, 2007). However, how important is EFN for ants? Several studies tried to address this question and found that EFN makes up a relevant part of the visiting ants' diet (Hölldobler & Wilson, 1990). As ant workers usually feed on carbohydrates while providing proteins and lipids to their larvae, a carbohydrate-biased food source such as EFN might even improve their activity and competitiveness (Davidson, 1997) and thus, indirectly, their need for proteins. Plants can obviously turn ants into even better predators by providing them with carbohydrates. However, no study has convincingly shown a fitness benefit of EFN for ants in facultative interactions (Bronstein, 1998).

Similarly, the character of VOC-mediated tritrophic interactions is not entirely clear. In this case, the benefit for the animal side is obvious, since many parasitoids and predators rely partly, or even completely, on plant-derived traits to localize their prey or host species (Vet & Dicke, 1992; Turlings & Wäckers, 2004), and since increased parasitation or predation rates directly affect the carnivore fitness. Specialists, in particular, often use very specific compounds released from the hosts of their hosts (Steidle & van Loon, 2003). In this case, the uncertainties remain with the plant. While laboratory studies indeed reported that feeding by parasitized caterpillars reduced *Arabidopsis* fitness to a lesser extent than feeding by unparasitized caterpillars (van Loon *et al.*, 2000), it is only during recent years that investigations have been conducted under realistic conditions. Planting an odorous grass into maize fields reduced herbivore damage. This grass, *Melinitis minutiflora*, constitutively emits a compound that is typically released by maize in response to caterpillar damage and that attracted parasitoids (Khan *et al.*, 1997). Field trials demonstrated that the specialist parasitic wasp *Cardiochiles nigriceps* used plant odours to localize plants infested by its host, *Heliothis virescens* (De Moraes *et al.*, 1998). Thaler (1999) found that caterpillars suffered higher parasitation rates when caged near tomato plants that were induced with JA to release VOCs, and more parasitic wasps were trapped near induced maize plants in a corn field (Bernasconi Ockroy *et al.*, 2001). Artificially applied (*Z*)-3-hexene-1-ol, Linalool, and (*Z*)-α-bergamotene increased predation rates of *Manduca sexta* eggs on *Nicotiana attenuata* plants (Kessler & Baldwin, 2001); methyl salicylate increased populations of predators and decreased populations of spider mites in grape vineyards and hop yards (James, 2003; James & Price, 2004); and (*E*)-β-caryophyllene released from corn plant roots in response to beetle damage was attractive to entomopathogenic nematodes (Rasmann *et al.*, 2005). Predators were also attracted to beetle-damaged bananas under field

conditions (Tinzaara *et al.*, 2005), and lima bean plants treated repeatedly with JA in nature released more VOCs and suffered less herbivory than controls, and they produced more leaves, flowers and fruits (Heil, 2004).

Lima bean, however, responds to JA with the induction of both VOCs and EFN (Heil, 2004). The latter study thus failed to nail down the observed defence effects to the VOCs. Net effects on plant fitness were not investigated by Kessler & Baldwin (2001), James (2003) or Rasmann *et al.* (2005), and the reduced herbivory in the intercropping experiment of Khan *et al.* (1997) could also have been caused by a repellent effect on the major maize pest rather than by an attraction of its parasitoids. As described for EFN, many factors can affect the outcome of a VOC-mediated tritrophic interaction. The previous experience of carnivores affects their behaviour towards particular VOCs (Turlings *et al.*, 1990; Petitt *et al.*, 1992; Krips *et al.*, 2001; De Boer *et al.*, 2005). Although VOC-mediated parasitation of herbivores can increase the fitness of the plants on which the herbivores feed (van Loon *et al.*, 2000; Fritzsche Hoballah & Turlings, 2001a), this does not represent a necessary outcome of the interaction, which for instance depends on the intensity of the parasitation and on the competition of herbivores for suitable hosts. Finally, the various herbivore or carnivore species present, with different responses to VOCs, may show complex interactions under natural conditions. For example, a parasitic wasp and an entomopathogenic nematode are both attracted to maize plants damaged by their respective host, but this attraction can be significantly reduced when both herbivores simultaneously feed on a plant (Rasmann & Turlings, 2007). Demonstrations of a VOC-mediated predation pressure on single herbivores (De Moraes *et al.*, 1998; Thaler, 1999; Kessler & Baldwin, 2001) are thus not sufficient to prove the defensive function of VOCs, and whether or not their role in tritrophic interactions represents a driving evolutionary force for the plants remains to be demonstrated.

Generalizations on the defensive role of EFN or VOCs are further complicated when the putative defenders exclude other predators (Mody & Linsenmair, 2004) or when these traits interact directly with the herbivores. Herbivore-induced VOCs can repel herbivores and then serve as direct rather than indirect defences (Dicke & Dijkman, 1992; Birkett *et al.*, 2000; De Moraes *et al.*, 2001; Kessler & Baldwin, 2001; Dugravot & Thibout, 2006). Less welcome for the plant is the attractive effect of cotton EFN on herbivorous moths (Beach *et al.*, 1985). VOCs, in particular, may simply signal the presence of a host plant and thus attract rather than repel searching herbivores, thereby forming a double-edged sword in plant defence (Loughrin *et al.*, 1996; Bolter *et al.*, 1997; Dicke, 1999; Kalberer *et al.*, 2001; Horiuchi *et al.*, 2003; Carroll *et al.*, 2006).

In summary, a positive effect on plant fitness has been shown repeatedly for ants attracted to EFN but never convincingly so for VOC-mediated interactions, while good evidence for positive effects on the attracted carnivores' fitness exists for parasitoids attracted to EFN or VOCs, yet not for the ant–EFN interaction. Research to date has left us with two 'half-supported mutualisms'.

5. When and where should plants exhibit indirect defence?

Plants should be under an evolutionary pressure to optimize their defensive investments according to abiotic growing conditions, herbivore pressure and the value of the defended organ. The optimal defence hypothesis (ODH; McKey, 1974, 1979; Rhoades, 1979) assumes herbivore pressure and fitness consequences of herbivory to constitute important evolutionary forces that vary among plant organs. Defensive needs are determined by an organ's value, the cost to the plant of its herbivore-inflicted damage or loss, and its vulnerability, the probability that the organ would be successfully attacked by herbivores in the absence of the defensive trait. In contrast, the resource availability hypothesis (RAH; Bryant *et al.*, 1985; Coley *et al.*, 1985) states that the optimal level of defence correlates negatively with the potential growth rate, since replacement of plant parts lost to herbivores is more costly when nutrients are limiting future growth, the relative impact of herbivory increases with decreasing inherent growth rate, and a percentage reduction in growth rate as a result of the cost of producing defences represents a greater absolute growth reduction for fast-growing species than for slow-growing species. Herms & Mattson (1992) pointed to the physiological trade-off between growth and differentiation processes, the latter also comprising defence. The growth-differentiation balance hypothesis (GDBH) assumes that nutrient-rich conditions favour growth and result in a low relative availability of carbon for secondary metabolism; inherently fast-growing species should thus have lower levels of defence than inherently slow-growing species (as also predicted by RAH). Most essentially, the described trade-offs restrict the possibilities to defend young, fast-growing plant parts, and in this respect GDBH directly contradicts ODH.

Induced defences allow plants to fine-tune their defensive investments according to the current herbivore pressure; their existence *per se* thus confirms the ODH. Even diurnal rhythms can be adapted to ODH requirements: EFN secretion by *Macaranga tanarius* peaks when herbivore activity is highest (Heil *et al.*, 2000), and VOCs released during the day and night can differ (Loughrin *et al.*, 1994; Kunert *et al.*, 2002) in order to elicit specific responses by certain arthropods (Turlings *et al.*, 1995; De Moraes *et al.*, 2001).

Induced defences are, moreover, generally regarded as cost-saving strategies, and empirical studies did indeed report low physiological costs of EFN or VOCs (O'Dowd, 1979; Fritzsche Hoballah & Turlings, 2001b). However, the production of VOCs can be limited by both light and soil nutrients (Gouinguené & Turlings, 2002) and thus is likely to incur considerable costs, at least under certain growing conditions. Since carnivores

represent a highly mobile defence (McKey, 1984), RAH and GDBH predict indirect defences to be particularly common in fast-growing species. These predictions are clearly confirmed in the case of obligate ant–plant interactions, which are most spectacularly evolved in pioneer trees (Heil & McKey, 2003). How good is the evidence for facultative interactions? Plant species known to employ VOC-mediated indirect defences comprise mainly fast-growing annuals from resource-rich sites, and even trees for which the phenomenon is reported are fast-growing species from secondary ecosystems (see lists of species in Dicke, 1999; Turlings & Wäckers, 2004; van den Boom *et al.*, 2004). However, this observation might be severely biased because of the general research focus on crop plants, and a wide screening of plant species is required to test whether the general distribution of VOCs can be satisfactorily explained by the RAH and/or the GDBH.

Water stress increased release of VOCs (Vallat *et al.*, 2005), as did nitrogen deficiency (Schmelz *et al.*, 2003), two observations that are in line with assumptions of the RAH. However, other studies reported contrasting results (Gouinguené & Turlings, 2002), and no generalizations as to the effect of abiotic factors on indirect defences can be drawn thus far. More is known about ontogenetic patterns, as EFN production generally depends on the developmental state of the secreting organ (Tilman, 1978; O'Dowd, 1979; Yokoyama & Miller, 1989; Heil *et al.*, 2000; Wäckers & Bonifay, 2004) with patterns fulfilling predictions of the ODH (Bentley, 1977; Heil *et al.*, 2000; Wäckers & Bonifay, 2004). Similarly, young leaves of *Glycine max* emitted more volatiles in response to herbivore feeding than older leaves (Rostás & Eggert, 2007). However, reproductive structures emitted no constitutive and very few inducible volatiles in the same study. This seeming contrast to the ODH might result from VOCs serving as long-distance signals for parasitoids rather than in host-finding at the within-plant level (Rostás & Eggert, 2007). ODH might generally apply more directly to spatial patterns in EFN secretion than in VOC release, since the distribution of EFN is more directly linked to its local efficiency. This might also explain why VOCs are generally induced systemically (Turlings & Tumlinson, 1992; Dicke, 1994; Paré & Tumlinson, 1999; Rostás & Eggert, 2007).

Hypotheses that have been formulated in the context of direct plant defence can help in understanding the within- and among-plant patterns in indirect defences, but more theoretical and empirical studies will be required to elucidate to what degree direct and indirect defences can be treated within the same theoretical frameworks.

III. Production of indirect defences

1. General production mechanisms

Volatile organic compounds are biochemically well characterized, and many genes and enzymes involved in their synthesis are known. The majority of VOCs are synthesized *de novo* after damage, and their metabolic origin is usually well defined (Paré & Tumlinson, 1997b; Dudareva *et al.*, 2006; Pichersky *et al.*, 2006). That selected biosynthetic pathways are completely known has been elegantly confirmed with plants that have been genetically engineered to alter their volatile release (Kappers *et al.*, 2005; Schnee *et al.*, 2006; Shiojiri *et al.*, 2006). However, experimental evidence for the biosynthetic origin of other plant volatiles is still missing (Pichersky *et al.*, 2006).

The scattered information on the chemical composition of EFN is mirrored by an even lower number of studies on its metabolic origin. Without consumers present, EFN secretion usually drops dramatically (Heil *et al.*, 2000, 2004b), and – being a herbivore-inducible trait – EFN is generally produced at very low rates by intact plants. However, the physiological and genetic mechanisms that underlie these phenomena remain unknown. Reabsorption has been described for floral nectar (Búrquez & Corbet, 1991; Stpiczyńska, 2003; Nepi *et al.*, 2007) but was never studied for EFN. Excised floral nectaries can secrete fructose, glucose and sucrose when only one of these sugars is provided as a substrate (Frey-Wyssling *et al.*, 1954). Starch is usually accumulating in secretory tissues of floral nectaries and is degraded when nectar secretion begins (Stpiczyńsa *et al.*, 2005; Ren *et al.*, 2007; Thornburg, 2007), but I am not aware of another study besides the one by Heil *et al.* (2005) that deals with mechanisms regulating the sugar composition in EFN. Nothing appears to be known about how amino acids, proteins, alkaloids and volatile compounds are secreted into the EFN. Many extrafloral nectaries have direct connections to xylem or phloem, or both (Elias, 1983), and the common opinion appears that EFN is directly derived from the contents of the vascular system. However, the nectaries' metabolic capacities (Frey-Wyssling *et al.*, 1954), the clear chemical differences between phloem sap and nectar, and the temporal secretion patterns along with its inducibility, make it clear that EFN production requires active, and thus far unidentified, synthetic and secretion processes.

2. Induction

Secretion rates or amino acid content of EFN increase in response to herbivory or mechanical damage (Mound, 1962; Stephenson, 1982; Koptur, 1989; Smith *et al.*, 1990). The first study demonstrating an increase in EFN secretion in response to herbivory (Stephenson, 1982) preceded the first reports on the role of odours of the damaged plant in the prey-searching behaviour of carnivores (Dicke, 1986) and therewith represents the first description of an induced indirect defence (Table 1). However, it was then shown rapidly for several species that damage dramatically changes the quantity and quality of VOCs released from plants (Turlings *et al.*, 1995; Paré & Tumlinson, 1997b; Tumlinson *et al.*, 1999; Farmer, 2001). These interactions are not restricted to the

Table 1 Keystone publications on indirect defence via tritrophic interactions

Year	Finding	Reference
1966	Myrmecophytes and their ant inhabitants are engaged in obligate defensive mutualisms	Janzen (1966)
1977	Meta-analysis shows that EFN attracts ants as 'pugnacious bodyguards'	Bentley (1977)
1982	EFN secretion is induced by herbivore feeding	Stephenson (1982)
1982	Distance to herbivore-damaged trees affects direct chemical defence of Sitka willow	Rhoades (1983)
1983	Herbivore resistance is induced in plants enclosed in the same air as damaged plants	Baldwin & Schultz (1983)
1986	Volatiles released from damaged plants facilitate host searching by carnivorous mites	Dicke (1986)
1990	Herbivore oral secretion on artificial wound sites induces terpenoid release from maize	Turlings *et al.* (1990)
1990	Methyl jasmonate is an airborne signal that induces neighbouring plants	Farmer & Ryan (1990)
1991	Systemin suggested as systemic signal released at wound sites of tomato leaves	Pearce *et al.* (1991)
1992	VOCs are induced systemically, i.e. they are also released from as-yet-undamaged organs	Turlings & Tumlinson (1992)
1992	VOCs can repel herbivores	Dicke & Dijkman (1992)
1995	VOCs released by plants carry all information required to attract parasitic wasps	Turlings *et al.* (1995)
1995	JA induces the release of VOCs by various plant species	Boland *et al.* (1995)
1997	VOCs are synthesized *de novo* in response to herbivore attack	Paré & Tumlinson (1997a)
1997	Volicitin acts as elicitor in *Zea mays* responding to caterpillar feeding	Alborn *et al.* (1997)
1998	VOCs attract parasitoidic wasps in nature	De Moraes *et al.* (1998)
1999	Induction of VOC release increases parasitation rates of herbivores in the field	Thaler (1999)
2000	Clipping of sagebrush leaves induces defence in neighbouring tobacco plants	Karban *et al.* (2000)
2001	VOCs repel herbivores in nature	De Moraes *et al.* (2001); Kessler & Baldwin (2001)
2001	Induction of EFN secretion is mediated by JA and benefits plants in nature	Heil *et al.* (2001b)
2004	VOCs prime resistance traits in neighbouring maize plants	Engelberth *et al.* (2004)
2006	Air flow from damaged to undamaged parts mediates systemic response in sagebrush	Karban *et al.* (2006)
2006	EFN secretion by lima bean is induced and primed by VOCs	Choh *et al.* (2006); Choh & Takabayashi (2006); Heil & Kost (2006); Kost & Heil (2006)
2007	VOCs mediate within-plant signalling and thus function as volatile plant hormones	Frost *et al.* (2007); Heil & Silva Bueno (2007)

VOCs, volatile organic compounds; JA, jasmonic acid; EFN, extrafloral nectar.
The development in research on indirect defensive traits is indicated by presenting a noncomprehensive listing of central publications that relate primarily to volatile-mediated tritrophic interactions (in yellow), volatile-mediated plant–plant signalling (in green) and EFN-mediated indirect defence (in red) in the temporal order of their publication.

aerial parts of plants, as roots of *Thuja* and maize can release VOCs in response to feeding by beetle larvae (van Tol *et al.*, 2001; Rasmann *et al.*, 2005). Feeding on roots even can induce changes in the volatile bouquet released from the aerial parts of a plant (Bezemer *et al.*, 2003; Soler *et al.*, 2007), although the ecological relevance of this observation remains elusive.

Less has been done on the induction of EFN secretion. The Euphorbiacea, *Macaranga tanarius*, responds to leaf damage with dramatically increased rates of EFN secretion. Within hours, this increases the number of ants showing up on the plant, which in turn reduces herbivore pressure (Heil *et al.*, 2001b). Experiments based on the exogenous application of JA and on the application of an inhibitor of endogenous JA synthesis demonstrated that the transient increase in endogenous JA that can be observed after mechanically damaging *Macaranga tanarius* plants (Fig. 3) is both required and sufficient to increase EFN secretion locally (Heil *et al.*, 2001b).

Increases in EFN secretion upon herbivory and/or mechanical damage were also demonstrated for cotton and castor (Wäckers *et al.*, 2001), the Bignoniaceae *Catalpa bignonioides* (Ness, 2003), and several species of the Faboideae (Heil, 2004) and the Mimosoideae (Heil *et al.*, 2004b). *Vicia faba* responds to mechanical leaf damage with increased numbers of nectaries (Mondor & Addicott, 2003), a phenomenon caused by the production of more nectary-bearing stipules on the growing shoot (Mondor *et al.*, 2006). The involvement of a long-distance signal has been demonstrated for cotton, since feeding on roots induced EFN secretion on above-ground parts (Wäckers & Bezemer, 2003).

Many excellent reviews exist on the signals involved in defence induction (Creelman & Mullet, 1997a; Wasternack & Parthier, 1997; Ryan, 2000; Farmer, 2001; Farmer *et al.*, 2003; Howe, 2004; Schilmiller & Howe, 2005), among these two Tansley reviews (Bennett & Wallsgrove, 1994; Gatehouse, 2002), and only a short overview is given here. The plant hormone, JA, plays a crucial role in the induction of both VOCs (Hopke *et al.*, 1994; Boland *et al.*, 1995) and EFN (Heil *et al.*, 2001b). What is the perceived signal initiating the octadecanoid signalling cascade, which terminates in the synthesis of JA? Eliciting compounds comprise cell wall

fragments such as oligosaccharins and pectins (Doares *et al.*, 1995; Creelman & Mullet, 1997b), compounds such as β-glucosidase (Hopke *et al.*, 1994; Mattiacci *et al.*, 1995) or cellulysin (Piel *et al.*, 1997) that cause such fragments to be formed, or fragments of plant proteins (Schmelz *et al.*, 2006). In short, many components that are released from a disrupted plant cell, and that may or may not be processed by insect-derived factors, are perceived as signals, and mere mechanical damage can therefore be sufficient to induce the majority of JA-dependent genes or defence traits, at least when the type of damage inflicted destroys many cells rather than leading only to a loss of leaf area (Heil *et al.*, 2001b, 2004b; Mithöfer *et al.*, 2005; Major & Constabel, 2006). In response to damage, the octadecanoid cascade (Fig. 3) starts with the release from cell membranes of the 18C-fatty acid linolenic acid, which is then converted to 13-hydroperoxylinolenic acid by lipoxygenase. 13-Hydroperoxylinolenic acid is a substrate for allene oxide synthase and allene oxide cyclase, which form 12-oxo-phytodienoic acid (12-OPDA). Following reduction and three steps of beta-oxidation, JA is formed (Creelman & Mullet, 1997a; Gatehouse, 2002). Jasmonic acid and several intermediates of the octadecanoid cascade have been identified as inducing different sets of VOCs (Koch *et al.*, 1999), and different components of the octadecanoid signalling cascade thus likely interact to determine the composition of the released volatile blend.

Jasmonic acid does not, however, directly induce gene activity. The search for its receptor and its mode of action in the regulation of gene expression has only recently seen a significant breakthrough with the discovery of a family of JAZ (jasmonate ZIM-domain) proteins (Chini *et al.*, 2007; Thines *et al.*, 2007). These proteins are repressors of MYC2 and similar transcription factors, which are important players in the regulation of jasmonate-sensitive genes (Boter *et al.*, 2004; Lorenzo *et al.*, 2004). Their discovery links these transcription factors to the long-known function of COI1 (coronatin-insensitive 1), an F-box protein (Xie *et al.*, 1998) forming part of an enzymatic complex (an E3 ubiquitin ligase) that targets JAZ-proteins for ubiquitination (Chini *et al.*, 2007). In short, jasmonates (most likely the JA–amino acid conjugate jasmonoyl–isoleucine; Staswick & Tiryaki, 2004) bind to the COI1-unit of an E3 ubiquitin ligase complex termed SCF^{COI1} (for Skip/Cullin/Fbox – COI1), thereby stabilizing the COI1–JAZ complex (Thines *et al.*, 2007). This allows the ubiquitination of JAZ-proteins and thus their rapid degradation. E3 ligases generally control the ubiquitination of proteins, and polyubiquitinated proteins are then recognized and degraded by the 26S proteosome (Devoto & Turner, 2005). Since JAZ-proteins are repressors of MYC2 and related transcription factors, their degradation deliberates these transcription factors and thus allows gene activation (Chini *et al.*, 2007; Farmer, 2007). Interestingly, JAZ-proteins themselves are MYC2-dependent and therefore rapidly induced by jasmonates (Chini *et al.*, 2007), which explains the only transient gene expression in response to jasmonates, which resembles the transient increase

in JA itself as generally found after wounding or short-term insect feeding (Fig. 3).

Although mechanical damage can induce the octadecanoid cascade in many plants, several studies identified specific elicitors, and hints were obtained on a systemic transport of at least some of them. For example, a primary wound signal in tomato was identified as an 18-amino acid peptide termed systemin. Systemin is released at wound sites by chewing herbivores (Pearce *et al.*, 1991) and is considered a systemically transported signal. The polypeptide is processed from a 200-amino acid precursor called prosystemin (Ryan & Pearce, 1998). Systemin is mainly discussed in the context of direct defences but also induces VOCs (Corrado *et al.*, 2007). Other elicitors are formed by a conjugation of plant- and herbivore-derived precursors. For example, the fatty acid–amino acid conjugate volicitin (N-[17-hydroxylinolenoyl]-L-glutamine) acts as an elicitor in *Zea mays* responding to caterpillar feeding (Alborn *et al.*, 1997) and it was then found that its fatty acid portion is derived from the plant while the 17-hydroxylation reaction and the conjugation with glutamine are carried out in the caterpillar (Paré *et al.*, 1998) by bacteria living in the caterpillar's gut (Spiteller *et al.*, 2000). Other plants in which fatty acid–mino acid conjugates act as elicitors are lima bean (Koch *et al.*, 1999) and native tobacco (Halitschke *et al.*, 2001). In general, such elicitors induce the octadecanoid cascade (Schaller & Ryan, 1995; Alborn *et al.*, 1997; Paré *et al.*, 1998; Ryan, 2000).

However, volicitin does not induce volatile release from lima bean (Koch *et al.*, 1999), and systemin was not active in all species of the Solanaceae (Schmidt & Baldwin, 2006). Amino acid conjugates of jasmonic acid can act as intermediates in the octadecanoid signalling pathway (Krumm *et al.*, 1995), JA-Ile is now discussed to interact with COI1 and thereby to directly induce jasmonate-responsive genes (Staswick & Tiryaki, 2004), and volicitin is an amino acid conjugate of linolenic acid (i.e. the fatty acid that forms the starting point of the octadecanoid signalling cascade; see Fig. 3). In spite of these structural similarities to the putatively general signals, elicitors such as systemin and volicitin function in a much more restricted range of species than does JA itself, which activates direct or indirect defences in many unrelated plant species.

3. Within-plant and among-plant signalling

Volatiles carry information on the status of attack of a plant, which can be used by other plants or parts of plants to adjust their defensive phenotype accordingly. In fact, the first reports on plant–plant communication date back to the early 1980s and preceded the first reports on plant–carnivore communication (Table 1). Rhoades (1983) reported that undamaged Sitka willow trees growing close to herbivore-infested conspecifics had a higher chemical defence to fall webworm larvae than controls from a more distant site. Shortly afterwards, Baldwin

& Schultz (1983) found that undamaged, potted plants kept in the same air with damaged plants had increased concentrations of phenolic compounds. Undamaged cotton seedlings became more attractive to predatory mites and less attractive to herbivorous mites when exposed to air from infested conspecific plantlets (Bruin *et al.*, 1992). Later field studies found that herbivory rates on alder trees were lower when growing close to damaged conspecifics (Dolch & Tscharntke, 2000), while others extended the phenomenon to the interspecies level by reporting that clipped sagebrush can induce polyphenol oxidase in wild tobacco plants (Karban *et al.*, 2000). Extrafloral nectar secretion by undamaged lima bean increased in response to volatiles from herbivore-damaged plants (Choh *et al.*, 2006; Kost & Heil, 2006). Even the mere exposure of lima bean to volatiles from beetle-damaged conspecific shoots increased EFN secretion and the number of ant visits, and it reduced herbivory rates in nature (Heil & Silva Bueno, 2007). Plant–plant communication mediated by VOCs thus appears to be a general phenomenon.

Responding to the neighbours' damage comes with the risk of investing in a defence that may not then be needed. Rather than being directly induced, many plants are therefore primed by VOCs, at least when these are present at low concentrations. Primed plants do not show detectable expression of resistance traits, but they respond more strongly once they are attacked or infected themselves (Zimmerli *et al.*, 2000; Conrath *et al.*, 2006). Exposing undamaged corn plants to VOCs from damaged conspecifics primed them to produce JA and terpenes more intensively and/or rapidly in response to caterpillar-caused damage than plants that were damaged without this pretreatment (Engelberth *et al.*, 2004), and it made them more attractive to parasitic *C. marginiventris* wasps (Ton *et al.*, 2007). Priming is also involved in the signalling between sagebrush and tobacco (Kessler *et al.*, 2006), in the response of EFN to VOCs (Choh & Takabayashi, 2006; Heil & Kost, 2006), and in the induction of direct and indirect defences of poplar (Frost *et al.*, 2007).

From the very beginning, the idea of a plant–plant communication was heavily discussed and criticized. Why should plants warn their neighbours (Karban, 2001; Baldwin *et al.*, 2006)? Plants usually compete with each other, and this type of communication would benefit the receiver at the cost of the emitter! How can such a signal evolve? One explanation would be that VOCs also serve plant-internal functions and, for instance, mediate signalling among different parts of the same plant individual, particularly in cases were direct vascular connections are restricted (Farmer, 2001; Orians, 2005).

In fact, airflow from damaged to undamaged parts appeared necessary for systemic resistance induction in sagebrush (Karban *et al.*, 2006). Similarly, VOCs released by damaged lima bean leaves primed and induced EFN secretion by neighbouring leaves of the same individual plant (Heil & Silva Bueno, 2007), an observation that was also found for the VOCs released from poplar saplings (Frost *et al.*, 2007). Volatile organic compounds can thus serve a hormone-like function,

mediating systemic induction in response to local damage. Volatile organic compounds identified so far as causing priming or induction of defence in undamaged plants include (*Z*3)-hexenyl acetate (Kost & Heil, 2006) and several structurally related C_6-volatiles (Bate & Rothstein, 1998; Engelberth *et al.*, 2004; Farag *et al.*, 2005; Ruther & Kleier, 2005), that is, substances that are released rapidly after damage. Volatile organic compound-mediated within-plant signalling might thus be faster in eliciting a systemic response than any signal that is transported in phloem or xylem, and it induces resistance in exactly those parts where it is most urgently needed: in the spatially, yet not necessarily anatomically, neighbouring organs (Heil & Silva Bueno, 2007).

IV. Interactions among different strategies

1. Myrmecophytes

Myrmecophytes are the only known cases of obligate, symbiotic mutualisms among plants and protecting animals, and they represent particularly sophisticated examples of plants combining more than one trait in order to optimize their indirect defence. Tropical plants from more than 100 genera house specialized ant colonies in domatia and usually provide their ants with food (Davidson & McKey, 1993). Being completely dependent on their host plant, the ants exhibit an intensive defending and cleaning behaviour. Since this comprises herbivorous insects and their eggs, competing vegetation, epiphytes and, in some cases, even pathogenic fungi, a highly effective defence of the host plant results (Heil & McKey, 2003; Rico-Gray & Oliveira 2007). After much discussion about whether or not the ants really have a protective function, long-term field experiments on Mesoamerican 'swollen thorn' *Acacia* myrmecophytes demonstrated beyond doubt that ant–plants and plant–ants can be engaged in obligate defensive mutualisms (Janzen, 1966). Myrmecophytes are highly successful pioneer trees and now build quantitatively relevant parts of secondary forests and the vegetation of ruderal sites in Southeast Asia, Africa and Meso- and South America (Davidson & McKey, 1993; Heil & McKey, 2003).

The trait making the interaction between myrmecophyte and ant an obligate one is usually the formation of domatia. The majority of ant domatia are caulinary, that is, hollow stems and shoots (Brouat & McKey, 2001), but ant domatia may be also localized in hollow thorns (Janzen, 1966) (Fig. 1f), in leaf pouches (Alvarez *et al.*, 2001; Bizerril & Vieira, 2002; Edwards *et al.*, 2006), in leaf petioles (Risch *et al.*, 1977; Clarke & Kitching, 1995), and even on fruits (Kato *et al.*, 2004). Besides nesting space, many obligate myrmecophytes strengthen the association with specialized ants by providing them with plant-derived food rewards, either as EFN or as FBs (Figs 1b,d,e). Owing to the high contents of lipids and proteins, FBs are being considered an 'expensive' form of defence. Their energy costs have been

estimated to be some 2% of leaf construction costs of Balsa, *Ochroma pyramidale* (O'Dowd, 1980), while *Macaranga bancana* invests *c.* 9% of above-ground tissue construction costs into FB production (Heil *et al.*, 1997).

That FBs are costly is underlined by the limitation of their production by nutrient supply (Folgarait & Davidson, 1995; Heil *et al.*, 2001a) or light (Folgarait & Davidson, 1994), and by the observation that myrmecophytes can reduce FB production in the absence of the consuming mutualist (Risch & Rickson, 1981; Folgarait *et al.*, 1994; Heil *et al.*, 1997). However, while they are comparably expensive, FBs produced by obligate myrmecophytes bear important benefits compared with other indirect defences: FBs are not as obviously produced according to 'optimal defence' requirements as is EFN involved in facultative interactions. In fact, instead of being produced on the surfaces of the youngest (defence-requiring) leaves, FBs of many myrmecophytes are localized in hollow petioles, at the leaf bases or under recurved stipules (Fig. 1d). In the case of myrmecophytes, the distribution of ants on the plant is independent of the distribution of FB production (Heil *et al.*, 2004a) and may be achieved by VOCs (Agrawal, 1998; Brouat *et al.*, 2000). Evolving an obligate defensive mutualism in which specialized predators receive a reliable food source enables the spatial separation of the plant parts where investment in defence takes place from those where defence is required, and thus the fulfilment of 'optimal defence' requirements without being compromised by 'growth differentiation' trade-offs (Heil *et al.*, 1997).

2. Interactions among facultative strategies

Myrmecophytes successfully combine different traits to achieve an optimum overall indirect defence, but, being obligate mutualisms, they may not be representative for the more common, facultative interactions. How good is the evidence for synergisms among indirect defensive traits that mediate facultative interactions? Unfortunately, the majority of studies have investigated isolated plant–herbivore–carnivore interactions or the induction of single defence traits. The attack by more than one enemy at a time has only recently been considered by researchers, although in nature it is the normal situation rather than the exception (Turlings & Wäckers, 2004). Such interactions are increasingly being considered, but the multiple functions of defence traits and their putative interactions remain virtually unexplored. Only scattered examples exist illustrating how plants can fine-tune the induction of direct and indirect defence traits in order to respond specifically to generalist vs specialist herbivores (Kahl *et al.*, 2000).

Volatile organic compounds serve as volatile hormones or pheromones, and their composition is affected by the emitter's status of attack by herbivores and pathogens. How common and how specific are such effects; and are there other forms of interaction among different types of indirect defences, and of indirect defences with other plant traits? Synergistic effects as

described earlier for myrmecophytes might be used by the plant to achieve optimal defence strategies when facing different sets of enemies: for instance, EFN and VOCs share parts of the same signalling pathway (Fig. 3), and VOCs can induce EFN secretion. The EFN induced by VOCs can then increase the time that carnivorous mites spend on the plant, which were attracted by these very VOCs (Choh *et al.*, 2006). Learning by parasitoids presents an important factor improving the synergisms among EFN and VOCs. Learning has been shown, in particular, for generalist parasitoids (Steidle & van Loon, 2003) and can turn nonspecialized interactions into short-term and locally restricted specialized interactions, since generalist parasitoids that have a positive association of a specific volatile bouquet with feeding (e.g. on EFN) will preferably choose plants with a similar or identical bouquet as experienced before. Extrafloral nectar might thus significantly strengthen VOC-mediated plant–parasitoid interactions.

Similarly, EFN-feeding predators such as ants and mites can be housed in domatia, and VOCs can help ants to localize both suitable host plants and the damaged plant parts where defence is most urgently needed (Fiala & Maschwitz, 1990; Agrawal, 1998; Jürgens *et al.*, 2006). Plant species having more than one indirect defence trait are common, and cotton, for instance, produces VOCs and EFN and bears leaf domatia (Fig. 1). Arimura *et al.* (2005) listed 10 species producing both VOCs and EFN and speculated that a co-occurrence of these two traits is widespread. Research on VOC-mediated tritrophic interactions has traditionally been conducted independently of indirect defence via EFN, FBs or domatia (Table 1). This situation hardly allowed the discovery of interactions among different traits involved in indirect defence, which are likely to be common and remain to be identified and investigated.

V. Outlook

Extrafloral nectar-producing plants are increasingly being discussed as additional food sources for carnivores in environmentally-friendly crop protection programmes (Pemberton & Lee, 1996; van Rijn & Tanigoshi, 1999; Gnanvossou *et al.*, 2005; Mathews *et al.*, 2007; Olson & Wäckers, 2007). Crops such as cotton (Limburg & Rosenheim, 2001; Röse *et al.*, 2006), cashew (Rickson & Rickson, 1998), cassava (Gnanvossou *et al.*, 2005), *Passiflora* (Labeyrie *et al.*, 2001), *Ricinus* (van Rijn & Tanigoshi, 1999), stone fruits, such as almond, cherry, peach and plum (Tilman, 1978; Yokoyama & Miller, 1989), and the majority of legumes bear extrafloral nectaries, which may contribute to the plants' natural defence against herbivores. An application in crop protection has always been an obvious and explicitly expressed goal of research into herbivore-induced VOCs (Walling, 2001; Degenhardt *et al.*, 2003; Shiojiri *et al.*, 2006; Turlings & Ton, 2006).

Alas, in spite of some promising attempts in that direction (Stapel *et al.*, 1997; Gnanvossou *et al.*, 2005), apparently only

one study has reported that EFN secretion by a crop can indeed have a defensive effect in the agronomic field (Mathews *et al.*, 2007). Demonstrations that VOC-mediated tritrophic interactions can benefit crop plants under realistic agricultural conditions are similarly scarce, as evidence for VOC-mediated attraction of predators and resulting plant protection appears overwhelming but in fact is mainly derived from laboratory studies (but see Thaler, 1999; Rasmann *et al.*, 2005). Yet, 'elegant and exciting as laboratory studies are, they cannot easily address the applicability of herbivore-induced volatile production to the protection of agricultural crops' (Hunter, 2002).

Which questions must be answered to understand the evolutionary importance of indirect defences and their potential relevance in crop protection? Most importantly, the question as to why VOCs and EFN have evolved as induced traits instead of being expressed constitutively needs to be answered, before crop plants transformed for a constitutive release of VOCs (Schnee *et al.*, 2006) can be regarded as a promising tool. Volatile organic compounds differ from EFN and FBs in not being a resource *per se*, but merely advertising the presence of prey. Signal reliability is thus an important aspect in this plant–predator mutualism, which would be highly unstable if plants would attract carnivores in the absence of herbivores (Turlings & Ton, 2006). Ecological and physiological costs of indirect resistance traits need to be quantified and, in fact, might be a factor severely compromising their application under certain growing conditions (Heil, 2007). Knowledge about how the production of VOCs and EFN depends on abiotic conditions is still in its infancy but will be highly important in order to understand how these traits can be used in environmentally-friendly crop protection programmes.

Plant physiology and molecular biology have benefited from the concentration on selected models such as Arabidopsis. Thousands of well defined mutants are available, the genome is fully sequenced, knowledge of the molecular basis of signalling cascades has greatly improved, and transgenic plants have been created that, for instance, release VOCs that are entirely new biosynthetic products for the whole plant family. Beyond doubt, this progress would have been impossible without using model species. However, no two single biological species are functionally identical. Focusing on a few model species therefore results in a biased picture of how plants cope with their environment and does not allow an understanding of the whole spectrum of plant traits, particularly when considering ecological interactions. Common traits such as EFN remain unexplored only because they are not expressed by the model species. This problem becomes even more pertinent when thinking about interactions among the different defensive traits, as they have now indeed been found to occur on the genetic, physiological and, in particular, ecological levels. Volatile organic compounds and EFN interact in many aspects, but these interactions were only recently made the subject of rigorous experimental investigations.

Their importance for the ecology of plants is still underestimated and thus underrepresented in the current research.

Indirect defences function via interactions among organisms that have evolved sophisticated means of regulation and communication to achieve an efficient and mutually beneficial cooperation. Scientists working on indirect defence via tritrophic interactions are best advised to copy their subjects' strategies and improve communication and cooperation among those who have thus far studied in isolation single aspects of a system that needs to be regarded and understood as a whole.

Acknowledgements

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