CHAPTER 3

PLANT–INSECT INTERACTIONS IN THE ERA OF CONSOLIDATION IN BIOLOGICAL SCIENCES

Nicotiana attenuata as an ecological expression system

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Abstract. The past decades have seen an intense development of organismal biology and genomics of individual species on the one hand, and population biology and evolutionary ecology on the other. While the great discoveries fuelled by the current model systems will continue over the next decades, more and more discoveries will occur at the interface between different biological disciplines. It is through such integrative approaches that the mechanisms of evolution and adaptation will be revealed. The study of plant–insect interactions, exemplary among such integrative research fields, unifies research efforts on the cellular and organismal level with those on the whole-plant and community level. Recent studies on the wild tobacco plant *Nicotiana attenuata* illustrate both the value of using genetic and molecular tools in ecological research and the importance of profound natural-history knowledge when studying plant–insect interactions.

Keywords: induced plant responses; herbivory; plant defence; jasmonate signalling

INTRODUCTION – THE MODERN CONSOLIDATION IN BIOLOGY

We are in the midst of what is widely regarded as the century of biology. Life science is already influencing multiple aspects of the modern economy and is expected to move to the forefront of all the sciences. Biotechnology, projected to become an unparalleled industrial mainstay, already touches everyone’s daily life. Its increasing importance even prompts university departments in the traditional engineering disciplines to offer life science as part of their curricula (Friedman 2001). The notion of the dominant role of life sciences in modern research and the economy is to a great extent based on the breathtakingly fast advances in genetics and molecular biology. It is projected that through this progress we will eventually be enabled to reveal how cells, organisms and ecosystems function. But will we?

The disproportionately high allocation of workforce and financial resources to genetics and molecular biology has led to an apparent under-representation of subjects such as natural history and organismal biodiversity in our biological curricula. Greene (2005) argues that scientific theories help us to study nature better

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through summarizing current knowledge and formulating hypotheses. Nonetheless, such theories cannot themselves replace discoveries of new organisms or new facts about organisms. The continuous study of species' natural history can help to reset research cycles and may change the hypothesis testing that underlies conceptual progress in science. Following this integrative notion, a profound understanding of ecological and evolutionary processes can be found only at the interface between different biological research domains. Scientific progress will ultimately be based on unification rather than fragmentation of knowledge (Kafatos and Eisner 2004).

On the threshold of the biological era, the life sciences are at the inception of a profound transformation by starting a process of consolidation. The life sciences have long formed two major domains, one reaching from the molecule to the organism, the other bringing together population biology, biodiversity study and ecology. Kafatos and Eisner (2004) argue that these domains, kept separate, no matter how fruitful, cannot deliver on the full promise of modern biology. Only the unification of the two research domains can lead to a full appreciation of life's complexity from the molecule to the biosphere or, indeed, maximize the benefits of biological research for medicine, industry, agriculture or conservation biology. Many researchers and academic institutions have recognized the necessity for unification and created research environments with integrative collaborations of researchers representing different disciplines and teaching programmes that emphasize multi-disciplinary approaches.

Chemical ecology is a discipline that emerged during the past half century and is by definition an integrative research field. It is driven by the recognition that organisms of diverse kinds make use of chemical signals to interact (Karban and Baldwin 1997). The original endeavour to decipher the chemical structure and the information content of the mediating molecules as well as the ecological consequences of signal transduction is now receiving a major directional addition, the modern domain of molecular biology (Eisner and Berenbaum 2002). It promises an understanding of the molecular and genetic mechanisms of biological signal transduction in species interactions, which can help to ultimately understand the evolution of complex species interactions.

The study of plant–insect interactions is an excellent example of the success of the modern approaches taken in chemical and molecular biology (e.g. Walling 2000; Berenbaum 2002; Kessler and Baldwin 2002; Dicke and Hilker 2003; Hartmann 2004). It is a fast-growing field within the research of organismal interactions to a great extent because the results can readily be applied in modern agriculture and therefore have a potentially high economic value (Khan et al. 2000). In the following sections I will summarize selected characteristics of induced plant responses to herbivory that at the same time define integrative focus directions in this research field, ranging from the physiological and ecological costs and consequences to the cellular signalling crosstalks that result in the elicitation of plant defences. In addition I will summarize studies of the complex multitrophic interactions of the wild tobacco plant *Nicotiana attenuata* (Torr. ex Watts) with its insect community that emphasize the potential role of induced plant defences in structuring arthropod communities and the value of using molecular and chemical analytical tools in ecological research.

PLANT–INSECT INTERACTIONS

Chemical communication can be studied at various levels of integration reaching from the expression of genes involved in biosynthesis of signal molecules to ecological consequences of the resulting organismal interactions on the community level. When studying plant–insect interactions we observe an exchange of signals that reciprocally influence the interacting partners and consequently include a complex crosstalk across all the levels of integration. Moreover, plant–insect interactions are played out in an arena that is much bigger than the plant itself. It includes interferences on the cellular level that have been extensively studied in plant–pathogen interactions (e.g. Lam et al. 2001; Van Breusegem et al. 2001) as well as interactions at the whole-plant and the community level. The latter result from multitrophic and inter-guild interactions, which are frequently mediated by the plants' chemical defences (Agrawal 2000; Dicke and Van Loon 2000; Karban and Agrawal 2002; Kessler and Baldwin 2002).

The fitness costs of plant defences

Plants have myriad ways to defend themselves against their attackers, including the production of defensive chemicals such as secondary metabolites and defensive proteins (Duffey and Stout 1996). The evolutionary arms race between plants and herbivorous insects has early on been suggested as one of the driving forces of the chemical diversity in the plant kingdom. Ehrlich and Raven (1964) coined the term 'coevolution' and stimulated entomological studies of how plants and insects influence each other's evolutionary trajectories. In the notion of their coevolutionary hypothesis a plant species' evolutionary innovation of new defensive compounds results in the exclusion of potential herbivores, which in turn will be strongly selected to tolerate or detoxify the new plant compounds. The counter-defences of insects are by no measure less diverse than the plant defences, and reach from the elicitation of changes in plant morphology (Sopow et al. 2003) to the sequestration of plant secondary metabolites and their use for the insects' own defences against natural enemies (Hartmann 2004).

The production of plant defence traits when they are not needed (e.g., in absence of herbivores) incurs significant fitness cost for a number of reasons (Agrawal et al. 1999; Heil and Baldwin 2002). First, the production of secondary metabolites can be costly if fitness-limiting resources are invested (Baldwin 2001; Heil and Baldwin 2002). For example, recent studies on nutrient-rich clay habitats and nutrient-poor white-sand habitats in the Peruvian Amazon region show that immature trees in nutrient-poor habitats are not able to compensate for severe herbivore damage. The nutrient-poor habitat therefore selects for plant species that invest more in defensive secondary-metabolite production at the cost of slower growth (Fine et al. 2004). However, resistance costs can also arise from higher-level ecological processes. For example, specialized herbivores may sequester defensive plant metabolites and use them for their own defence against predators (Karban and Agrawal 2002; Reddy and Guerrero 2004; Hartmann 2004), or compounds that provide defence against generalist herbivores may attract specialist herbivores, which use them as host location signals (Turlings and Benrey 1998). In addition plant defences may disrupt important mutualistic interactions with other insects such as pollinators (Adler et al. 2001) and parasitoids (Campbell and Duffey 1981; Barbosa et al. 1991), and may differently affect the performance of interacting organisms across several trophic levels (Orr and Boethel 1986; Harvey et al. 2003).

Induced plant defences

Constitutively high production of costly defences could only be beneficial for a plant if herbivore pressure is a predictable environmental factor. Unpredictable environments would select for plants that are able to produce a defence only when needed, in the presence of herbivores. Such phenotypically plastic plant responses are referred to as induced defences (Karban and Baldwin 1997). The fitness costs of the production of defensive compounds probably provide the selection pressure behind the evolution of inducible defences. Herbivore-induced plant defences have received a considerable attention in the past few decades, in part because the ecological implications for the plant and its arthropod community are different from those that derive from purely constitutive defences. Induced defences extend plant–insect interactions from the cell and whole-plant level to the community level.

Plants can respond to herbivore damage with the increased production of secondary metabolites or defensive proteins that are categorized by their mode of action (Duffey and Stout 1996). Compounds such as alkaloids, glucosinolates (in combination with myrosinase) and terpenoids function as toxins while proteinase inhibitors and polyphenol oxidases function as anti-digestive or anti-nutritive compounds, respectively. A plant inducing such defences in response to herbivory has a lower nutritive value for subsequently arriving herbivores and therefore reduces the probability of secondary attacks. The plant's metabolic changes may thereby not only affect insects of the same species but may result in cross-resistance effects that affect the herbivore-community composition of this plant (Agrawal 1998; Kessler and Baldwin 2004).

In addition to direct defensive secondary metabolites, plants produce volatile organic compounds (VOCs) in response to herbivore damage. These can function as signals for organisms able to receive and respond to changed odour bouquets. The most studied function of herbivore-induced VOC emission is the attraction of natural enemies such as parasitoids and/or predators to the damaged plant, a process referred to as indirect plant defence (Dicke and Van Loon 2000; Turlings and Benrey 1998). The VOC signal increases the natural enemy's foraging success and therefore facilitates top-down control of the herbivore population. The VOC response can be highly specific. For example, parasitoid wasps can use the specificity of the signal to locate particular hosts or even particular instars of their hosts (Turlings and Benrey 1998). On the other hand, generalist herbivores can also be attracted by single compounds of the VOC bouquet, which are commonly emitted after attack from a diverse set of herbivore species (Kessler and Baldwin 2001). In addition to attracting natural enemies, VOCs can function as direct defences by repelling ovipositing herbivores (De Moraes et al. 2001; Kessler and Baldwin 2001;

2004) or they may be involved in plant–plant interactions (Arimura et al. 2000; Karban et al. 2000).

Indirect plant defences may be compromised by direct plant defences if herbivores are able to sequester secondary plant metabolites and use them for their own defence. A number of studies have shown negative effects of plant secondary metabolites on the third (Campbell and Duffey 1981; Barbosa et al. 1991) and the fourth trophic level (Orr and Boethel 1986; Harvey et al. 2003), and suggest trait-offs between direct and indirect defences. However, direct and indirect defences have rarely been manipulated or characterized in the same experiment. Moreover, the parasitoid performance was only investigated in non-choice experiments. Since herbivore-induced VOC emission is a signal that is very specifically associated with host/prey (Turlings and Benrey 1998) it may provide information not only about the spatial distribution of potential hosts/prey species but also about their quality. Parasitoids or predators of the third trophic level may well be able to differentiate between good and bad hosts and may, in nature, actively avoid hosts which sequester plant metabolites that the natural enemies can not detoxify. Therefore we may more commonly observe a synergism rather than a trade-off between direct and indirect defences in nature because the plant’s direct defences may amplify the effects of parasitoid/predator attraction (Kessler and Baldwin 2004, see example below). There is an urgent need to approach this question for the apparent trade-off between direct and indirect defences in native systems without artificial human selection, because answering it provides one of the most important building blocks for utilizing plant defences in sustainable agriculture.

Defensive function of plant secondary metabolites

The biosynthetic pathways involved in the production of secondary metabolites have been or are currently elucidated with impressive speed, and the progress in identification of the underlying genetic and transcriptional mechanisms will only enhance this exploratory process. However, the knowledge about the ecological consequences of induced direct and indirect defences is sketchy and we are far from appreciating the complexity of the arena of plant–insect interactions to its full extent. The defensive function as well as direct physiological or indirect ecological costs of secondary-metabolite production can only be evaluated when the defensive traits can be experimentally manipulated and tested in comparative experiments, ideally in the plants’ natural habitats. This can be accomplished by both using chemical elicitors to induce specific plant responses and using mutants or transgenic plants that are not able to produce or over-express a particular defence (Thomas and Klaper 2004). This latter approach is largely restricted to a few model plant species, such as *Arabidopsis thaliana* (e.g. Van Poecke and Dicke 2004; D'Auria and Gershenzon 2005) or a limited number of agricultural crops (e.g. tomato, maize, rice). Thereby the current widespread exposure of genetically modified crop plants that express new defensive compounds, such as *Bacillus turingiensis*-toxin (Bt-toxin), to the natural arthropod community could be used to elucidate principal patterns in the plant–insect coevolutionary process (e.g. the evolution of the insect’s resistance to plant toxins) (Tabashnik et al. 1998). However, the conclusions derived from studies in agro-ecosystems may be limited because frequently neither the crop plant nor their herbivores are studied in their native habitats where coevolutionary processes occur or occurred. Similarly limiting is the study of single native species. The induction mechanisms of plant defences may differ among species specifically depending on internal factors, such as signal perception and transduction (elicitation), and external factors, such as the frequently complex web of interacting species on multiple trophic levels and abiotic factors. Thus, the inclusion of additional, preferentially native study systems to survey the diversity of internal and external factors influencing plant–insect interactions, will eventually reveal the general underlying mechanisms, which would allow a sustainable utilization of plant defences in agriculture.

Elicitation of plant responses

Any compound that comes from herbivores and interacts with the plant on a cellular level is a potential elicitor. A series of herbivore-derived elicitors have been isolated from the oral secretion of lepidopteran caterpillars and the oviposition fluid of weevil beetles. The elicitors represent three classes of compounds; lytic enzymes (Mattiacci et al. 1995; Felton and Eichenseer 1999), fatty-acid–amino-acid conjugates (FACs) (Halitschke et al. 2001; Alborn et al. 1997; Pohnert et al. 1999) from caterpillar regurgitant, and bruchins from the oviposition fluid of *Callosobruchus maculatus* (Doss et al. 2000).

Both herbivore feeding and mechanical damage induce plant responses that are systemically propagated throughout the plant or remain locally restricted to the wound site. As a consequence, the plant's response to herbivore damage must integrate the responses to the herbivore-unspecific mechanical wounding and the herbivore-specific application of insect-derived chemical elicitors. Wound-induced resistance is to a large extent mediated by products of the octadecanoid pathway, which includes linolenic acid-derived compounds, such as 12-oxophytodienoic acid, jasmonic acid and methyl jasmonate (Creelman and Mullet 1997; Wasternack and Parthier 1997). However, at least two more signalling pathways, to ethylene and salicylic acid, are involved in the plant response to herbivores. Although it is becoming increasingly clear that single signal cascades, such as the oxylipins, can alone produce a bewildering array of potential secondary signal molecules with a diversity of functions (Creelman and Mullet 1997; Farmer et al. 1998; Wasternack and Parthier 1997), it has also become apparent that herbivore attack frequently involves the recruitment of several signalling cascades. The interaction between these different signalling pathways, widely referred to as 'signalling crosstalk', may explain the specificity of responses. Reymond and Farmer (1998) proposed a tuneable dial as a model for the regulation of defensive gene expression based on the crosstalk of the three signal pathways for jasmonic acid, salicylic acid and ethylene. How the responses are fine-tuned to optimize the defence against particular herbivore species or the attack by multiple species or guilds is the subject of a series of recent investigations (Bostock et al. 2001; Walling 2000; Thaler and Bostock

2004). Genoud and Metraux (1999) summarized examples of crosstalks between different signal pathways and modelled them as Boolean networks with logical linkages and circuits. The model complements earlier crosstalk models and makes concrete predictions regarding the outcome of the interactions between different signalling pathways. Currently such models are limited by our incomplete understanding of all the signalling cascades that are involved and sketchy knowledge about the biochemical consequences of the expression and interactions of these pathways. Also sketchy is the understanding of how signal crosstalk translates to ecological interactions among players of the second and the third trophic levels and how compromised plant defence responses translate into plant fitness and eventually influence the coevolutionary process between plants and insects. An understanding of the functional consequences of signal crosstalk and the resulting expression of the various plant defences requires a sophisticated understanding of the whole plant function and natural history of the involved multitrophic interaction networks in the plants' native habitats (Kessler et al. 2004; Steppuhn et al. 2004).

The wild tobacco plant *Nicotiana attenuata* (Torr.ex Watts) is a study system in which modern molecular and chemical-analytical tools are being applied in field and laboratory experiments to understand the complex plant–insect interactions. The system, propagated by Ian T. Baldwin and his co-workers at the Max Planck Institute for Chemical Ecology in Jena, Germany, is a prime example of the modern consolidation of different research domains. In the following paragraph I will give a brief introduction into the study system and highlight studies that illustrate the complexity of species interactions that result from herbivore-induced plant responses and the potential importance of inducible plant defences for structuring the plant's arthropod community.

THE WILD TOBACCO NICOTIANA ATTENUATA

The wild tobacco plant *N. attenuata* grows ephemerally in Great Basin desert habitats in the southwestern USA. It germinates from long-lived seed banks in response to chemical cues in wood smoke (Preston and Baldwin 1999). One such compound, the butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, has recently been identified and found to promote seed germination in a number of plant species (Flematti et al. 2004). The 'fire-chasing behaviour' of *N. attenuata* forces the plant's arthropod herbivore community to re-establish with every new plant population. Inducible plant defences are thought to be an adaptation to such unpredictable herbivore pressure (Karban and Baldwin 1997). Wild tobacco increases its production of secondary metabolites (nicotine, phenolics, diterpeneglycosides, VOCs) and defensive proteins (trypsin proteinase inhibitors (TPI)) after attack by herbivores such as *Manduca* hornworms, *Tupiocoris notatus* bugs or *Epitrix hirtipennis* beetles (Kessler and Baldwin 2001; 2004), as well as in response to mechanical damage, or by elicitation with methyl jasmonate (Halitschke et al. 2000; Keinanen et al. 2001; Van Dam and Baldwin 2001).

Although the responses to these different elicitors frequently differ qualitatively and quantitatively, they diminish the plant's palatability to herbivores (direct defence) (Steppuhn et al. 2004; Van Dam et al. 2000; Zavala et al. 2004b) and/or increase its attractiveness to the natural enemies of the herbivores (indirect defence) (Kessler and Baldwin 2001; 2004). Anti-digestive proteins such as TPIs are known from several plant species to play a direct defensive role (Koiwa et al. 1997; Tamayo et al. 2000). Recent studies with natural mutants and antisense-transformed *N. attenuata* plants that are deficient in the induced production of TPIs, provide striking evidence for the defensive function of these anti-digestive enzymes. *Manduca sexta* caterpillars grow significantly faster and suffer from lower mortality rates on TPI-deficient plants than on plants with an intact TPI response or on plants that constitutively produced TPIs (Zavala et al. 2004b). The production of TPIs results in significant physiological fitness costs for the plant (Zavala et al. 2004a). In nature, the induction of plant defences in the absence of herbivores causes a significant reduction in lifetime seed production (Baldwin 1998). In contrast to the nitrogen-consuming production of defences such as nicotine or TPIs, the herbivore-induced production of VOCs is thought to be less costly (Halitschke et al. 2000). However, their indirect defensive effects may be not less important for the plant's fitness.

N. attenuata produces a series of VOCs, which derive from at least three different biochemical pathways (terpenoids, oxylipins, shikimates), in response to herbivore damage (Halitschke et al. 2000; Kessler and Baldwin 2001). Interestingly the four herbivore species (*M. sexta*, *M. quinquemaculata*, *T. notatus* and *E. hirtipennis*) that had been used in experiments elicited the emission of similar VOCs from *N. attenuata* plants (Kessler and Baldwin 2001). However, the quantities of the specific compounds, produced by the plant, differed significantly after the elicitation by different herbivore species. Some of the commonly emitted compounds have also been identified in the headspace of other plant species (Pare and Tumlinson 1998; Takabayashi and Dicke 1996; Turlings and Benrey 1998). Therefore it had been hypothesized that they may function as universal signs of herbivore damage and should, if singly emitted in the background of the plants' natural emissions, attract generalist predators in nature. The hypothesis proved right in that a generalist predator, the big-eyed bug *Geocoris pallens*, was attracted by the entire herbivore-induced VOCs bouquet as well as by single compounds (Kessler and Baldwin 2001; James 2005). In addition, adult *Manduca* moths used the same VOC signal to avoid already damaged plants for oviposition and thereby avoid increased predation pressure and reduced food quality as a result of induced direct defences. As a consequence, the multiplicative effect of the bottom-up and top-down components of herbivore-induced VOC emission was significant. It could reduce the numbers of *N. attenuata*'s most damaging herbivore, *M. quinquemaculata* by over 90% (Kessler and Baldwin 2001).

N. attenuata is attacked by many herbivore species from different feeding guilds in nature. However, these species may not always co-occur on the same plant due to plant-mediated effects. For example, the leaf-chewing larvae of the sympatric sibling species *M. sexta* and *M. quinquemaculata* tend not to co-occur with the sap-sucking mirid *T. notatus*, even when both species are found in adjoining host populations. Moreover, in plant populations with high numbers of plants infested by *T. notatus* the mortality of *Manduca* larvae and the seed-capsule production of *N.*

attenuata plants was higher than in plant populations without *T. notatus* (Kessler and Baldwin 2004). The apparent mechanism of this antagonistic relationship between two herbivore species and its fitness consequences for the plant reflects the complexity of plant–insect interactions and the size of the arena in which the interaction is played out (Figure 1).

That the two hornworm species and the mirid bugs seemed not to interact directly led us to hypothesize that plant-mediated effects caused the seemingly competitive interaction between the herbivores. Indeed, *M. sexta* and *M. quinquemaculata* hornworms grew much more slowly on plants that previously had been damaged by *T. notatus* than on undamaged plants. Interestingly, the metabolic responses to the damage by leaf-chewing hornworms and piercing-sucking mirids seemed very similar. The concentrations of a series of plant resistance-related secondary metabolites (phenolics and diterpene glycosides) and TPI were similarly increased in hornworm and mirid-damaged plants compared to undamaged plants. In confirmation with this result the *Manduca* larvae grew slower on plants that had been damaged by both conspecific caterpillars and mirids than on undamaged plants. Moreover, the emission of VOCs as well as the production of direct defensive compounds was increased after the damage by both herbivore species. Herbivore-induced VOCs in turn can function as indirect defences by attracting predators, such as *G. pallens,* to the damage site. As attack from both species elicits rather similar direct and indirect defensive plant responses, it was likely that the ecological context of these similar responses determines fitness consequences of the interaction for the *Manduca* hornworms and as a consequence for the plants (Kessler and Baldwin 2004).

One fitness benefit for the plant arises from the natural history of its interactions with herbivores. *Manduca* hornworms can consume three to five plants before they reach the pupal stage and therefore are considered the most damaging insect herbivores on *N. attenuata*. The hornworms usually depart before the plant is completely consumed, but the amount of leaf tissue lost to hornworm feeding is negatively correlated to the lifetime seed-capsule production of *N. attenuata*. Therefore, the plant's fitness costs from hornworm damage depend strongly on the developmental stage in which the hornworm leaves the plant or is removed by natural enemies such as parasitoids or predators. The growth-reducing effect of TPIs and secondary metabolites elicited by previous hornworm and mirid attack causes subsequently feeding hornworms to remain longer in the first two larval instars. As a consequence, the younger, more vulnerable hornworms are exposed longer to the dominating predator, the big-eyed bug *Geocoris pallens*, which is additionally attracted by the herbivore-induced VOCs (Kessler and Baldwin 2004). The direct effects of mirid-induced plant responses amplify the indirect defensive effects of predator attraction with negative fitness effects for the hornworms. Moreover, the predators prefer young hornworms over mirids as prey, which adds yet another factor contributing to the outcome of the interaction between the plant and its insect community.

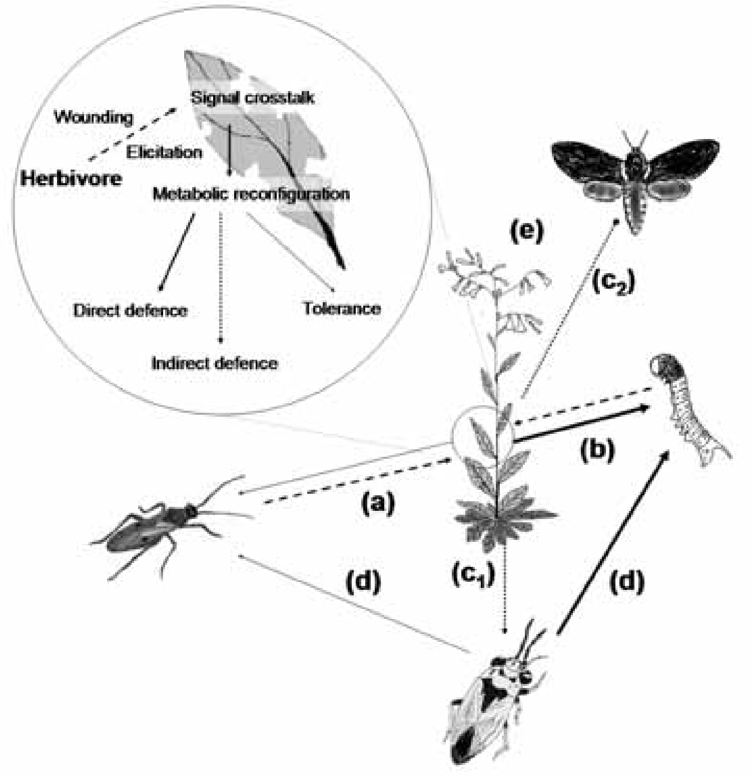

***Figure 1**. The herbivorous mirid bug Tupiocoris notatus vaccinates the wild tobacco plant, Nicotiana attenuata against the more damaging tomato hornworm, Manduca quinquemaculata. **(a)** T. notatus damage (leaf-tissue wounding in combination with the application of salivary excretions) elicits a reconfiguration of the plant's secondary and primary metabolism. **(b)** The resulting mirid-induced production of toxic and anti-digestive plant compounds functions as **direct defence** and reduces the growth of the more damaging herbivore M. quinquemaculata, which therefore remains longer in the for predators vulnerable first two instars. **(c)** In addition the plant releases volatile organic compounds in response to mirid and hornworm damage, which attract the predatory bug Geocoris pallens to the plant (**indirect defence**) (c_1) and repel adult M. quinquemaculata moths from oviposition (c_2). **(d)** The predator G. pallens prefers young Manduca hornworms over Tupiocoris bugs as prey. The direct effects of mirid-induced plant responses amplify the indirect defensive effects of predator attraction with negative fitness effects for the hornworms. **(e)** Mirid-damaged plants, in contrast to hornworm-damaged plants, seem to compensate metabolically for the allocation of resources (**tolerance**) into defences and produce the same number of seeds as undamaged control plants. With the elicitation of induced direct and indirect responses and the neutral effect on plant fitness, T. notatus attack 'vaccinates' N. attenuata plants against the more severely damaging Manduca hornworms. Manduca damage also induces the production of toxic and anti-digestive plant compounds but results in a significant fitness loss for the plant. The effects of the herbivore-induced plant responses on Tupiocoris fitness remain unknown*

Interestingly, the reproductive consequences of hornworm and mirid attack are very different for the plant. While the plant metabolically responds very similarly to hornworm and mirid attack and gains resistance to hornworms, attack by mirids (in contrast to attack by hornworms) does not reduce the reproductive success of the plant, although the damage from these piercing-sucking insects can be substantial. Thus, mirid-damaged plants seem to compensate metabolically for the allocation of resources into defences. A differential display-reverse transcriptase PCR and subtractive library study of mirid-attacked *N. attenuata* plants (Voelckel and Baldwin 2003) revealed a series of mirid-specific transcriptional responses, which suggest that an adjustment of the primary metabolism is involved in the plant's ability to tolerate mirid attack. Particularly interesting is the mirid-induced increase in ribulose-1,5 bisphosphate carboxylase (RuBPCase) activase transcripts, which code for a stromal, regulatory protein that regulates the activity of the key enzyme in CO_2 assimilation, RuBPCase (Portis 1995). In addition, a cDNA microarray analysis that compared the transcription patterns induced by mirids and hornworms, respectively, identified that herbivore-specific changes occur largely in the primary metabolism and signalling cascades rather than secondary metabolism (Voelckel and Baldwin 2004). Experiments with *Datura wrightii* reported similar neutral effects of *T. notatus* attack on plant fitness and suggested that damage by *T. notatus* may reduce photosynthetic capacity less than equivalent damage by chewing insects does (Elle and Hare 2000; Hare and Elle 2002). With the elicitation of induced direct and indirect responses and the neutral effect on plant fitness, *T. notatus* attack literally 'vaccinates' *N. attenuata* plants against the more severely damaging *Manduca* hornworms. The neutral effects on plant fitness and herbivore-induced plant defences in the context of the particular life-history traits of the interacting species provide the mechanism for the plant vaccination phenomena (Figure 1). The study shows that a suite of rather similar responses to attack from different herbivores can result in dramatic differences in plant fitness and illustrates the importance of studying plant–insect interactions in the rough and tumble of the natural environment (Kessler and Baldwin 2004).

Herbivore-induced responses as the one described above depend to a great extent on a functioning oxylipin signalling in the plant. In *N. attenuata* the wounding of leaf tissue is recognized by an endogenous jasmonic-acid (JA) burst (Baldwin et al. 1997; Schittko et al. 2000) that results in the expression of a series of defence-related genes (Halitschke et al. 2001) and eventually in the increased production of defensive compounds such as nicotine and TPIs (Baldwin et al. 1997; Van Dam et al. 2000). However, the plant response to herbivory frequently differs from the response to mechanical damage of the leaf tissue. For example, the attack from *Manduca* caterpillars is recognized by the plant as evidenced by a JA burst that is far greater than that produced by mechanical wounding (Halitschke et al. 2001; Schittko et al. 2000). This JA burst is associated with the expression of wound-responsive and JA-independent genes, and the introduction of oral secretions from the feeding caterpillar account for the differences (Halitschke et al. 2003). Interestingly, the specific elicitation by caterpillar oral secretions accounts also for an ethylene burst (in addition to the JA burst) in response to herbivore damage, which attenuates the damage-induced accumulation of nicotine (Kahl et al. 2000). The ethylene burst antagonizes the wound-induced transcriptional increase in the nicotine biosynthetic genes NaPMT1 and NaPMT2 (Winz and Baldwin 2001).

Current experiments with genetically transformed *N. attenuata* plants in their native habitat emphasize the crucial role of oxylipin signalling for the plant's herbivore defence and the impact of induced plant defences on the arthropod community composition. Halitschke and co-workers (Halitschke and Baldwin 2003; Halitschke et al. 2004) generated transformed *N. attenuata* lines, which expressed *N. attenuata* lipoxygenase 3 (NaLOX3), hydroperoxide lyase (NaHPL) and allene oxide synthase (NaAOS) in an antisense orientation. All three enzymes are key regulators in two distinct oxylipin pathways and play a major role in the plant's wound recognition. In laboratory studies, plants deficient in the expression or recognition of octadecanoids, derived from LOX3, are unable to elicit defence compounds and are more susceptible to herbivore attack. The herbivore resistance can be restored by externally treating the LOX3-deficient plants with methyl jasmonate (the methyl ester of jasmonic acid) (Halitschke and Baldwin 2003). Interestingly, AOS-deficient *N. attenuata* plants partially reduced JA and defence-compound accumulation but this did not attenuate the resistance to herbivores, which was attributed to a leaky genotype and is currently under further investigation. HPL-deficient plants did not produce C_6-aldehydes and alcohols (green-leaf volatiles), which can function as defences (antimicrobial and as direct defences against some herbivores) or as wound signals to transmit information within (Sivasankar et al. 2000) and between plants (Arimura et al. 2000). However, HPL-deficient plants retained their resistance against hornworm damage despite their potential signal function. In fact hornworms in the laboratory consumed and grew more slowly on HPL-deficient plants than on wild-type control plants. The hornworms' growth rate could be restored to the levels of wild-type plants if GLVs were added to the HPL-deficient transformants, which suggests that GLVs stimulate feeding by *Manduca* hornworms (Halitschke et al. 2004).

The example of plant vaccination by mirid herbivores illustrates how important the ecological context is when interpreting the function of an interaction-mediating trait. Therefore we exposed the same transformants that so convincingly confirmed the crucial function of oxylipin signalling in the laboratory to the natural arthropod community in their native habitat in southwestern Utah (Kessler et al. 2004). First, the plants responded to standard bioassays with *Manduca* caterpillars in the field much as they did in the laboratory. The most pronounced effect was the loss of resistance in LOX3-deficient plants. The plants were more susceptible to *Manduca* damage in the standardized bioassay and received more damage from natural herbivory than wild-type control plants and the two other transformed plant lines. However, a more detailed analysis of the herbivore community that had established on the plants revealed that the herbivore-induced plant responses can alter the host spectrum of generalist herbivores. We found two new herbivore species on the LOX3-deficient plants that do not usually feed on *N. attenuata*: a leafhopper *Empoasca* sp. and the western cucumber beetle *Diabrotica undecimpunctata tenella* (Figure 2). In fact, most of the observed damage on LOX3-

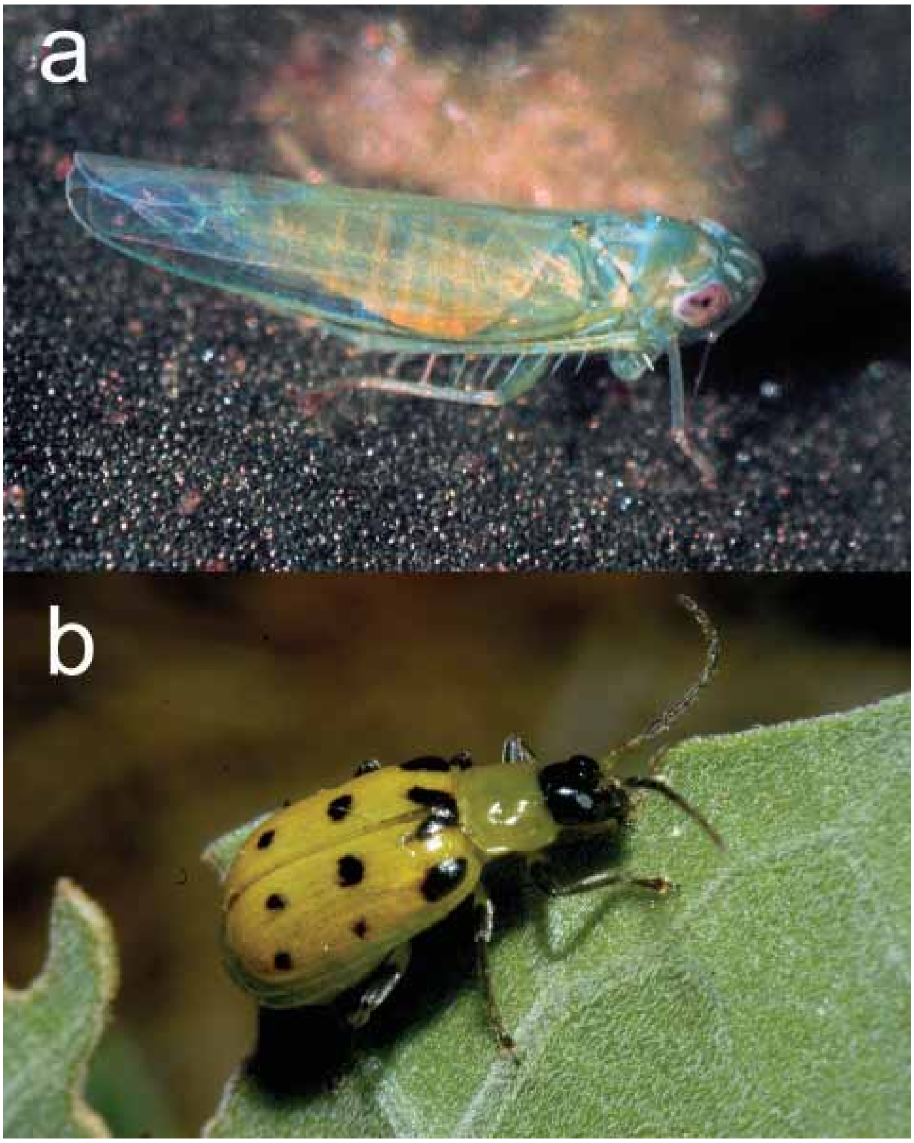

Figure 2. *New herbivores on lipoxygenase3 (LOX3)-deficient Nicotiana attenuata plants.* ***(a)*** *The leafhopper Empoasca sp. and* ***(b)*** *the leaf beetle Diabrotica undecimpunctata tenella do not usually feed on wild tobacco N. attenuata, but use plants, transformed with an antisense construct of N. attenuata LOX3 as new host plants in nature*

deficient plants resulted from one of the new herbivores, *Empoasca* sp., which successfully reproduced on the new, undefended host plants. The results of this study demonstrated that the LOX3-mediated inducibility of plant responses is crucial for the oviposition decision and the opportunistic host selection behaviour of generalist herbivores such as *Empoasca* sp. and *D. undecimpunctata*. Host selection thus seems determined not only by the plant's constitutively expressed chemical phenotype and external mortality factors but also by the plant's ability to induce responses to herbivory (Kessler et al. 2004). As with the discovery of the plant vaccination effect of mirid damage, the study with plants that are not able to induce responses to herbivory emphasizes the role of induced plant defences in structuring arthropod communities. Moreover, the few selected examples from the *N. attenuata* system point to the value both of using genetically silenced plants and molecular tools in ecological research and of studying plant–insect interactions in the full complexity of the natural environment.

PLANT–INSECT INTERACTIONS AND GENOME PROJECTS – A CONCLUSION

Genomic and molecular technologies have expanded the types of questions that can be addressed in the research of plant–insect interactions and ecology as a whole. Modern genomic and molecular approaches provide ways to examine physiological mechanisms of biological interactions including elicitation of responses, signal perception and transduction by the plant at the cellular level, and the ecological function of traits, such as the fitness effects of plant defences on the whole-plant and community level. However, ecologists interested in using genomic tools are currently restricted to the limited number of model organisms that already have significant genomic resources available (Thomas and Klaper 2004). The development of genomic tools for research on ecological study systems with well characterized natural histories appears to be too time- and resource-consuming to be achieved in our rather ephemeral and resource-limited research environments. On the other hand ecologists have only begun to utilize the already available genetic and molecular model systems to answer ecological questions although the current success of this approach is promising. For example, the list of secondary metabolites isolated from the genetic model plant *A. thaliana* has grown more than five-fold in the last ten years and the biosynthetic pathways resulting in these compounds as well as their ecological function are revealed with breathtaking speed (D'Auria and Gershenzon 2005). The vast diversity of available *Arabidopsis* mutants and the applicability of the developed genetic tools for studies on related species have inspired a number of ecological studies (e.g. Clauss et al. 2002; Cipollini et al. 2003; Van Poecke and Dicke 2004; Cipollini et al. 2005) which provide a basic building block for future research in the study of species interactions.

The constantly growing number of plant species whose genome will be partially or fully sequenced will allow these tools also to be applied to ecological (supplementary to genetic) model systems in two ways: a) the increasing number of genetic model systems also increases the number of wild relatives to which the developed tools can readily be applied; b) the development of genetic tools for new systems will be faster and more cost-efficient. For example, comparative genomics provides a tool to utilize the increasing sequence information from model plant species to clone genes that mediate the plants' resistance to herbivores from less studied native species (Mueller et al. 2005). Comparative approaches have already been used in modern plant breeding to identify genes that are involved in plant development and resistance to abiotic and biotic stresses (King 2002; Shimamoto and Kyozuka 2002). In addition, manipulative techniques such as genetic transformation methods can help to reveal the function and ecological relevance of defensive traits in nature (Kessler et al. 2004; Steppuhn et al. 2004).

The recently launched Solanaceae Genome project, although focusing on the genome sequence of the domesticated tomato *Lycopersicon esculentum* (*Solanum lycopersicon*), promotes the parallel sequencing and comparative biology of a number of species in the Solanaceae family, including wild species (www.sgn.cornell.edu). That way it will supplement and extend the opportunities given by the classical genetic model plants and increase the number of potential systems to study multi-species interactions in nature. Utilizing the new genetic tools and information and apply them in native plant systems to answer ecological and evolutionary questions will be crucial to understand the mechanisms of species interactions. And, it is through this integrative approach that we will be enabled to reveal how cells, organisms and ecosystems function.

With the growing appreciation of the importance of species interactions in natural as well as in agricultural systems, the success of the genome projects will be increasingly measured by their contributions to integrative biological research fields. Therefore, the modern consolidation of the once-separated biological research domains becomes a research necessity as well as a logical consequence of these domains' conceptual interdependence. The *N. attenuata* example nicely illustrates the multiple spatial scales on which plant–insect interactions are played out. In addition it emphasizes both the value of using genetic and molecular tools in ecological research and, more importantly, the value of profound natural-history knowledge when studying multi-species interactions. *N. attenuata* is only one out of the estimated 230-422,000 flowering plant species interacting with only a few of the estimated 2 to 30 million insect species. In order to understand the patterns in community ecology and biodiversity we may not need to study all the possible interactions. But in order to apply our knowledge in agriculture and species conservation we will need at least a few well studied examples derived from a good number of different habitats. In short, and most importantly: we must never stop exploring in the old naturalist's way.

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