Inducible defence of plants against insects: from molecular to evolutionary biology

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Insects are one of the dominant forms of life in terms of the number of species and of individuals. Plants can have different types of interactions with insects, such as antagonistic interactions with herbivores and mutualistic interactions with carnivorous and pollinating insects. Plants can defend themselves against insects by employing a ‘do-it-yourself’ strategy and/or by enlisting ‘bodyguards’ that attack herbivores. These plant strategies can be present constitutively or they can be induced by herbivory. Inducible defences result in variable plant phenotypes and consequently in variable types of interactions with insects. To make maximal progress in and to gain a thorough understanding of inducible plant defences both mechanisms and ecological aspects should be studied in conjunction. Mechanistic studies can explain how plants cope with ecological constraints and they can provide tools for manipulative approaches in ecological studies. In their turn, evolutionary ecological studies can elucidate the most important mechanisms to be investigated to make significant progress in the understanding of the intriguing complexity of active defences of plants, and their effects on insects at different trophic levels. As a result, the field of insect-plant interactions represents studies on highly dynamic systems that should be investigated through a multidisciplinary approach.

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Circa 50% of all insect and mite species are herbivores and thus exploit plants as resource and microhabitat (Strong et al. 1984). The other 50% of insect and mite species comprises carnivores and saprophages (Strong et al. 1984). The carnivores feed on many of the herbivores and often inhabit plants on which their victims may occur (Walter 1996). As a consequence, many plant characteristics can affect carnivorous insects. Thus, the field of insect-plant interactions relates to the majority of insect species. Given that insects and plants together make up more than three quarters of all species (Strong et al. 1984) and the dominance of insects in terms of individuals (Berenbaum 1995), the study of insect-plant interactions is important for a thorough understanding of ecosystems. This should relate to both mechanisms of insect-plant interactions and evolutionary ecological aspects. These are two sides of the same coin. Understanding the mechanisms of insect-plant interactions is essential for gaining understanding of evolutionary ecological aspects and vice versa evolutionary ecological knowledge is important to guide important developments in mechanistic studies.

Individual plants cannot run away from their enemies and so they should have other defences to avoid succumbing to mobile insects. Indeed plants have a wide range of defences, ranging from ‘do-it-yourself’ strategies that employ characteristics that negatively affect herbivores (direct defence) to the enlistment of ‘bodyguards’ that attack herbivores (indirect defence). In addition to being constitutively present, many of these defences can be induced by herbivory (Karban & Baldwin 1997; Agrawal et al. 1999; Chadwick & Goode 1999; Dicke & Vet 1999; Sabelis et al. 1999).

INDUCIBLE DEFENCES

Inducible defences have been studied since the 1970s and initially the emphasis was exclusively on direct defences. For instance, Ryan and colleagues showed that potato plants that were damaged by herbivores or by mechanical means had an induced production of proteinase inhibitors that reduced the digestibility of the plant for the herbivore (Green & Ryan 1971). This inducible defence mechanism has been investigated in great depth until the present day and this project has yielded many incentives for studies on other inducible defences (e.g. Pearce et al. 1991; Farmer &
Ryan 1992; McCloud & Baldwin 1997; Constabel et al. 1998; Baldwin & Preston 1999). Studies by Haukioja and colleagues on population dynamics of the geometrid moth *Epirrita autumnata* showed a strong cycling and this appeared to be influenced by cyclic changes in host plant (*Betula pubescens*) quality related to inducible defence. This phenomenon has been intensively studied from an ecological point of view (e.g. Haukioja & Hakala 1975; Haukioja & Hanhimaki 1985; Haukioja *et al.* 1985; Haukioja & Neuvonen 1987), and recently the mechanisms involved are being studied in addition (Kause *et al.* 1999; Salminen *et al.* 1999).

Inducible indirect defence of plants has been studied since the 1980s (Sabelis & van de Baan 1983; Sabelis & Dicke 1985; Dicke & Sabelis 1988; Dicke *et al.* 1990b; Turlings *et al.* 1990). Plants that were damaged by herbivores were shown to emit an odour blend that is qualitatively different from the odour of undamaged or mechanically damaged plants and the induced odour blend attracted natural enemies (predators or parasitoids) of the herbivores. This phenomenon has been intensively studied since the 1990s, both with regard to mechanisms (e.g. Turlings *et al.* 1998; Boland *et al.* 1999; Dicke *et al.* 1999) and evolutionary ecological aspects (e.g. Vet 1998; Dicke & Vet 1999; Sabelis *et al.* 1999).

Both forms of inducible defence appear to be widespread in the plant kingdom (Table 1). In this paper I will concentrate on induced indirect defence of plants and emphasize the multidisciplinarity of the research on this type of induced defence (Fig. 1).

**MECHANISMS**

Inducible indirect defence has been found in all plant species investigated so far (for review see Dicke 1999). Mechanisms of inducible indirect defence have been intensively studied. In fact the investigations of the mechanism provided the basis of this research field. Only by elucidating that herbivore-induced volatiles produced by plants are most important in attracting carnivores to herbivore-infested plants, studies of inducible indirect plant defence got off the ground (Sabelis & Dicke 1985; Dicke *et al.* 1990b; Turlings *et al.* 1990).

**Analytical chemistry**

An analytical chemical approach was instrumental in identifying the plant as the source of volatiles that attracted carnivorous arthropods to herbivore-infested plants. The volatiles recovered were all well-known to be plant products (Dicke *et al.* 1990b; Turlings *et al.* 1990) and were even recorded to be produced systemically from uninfested leaves from infested plants (Turlings & Tumlinson 1992; Röse *et al.* 1996). Plant species may vary in the mechanism of indirect defence. For instance, plants may respond to herbivory with the production of chemicals that are not emitted in response to mechanical damaged. This has been found for *e.g.* lima bean (Dicke *et al.* 1990b), corn (Turlings *et al.* 1990) or gerbera (Krips *et al.* 1999). Alternatively, they may respond with the emission of a qualitatively similar blend to that emitted after mechanical damage, albeit that the amounts are much bigger in response to herbivory and the emission lasts longer after the damage stops. Examples are *e.g.* potato (Bolter *et al.* 1997), tomato (Dicke *et al.* 1998) or cabbage (Mattiacci *et al.* 1994). The analytical chemical approach was an essential to elucidate these different mechanisms of induced volatile production (Dicke *et al.* 1990a,b; Turlings *et al.* 1990; Turlings & Tumlinson 1992) that have important consequences for foraging carnivores (Dicke, 1999).

**Chemical ecology and neurobiology**

The identification of those induced volatiles within the complex blend of herbivore-induced plant volatiles, that result in the attraction of carnivores has been a difficult task. For only a few systems some behaviourally active components of the total blend are known, *i.e.* for two parasitoid wasps, one specialized predatory mite and one anthocorid predatory bug (Dicke *et al.* 1990b; Turlings *et al.* 1991; Scutareanu *et al.* 1997; Du *et al.* 1998; Turlings & Fritzsch 1999). However, given that the induced blends of volatiles are complex mixtures of up to 200 components complicates a behavioural approach because of the many combinations of compounds that may be tested. Fractionation of the total blend may provide an option (Turlings & Fritzsch 1999), but when very different compounds have a synergistic or antagonistic effect this may lead to new complications.
An approach that combines gas chromatography with electrophysiology can elucidate those compounds that are perceived by chemoreceptors at the periphery. For some systems such knowledge on electrophysiological activity of blend components is available (Du et al. 1996; Weissbecker et al. 2000). However, the knowledge on how insects perceive, evaluate and respond to complex and variable mixtures of volatiles is still rudimentary. A neurobiological approach has recently been initiated in several laboratories (e.g. Bleeker & Smid 1999; Van Wijk et al. 2000) and this will significantly advance our knowledge on how carnivorous arthropods cope with the complex information that plants emit (cf. Laurent et al. 1999).
Biochemistry and plant physiology

In recent years a lot of knowledge has been gained on the induction mechanism. Herbivore elicitors have been identified (Mattiacci et al. 1995; Alborn et al. 1997; Eichenseer et al. 1999; Engelberth et al. 2000) and signal-transduction pathways have been analysed (Boland et al. 1999; Dicke & Van Poecke 2000; Ozawa et al. 2000). The major phytohormone involved appears to be jasmonic acid (Boland et al. 1995; Krumm et al. 1995; Dicke et al. 1999; Thaler 1999; Ozawa et al. 2000), which is also involved in the induction of direct defence (Farmer & Ryan 1992; Stanjek et al. 1997; Zhang et al. 1997), but there seems to be an involvement of salicylic acid as well (Ozawa et al. 2000). The latter is very interesting because salicylic acid is also involved in induced resistance against pathogens (Malamy et al. 1996). In some plants, the methyl ester of salicylic acid, methyl salicylate, is emitted in response to herbivory (Dicke et al. 1990b; Bolter et al. 1997; Scutareanu et al. 1997; Dicke et al. 1998) and attracts the predatory mite Phytoseiulus persimilis (Dicke et al. 1990b). Finally, (Z)-jasmone, a product from the lipoxygenase pathway has also been reported to be involved (Birkett et al. 2000). Jasmonic acid is also involved in induced systemic resistance (Pieterse et al. 1998) and thus it appears that induced defences against herbivorous arthropods and microbial pathogens share components of signal-transduction pathways. This opens up the exciting research field on cross-talk between arthropod-induced and microbe-induced responses of plants (Stout et al. 1999; Thaler et al. 1999). Cross-talk between different signal transduction pathways has been recorded in several instances (O'Donnell et al. 1996; Stout et al. 1999; Thaler et al. 1999) and this may provide an important mechanism for plants to integrate defences against very different types of attackers.

The mixtures of volatiles that plants emit consist of compounds from various groups that are produced through a few main biosynthetic routes (Paré & Tumlinson 1997). Although the general aspects of the biosynthetic routes are known, specific aspects, especially those related to the induced aspect of biosynthesis remain poorly known. For some of the induced terpenes, i.e. the homoterpenes (E)-4,8-dimethyl-1,3,7-nonatriene and (E,E)-4,8,12-trimethyl-1,3,7,11-tetradecaene some more knowledge has been gained on the biosynthesis (Donath & Boland 1994, 1995; Bouwmeester et al. 1999; Degenhardt & Gershenzon 2000). This has indicated that a nerolidol synthase is likely to mediate the first dedicated step in the induced biosynthesis (Bouwmeester et al. 1999; Degenhardt & Gershenzon 2000). Based on this knowledge and information on genes encoding for terpene synthases (Bohlmann et al. 1998) one may isolate the gene encoding for nerolidol synthase which will provide an interesting tool to investigate the role of (E)-4,8-dimethyl-1,3,7-nonatriene in the ecology of plant-carnivore interactions.

Molecular biology

The identification of genes that are central in the induction of herbivore-induced plant volatiles allows to generate plants that overexpress the gene or in which the gene is silenced. This provides novel tools for evolutionary ecologists. Such plants differ from the wildtype plant in just a single gene and this allows an investigation of the importance of this gene’s product in the evolutionary ecology of the plant. Mechanisms of inducible defence are more and more being studied at the molecular level (Mitchell-Olds et al. 1998) and this will soon lead to information on transcriptome changes in response to herbivory (Arimura et al. 2000ab; Reymond et al. 2000) and consequently to knowledge on the choreography of gene activation changes in time and space. This will open many interesting new avenues to the study of inducible indirect defence. It will allow insight in the complex changes that are the result of different types of herbivory, relative to pathogen attack or mechanical damage.

EVOLUTIONARY ECOLOGY

Carnivorous arthropods can have important effects on herbivore population dynamics. For instance, predatory arthropods can exterminate local populations of their prey and this obviously benefits individual plants (Sabelis & Dicke 1985). In the evolution of inducible versus constitutive defences, the costs and benefits as well as the predictability of plants as resources for herbivores have been addressed (Adler & Karban 1994; Agrawal & Karban 1999; Dicke 1999; Dicke & Vet 1999; Sabelis et al. 1999). Inducible indirect defence has been studied for plant-predator and plant-
parasitoid interactions. Both for predators and parasitoids there is ample evidence for attraction to herbivore-induced plant volatiles. The benefits of induced volatiles for the carnivores are obvious because these volatiles allow them to effectively find their herbivorous victim, while the herbivore itself hardly disseminates information that can be exploited by their carnivorous enemies (Vet & Dicke 1992).

However, what are the costs and benefits to the plant? It seems that biosynthetic costs are low, but ecological costs may be considerable (Dicke & Sabelis 1989). For instance, herbivores may be attracted to the induced volatiles (Dicke & Vet 1999) and consequently, if no (effective) carnivores are in the habitat, the induced volatiles may have a severe cost. Furthermore, neighbouring plants may exploit the volatiles to their own benefit (Bruin et al. 1995) and if this results in a competitive advantage (Van Dam & Baldwin 1998) this may be an important cost to the volatile-emitting plant as well.

Apart from these ecological costs, the benefits to plants of attracting carnivores have been recently questioned. More specifically, this related to the benefits of attracting parasitoids (Coleman et al. 1999; Van der Meijden & Klinkhamer 2000). The argument for this is that parasitoids, in contrast to predators, do not remove the herbivore from the plant but after parasitization the herbivore continues to feed and this may occur at a rate higher than for unparasitized herbivores (Slansky 1978; Brewer & King 1980, but see Harvey et al. 1999; Harvey 2000). These studies all relate to herbivore consumption rates. However, until recently not a single study had investigated the effect of herbivore parasitization on plant fitness. Recently, a first investigation on the effect of parasitization of herbivores revealed a clear positive effect of parasitization on plant fitness, i.e. seed production. *Arabidopsis thaliana* plants on which a single unparasitized caterpillar had fed had a significantly lower seed production than uninfested plants or plants on which a parasitized caterpillar had been feeding. Seed production of undamaged plants and plants on which a parasitized caterpillar had been feeding was similar, while plants on which an unparasitized caterpillar had been feeding had (Van Loon et al. 2000).

**Interconnection between above-ground and below-ground multitrophic interactions**

The study of inducible indirect defence has, just as the study of inducible direct defence, been restricted almost completely to interactions between above-ground plant tissues and herbivorous and carnivorous arthropods. However, herbivores may also attack plant roots and these are vital organs to organisms that are rooted in the soil that use their roots for support and the uptake of resources. Very recently, first studies suggest that inducible indirect defence may also occur below-ground (Boff et al. 2001; Van Tol et al. 2001). For phytopathological studies such evidence has been presented during a longer period already (Van Loon et al. 1998). Above-ground interactions between plants and herbivorous arthropods may also result in below-ground interactions between the attacked plant and neighbouring plants (Chamberlain et al. 2001; Dicke & Dijkman 2001). Thus, above-ground multitrophic interactions are likely to be linked with below-ground multitrophic interactions (Van der Putten et al. 2001). This implies that interactions between plants and other organisms are even more complex than often considered and this should further incite multidisciplinary studies.

**Functional genomics and inducible indirect defence of plants**

In the era of molecular biology, the study of multitrophic interactions and inducible defences should take advantage of the wealth of knowledge that is gained by genomic projects. Recently the first complete plant genome has been unravelled, i.e. for *Arabidopsis thaliana* (Kaul et al. 2000). Thus, this brassicaceous plant, of which many mutants and transgenes have been characterized that are modified in signal transduction pathways involved in induced defence, should lend itself for a functional genomics approach to inducible defences against insects. It is becoming clear that Arabidopsis can be a suitable plant to study insect-plant interactions (Grant-Peterson & Renwick 1996; McConn et al. 1997; Stotz et al. 2000) and this plant species has also proven to be a suitable model for the study of inducible indirect defence against insects (Van Loon et al. 2000; Van Poecke et al. 2001). Therefore, the adoption of Arabidopsis as a model species is likely to significantly advance our understanding of insect-plant interactions. A functional genomics
approach should be followed by an environmental genomics approach in which the function of certain plant genes is investigated with respect to the effect on a plant’s phenotype related to its interactions with biotic and abiotic environmental agents. The knowledge gained on Arabidopsis may be extrapolated to other brassicaceous plant species (Van Poecke et al. 2001; Agelopoulos & Keller 1994; Blaakmeer et al. 1994; Geervliet et al. 1994; Mattiacci et al. 1995) and most likely to other plant species as well.

CONCLUSIONS
The field of induced indirect defence of plants has made tremendous progress over the past 15 years (Sabelis & Dicke 1985; Dicke et al. 1990a; Karban & Baldwin 1997; Baldwin & Preston 1999; Chadwick & Goode 1999; Dicke & Vet 1999; Sabelis et al. 1999; Dicke & Van Poecke 2000). The information comprises detailed insight in mechanisms and more and more knowledge at the level of the changes in transcriptome is being gathered. In addition, there is extensive knowledge of ecological aspects. Moreover, the most exciting observation is that multidisciplinary approaches to induced indirect defence of plants are being developed, that combine a molecular ecological approach to a biochemical, molecular genetical, analytical chemical and behavioural ecological approach. Such multidisciplinary approaches are likely to significantly increase the rate of development within this research field and allow to tackle complex research questions that remained difficult to answer so far.

REFERENCES


