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Mechanisms and ecological consequences of plant defence induction and suppression in herbivore communities

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Introduction

At a first glimpse, plants seem subject to the caprices of their environment, being constantly confronted with stresses such as drought, heat or ultraviolet radiation while frequently being challenged by pathogens or herbivores. However, these stresses have posed selection pressures on plants that have resulted in numerous adaptations, ranging from stress tolerance and resistance to the ability to manipulate their environment. Many of these adaptive traits have multiple functions. For example, plants are equipped with a waxy cuticle that prevents evaporation of water, but also forms an efficient barrier against phyto-pathogens (Eigenbrode and Espelie, 1995). Moreover, many...
species have epidermal leaf hairs (trichomes), some of which carrying a special gland at the tip, and for which a broad diversity of functions have been reported. For example, they form structural barriers that hinder small arthropods in their mobility (Simmons and Gurr, 2005), but also guide light to the leaf surface of the leaf so that it can be used optimally for photosynthesis (Wagner et al., 2004) while filtering out UV-A and -B (Karabourniotis and Bornman, 1999). Moreover, glandular hairs also secrete protective coatings that prevent fungal spores from germinating (Shepherd et al., 2005), and contain glues and toxins that obstruct and intoxicate plant-surface-dwelling arthropods when ruptured (Glas et al., 2012). Preformed structural barriers are referred to as ‘constitutive defences’ and they augment the so-called induced defences, which become operational only in response to an attack.

Defence against herbivores requires measures different from defence against pathogens. In general, phytopathogens are less mobile than herbivores, and migration across or through their host plant is often passive and occurs over relatively short distances. For example, plant-infecting viruses usually need plant proteins for tissue-to-tissue transport, plant-infecting fungi can only relocate by growing longer hyphae and plant-infecting flagellar bacteria can only travel short distances by taxis in fluid media. Hence, the infected host can attack such relatively immobile pathogens on the spot; at the site of infection, plants will often respond by producing structural reinforcements (e.g. cell wall thickening and callose deposition) or toxins (e.g. phytoalexins or alkaloids), or by initiating programmed local cell death (apoptosis) to isolate and possibly kill the pathogen (Dangl and Jones, 2001). Programmed cell death, orchestrated by the hypersensitive response, is highly efficient in preventing pathogens from spreading and is therefore one of the most common anti-parasite defence strategies found in nature. Herbivores, however, are not very susceptible to this isolate-and-kill strategy because they are mobile. Therefore, anti-herbivore defences generally come down to a go-away-or-die strategay or a slow-them-down strategy, and these two strategies share many physiological characteristics. In both instances, plants will mount a sequence of defence programmes that serve to interfere with herbivore growth and development on the one hand and to reallocate resources on the other, in order to delay growth of the herbivores into larger individuals, stages or populations, which consume more plant tissue. For example, well-known anti-herbivore defence proteins include proteinase inhibitors (PIs) and polyphenol oxidases (PPOs), both believed to interfere with digestive processes in the herbivore gut (Zhu-Salzman et al., 2008). Hence, the simultaneous reallocation of resources may not only serve to rescue resources so that the plant can use them later for growth and reproduction, but may also serve to deprive the herbivore of food. This may be an effective strategy, especially when the plant is attacked by small herbivores or relatively immobile (immature) stages. Resource allocation is characterized by reallocation of nutritious carbon-containing (Schwachtje et al., 2006; Babst et al., 2008) and nitrogen-containing (Newingham et al., 2007; Gomez et al., 2012) substances to either the reproductive tissues or the storage organs, such as roots (Anten and Pierik, 2010). Hence, it can give rise to rapid flowering or to a period of dormancy (Stowe et al., 2000) to regrow later, depending on the life-history characteristics of both plant and herbivore. Consequently, herbivores that decide to stay on defended plants select plant tissues where defences are lower (Paschold et al., 2007; Shroff et al., 2008; Stork et al., 2009) and/or increase their feeding intensity to gain sufficient biomass, thus compensating for the decreased efficiency of food conversion (Gomez et al., 2012). Plants, in turn, often initiate systemic responses (Pieterse et al., 2009) to decrease the chance that the herbivore will simply move to undefended tissues (Paschold et al., 2007) and furthermore produce secondary metabolites to constrain compensatory feeding responses (Steppuhn and Baldwin, 2007).

The sequence of defence programmes executed by plants under attack appears not to be fully hard-wired, suggesting a certain degree of herbivore-specific tailoring by the plant, and while the early responses seem usually aimed at rescuing the attacked tissue, they may shift towards senescence and tissue death (reminiscent of the hypersensitive response) after a couple of days (Steinbauer et al., 2014). It is unknown whether, how and when the plant decides to switch from the ‘rescue’ response to the ‘scorched earth’-like tactic and whether this is characterized by a partial (Kahl et al., 2000) or complete induced shut-down of local metabolic activity, e.g. to initiate senescence (Gross et al., 2004). Moreover, it is unclear whether, and if so for how long, tissues that are being sacrificed are supported by photosynthates (Ferrieri et al., 2013) and by defensive products from distal tissues (Nabity et al., 2009). From the herbivore’s point of view, resource depletion at the feeding site may represent a more difficult problem to deal with than toxins, because they cannot develop resistance to an absence of nutrients. However, some herbivores such as gallmakers have evolved abilities to manipulate plant resource flows and turn their feeding site into a sink for resources (Tooker et al., 2008), but also leaf-cutters and trenchers (Dussourd and Denno, 1991) that prevent plants from transporting defence compounds to the feeding site and possibly also from transporting resources away from it.

Whereas the role of resource allocation remains under-studied, induced plant defences and their effects on herbivores have been analysed in great detail. In this review, we evaluate what determines the susceptibility of arthropod herbivores to plant defences and which herbivore traits counteract these defences. We focus on the induction and suppression of plant defences and outline how traits determining the susceptibility of parasites to plant defences can give rise to exploitative competition and facilitation in the ecological communities inhabiting the plant. First, we describe the diversity of herbivore feeding modes and life styles with which plants have to cope, and how plants detect the presence of herbivores through salvia and oral secretions.

**HERBIVORE FEEDING AND PLANT DEFENCE STRATEGIES**

*Herbivore feeding styles*

Early in evolution, herbivorous arthropods were mandibulate and chewed on vegetation (Budd and Telford, 2009). After vascular plants had emerged, many different forms of feeding evolved, such as sap-sucking, leaf-mining, gall-forming and nectar-feeding (Grimaldi and Engel, 2005). Among the arthropods, many differently shaped mouthparts can be found which are suitable for chewing, siphoning, piercing–sucking and...
sponging, or a combination of these. Chewing insects, such as many lepidopteran larvae, but also beetles and grasshoppers, have two mandibles, surrounded by sensory organs, which they use for crushing or cutting food while mixing it with salivary secretions before swallowing it. This type of feeding removes relatively large quantities of leaf material. Siphoning insects, typically adult lepidopterans, feed by sucking without piercing. Often, these insects have a long coiled proboscis that can be extended to reach food such as nectar. Siphoning itself does not cause much damage, although flowers may be damaged severely by an agile siphoning insect (Kessler et al., 2008). The piercing–sucking arthropods feed by means of styli, which are needle-shaped mouthparts. Styli are common among insects and mites, although they probably evolved independently in these groups and possibly even more than once among mites (Manton and Harding, 1964; Caravas and Friedrich, 2010). The insect stylet is a single anatomical structure, often equipped with two separate channels, one for releasing saliva and the other for ingesting food (Miles, 1999). Mite mouthparts are composed of two or more styli, which can be grouped together in pairs to form a piercing structure with an internal duct, which probably serves to release saliva (Ragusa and Tsolakis, 2000). While stylet-feeding insects such as whiteflies, psyllids and aphids usually feed from vascular tissue (Miles, 1999), herbivorous mites feed on the contents of mesophyll cells (Park and Lee, 2002). Insects such as thrips are usually also considered stylet–mesophyll feeders, but have an asymmetrical mouth cone, i.e. they have a single mandibular stylet, which is used for piercing and rasping plant tissue, after which their acinial stylet is inserted in the wound to take up food (punch-and-suck feeding) (Coll and Guershon, 2002; Kindt et al., 2003). Finally, sponging insects have non-functional mandibula but an extended food channel that ends in a sponge-like labellum. They secrete saliva, which is dabbed onto their (solid) food to liquefy it, after which the labium channels the food to the oesophagus. This mode of feeding is typical of many species of flies (Vijaysegaran et al., 1997). Thus, arthropods have diverse feeding styles and, especially among insects, different life stages can make use of distinct modes of feeding.

Different herbivore life stages feed differently

Different feeding styles accompany diverse life styles of herbivorous arthropods. These can differ considerably across stages of the life cycle, especially in holometabolous arthropods, but also in many hemimetabolous species. For example, adult thrips are winged and feed mostly from plant tissues. They deposit their eggs under the epidermis of the host plant and the wingless larvae that emerge from these eggs move across the plant surface to more protected areas and feed from pollen, nectar and/or leaf tissue until they develop into the nympha (the third larval stage) and move into the soil to pupate (Broadbent et al., 2003). Many other herbivores also spend part of their life cycle inside the host plant and/or the soil. Many lepidopteran and dipteran species have larval stages called leaf miners; they live inside a leaf or in needle tissue, where they feed and find protection. The adults of leaf miners are free-living but deposit their eggs underneath the leaf cuticle, where the larvae usually stay to graze the mesophyll until they develop into pupae. This feeding behaviour causes the formation of distinct blotches or serpentine tunnels on plant leaves (Connor and Taverner, 1997). In addition, leaf-rollers are moth larvae that feed and pupate within the protection of rolled-up leaves; these leaves are either cut and folded or rolled up and wrapped by the larvae in their silk to create a shelter and feeding site (Gaston et al., 1991). Leaf miners and leaf-rollers usually cause minor damage, i.e. mostly aesthetic, but can cause defoliation and fruit malformation when densities are too high (Witzgall et al., 2008). Another group of herbivores are the stem and root borers, which deposit their eggs in stems or roots and whose larvae consume these tissues. They can be very damaging, not only because they often cause stunted growth, but also because they weaken the plant structure. Some species of borers have specialized in specific plant tissues such as fruits and seeds (frugivores) or bark (Mainali, 2014). Whereas leaf rollers physically create their shelter, galling insects induce somatic plant tissues to form domatia (Stone and Shonrogge, 2003). Many species of gall midges (Harris et al., 2003) and gall mites (Van Leeuwen et al., 2010a) manipulate the plant into producing tumorous outgrowths by mitosis to form hollow structures in which they spend a substantial part of their life cycle and which often serve as a feeding site, reminiscent of galls induced by root-knot nematodes (Caillaud et al., 2008) or Agrobacterium (Zhu et al., 2000). Many eriophyids do not induce protective cavities but other types of external malformations, like tufts or hairy outgrowths (erineum), in which they can seek shelter, while others induce deterioration rather than formation of plant leaf hairs (Karioti et al., 2011; Van Houten et al., 2013). Finally, most sap feeders, such as aphids, whiteflies and psyllids, spend their whole life cycle on the plant surface. However, the juvenile stages of these homopterans can be relatively immobile and sometimes cover themselves with honeydew for protection (VanDoorn et al., 2015). Because phloem lacks essential amino acids, many homopteran species possess bacteriomes which harbour symbiotic bacteria that provide these and vitamins in return for nutrients (Schwemmler, 1989). Thus, plants encounter a wide range of different attackers and are therefore in need of defence systems that allow a degree of tailoring.

Plant defence theories

Understanding the mechanisms that plants have evolved to defend themselves and identification of the ecological drivers of this evolution have been major challenges during recent decades. Whereas research focusing on plant physiological aspects of these defences mostly worked from a scenario in which a single plant species is attacked by a single species, it has become increasingly clear that the diversity of ecological interactions within plant-inhabiting communities is an important determinant of the evolution of plant defence strategies. This notion gave rise to several theoretical frameworks revolving around the central dilemma that plant defences require resources that would otherwise be available for growth and reproduction (Mattson, 1980; Stamp, 2003). These frameworks can be classified into two groups: one of these attempts to explain the distribution of plant defences based on defensive function and plant life history, whereas the other group attempts to
explain this based on resource availability. The first theoretical framework is the optimal defence theory, which hypothesizes that plants with limited resources will defend different tissues differently, depending on the chance they will be attacked, the fitness value of the tissue and the cost of the defence. It was suggested that such a strategy will impose an ‘evolutionary dilemma’ leading to selection for defences at intermediate levels when plants are frequently attacked by generalists and specialists, because the latter will generally be more resistant to the plant’s defences than the former (Zangerl and Rutledge, 1996). This theory is related to the plant apparency hypothesis (Feeley, 1976), which posits that plants that are attacked by a relatively large diversity of herbivores will need to display a larger diversity of defences than plants that are targeted by few species. It assumes that, compared with short-lived species, long-lived species are more likely to encounter generalists as well as specialists during their lives and hence have been under selection to display a broader range of defences. Third is the carbon:nutrient balance hypothesis, which hypothesizes that plant defences are constrained by nutrient variation in the environment and posits that the C:N ratio will dictate which secondary metabolites are synthesized (Bryant et al., 1983). This hypothesis predicts that changes in available nutrients will change the pallate of defences. The fourth is the resource availability hypothesis, which posits that defence strategies are determined by the inherent growth rate of the plant, which is assumed to be constrained by resource availability (Coley et al., 1985). This theory implies that a trade-off between growth rate and defences will restrict species to particular habitats. Finally, the fifth theory is based on the growth–differentiation balance hypothesis and it subsumes elements of the previous hypotheses (Hermes and Mattson, 1992). It posits that the ecological costs of the physiological trade-off between growth and secondary metabolism (defence) vary across environments. Since plants must protect their acquired resources, which are needed for growth in order to be able to compete for new resources, natural selection has shaped their secondary metabolism to be flexible and their life histories to vary across environments. Hence, this theory aims to explain patterns of phenotypic and genetic variation in secondary metabolism in response to environmental variation and resource gradients. It needs to be said that it has been difficult to generate testable hypotheses from these defence theories, not only because the magnitudes of the costs and benefits of defences have proved to be very difficult to measure, but also since they often fail both to distinguish among evolutionary, ecological and physiological levels of analysis and to clearly distinguish between genetic and environmental influences.

Plant defence strategies

Plant defences are often divided into three basal strategies: deterrence (antixenosis), resistance (antibiosis) and tolerance (a compensation strategy to reduce the detrimental effects of herbivory). Deterrence traits are usually constitutively expressed and can emanate from colours, odours or textures (such as hairs) that demotivate a herbivore from feeding on the plant, or from the absence of feeding stimuli that otherwise would stimulate the attacker. Resistance traits are those that can injure or kill a herbivore or slow its development and reproduction. Finally, tolerance comes from those traits that do not primarily serve to negatively interact with the herbivore, but to compensate for damage through changes in assimilation rate, compensatory growth, phenological shifts, resource allocation or morphological changes. These three strategies are not mutually exclusive and can overlap mechanistically and functionally. Hence, it will often be difficult to tell these three strategies apart (Stout, 2013) and it is doubtful whether deterrence as a stand-alone defence strategy will be evolutionarily stable since it offers ample opportunities for herbivores to adapt.

Constitutive plant defences

Plants cannot simply accumulate all the defences that have emerged during the course of evolution within a ‘super-genotype’ because defensive structures, compounds or processes such as the inducible defences (Baldwin, 1998) cost energy to form and maintain. Hence, only those defences for which selection pressure has been constant and strong enough have been retained. Moreover, the optimal defence theory predicts that plants with limited resources are selected to arrange the relatively costly and less costly defences across tissues based on the fitness value of these tissues. Moreover, assuming that the induced defences are overall less costly than constitutive defences, this theory predicts that tissues with a higher probability of being attacked will rely more on constitutive defences, whereas tissues with a lower probability of being attacked will depend more on induced defences. Indeed, the reproductive parts of wild parsnip (Pastinaca sativa) had the highest levels of constitutive furanocoumarins with low inducibility and had the highest probability of being attacked, while in the roots, which were less frequently attacked, constitutive levels were relatively low but highly inducible (Zangerl and Rutledge, 1996).

Discriminating experimentally between constitutive and induced defences is often not easy since there can be considerable overlap. For example, the size and density of physical barriers such as spines and plant hairs (Glas et al., 2012), which are commonly considered to function as constitutive defences, can also be increased by induction (Traw and Dawson, 2002). For example, formation of more and longer thorns was induced by giraffes feeding on acacia (Acacia seyal) trees (Milewski et al., 1991). Moreover, not only are leaf hairs mechanical barriers (Pott et al., 2012), but glandular trichomes (Tissier, 2012) are important production sites of a wide variety of constitutive and induced secondary metabolites with defensive functions, including terpenoids, phenylpropenes, flavonoids, methylketones, acyl sugars and defensive proteins, and some of these compounds are inducible. For instance, the emission of terpenoids (Van Schie et al., 2007) and the production of acyl sugars and defensive proteins (Hare and Walling, 2006) can be induced in trichomes by treating plants with methyl jasmonate, a compound that activates the jasmonate (JA) signal transduction cascade, also induced by herbivores. Even mere contact of insects with a plant was found to suffice to induce the expression of PIs in glandular trichomes (Peiffer et al., 2009). The contents of glandular trichomes, such as sticky acyl sugars and polyphenols, can be excreted or released after they are ruptured by insect movement, and cause entrapment of small herbivores.
often followed by death (Simmons et al., 2004). Trichomes of tobacco (Nicotiana tabacum) also produce defensive proteins that are secreted to the leaf surface and inhibit germination of oomycete spores (Shepherd et al., 2005). Conversely, some pathogens, like Pseudomonas syringae on tomato, have adapted to use trichomes as their habitat (Schneider and Grogan, 1977), and damaged trichomes may be used as an entry point by these bacteria (Huang, 1986). Thus, trichomes are important components of both the constitutive and the inducible defence system, and trichome secretions function to hinder herbivore feeding and the germination of fungal spores. Other forms of constitutive defence involve the plant’s waxy cuticle; this primarily serves to prevent the evaporation of water (Buschhaus and Jetter, 2012), but wax morphology and chemistry contribute to a plant’s resistance by restraining herbivore foraging behaviour (Eigenbrode and Shelton, 1990). Also, leaf toughness, which includes cell wall lignification, is known to deter herbivore feeding (Choong, 1996) and is positively correlated with resistance to pathogens (Bhuiyan et al., 2009).

Induced plant defence

Induced defences are often subdivided into direct and indirect defences. Direct defence includes the activation or production of antifeedants, such as toxins and inhibitors of digestion, which negatively affect the growth and/or survival of herbivores (Howe and Jander, 2008). The existence of induced defences has been known for more than 100 years (early work is reviewed by Chester, 1933). A well-known example of a herbivore-induced plant defence is increased PI gene expression and enzyme activity. Although most plant PIs have regulatory roles in the plant’s endogenous protein metabolism, the herbivore-induced PIs inhibit proteases in the gut of herbivores, thereby decreasing the plant’s palatability and increasing its resistance (Hartl et al., 2010). Defences may also be induced in the phloem (Will et al., 2013). For instance, feeding on rice by the brown planthopper (Nilaparvata lugens) induces the deposition of callose on the plant’s sieve plates to block further transport of sap through the attacked phloem tissues (Hao et al., 2008). Another mechanism of such sieve tube occlusion is dependent on large protein bodies called forosomes, which can seal off sieve elements in a Ca²⁺-dependent manner (Furch et al., 2007) to obstruct herbivore feeding.

Indirect defence refers to plant traits that enhance attraction or arrestment of natural enemies of the herbivore, such as predators and parasitoids (Sabelis et al., 2001). Often, this type of defence is inducible. That natural enemies of herbivores use plant odours for locating prey has been suggested several times (reviewed by Vinson, 1976), and Dicke and Sabelis (1988) outlined a framework for the mode of action and the evolution of indirect defence strategies, mediated by so-called infochemicals, which forms the basis for our current view of the phenomenon. Since then, induced indirect defences have been reported for many plant species under laboratory conditions, including Arabidopsis (van Poecke et al., 2001), cotton (De Moraes et al., 1998), tomato (Kant et al., 2004) and maize (Schnee et al., 2006). In 1999, Thaler showed that indirect defences can act in the field while, in 2001, Kessler and Baldwin showed that plant volatiles can establish indirect defences under natural conditions. They supplemented Nicotiana attenuata plants with synthetic volatiles and some of these increased the natural predation of herbivore eggs and repelled adult moths. In a later study with transgenic plants that were silenced for genes involved in volatile production, the same group showed that indirect defences can actually promote a plant’s fitness under natural conditions (Schuman et al., 2012). Moreover, it was found that hyperparasitoids also respond to herbivore-induced plant volatiles; volatiles released by plants infested with parasitized caterpillars attracted more hyperparasitoids than volatiles emitted by plants infested with healthy caterpillars (Poelman et al., 2012). Indirect defence is known to occur below ground as well. A well-known example is the release of the volatile β-caryophyllene by maize roots into the soil when attacked by larvae of the beetle Diabrotica virgifera virgifera; this compound was shown to function as an attractant for entomopathogenic nematodes that attack the beetle larvae (Rasmann et al., 2005). Finally, restoring this function in maize varieties deficient in the release of β-caryophyllene from roots also increased attraction of the nematodes (Degenhardt et al., 2009).

Volatiles are not the only means by which plants can increase the abundance of natural enemies in their vicinity. Natural enemies can be arrested by providing them with food, e.g. extrafloral nectar (Pemberton and Lee, 1996) or food bodies (Fischer et al., 2002). Also, dead insects entrapped on sticky plants were shown to attract predatory insects such that overall herbivore damage decreased and fruit production increased (Krimmel and Pearse, 2013). Finally, an alternative means by which plants establish indirect defence is to provide shelter (domatia) such as cavities or tufts of hair, for small natural enemies, which these can use to moult and/or to protect their eggs (Walter, 1996).

HERBIVORE DIGESTION, SALIVA AND REGURGITATION

A herbivore’s host-plant range is closely linked to its digestive physiology (Pearse et al., 2013). The midgut and salivary glands are important organs for establishing interaction between a phytophagous insect and its host plant (Shukle et al., 2010). For example, the ability of insects to tolerate ingested tannins, which are among the most abundant secondary plant metabolites, is determined by a variety of biochemical and physiological features of their midgut (Fig. 1). These include the release of surfactants and antioxidants, the maintenance of a high pH and the formation of a protective peritrophic membrane envelope lining the midgut such that it functions as a barrier (Barbehenn and Constabel, 2011). The more a herbivore can maintain flexible and diverse digestion and detoxification pathways, the more host plants may eventually become available to it, as is the case for the spider mite Tetranynchus urticae, which has been found to live on well over 1000 plant species (Dermauw et al., 2013a).

Herbivore symbionts to aid digestion

In many cases, herbivores rely on the help of microorganisms to overcome some of the problems that come with feeding on plant tissues. Phloem is a troublesome food source for insects,
not only because it lacks essential components, but also because its sugar content and osmotic pressure are high. Many phloem feeders have adapted tolerance to this and display sucrose trans-glucosidase activity in their gut, which can transform the excess ingested sugar into long-chain oligosaccharides, which in turn can be excreted as honeydew (Douglas, 2006). Especially homopteran insects such as aphids, which live solely on plant sap (i.e. predominantly phloem), have evolved associations with microbes to compensate for the deficiencies in this food source. Phloem is not only rich in sugars but also lacks several amino acids and vitamins. Hence, insects such as aphids have evolved intimate endosymbiotic relationships with bacteria, which supply them with essential amino acids and vitamins and receive other nutrients in return (Engel and Moran, 2013). These bacteria seldomly reside within the gut, but instead are usually found in specialized cells called bacteriocytes (Schwemmler, 1989).

Chewing insects in particular have to deal with the poorly digestible cell wall components that they ingest. The major structural components of the primary plant cell wall are cellulose, hemicellulose and pectin, forming a complex and organized structure, and proper degradation of these components requires a range of enzymes, including cellulasmes, hemicellulasmes and pectinases (Cosgrove, 2005; Vilanova et al., 2012). Microbial symbions present in the digestive tract of insects are known to contribute significantly to the digestion of plant cell wall components. The prevailing view was that herbivorous insects are completely dependent on these symbions, but it was found that some herbivorous insects also produce their own endogenous plant cell-wall-degrading enzymes (Caldéron-Cortés et al., 2012). These enzymes are usually secreted by the epithelial cells of the insect’s midgut and move forward to the foregut or are secreted by the salivary glands (Terra and Ferreira, 1994). In some cases, these endogenous cell-wall-degrading enzymes have been acquired by insects from microbes by horizontal gene transfer. Xylanases, for example, were transferred from gammaproteobacteria to the mustard leaf beetle Phaedon coehleariae or its ancestor (Pauchet and Heckel, 2013). Other cases of horizontal gene transfer that enable arthropods to feed on plants or plant tissues with an unfavourable nutrient composition show that such transfers may be important drivers of herbivore evolution. Examples of these have been found for the spider mite T. urticae (Grbic et al., 2011; Wybouw et al., 2012) and the coffee berry borer beetle Hypothenemus hampei (Acuña et al., 2012).

The importance of amino acid availability

Herbivorous animals have a lower tissue carbon-to-nitrogen ratio than plants. Hence, they must eat an excess of carbon-rich plant material to acquire sufficient nitrogen, making nitrogen one of the central determinants of herbivore foraging behaviour and population growth (Fagan et al., 2002). Proteins (i.e. amino acids) are the main macronutrients containing nitrogen and are commonly considered to strongly affect the growth rates of arthropod herbivores (Mattson, 1980). The nutritional value of a host plant is not solely based on its protein quantity, as protein quality is hypothesized to be equally important. Protein quality depends on the essential amino acid composition and can be quantified based on which essential amino acids have the lowest abundance relative to the composition required by a herbivore (Barbehenn et al., 2013).

Dietary protein is broken down into peptides and amino acids in the midgut region of the insect digestive tract, a process catalysed by the abundant gut proteases. There are two types of proteases: proteinases, which cleave protein chains at specific peptide bonds, and exopeptidases, which remove amino acids from the C- or N-terminus of a protein (Jongsma and Bolter, 1997). Such free amino acids, but also di- or tripeptides, are subsequently absorbed via transporter proteins in the midgut. Plants, in turn, have evolved mechanisms to disturb the uptake of amino acids by herbivores as an integral part of their defence system (Chen et al., 2005; Yang et al., 2013). For example, essential amino acids in the insect gut can be bound to quinones, highly reactive molecules generated by the ingested plant material. This compromises the plant’s nutritional value, thereby reducing insect performance (Felton, 2005). In addition, the digestion of dietary plant proteins by the insect’s gut proteases may be hampered by ingested plant PIs, which rapidly accumulate in herbivore-damaged plant tissues. In turn, some herbivores have evolved novel proteases that are largely insensitive to plant PIs or have adapted by increasing their overall protease gene expression levels and thereby the total amount of gut protease activity (Jongsma and Bolter, 1997).

Herbivore saliva and regurgitant

Herbivore oral secretions play an important role in plant–herbivore interactions. Oral secretions are a mixture of secretions from the labial and mandibular salivary glands and regurgitant (Vadassery et al., 2012). Regurgitation is the expulsion of material from the herbivore’s oesophagus and this gut reflux is usually composed of partially digested food and gut juices. Some species regurgitate to digest food, e.g. some species have life
stages that cannot chew and that repeatedly draw regurgitated liquid in and out of their proboscis, whereas for others regurgitation may have evolved as a defence mechanism against natural enemies (Rhaïnds et al., 2011). Chewing caterpillars may regurgitate during feeding (Vadassery et al., 2012), but their tendency to do so differs across species of herbivore and host plant. Regurgitant can be collected from caterpillars by gently squeezing them (Peiffer and Felton, 2009) and regurgitant of several herbivore species has been shown to contain components that alter the plant defence response when applied to wounded tissues. Digestive enzymes in regurgitant may emanate from the gut or from the saliva (Afshar et al., 2013; Chen et al., 2013) and some constituents of the saliva of caterpillars (Musser et al., 2002) and aphids (Rodríguez and Bos, 2012), were found to modulate the host’s defence responses.

**Elicitors of defences from herbivore saliva and regurgitant**

An elicitor of plant defences can be any substance that provokes a specific defence-related response in a host plant after exposure to it. Four groups of elicitors have been distinguished in the literature: plant-derived (endogenous) elicitors, herbivore-derived elicitors, conjugates of these two, and synthetic elicitors.

Plant-derived elicitors are molecules produced or released upon injury or infection and are responsible for induction or amplification of the plant’s defence responses (either local or systemic) against the attacking organism. Such molecules can include cell wall fragments, phytohormones, reactive oxygen species (ROS) and peptides (Albert, 2013; Gozzo and Faoro, 2013). Many elicitors act in concert or in sequence with other elicitors or signalling molecules (Kessler and Baldwin, 2002). Six groups of plant-borne peptide elicitors that play a role in plant–herbivore interactions have been identified: (1) peptides derived from preproteins such as systemin; (2) hydroxyproline-rich systemin (HypSys); (3) a group referred to as ‘plant elicitor peptides’ (Peps); (4) cryptic peptides, derived from ‘preproteins’ that have their own (unrelated) primary functions, such as inceptin, which is derived from a chloroplastic ATP synthase; (5) SubPep, which is derived from a subtilisin-like protease (Yamaguchi and Huffaker, 2011); and (6) CAPE1 (CAP-derived peptide 1), which is derived from PR-1 (Pathogenesis-related protein 1) (Chen et al., 2014). All these peptide plants were shown to act upstream of a subset of defence responses and to modulate these responses. Receptors for the Peps have been identified (Yamaguchi et al., 2010). Inceptors are disulphide-bridged peptides, originally described from cowpea (Vigna unguiculata), and are derived from the chloroplast ATP synthase γ subunit following digestion by proteolytic enzymes in the gut of the fall armyworm (Spodoptera frugiperda) larva. Hence, inceptin is considered a plant-derived elicitor and it induces and amplifies local and systemic defence responses (Yamaguchi et al., 2011). Inceptin recognition shows that plants have evolved the means to sense insects not only directly via their secretions or movements, but also indirectly by monitoring the emergence of catabolic products indicative of an insect that successfully feeds and digests (Schmelz et al., 2009). Elicitor activity of inceptors seems to be specific to legumes in the genera Phaseolus and Vigna (Yamaguchi et al., 2011). Interestingly, the inceptin-related peptide of the velvet bean caterpillar, *Anticarsia gemmatalis*, has a C-terminal truncation and does not induce but rather antagonizes defences (Schmelz et al., 2012). This indicates that plants and herbivores may be involved in an arms race reminiscent of plants and pathogens. The elicitor 2-hydroxyoctadecatrienoic acid (2-HOT) is generated during *Manduca sexta* feeding on *N. attenuata* by a dioxygenase from the plants using plant linolenic acid as a substrate. However, this conversion occurs mainly locally at the feeding site, possibly because the dioxygenase has a high pH optimum and may increase its activity under the alkaline conditions at the feeding sites or potentially in the insect’s mouth during chewing and regurgitation (Gaquerel et al., 2009). Also, plant hormones, such as the linolenic acid-derived jasmonic acid (JA) and the phenolic salicylic acid (SA), elicit specific defences when applied to plants as pure compounds. Moreover, plants that are attacked by herbivores release distinct volatile blends that can contain methyl jasmonate (the volatile form of JA) and methyl salicylate (the volatile form of SA), which, together with a small subset of additional volatiles, are potent elicitors of defences in systemic uninduced leaves of the same plant or in nearby plants (Kessler and Baldwin, 2002). It may not come as a surprise that pure (synthetic) plant defence signalling molecules, or substances closely related to them, elicit such defences when applied manually to uninduced plants. However, molecules derived directly from herbivores have also been found to elicit specific defence-related processes. Such elicitors can originate from organs associated with feeding (gut, salivary glands, etc.) and can be present in saliva, regurgitant or other secretions such as honeydew. In lepidopterans, saliva is proposed to be a more important source of elicitors than regurgitant, whereas the opposite may be true for Coleoptera (Kim et al., 2011). Herbivore saliva has been studied in detail (Miles, 1999). Some lepidopteran larvae also possess a ventral eversible gland, whose secretions have been associated with silk strengthening, defences against predators and the production of anti-aggregation pheromones (Zebelo and Maffei, 2012). The secretions may, however, also interact with the plant host because the tip of the everted gland can reach the mouthparts of the larvae, allowing them to mix with the oral secretions. Research by Zebelo and Maffei (2012) suggests that the ventral eversible gland of *Spodoptera littoralis* might contain elicitors that are able to trigger early plant defences in *Arabidopsis*. Phloem-feeding herbivores deposit secretions onto the leaf surface when attaching their stylet and coat the stylet trajectory with a protective sheath. Subsequently, they inject saliva in a pierced vascular bundle (Hogenhout and Bos, 2011). Two salivary proteins (Mp10 and Mp42) of the green peach aphid *Myzus persicae* were found to act as elicitors in *Nicotiana benthamiana* and to reduce aphid fecundity when expressed in plants. Furthermore, Mp10 overexpression in *N. benthamiana* resulted in chlorosis and local cell death. The involvement of the plant chaperone protein SGT1 suggest that aphid elicitor recognition is mediated by proteins encoded by R (Resistance) genes: these are sensory proteins that are known to recognize pathogen elicitors. Induction of chlorosis was not observed in tomato, implying that the Mp10 response may be specific for *N. benthamiana*. Mp10 was also able to suppress the ROS response induced by the well-known bacterial elicitor flagellin 22, but not by the putative insect elicitor chitin.
Hence, mechanisms of recognition and signal transduction of aphid salivary proteins are unclear at this stage (Hogenhout and Bos, 2011).

The interface between the plant and the feeding insect will often contain a mixture of substances of both herbivore and plant origin. Sometimes plants specifically recognize substances that originate from the plant but only after they are processed by the herbivore, reminiscent of incepstin. Some lepidopteran larvae harbour fatty acid amino acid conjugates (FACs) in their digestive system. The fatty acid moiety of these conjugates originates in the plant (Felton and Tumlinson, 2008) and is conjugated to amino acids, such as glutamine, in the insect gut. These conjugates may play a primary role in the regulation of glutamine supply for nitrogen assimilation (Yoshinaga et al., 2010). The oral secretions and regurgitant of caterpillar larvae may also contain FACs, which can act as elicitors of specific defence responses after recognition by the host plant (Bonaventure et al., 2011). The FACs have a broad taxonomic distribution in insects (Felton and Tumlinson, 2008). The first FAC found was named volicitin [N-(17-hydroxylinolenoyl)-L-glutamine]. Together with several related substances, such as N-linolenoyl-L-glutamine and N-linolenoyl-glutamic acid, it constitutes a significant fraction of the elicitor pool of lepidopteran larvae, responsible for the production and release of induced plant volatiles (Alborn et al., 1997). Apart from inducing volatiles, FACs are also known to induce an increase in activity of the salicylic-induced protein kinase and the wound-induced protein kinase in N. attenuata leaves when attacked by caterpillars or treated with oral secretions (Wu et al., 2007). Other elicitors of plant volatiles with a basal fatty acid moiety are caeliferins. These are disulphooxy fatty acids originally isolated from Schistocerca americana regurgitant and were found in grasshoppers of the suborder Caelifera. These compounds elicit defence responses in corn (Alborn et al., 2007) and Arabidopsis (Schmelz et al., 2009). Furthermore, synthetic caeliferin A16:0 was shown to strongly induce ethylene production in Arabidopsis (O’Doherty et al., 2011). However, application of synthetic caeliferin A16:0 to puncture wounds in Arabidopsis did not induce any of the responses observed on treatment with grasshopper oral secretions (Schäfer et al., 2011). Regurgitant of Pieris brassicae caterpillars also contains the enzyme β-glucosidase. This molecule is the first reported herbivore-associated elicitor and triggers the same emission of volatiles in cabbage plants as that induced by feeding caterpillars (Mattiacci et al., 1995). Another well-known enzyme from caterpillar saliva with elicitor properties is glucose oxidase (GOX). In some cases, however, GOX was found to act neutrally or in favour of the herbivore (Tian et al., 2012; Musser et al., 2002).

Together, these elicitors activate defensive responses of the host plant. Hence, they are referred to as herbivore-associated molecular patterns (HAMPs), a term that covers all herbivore-derived signalling compounds that might come into contact with host plants during any stage of their life cycle and elicit defence reactions (Felton and Tumlinson, 2008). The HAMPs are presumably recognized by pattern recognition receptors that evolved to recognize conserved, generally occurring pathogen- and herbivore-derived molecules or motifs, but so far no specific HAMP receptors have been identified (Erb et al., 2012), with the exception of a putative volicitin receptor (Truit et al., 2004). However, the additional perception of specific individual herbivore-associated elicitors may allow the plant to distinguish the type of attacking herbivore (Poelman et al., 2011).

**Non-oral elicitors of defences**

HAMPs also include those elicitors that do not directly result from feeding activities. These include the secretions from the ventral eversible gland of S. littoralis, but the fluids secreted by female pea weevils (Bruchus pisorum), which are used to attach the eggs to the plant surface and can also contain substances perceived by the plant. These fluids contain mono- and bis-(3-hydroxypropanoate) esters of long chain α,α-diols (‘bruchins’) and increase cell division and induce neoplasms formation in several legume hosts. Also, benzyl cyanides from the oviposition fluids of mated female Pieris rapae can elicit transcriptional changes in defence-related genes (Fatourou et al., 2008). The presence of feeding herbivores can also be detected by the plant through components present in the excreted honeydew (VanDoorn et al., 2015). In addition to HAMPs, non-molecular signals can also alert a host plant. For example, herbivore larvae can betray their presence to plants by their crawling, which stimulates the synthesis of 4-aminobutyrate (GABA), while imprints of their footsteps lead to increases in chlorophyll fluorescence or superoxide production. This possibly represents early defence signalling events (Hall et al., 2004). Plant trichomes can also operate as sensors of herbivore movements after being touched (Peiffer et al., 2009).

**REGULATION OF PLANT DEFENCES AGAINST HERBIVORES**

**Detection of herbivores**

Plants recognize herbivores by their molecular patterns or their elicitors. It is hypothesized that polyphosphoinositides generated at the plasma membrane play an important role as second messengers, just as they do during pathogenesis (Munnik and Nielsen, 2011). The most rapid measured responses are ion (e.g. Ca2+ and K+) fluxes across the plasma membrane, followed by changes in the plasma membrane potential. Subsequently, a protein kinase cascade can activate the production of ROS such as hydrogen peroxide by activating an NAPDH-dependent oxidase. Hydrogen peroxide can have a direct effect on herbivores or enter the cell, thus changing its redox status. The rapid increase in cytosolic Ca2+ can also give rise to increased nitric oxide-mediated processes that precede the upregulation of JA levels (Zebele and Maffei, 2015). These responses occur not only locally, but also in unattacked neighbouring cells and in distal tissues. Herbivory, the application of oral secretions to wounded leaves and aphid probing have been shown to give rise to membrane depolarization due to an electrochemical gradient between the interior and the exterior of the attacked plants cells. This membrane depolarization can travel with a speed of up to 40 cm s−1 through the entire plant and mutant plants with attenuated wound-induced surface potential changes exhibit a reduced JA response in distal leaves (Mousavi et al., 2013). Moreover, ablation of the ventral
eversible gland of *S. littoralis* reduced this depolarization as well as the Ca\(^{2+}\) and hydrogen peroxide bursts and downstream defence responses (Zebelo and Maffei, 2012). The relationship between Ca\(^{2+}\) levels, ion channel activity and the oxidative burst is correlative but may depend more strongly on K\(^+\) than on Ca\(^{2+}\). Additional to these rapid electric signals (Mousavi et al., 2013), slower chemical signals are also transmitted to distal tissues, either via the vascular tissues (Schilmiller and Howe, 2005) or via the air (Sugimoto et al., 2014) (Fig. 2).

Defence-regulating plant hormones

Three phytohormones play a primary role in regulating defence responses (Pieterse et al., 2009); JA (Wasternack and Hause, 2013), SA (Vlot et al., 2009) and the volatile ethylene (Adie et al., 2007). The central roles of JA and SA are substantiated by the fact that biosynthesis mutants are hypersensitive to a wide range of attackers. Several other phytohormones are known to play a secondary role in plant defence by modulating it, including abscisic acid (ABA) (Dinh et al., 2013), auxin (Kazan and Manners, 2009), cytokinin (Choi et al., 2011), gibberellic acid (GA) (Yang et al., 2012) and brassinosteroids (Nakashita et al., 2003) and possibly strigolactones (Torres-Vera et al., 2013). In concert with these hormones, a small set of signalling peptides, such as systemin (Ryan, 2000) and the Peps from *Arabidopsis* (Huffaker et al., 2006), are also involved in orchestrating plant defences. The peptide system of tomato, *Solanum lycopersicum* (Pearce et al., 1991), functions upstream of JA biosynthesis and may facilitate priming of the plant’s JA response (Kandoth et al., 2007). Moreover, homologues from different plant species were found to have different (non-defence related) functions (Schmidt and Baldwin, 2006). In contrast, some of the Peps appeared to have similar functions across different plant species, because Pep3 from maize (*Zea mays*) induces accumulation of JA and ethylene as well as their downstream responses, including the emission of volatiles (Huffaker et al., 2013).

Jasmonate as a regulator of plant defences against herbivores

Jasmonic acid regulates the core defences of dicots against herbivorous arthropods (Howe and Jander, 2008) and necrotrophic pathogens (Glazebrook, 2005). The biosynthesis of JA was elucidated by Vick and Zimmermann (1984) and seems quite conserved across species. In tomato, JA biosynthesis was shown to take place in the chloroplast and peroxisomes of the phloem companion cells (Howe, 2004).Briefly, the first step in JA biosynthesis comprises the formation of \(\alpha\)-linolenic acid, which is released from the galactolipids of chloroplast membranes by the action of one or more phospholipases, although it...
JA accumulation can also occur in distal leaves (Glauser et al., 2008). Spatialtemporal variability in JA accumulation may be a defensive tactic by itself because it makes it difficult for herbivores to anticipate which tissues are defended poorly and which strongly (Stork et al., 2009). Finally, it has been shown that plants synchronize the JA response with the feeding activities of a generalist herbivore across day–night cycles (Goodspeed et al., 2012), although different plant ecotypes challenged by different kinds of herbivores may exhibit different circadian interactions (Jander, 2012).

**Ethylene as a regulator of plant defences against herbivores**

Ethylene is a gaseous hormone and is involved in development, senescence and defence against necrotrophic pathogens (Chen et al., 2005). Endogenous ethylene concentrations in plant tissues depend on the activities of two biosynthetic enzymes, 1-aminoacyclopropane-1-carboxylic acid synthase (ACS) and 1-aminoacyclopropane-1-carboxylic acid oxidase (ACC oxidase), which convert S-adenosyl-Met to ethylene, but also on the rates of outward diffusion and metabolization (Wang et al., 2002). Transcription factors that control ethylene-responsive genes are constitutively repressed by proteins such as JAZ (Zhu et al., 2011) and ethylene perception controls the ethylene response. *Arabidopsis* contains five ethylene transmembrane receptors, located in different organelles (Kendrick and Chang, 2008). These receptors are active in the absence of ethylene (Hua and Meyerowitz, 1998), and suppress the ethylene response by constitutively stimulating phosphorylation of the ethylene signalling hub EIN2 (ETHYLENE INSENSITIVE2), leading to its degradation (Qiao et al., 2009). Upon binding to ethylene, the receptors become inactive, allowing unhindered accumulation of EIN2 in the cytosol. This initiates degradation of the ethylene transcriptional regulators and thus the activation of ethylene-responsive genes in the nucleus (An et al., 2010).

**Salicylate as a regulator of plant defences against herbivores**

Salicylate mediates defences against biotrophic pathogens (Glazebrook, 2005) and phloem-feeding herbivores (Kaloshian and Walling, 2005). During pathogen infections, defence responses can spread systemically, but are also expressed in uninfected tissues, and this is referred to as systemic acquired resistance. Several candidate signals have been reported to play a role in systemic acquired resistance, including the SA-derivative methyl salicylate. However, SA is the central local regulator because plants that are unable to accumulate SA are often highly susceptible to pathogen infections (Klessig, 2012). In rice (a monocot), the JA and SA pathways are thought to regulate a common set of defence genes that are effective against both biotrophic and necrotrophic pathogens (De Vleesschauwer et al., 2013). Salicylate is derived from chorismate, the end-product of the shikimate pathway. From there it can be synthesized in plants via at least two distinct biosynthetic routes. The first route delivers SA in two steps and depends on the enzymes isochorismate synthase, which is induced upon pathogen infection (Wildermuth et al., 2001), and isochorismate pyruvate lyase. The second route depends on the phenylpropanoid pathway. This is a pathway responsible for a variety
of products, such as flavonoids and lignins, but also for SA, and there may be parallel sub-branches within the branch leading to SA (Boatwright et al., 2013). Which of these pathways or branches determines induced SA levels most strongly may also differ across plant species. Once formed, SA may be modified further by glucosylation, methylation or amino acid conjugation. Most of these derivatives are inactive and may serve to fine-tune local and systemic SA accumulation and function or may provide safe storage. Methyl salicylate is inactive but easier to transport to distal tissues, either actively via the phloem or passively via the air (Dempsey et al., 2011).

A central role is played by the NONEXPRESSOR OF PR GENES (NPR) protein family. It has recently been discovered that NPR3 and NPR4 are SA receptors, whereas NPR1 acts as a master regulator of SA-mediated responses (Yan and Dong, 2014). NPR1 proteins are constitutively present in the cytosol of the cell as oligomers (Tada et al., 2008) and their concentration increases upon induction (Spoel et al., 2009). Accumulation of SA causes an increase in the levels of reduced glutathione (the antioxidant form of glutathione), thereby changing the redox status of the cell, i.e. the balance between oxidants and antioxidants (Spoel and Loake, 2011), and this generates NPR1 monomers by the thioredoxin-catalysed reduction of monomeric disulphide bridges. Subsequently, NPR1 monomers migrate into the nucleus (Mou et al., 2003; Tada et al., 2008). Without NPR1, the expression of the SA-responsive genes is repressed by TGA transcription factors. After NPR1 has arrived in the nucleus, a portion of it is phosphorylated. Phosphorylated NPR1 binds to the TGA transcription factors and this complex allows the expression of target genes such PR-J. Unphosphorylated NPR1 may assemble together with different transcription factors and give rise to TGA-independent expression of other target genes. After a round of transcription initiation, the NPR1 protein complexes are degraded via the proteasome and new monomeric NPR1 proteins need to enter the nucleus from the cytosol to keep the response going (Mukhtar et al., 2009).

**Hormonal crosstalk in plant defences against herbivores**

The distinct defence signalling pathways that are regulated by phytohormones interact directly and indirectly, forming complex networks, and these interactions can be additive, antagonistic or synergistic (Koomen Me and Pieterse, 2008). Of all the interactions that occur between hormonal defence signalling pathways, crosstalk between the JA and SA pathways has received most attention, after it was discovered that SA can inhibit the plant’s wound response (Doherty et al., 1988) and indications were found for the opposite (Sano et al., 1994). Under most conditions, crosstalk between SA and JA is antagonistic (Thaler et al., 2012), but when applied to plants in specific ratios synergistic interactions were also observed (Mur et al., 2006), and other plant hormones may modulate this crosstalk (Robert-Seilaniantz et al., 2011). Suppression of the JA response by SA occurs downstream of JA-Ile perception, depending on ORA59 (Van der Does et al., 2013). Other regulators include NPR1 and some of its interacting partners, including the TGA and WRKY transcription factors (Pieterse et al., 2012). Nuclear localization of NPR1 is not required for the suppression of JA signalling, and it has therefore been suggested that the role of NPR1 in mediating JA/SA crosstalk depends on a function executed in the cytosol (Spoel et al., 2003). The adaptive value of the JA/SA (antagonistic) crosstalk is not clear. It has frequently been suggested that it allows plants to fine-tune the balance between different defensive strategies, depending on the type of attacker, or in case the plant is attacked by multiple attackers, depending on the timing and sequence of infestation (Pieterse and Dicke, 2007). However, whether JA/SA antagonism is adaptive remains an open question (Thaler et al., 2012).

**MOLECULES USED BY PLANTS TO RESIST HERBIVORES**

The collective hormonal responses and their interactions induced by herbivores determine which defences are established in which host plant tissues and to what extent. Induced plant defences upon herbivory are seldom lethal: the fact that herbivores can move away from defended tissues will usually prevent them from ingesting a fatal dose. Hence, plant defences induced by herbivores will cause them to depart or, alternatively, slow down their development and population growth because bigger herbivores, or higher herbivore densities, consume and reproduce more, thus causing more damage. Many of these herbivore-induced plant defences rely on the direct antagonistic action of enzymes that interfere with feeding activities, (gut) digestive processes and gut integrity (Carlini and Grossi-de-Sa, 2002) (Fig 2).

**Defence proteins**

**Protease inhibitors** Most organisms produce a range of enzymes belonging to the protease class (also called ‘peptidases’). Proteases are enzymes that perform proteolysis on target proteins and can thereby regulate enzymatic activities (Rawlings et al., 2014). A subset of these proteases are the endopeptidases, which cleave the chain of amino acids of the target protein, and are referred to as ‘proteinases’. The active site of a protease can be centred on a particular amino acid, e.g. a serine protease has its active site at a serine, and these ‘active site’ amino acids are used for their classification. Proteases themselves are regulated by PIs, which are also commonly occurring enzymes across the tree of life. Most plants upregulate a subset of their PIs, often measured as PI activity, upon herbivory, and some of these are associated with resistance to herbivores (Ryan, 1990; Lison et al., 2006). These typical herbivore-induced plant PIs are believed to act on proteases in the herbivore’s gut and have been suggested to have a dual role: to reduce the efficiency of proteinase activity during the herbivore’s digestion of plant proteins and to protect co-ingested defensive proteins of the plants against herbivore proteases (Macintosh et al., 1990). Proteases and their inhibitors often form couples: a serine protease can be inhibited by a serine PI, although these functional annotations can be less strict than the name suggests. PIs can have different modes of action, but in general mimic the substrate of the protease and establish a strong bond with the enzyme, thus creating a protease–PI complex and delaying or blocking its proteolytic activity (Bateman and James, 2011). Different plant PI families...
have been associated with defence against specific families of herbivores (Ryan, 1990). For instance, serine PIs have a primary role in defence (Hartl et al., 2010) and affect the performance of some lepidopteran species (Duan et al., 1996; Yeh et al., 1997). In contrast, cysteine PIs are effective against coleopteran species such as the southern corn rootworm, Diabrotica undecimpunctata howardi (Fabrick et al., 2002), and the spider mite T. urticae (Santamaria et al., 2012).

Peptidases Several peptidases/proteases are associated with plant defence responses, but it is not always clear what their functions in defence responses are and whether they directly interact with the physiology of attackers or have regulatory roles within the plant’s defence network (Harrison and Bonning, 2010). While subtilisin-like proteinases are associated with anti-pathogen defences and depend on SA signalling (Jorda and Vera, 2000), cysteine proteases (such as papain) are typically induced by herbivory and are associated with disruption of the peritrophic matrix in the insect gut (Pechan et al., 2002; Fescemeyer et al., 2013). Some insects have evolved adaptations to protect the peritrophic membrane against these proteases (Li et al., 2009; Zhu-Salzman and Zeng, 2013). Interestingly, overexpression of a cotton cysteine protease in Arabidopsis improved the effect of plant-delivered small RNAs, designed to induce expression of leucine aminopeptidase (LAP) genes. The LAPs are exopeptidases that hydrolyse the terminal amino acid (leucine, methionine and arginine) residues of proteins (Gu et al., 1999). Herbivore-induced accumulation of LAPs in the chloroplasts depends on the JA pathway and it is assumed that they have a direct role in defence against plant consumers, probably by inactivating herbivore digestive enzymes by proteolysis (Fowler et al., 2009; Lomate et al., 2013). However, they may also have regulatory roles in the plant itself and modulate the expression of defence genes (Scranton et al., 2013).

Amino acid degrading proteins Digestion of proteins in the intestinal lumen results in free amino acids and peptides, available for absorption by the insect. Transepithelial transport of (essential) amino acids in the alimentary canal (predominantly the midgut) often requires energy. Some plant defensive enzymes degrade such amino acids in the arthropod gut before they can be taken up. Mandraea sexta feeding on tomato (S. lycopersicum) was found to induce an arginase and a threonine deaminase, which degrades the amino acids arginine and threonine respectively, which could be found back in the gut of the larvae. Interestingly, the optimal pH for enzymatic activity of both enzymes was found to be similar to the pH found in the midgut of M. sexta. Moreover, threonine deaminase was inactive in tomato itself due to an activation domain that was cleaved off in the herbivore gut, thereby activating the enzyme (Chen et al., 2005; Gonzales-Vigil et al., 2011). This suggests that some plant-defensive enzymes have evolved to be activated only after having reached the herbivore gut.

Polyphenol oxidases The PPOs have been associated with plant defences: they are among the major gene/enzyme families induced by wounding, herbivory or after application of JA or JA derivatives (Thaler et al., 1996; Constabel and Ryan, 1998; Stout et al., 1998). While PPOs can be found in diverse phylogenetic groups (Tran et al., 2012), such as green algae, mosses and gymnosperms, Arabidopsis does not have them. Their mode of action and their site of action during plant–herbivore interactions is still under debate. Polyphenol oxidases catalyse the oxidation of ortho-oriented dihydroxy phenolic compounds, thereby generating quinones, which are highly reactive molecules that can either spontaneously polymerise or damage proteins, amino acids and nucleic acids via an alklylation reaction (Constabel and Barbehenn, 2008). Polyphenol oxidases have been implicated in at least two distinct defence responses. First, polyphenolics on the plant surface hinder crawling herbivores. For instance, Yu et al. (1992) and Kowalski et al. (1993) found a correlation between PPO activity, i.e. phenol polymerization activity, in glandular trichomes of different solanaceous species and the entrapment of small arthropods between such trichomes. Second, PPO-mediated generation of quinones in the herbivore’s gut, i.e. after ingesting and mixing plastidial plant PPOs in combination with vacuolar plant phenolics, damages amino acids and proteins and thus interferes with food digestion (Constabel and Barbehenn, 2008).

Activity of PPOs has been associated with defence against herbivores such as coleopterans (Castañera et al., 1996) and lepidopterans (Felton et al., 1992a). Moreover, PPO overexpression in different plant species was also shown to increase resistance to several herbivores. Overexpression of a potato PPO in tomato increased resistance to the common cutworm Spodoptera litura (Mahanil et al., 2008) and overexpression of a poplar (Populus trichocarpa) PPO in Populus tremula increased resistance to the forest tent caterpillar Malacosoma disstria. Interestingly, this poplar PPO is latent in plant cells, whereas it was fully active when isolated from the caterpillar’s frass, suggesting activation in the insect’s gut (Wang and Constable, 2004). However PPO overexpression does not always have an effect on herbivores (Barbehenn et al., 2007), indicating that PPO effectiveness could depend on specific physiological conditions in the herbivore gut (Felton et al., 1992b) or on the availability of specific PPO substrates. Finally, other oxidases, such as peroxidase and lipoxygenase, may play a similar functional role in plant defences by creating potent electrophiles or interfering with the accumulation of essential nutrients (Zhu-Salzman et al., 2008).

Lectins Plant defensive lectins are associated with the disruption of several processes involved in the digestion of food and nutrient uptake in herbivores (Michiels et al., 2010). Lectins comprise a diverse family of proteins that bind specifically with mono- and oligosaccharides (Komath et al., 2006). Although the mode of action is not well understood, there are several cases in which herbivorous insects that propagated on artificial diets with lectins or on plants that overexpressed lectin genes showed delayed or impaired development (Gatehouse et al., 1996; Sauvion et al., 2004). Due to their high affinity with oligosaccharides, it is assumed that lectins can interact with glycoproteins in the digestive tract of the herbivore and bind to the insect’s intestinal epithelial cells or its peritrophic membrane and disrupt these tissues (Macedo et al., 2004).

Pathogenesis-related proteins Pathogenesis-related (PR) proteins include a wide variety of proteins with diverse functions,
predominantly associated with resistance to pathogens. These proteins can be classified into 17 families and they are often used as defence marker genes, though not all of them are functionally understood: most of them are classified as a glucanase, chitinase, thaumatin, PI or peroxidase. They are defined as pathogen-induced proteins and for the majority of them evidence is largely lacking that they play a significant role in anti-herbivore defences. For example, PR-2 proteins have $\beta$-1,3-endoglucanase activity and are associated with (fungal) cell wall degradation. However, some of these proteins could also be active against herbivores. The families PR-3, 4, 8 and 11 are chitinases (Sels et al., 2008). Insect chitinases, key enzymes for arthropod morphogenesis, have insecticidal activity when delivered via the plant (Kramer and Muthukrishnan, 1997). However, the effects of plant chitinases on insects are not clear. Carnivorous plants secrete chitinases to digest arthropod prey (Paszota et al., 2014) and purified chitinases from mulberry latex were found to have insecticidal activity (Kitajima et al., 2010). In addition, overexpression of a poplar chitinase in tomato inhibited the development of Colorado potato beetles (Lawrence and Novak, 2006). Moreover, larvae of Orzya antiqua showed a lower growth rate when feeding on transgenic birch (Betula pendula) expressing a sugar beet chitinase (Vihervuo et al., 2013), but transgenic plants in the field were more susceptible to aphids (Vihervuo et al., 2008). Thus, there are indications that plant chitinases are active against insects and have the potential to damage the exoskeleton and chitin-rich peritrophic membrane of arthropods, but these activities could be limited to some species and life stages of herbivores. Other PR proteins could also have insecticidal activities. PR-1 is found in the digestive fluids of picker plants (Buch et al., 2014) and PR-2 in the digestive fluid of sundew, although possibly in order to utilize pollen grains, fungal spores or detritus as nutritional source (Michalko et al., 2013). However, there is not much evidence for herbivore-induced foliar PR proteins other than chitinases that affect herbivores directly, although PR proteins are ingested by them and can be found in their frass (Chen et al., 2007).

**Small cysteine-rich defence proteins**

There are two families of small cysteine-rich proteins that are suggested to play a role in plant defence against herbivores: defensins (e.g. PR-12) and cyclotides. Defensins are small cysteine-rich proteins, commonly synthesized in plants but also by animals. They are proteins of 45–54 amino acids that contain eight conserved cysteine residues and are similar to thiopins (Thomma et al., 2002). Most defensins operate by binding to cell membranes, resulting in pore-like membrane defects, causing efflux of essential ions and nutrients. Plant defensins are predominantly active against fungi (Stotz et al., 2009), but some defensins inhibit $\alpha$-amylase activity and have no effect on fungi (Osborn et al., 2009). $\alpha$-Amylase is a typical insect gut enzyme and there are indications that particular defensins reduce the digestibility of plant material (Shade et al., 1994; Shiau et al., 2006). Cyclotides are peptides of typically 28–37 amino acids, derived from longer precursor proteins, such as metallocarboxy peptidase inhibitors (Cavallini et al., 2010). They contain six conserved cysteine residues connected by three intermolecular disulphide bonds that form a knotted structure. Their mode of action is poorly understood, but is also associated with disrupting membrane integrity. Several cyclotides were found to exhibit insecticidal activities (Poth et al., 2011; Pinto et al., 2012), although they may also find applications as therapeutic drugs (Smith et al., 2011) (Fig. 3).

**Defence metabolites**

While defensive plant proteins play a significant role in direct interactions between herbivores and plants, the role of non-protein secondary metabolites is just as big. The term ‘secondary’ is used to contrast them with metabolites that are directly involved in growth, development or reproduction, although it is not always possible to determine the precise physiological role of each metabolite. Across the plant kingdom, there is a staggering diversity of secondary metabolites, and they can be distinct for small phylogenetic groups. Many secondary metabolites have been implicated in plant defences or are stress-related. Despite the rich diversity of secondary metabolites, their biosynthetic origins allow them to be classified into three basal groups: (1) the phenolics; (2) the isoprenoids; and the (3) N-containing compounds (Fig. 2).

**Phenolics**

Phenolics consist of an aromatic ring with one or more hydroxyl groups. Two pathways are responsible for the majority of plant phenolics: (1) the phenylpropanoid pathway (Cheynier et al., 2013), which converts the aromatic amino acid phenylalanine into phenolics, and (2) the acetate/malonate (polyketide) pathway (Quideau et al., 2011). Single phenolics can be polymerized to form polyphenols and both can be subjected to additional modifications, giving rise to a vast quantity (>9000) of chemically diverse metabolites, which include benzoxinones, phenolic acids (such as SA), coumarins, flavonoids, lignins and tannins (Balasundram et al., 2006). Phenolics have various functions in primary metabolism; for instance, they protect plants from UV radiation (Landry et al., 1995) or form, as lignins, an integral part of the secondary cell wall in vascular plants (Boerjan et al., 2003). Furthermore, flavonoids are crucial for reproduction because they are required for pollen development (Van der Meer et al., 1992) and provide many of the visual and volatile cues used by flowers (Hoballah et al., 2013). However, there is a staggering diversity of secondary metabolites, and they can be distinct for small phylogenetic groups. Many secondary metabolites have been implicated in plant defences or are stress-related. Despite the rich diversity of secondary metabolites, their biosynthetic origins allow them to be classified into three basal groups: (1) the phenolics; (2) the isoprenoids; and the (3) N-containing compounds (Fig. 2).

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such as methyl salicylate, are involved in the indirect defence response (Ament et al., 2004, 2010).

**Isoprenoids** Isoprenoids constitute the largest and structurally most diverse class of metabolites with over 55,000 known structures. The universal isoprene [C5] precursors isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are produced via two pathways in plants: the cytosolic/peroxisomal mevalonate (MVA) and the plastidial 2-methylenetetrahydrofolate (MEP) pathway (Lange et al., 2000; Sapir-Mir et al., 2008). DMAPP (C5) is a precursor for the synthesis of cytokinins and isoprene. It also serves as the substrate for successive head-to-tail condensations with one or more IPP units to obtain precursors of longer-chain isoprenoids (Akhtar et al., 2013). Isoprenoids are building blocks for several phytohormones and are essential components of membranes (Schaeffer et al., 2001) and the photosynthetic (Fraser et al., 2000) and respiratory (Ducluzeau et al., 2012) machinery. Terpenoids are derived from isoprenoid precursors through the action of terpene synthases and subsequent modifications lead to a large diversity of molecules (Schilmiller et al., 2009), some of which are volatile and emitted in large quantities (Kant et al., 2009). Volatile terpenoids are present in relatively large quantities in most plant-derived scent bouquets (Knudsen et al., 2006). As such, they are cues for pollinators (Wright et al., 2005) and seed dispersers (Hodgkinson et al., 2007), but also function in responses against biotic and abiotic stresses (Gershenzon and Dudareva, 2007). Since volatile terpenoids are highly lipophilic, they can penetrate plasma membranes and increase their permeability (Sikkema et al., 1995) to exert direct toxic (and repellent) effects on arthropods (Bleeker et al., 2012; Vaughan et al., 2013). Moreover, terpenoids serve as key compounds in the indirect defence against herbivores as attractants of their natural enemies both above- and below-ground (Kant et al., 2009). Their production and release is regulated in a spatiotemporal manner (Köllner et al., 2004) through defence-associated phytohormones such as JA and SA (Van Poecke and Dicke, 2002). Although considered highly effective (Kessler and Baldwin, 2001), terpenoid-mediated indirect defence can also backfire as it might attract even more herbivores (Carroll et al., 2006) and can prime defence responses in competing neighbouring plants (Kessler et al., 2007) and guide parasitic plants to a new host (Runyon et al., 2006).

**Nitrogen-containing compounds** Nitrogen-containing secondary metabolites are thought to have primarily defensive purposes. Alkaloids constitute the biggest subgroup (>12,000 compounds). They bear at least one nitrogen atom in a heterocyclic ring and have been identified in ~20% of all plant species (Levin, 1976). Several amino acids as well as purine nucleotides and isoprenoids are precursors of alkaloids (Itkin et al., 2013). Their biosynthesis is often initiated in roots, followed by phloem and usually xylem transport (Coudravault et al., 2014). Alternatively, the final steps of their de novo biosynthesis can take place above ground (Miettinen et al., 2014). Alkaloids are constitutively present in plants, but their production and transport can increase upon herbivory (Baldwin, 1988) and exogenous application of methyl jasmonate (Baldwin, 1996). They can react with DNA, membranes and enzymes, and are therefore potent toxins for many organisms, including arthropods.

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**Fig. 3. Mode of action of plant defence proteins in the herbivore gut.** Herbivores utilize plant material predominantly to obtain sugar and amino acids. They digest proteins into amino acids via proteases and starch/sucrose into free sugars via amylases/invertases. Plants produce special proteins that are co-ingested and interfere with digestive processes in the gut. Defensins inhibit \( \alpha \)-amylase activity. Deaminases degrade amino acids. Proteinase inhibitors and peptidases inhibit the arthropod’s proteases. Peptidases, lectins and possibly some PR proteins damage the peritrophic membrane. Plant polyphenol oxidases in combination with plant phenolics are believed to generate quinones in the arthropod gut. These quinones may damage soluble and membrane proteins and DNA. Digestive processes of the herbivore are shown in red and counter-measures of the plant in green.
Interestingly, the nectar of some plants contains sublethal amounts of alkaloids, which not only protects them from nectar robbers (Kessler et al., 2008), but also improves their reproductive success by manipulating the behaviour of their natural pollinators (Kessler et al., 2012a).

Many plant species accumulate cyanogenic glucosides (α-hydroxynitrile glucosides), which protect them against herbivores because they can release volatile hydrogen cyanide (HCN), which inhibits cellular respiration (Way, 1984). Because plants are vulnerable to high concentrations of HCN as well, cyanogenic glucosides are stored in vacuoles, whereas the HCN-liberating enzymes β-glucosidase and α-hydroxynitrile lyase are localized in plastids (Thayer and Conn, 1981), the apoplast (Frehner and Conn, 1987) or in intracellular protein bodies (Swain et al., 1992). Upon herbivory, the cyanogenic glucosides become exposed to β-glucosidases. Depending on the pH, the resulting α-hydroxynitrites will either dissociate spontaneously into HCN or will be enzymatically converted to it by α-hydroxynitrile lyases (Siritunga et al., 2004).

Like cyanogenic glucosides, glucosinolates are derived from amino acids (Hansen et al., 2001). They are N- and S-containing defensive metabolites mainly found in Brassicaceae (Halkier and Gershenzon, 2006). Glucosinolates can be activated by the enzyme myrosinase (Husebye et al., 2004), from which they are separated by compartmentalization. Herbivory mixes the two (Barth and Jander, 2006), thereby triggering the production and release of various reactive hydrolysis products, mainly isothiocyanates and nitriles (Bones and Rossiter, 1996), which can be directly toxic and repellent to herbivores (Lazzeri et al., 2004) but also attract specialist herbivores (Beran et al., 2011) and serve as attractants of parasitoids (Mumm et al., 2008). Consistently, glucosinolate biosynthesis can be induced by herbivory (Hopkins et al., 2009) and is controlled by JA, SA and ethylene (Schweizer et al., 2013) (Fig. 3).

**PLANT DEFENCES IN NON-ATTACKED TISSUES**

Induced plant defensive substances accumulate not only locally at the feeding site, but also in undamaged tissues, and these ‘systemic’ responses have distinct spatiotemporal dynamics. There are several cases showing that distinct defence-associated changes can be observed in tissues neighbouring the damaged area within 1 min after wounding or herbivory (Schittko et al., 2000; Glauser et al., 2009), and these changes are typically the signalling events upstream of the actual defence response. A steady increase in the levels of JA and JA-Ile can be observed after a few minutes of damaging, with a peak after ~30 min (Glauser et al., 2008; Stork and Baldwin, 2009; VanDoorn et al., 2011). The extent to which the JA from this JA burst is synthesized de novo or released from storage is not always clear; it probably differs across different plant species and with the number of elicitations. The JA burst is preceded by a fast signal (Schittko et al., 2000), which most probably is electric, i.e. transmitted via membrane depolarizations, and which also travels to distal leaves (Glauser et al., 2009; Koo et al., 2009; Mousavi et al., 2013). However, molecular signals also travel from leaf to leaf, via either the phloem or the xylem (Malone and Alarcon, 1995; Rhodes et al., 1999). However, the identity of these signals is still elusive. Grafting experiments with tomato plants showed that JA biosynthesis, but not perception, was required for initiating the systemic signal while the downstream defence responses required perception (Schilmiller and Howe, 2005). Peptides such as systemin and PepS, and phytohormones such as ABA, auxin and cytokinins (Soler et al., 2013), are closely associated with local and systemic signalling, but especially JA and usually JA derivatives play a critical role (Wu and Baldwin, 2010).

**Temporal and spatial heterogeneity of signalling within and between leaves**

The spatiotemporal patterns of systemic responses differ across plant species, depending on differences not only in their size or age but also in plant-specific (vascular) architecture. Because not all leaves of a plant are connected to the same degree, the induction of defensive compounds is higher in undamaged leaves with the most direct vascular connection to the attacked leaf, the so-called orthostichous leaves (Orians et al., 2000), and these are less vulnerable to herbivores than leaves with a less direct connection (Viswanathan and Thaler, 2004). Heterogeneity in the levels of defensive compounds in damaged tissues can stimulate herbivores to move to other areas in an attempt to avoid the induced defences (Shroff et al., 2008). For example, caterpillars of *M. sexta* might be able to escape from the induced resistance of a plant through ‘induced movement’ (Paschold et al., 2007). Also, the foraging behaviour of the generalist insect *Helicoverpa armigera* depends on JA: the larvae leave induced areas quickly and move to non-induced distant parts. Interestingly, *Plutella xylostella*, which is known to be resistant to defensive chemicals in some Brassicaceae plants, did not display this behaviour (Perkins et al., 2013). Finally, heterogeneity of leaf systemic response (Stork et al., 2009) or plant systemic response due to heterogeneity of vascular connections was suggested to be adaptive because it makes it more difficult for herbivores to learn which tissues will be least defended. Rodriguez-Saona and Thaler (2005) used normal tomato plants and JA-deficient (def-1) tomato plants to analyse local and systemic induced JA responses in relation to patterns of caterpillar feeding damage. They observed that the systemic response in leaves with a stronger vascular connection was stronger than in leaves with a weaker connection, but at a similar physical distance from the damaged area. Hence the extent to which a herbivore can avoid induced defences is determined by the strength of the vascular connection of the induced leaf and the newly selected (distal) leaf.

It was shown that electric and vascular signals can act in synergy with airborne signals to optimize the systemically expressed resistance within a plant (Heil and Ton, 2008). Moreover, exposure of plants to relatively high amounts of synthetic volatiles was shown to induce defences. For example, exposure of tomato plants to methyl jasmonate results in the accumulation of PIs in systemic leaves of the plant, in neighbouring tomato plants and even in plants of different species, such as tobacco and alfalfa (Farmer and Ryan, 1990). Similarly, methyl salicylate may act as an airborne signal to activate resistance in uninfected tissues of an infected plant and in neighbouring plants (Shulaev et al., 1997). In contrast, corn...
Plants and herbivores have coevolved for over 400 million years. While plants have evolved signalling networks to regulate induced defences and diversity in their palette of secondary metabolites, herbivores have been under pressure to evade defences (reviewed in Alba et al., 2011). Hence, behavioural adaptations have evolved that allow herbivores to avoid defended plant tissues (Pasehold et al., 2007; Shroff et al., 2008; Perkins et al., 2013) as much as possible or to dismantle defensive structures such as trichomes (Cardoso, 2008) and latex channels (Rodrigues et al., 2010). However, herbivores have also evolved a variety of mechanisms to cope with deterrent substances produced by their host plants. Given the enormous economic impact of herbivore resistance to agrochemicals, a large part of our knowledge of adaptations to xenobiotics comes from the field of pesticide resistance (Despres et al., 2007). However, the mechanisms at play are similar to those that enable them to resist defensive phytotoxins, and a functional overlap between adaptation to agrochemicals and to phytotoxins has been suggested (Dermauw et al., 2013a).

Two general mechanisms allow herbivores to cope with the xenobiotics from their environment: mechanisms that decrease exposure (pharmacokinetic responses) and mechanisms that decrease sensitivity (pharmacodynamic responses). Pharmacokinetic responses comprise a variety of adaptations that reduce uptake, increase catabolism and allow sequestration, whereas the pharmacodynamic response types comprise adaptations at the level of interactions between allelochemicals and their target-site(s) (Taylor and Feyereisen, 1996; Kennedy and Tierney, 2013). Together, these mechanisms determine the level of tolerance of herbivores to xenobiotics (Fig. 4).

**Pharmacokinetic responses**

**Mechanisms of decreased exposure** The detoxification of xenobiotics usually occurs in three phases. In phase I, the xenobiotic is modified by reactions that incorporate a nucleophilic functional group (a hydroxyl, carboxyl or amine group) and this often results in a more polar/hydrophilic substance. In phase II, the metabolite resulting from phase I is conjugated to endogenous molecules such as glutathione or a sugar molecule, which further increases the compound’s polarity/hydrophilicity. In phase III, the phase II conjugated xenobiotic is transported out of the cell by cellular transporters. In several cases, these transporters can also act as a first line of defence, preventing allelochemicals entering the cell by rapid efflux without the need for modifications (sometimes this is referred to as phase 0).

Enzymes that operate during phase I are often cytochrome P450 monooxygenases (P450s) and carbonyl/choline esterases, whereas enzymes such as glutathione-S-transferases (GSTs) and UDP-glycosyltransferases (UGTs) typically operate during phase II. Finally, transport of phase II metabolites out of the cell is often performed by ATP-binding cassette (ABC) and solute carrier (SLC) family proteins. Adaptations that allow herbivores to enhance their detoxification of particular target xenobiotics often occur via mutations that increase the production of specific detoxification enzymes and transporters as well as by mutations that improve their catalytic or transport properties (Brattsten, 1992; Despres et al., 2007; Kennedy and Tierney, 2013).

**Phase I detoxification** A wide range of allelochemicals, including furanocoumarins, terpenoids, glucosinolates, flavonoids and alkaloids can be metabolized by P450s of herbivorous arthropods (Despres et al., 2007; Feyereisen, 2012). The involvement of P450s within the lepidopteran genus Papilio in furanocoumarin resistance is one of the first documented examples relating to plant–arthropod interactions (Berenbaum, 1982). The P450s are by far the best studied enzymes of phase I, and have been associated with resistance to plant allelochemicals and pesticides in a wide range of species (Feyereisen, 2012; Schuler, 2012). Although esterases have been linked to pesticide resistance in a number of cases, their role in plant allelochemical defence remains elusive (Despres et al., 2007; Li et al., 2007). Notably, esterases were found not always to operate via hydrolysis but also to confer resistance to pesticides by sequestration, i.e. by binding to the target substance without modifying it. Remarkably, esterase genes were duplicated in some aphid species, which gave rise to elevated expression, such that 3% of their total protein content consisted of these enzymes (Devonshire and Sawicki, 1979; Devonshire and Moores, 1982). It is well imaginable that such esterases are involved in resistance or tolerance to phytotoxins as well.

**Phase II detoxification** Conjugation of xenobiotics by GSTs has been linked to allelochemical tolerance in a number of cases, although most evidence has been obtained from in vitro assays. The expression of GSTs in arthropod herbivores can be induced...
by a number of allelochemicals, and these enzymes are generally believed to make up an important component of the overall pharmacokinetic response. Some GSTs of the polyphagous insect *Spodoptera frugiperda* are able to metabolize a variety of thiocyanate conjugates. Moreover, the diversity of glucosinolate-derived thiocyanates that insects such as the larvae of *S. frugiperda*, *Trichoplusia ni* and *Anticarsia gemmatalis* can metabolize correlates with their host plant range (Li et al., 2007), whereas the major glucosinolate-derived thiocyanate was found to be a glutathione-conjugated derivative in several generalist insect herbivore species (Schramm et al., 2012). Recently, a role for GSTs in tolerance to glucosinolates was also suggested for the whitefly *Bemisia tabaci* and in the mustard-feeding specialist *Scaptomyza nigrita* (Elbaz et al., 2012; Gloss et al., 2014). Finally, an enzyme called GST16 inactivates the phytohormone OPDA in the gut of *Helicoverpa armigera* by isomerization to inactive iso-OPDA (Dąbrowska et al., 2011). However, it is unclear whether this OPDA modification has adaptive significance (Shabab et al., 2014).

The second important class of conjugation enzymes is that of the UGTs, which convert lipophilic aglycones into more hydrophilic glycosides by conjugation with UDP-glucose. UGTs have been shown to be involved in resistance of lepidopterans to the alkaloid capsacain and the detoxification of benzoxazinoids (Ahn et al., 2011; Wouters et al., 2014), and they may be widespread enzymes that allow herbivorous arthropods to adapt to xenobiotics (Ahn et al., 2014). Some insects have evolved traits that in principle allow them to prevent the activation of protoxins, such as glucosinolates, by plant enzymes. For example, aphids prevent glucosinolates from mixing with myrosinases by avoiding rupturing myrosinase-containing plant cells. Moreover, the insect degrades these glucosinolates in the gut and conjugates the breakdown product to ascorbate, glutathione and cysteine. However, artificial feeding assays suggested that these conjugates also have an antifeeding effect in *M. persicae*. Hence, glucosinolates may also have defensive functions independent of myrosinase, via post-ingestive digestive processes occurring in the aphid (Kim et al., 2008).

**Phase III detoxification** While phases I and II have been characterized in some detail, phase III has been documented in far less detail (Sorensen and Dearing, 2006; Dermauw and Van Leeuwen, 2014). Gaertner *et al.* (1998) suggested that the efflux of nicotine and other alkaloids by the Malpighian tubules, the main excretory organs of insects, of *M. sexta* is mediated by an ABC transporter. This finding was based on the observation that verapamil, a known inhibitor of the ABC transporter, blocks nicotine transport in the Malpighian tubules of *M. sexta*. In addition, Petschenka *et al.* (2013) found an ABC transporter that probably functions as cardenolide efflux carrier, thereby protecting the nervous tissues of lepidopterans. Other transporter families are also implicated in mediating the efflux of plant allelochemicals. Govind *et al.* (2010) showed that transporters of the major facilitator superfamily, which include many transporters of the SLC family, were downregulated in *M. sexta* larvae after feeding on wild tobacco mutants that were unable to produce JA. Interestingly, other members of the same superfamily were upregulated in arthropod herbivores that were

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**Fig. 4.** The herbivore resistance response. Plant secondary metabolites can have diverse target sites. Two general mechanisms allow arthropod herbivores to cope with plant secondary compounds: mechanisms that decrease exposure via detoxification, transport and/or sequestration (shown in this figure) but also mechanisms that decrease sensitivity (see main text). Detoxification (indicated as ‘Detox’ in this figure) usually occurs in three phases: in phase I, enzymes such as P450s and CCEs (carboxyl/cholinesterases) modify the metabolite; in phase II GSTs and UGTs conjugate it and in phase III the conjugated metabolites are transported out of the cell, typically by ABC transporter and SLC family proteins. The midgut, fat body and Malpighian tubes are the insect tissues where these detoxification phases occur (Harrop *et al.*, 2014). Metabolites are excreted via faeces and urine. Some metabolites, or modified metabolites, are stored in the cuticle or in other organs and are used by the arthropod for its own protection. Plant substances are indicated in green and arthropod responses in red.
transferred from their preferred host to a more challenging, less suitable host (de la Paz Celorio-Mancera et al., 2013), e.g. in the non-insect arthropod T. urticae (Dermauw et al., 2013b). Regardless of the identity of the transporters or transport mechanisms, a number of cases have been described in which the evolution of transport systems has determined herbivore success. For example, the ability to selectively transport plant glycosides has been suggested to stand at the basis of the evolution of life styles and host ranges of leaf beetles (Kuhn et al., 2004; Strauss et al., 2013).

Miscellaneous resistances to xenobiotics

Some insects, especially host-plant specialists, have developed resistance mechanisms that differ from those described above. For example, a flavin-dependent mono-oxygenase in arctiid moths was shown to be involved in detoxifying pyrrolizidine alkaloids (Sehlmeyer et al., 2010; Wang et al., 2012). These enzymes usually have general functions in an insect’s primary metabolism but here evolved, after gene duplication, to catalyse N-oxidation of pyrrolizidine alkaloids (Langel and Ober, 2011). Moreover, specialist crucifer-feeding insects have developed the means to redirect the formation or to overcome the toxic action of glucosinolate breakdown products (Pentzold et al., 2013) by evolving novel enzymes and proteins into general detoxification pathways. Pieris rapae was shown to use a nitrile-specifier protein to divert the degradation of glucosinolates in the toxic isothiocyanates to less toxic nitriles (Wittstock et al., 2012; Wybouw et al., 2014). Another specialist, the diamondback moth, Plutella xylostella, desulphates glucosinolates and thereby generates inactive metabolites (Ratzka et al., 2002).

Other examples of adaptations to xenobiotics in a number of lepidopterans include the detoxification of cyanogenic glucosides by converting cyanide into β-cyanoalanine (Zagrobelny and Moller, 2011). It was shown recently that the β-cyanoalanine synthase genes of lepidopterans as well as mites stand at the basis of this adaptation and were originally obtained from bacteria by horizontal transfer into the ancestral genomes (Wybouw et al., 2014). This enzyme might also be crucial for countering the negative effects of glucosinolate in P. rapae because their breakdown also generates cyanide (Stauber et al., 2012; Wybouw et al., 2014). In addition, some lepidopterans have evolved the means to convert cyanide into nitrogen (Engler et al., 2000; Pentzold et al., 2014).

Sequestration

Some herbivores make use of plant allelochemicals for their own defence against predators by storing ingested chemicals in specialized tissues or in the integument. A variety of mechanisms have been described that allow herbivores to exploit these toxic substances without suffering from their latent detrimental effects. Compounds can be sequestered either directly or after biotransformation, such as by oxidation and conjugation. More than 250 insect species have been shown to sequester plant metabolites covering a wide range of chemical classes, such as alkaloids, cyanogenic glucosides, glucosinolates, isoprenoids and cucurbitacins (Nishida, 2002; Opitz and Müller, 2009). In some cases, insects have evolved the ability to synthesize these compounds de novo by convergent evolution of the biosynthetic pathways (Jensen et al., 2011). Also, some insect species have evolved additional means to efficiently utilize sequestered compounds for their own defence. For example, some aphid and flea beetle species have evolved a specific myrosinase that allows them to convert glucosinolates into their toxic breakdown products in a similar way as plants do and to release these as toxic and repellent volatiles into the air (Beran et al., 2014; Rahtfeld et al., 2014).

Pharmacodynamic responses: mechanisms of decreased sensitivity

While many adaptations of herbivores to xenobiotics depend on mechanisms that directly or indirectly divert these substances from their target sites, some adaptations are due to reduced target-site sensitivity. Although alterations in the target site of pesticides have been associated with herbivore resistance to pesticides (French-Constant, 2013; Van Leeuwen et al., 2010b; Feyereisen et al., 2015), few studies have linked such mutations to insect resistance to plant allelochemicals. This lack of documented target-site resistance to phytochemicals is probably due to the fact that many of these have multiple modes of action and our knowledge of these mechanisms is limited (Berenbaum, 1987; Despres et al., 2007). The best documented example of target-site insensitivity to phytotoxins is that of amino acid substitutions in the α subunit of Na+/K+-ATPase from four different insect orders (Lepidoptera, Coleoptera, Heteroptera and Diptera), conferring resistance to the plant toxin ouabain, a cardenolide produced by several members of the Apocynaceae. Remarkably, the same substitution was found in different insect species, all of which specialized in cardenolide-containing plant species, suggesting that this replacement must have evolved independently several times (Agrawal et al., 2012; Dobler et al., 2012; Zhen et al., 2012; Dalla et al., 2013). Finally, tolerance to l-canavanine, a non-proteinogenic amino acid of leguminous plants, can also be considered as a target-site-based resistance mechanism in some insect species. The toxicity of l-canavanine is caused by its incorporation into proteins, replacing l-arginine. Insects such as the bruchid beetle Caryedes brasiliensis have specialized in feeding on the l-canavanine-rich seeds of Dioclea megacarpa and have evolved an arginyl-tRNA synthetase that can discriminate between l-canavanine and l-arginine, thereby effectively avoiding the adverse biochemical effect of l-canavanine (Rosenthal et al., 1976; Leisinger et al., 2013). In addition to target-site insensitivity, mechanisms of decreased exposure to l-canavanine have also been reported for the tobacco budworm Heliothis virescens (Melangeli et al., 1997) (Fig 4).

Plant defence suppression by herbivores

Because many defensive actions of plant are induced or maintained by ongoing physiological processes, some herbivores have evolved the means to interfere with these processes. In this way, these herbivores may manipulate resource flows (Clark and Harvell, 1992) or suppress defences (Musser et al., 2002). The suppression of defences is distinct from defence
avoidance, such as 'stealthy feeding' or vein cutting, which serve to prevent detection or to prevent physical contact with defensive substances. However, these two processes will not always be easy to separate experimentally (Karban and Agrawal, 2002; Stireman and Cipollini, 2008; Alba et al., 2011). Suppression of defences is characterized by lowering the rate of production of defensive compounds. Hence, suppression can operate upstream or downstream of a defensive pathway and block it altogether or dampen it to intermediate levels, although assessing the latter can be difficult without having a suppression-free control experiment as a benchmark (Alba et al., 2015). Finally, downregulation of plant defences qualifies as suppression when it is paralleled by an increase in (reproductive) performance of the herbivore (Table 1).

Suppression of plant defences is a well-known phenomenon in plant pathogens such as pathogenic bacteria (Abramovitch et al., 2006), rust fungi (Voelge and Mendgen, 2003), oomycetes (Kamoun, 2006) and viruses (Kasschau and Carrington, 1998). However, nematodes and mites were also found to suppress defences. Several phytophagous nematode species interfere with host plant resistance (Haegeman et al., 2012) and the cyst nematode Meloidogyne incognita was found to suppress SA- and JA-dependent systemic acquired resistance in Arabidopsis thaliana (Hamamouch et al., 2011). In addition, two spider mite species were found to suppress the defences downstream of both JA and SA simultaneously in tomato (Kant et al., 2008; Sarmento et al., 2011a; Alba et al., 2015), whereas an eriophyid mite was found to suppress only the downstream JA defences, independently from hormonal crosstalk (Glas et al., 2014). Other examples of suppression are from insects and can be attributed to hormonal crosstalk in the majority of cases. Hemipteran phloem feeders such as the mealybug Phenacoccus solenopsis (Zhang et al., 2011) and the whitefly B. tabaci were found to suppress JA defences (Zhang et al., 2009), possibly by inducing antagonist SA defences (Zarate et al., 2007; Walling, 2009). In addition, the aphid Megoura viciae inhibits defensive phloem clogging (Will et al., 2007), whereas other aphid species were found to suppress the oxidative burst (Bos et al., 2010). The leafhopper Macrosteles quadrilineatus suppresses JA defences indirectly via an effector derived from a vectored phytoplasma (Sugio et al., 2011). Also, chewing larvae of several lepidopteran species have been found to interfere with induced defences (Bede et al., 2006). On A. thaliana, larvae of Spodoptera exigua inhibit JA-mediated defence responses via the systemic acquired resistance pathway (Weech et al., 2008) whereas Pieris brassicae suppresses defences independently of the JA and SA pathways (Consales et al., 2012). In addition, Helicoverpa zea was found to suppress nicotine accumulation in N. tabacum (Musser et al., 2002, 2005) and to suppress JA- and ethylene-regulated genes via salivary enzymes in tomato (Wu et al., 2012). This is reminiscent of the downregulation of nicotine by M. sexta feeding on N. attenuata, although it may reflect a plant-adaptation rather than a herbivore-adaptive trait (Kahl et al., 2000; Voelckel et al., 2001). Moreover, the Colorado potato beetle, Leptinotarsa decemlineata, suppresses transcription of PI genes in tomato (Lawrence et al., 2011). Finally, larvae of virulent strains of the hessian fly, Mayetiola destructor, secrete substances via their vestigial mouthparts into plant tissues, thereby suppressing the expression of PI and lectin genes (Stuart et al., 2012).

Thus, evidence for suppression of plant defensive processes is found across herbivorous insects, plant-eating mites and nematodes.

Whereas induction of plant defences often results from elicitor or HAMP recognition, the suppression of induced or constitutive defences is often attributed to so-called effector molecules. These molecules are especially well known from phytopathogens and had been discovered already in the 1970s (Shiraiishi et al., 1978), but the notion that herbivores may secrete molecules with similar properties arose after the discovery that GOX from the saliva of H. zea caterpillars counteracts the production of nicotine in N. tabacum (Musser et al., 2002). Although our knowledge of herbivore effectors is still limited, a staggering diversity of effectors from pathogens (Deslandes and Rivas, 2012; Rovenich et al., 2014) and nematodes (Mitchum et al., 2013; Kazan and Lyons, 2014) has been discovered. Roughly, these effectors comprise the following functional groups (although these are not mutually exclusive).

- Metabolites secreted into the host to manipulate particular physiological processes such as hormonal signalling. Some strains of P. syringae produce the JA mimic coronatine, which puts JA defences into overdrive to suppress SA defences (Zhao et al., 2003).
- Enzymes that interfere with the host’s ability to control infected tissues encoded by transgenes inserted into the host’s genome by the pathogen. The best known example is Agrobacterium tumefaciens, which injects its transfer DNA (which carries genes that facilitate gall formation) into plant cells, where it integrates into the host’s genome and is expressed by the host (Zhu et al., 2000).
- Enzymes secreted by pathogens to perform a metabolic conversion in the host that affects its defences. Fungi such as Septoria lycopersici produce tomatinase, which not only detoxifies the defensive alkaloid tomatine but also generates hydrolysis products that suppress the hypersensitive response (Bourrarab et al., 2002).
- Secreted proteins that interfere with transcription factors or that act as transcription factors of defence genes of the host. The first is the case for an effector called SAP11, produced by phytoplasmas vectored by leafhoppers. SAP11 destabilizes the host’s CIN-TCP transcription factors, leading to downregulation of the JA response (Sugio et al., 2011). The second is the case for the transcription activator-like (TAL) effectors produced by the Xanthomonas bacterium. TAL effectors are translocated to the host’s nucleus, where they modulate the expression of specific target genes to facilitate the infection (Boch and Bonas, 2010).
- Secreted proteins that interfere with host receptors involved in defences. For example, the P. syringae HopF2 effector suppresses plant immunity by targeting the R gene co-receptor BAK1 (Zhou et al., 2014).
- Secreted proteins that interfere with defence signalling cascades downstream of receptor recognition. This is the case with AvrB of P. syringae, which phosphorylates the signalling hub RIN4 to block PAMP (Pathogen-Associated Molecular Pattern) triggered immunity (Mackey et al., 2002).
- Secreted proteins that manipulate proteasome functioning in defensive processes. This is the case for the E3 ubiquitin
<table>
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<th>Arthropod Common name</th>
<th>Plant Common name</th>
<th>Defence suppressed</th>
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<td>Unknown</td>
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<td>Bemisia tabaci (Insecta) Silverleaf whitefly</td>
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<td>Salivary effector protein C002</td>
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<td>Myzus persicae (Insecta) Green peach aphid</td>
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<td>Oxidative burst</td>
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<td>Macrophosphus euphorbiae (Insecta) Potato aphid</td>
<td>Nicotiana benthamiana, Solarium lycopersicum</td>
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<td>Nicotiana tabacum, Nicotiana benthamiana, Arabidopsis thaliana</td>
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<td>Brevicoryne brassicae (Insecta) Cabbage aphid</td>
<td>Brassica oleracea</td>
<td>JA defence</td>
<td>Interference with JA signal transduction</td>
<td>Caterpillar developmental speed, caterpillar weight gain</td>
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<td>Macrosphus quadrilineatus (Insecta) Astar leafhopper</td>
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<td>Reproductive performance, oviposition rate</td>
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<tr>
<td>Phenacoccus solenopsis (Insecta) Cotton mealybug</td>
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<td>Gossypol production, JA defence response</td>
<td>Unknown</td>
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<td>Leptinotarsa decemlineata (Insecta) Colorado potato beetle</td>
<td>Solanum lycopersicum</td>
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<td>Unknown</td>
<td>Larval weight gain</td>
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</tr>
<tr>
<td>Leptinotarsa decemlineata (Insecta) Colorado potato beetle</td>
<td>Solanum lycopersicum</td>
<td>JA defence</td>
<td>Ora1 bacteria</td>
<td>Larval weight gain</td>
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<td>Tetranchus urticae (Acari) Two-spotted spider mite</td>
<td>Solanum lycopersicum</td>
<td>JA and SA defence, terpenoids and methyl salicylate</td>
<td>Unknown</td>
<td>Oviposition rate</td>
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<td>Tetranychus evansi (Acari) Red spider mite</td>
<td>Solanum lycopersicum</td>
<td>JA and SA defence, terpenoids and methyl salicylate</td>
<td>Unknown</td>
<td>Oviposition rate, population growth</td>
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<tr>
<td>Acalyp f lycopersici (Acari) Tomato russet mite</td>
<td>Solanum lycopersicum</td>
<td>JA defence</td>
<td>Unknown</td>
<td>Population growth</td>
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</tr>
</tbody>
</table>
ligase AvrPtoB of *P. syringae*, which initiates degradation of a kinase that is essential for innate immunity (He et al., 2006).

- Secreted proteins that perform proteolysis of plant defence proteins. This was found for an extracellular PI of *Phytophthora infestans*, which targets a tomato PR protein (Tian et al., 2004) and for AvrRpt2 of *P. syringae*, which acts as protease to eliminate RIN4 (Axtell et al., 2003).
- Secreted proteins that interfere with host vesicle trafficking during immune responses. This is the case for HopM1 of *P. syringae*, which mediates degradation of the vesicle trafficking regulatory protein MIN7 (Nomura et al., 2011).
- Secreted proteins that interfere with RNAi, such as the 2b protein of the cucumber mosaic virus. This protein protects the virus against RNAi-mediated degradation and also suppresses SA defences (Ji and Ding, 2001) and JA defences induced by their aphid vectors (Westwood et al., 2014).
- Secreted small RNAs that manipulate a host’s RNAi machinery, such as a small RNA transferred by *Botrytis cinerea*, which silences the protein Argonaute 1 in *Arabidopsis* and tomato and thereby suppresses host immunity (Weiberg et al., 2013).

Effectors may be powerful weapons whereby pathogens and nematodes interfere with plant defences, but plants have evolved a range of R genes in return, which specifically serve to recognize effectors and bypass suppression (Bergelson et al., 2001). These molecular sensors are used in plant breeding to obtain pathogen-resistant crops but R gene resistance is often broken again shortly after its introduction due to counter-adaptations in pathogens (Bent and Mackey, 2007). Hence, plant breeders have redirected their focus to the effector targets, also referred to as susceptibility genes or S genes (van Schie and Takken, 2014), since effector-resistant breeding targets are promising tools for obtaining more durable resistance (Gawe lhs et al., 2013).

**Effectors involved in plant defence suppression by herbivores**

There are indications that plant-defence-suppressing herbivorous arthropods also secrete effectors via their saliva into their host, similar to pathogens and nematodes. The first of such salivary components that was discovered was the enzyme GOX, which is the most abundant molecule in the oral secretions of the caterpillar of *H. ze a* (Musser et al., 2002). This enzyme catalyses the oxidation of glucose to D-gluconic acid and thereby generates hydrogen peroxide. The amount of GOX applied to *T. tabacum* plants correlates with an increase in SA and a decrease in the accumulation of nicotine. Possibly, GOX suppresses or attenuates JA and ethylene responses by crosstalk with SA (Diezel et al., 2009; Eichenseer et al., 2010). GOX has been found in many more caterpillar species (Eichenseer et al., 2010) and other herbivorous insects, such as aphids and non-herbivores such as honeybees (Iida et al., 2007; Harmel et al., 2008). A comprehensive study by Eichenseer et al. (2010) showed large variation in GOX activity within families and subfamilies of 88 caterpillar species, but these activities depended on the host plant species as well. Moreover, a recent report showed that *H. ze a* GOX elicits the JA pathway in tomato (Tian et al., 2012). Taken together, these studies suggest that some plants, such as tomato, may have evolved a recognition mechanism for GOX, resembling R-gene-mediated recognition of effector proteins in plant–pathogen interactions.

Advances in genomics and proteomics have greatly facilitated the discovery of more effector proteins in insects. After the *Acr y th o siph on pisum* (peach aphid) salivary glands were sequenced, the first aphid effector was discovered. This protein is a 22-kDa salivary-secreted protein of unknown function called C002 (Mutti et al., 2008). RNAi-mediated knockdown of C002 expression affected *A. p is um* foraging and feeding behaviour and reduced aphid fitness. Bos et al. (2010) used *N. benthamiana* to ectopically express C002 from *M. persic a* (green peach aphid) and showed that aphid fecundity increased on these plants. Transient overexpression of a second aphid protein, Mp10, sufficed to suppress the flagellin-triggered oxidative burst in *N. benthamiana*, but aphid reproduction was lower on these plants. A subsequent study characterized two additional aphid proteins, Mp1 (Plt01) and Mp2 (Plt02), which correlate positively with aphid fecundity on *A rabidopsis*. Interestingly, the performance of *M. persic a* did not improve on *A rabidopsis* plants expressing the A. *p is u m* orthologues of both effectors (Pitino and Hogenhout, 2013). Finally, two putative effectors of *Mac os pi um eup hori bia e* were found, Me10 and Me23, both of which increased aphid fecundity on *N. benthamiana*, whereas only Me10 increased their fecundity on tomato (Atamian et al., 2013).

Research on gall midges has provided independent evidence for a role of effector proteins in plant–herbivore interactions. Early larval stages of the hessian fly, *M. destructor*, are plant parasites. When they colonize wheat (*Triticum spp.*), the larvae induce feeding cells in their host, which provide them with food until they develop into adults (Harris et al., 2006). More than 30 hessian fly resistance genes have been found in wheat, some of which are predicted to encode typical R proteins (Liu et al., 2005). On resistant wheat, hessian fly larvae are unable to induce feeding cells, but instead induce a hypersensitive-like response that prevents them from eating (Harris et al., 2010). One *M. destructor* gene, vH13, functions as an avirulence factor on wheat carrying the *H13* resistance gene. In contrast, larvae from populations that are virulent on *H13* wheat did not express vH13, while RNAi-mediated knockdown of vH13 in avirulent larvae made some of them virulent (Aggarwal et al., 2014). These data suggest that vH13 may function as an effector on non-resistant wheat varieties.

Thus, there are indications that herbivores may make use of effectors, just as pathogens do. This notion is strengthened by the existence of anti-herbivore R genes such as *Mi-1, Vat* and *Bph14* (Rossi et al., 1998; Dogimont et al., 2008; Du et al., 2009). The high diversity found among pathogen effectors discourages the use of protein homology as a strategy to identify herbivore effectors (Rep, 2005). Nevertheless, most effector proteins share structural features that can be easily recognized, such as an amino-terminal signal peptide, the absence of transmembrane domains and a small protein size. Furthermore, effectors that operate in the plant apoplastic space are usually rich in cysteine residues (Rooney et al., 2005). Several studies have exploited these common properties to find novel effector-encoding genes from sequenced pathogen genomes or transcriptomes. Comprehensive datasets on herbivore transcriptomes and proteomes (Grbić et al., 2011; Su et al., 2012;
DeLay, 2012) will probably give rise to the discovery of new effectors in the near future (Table 1).

HOW COMMUNITY INTERACTIONS SHAPE THE ADAPTIVE PROCESS

Plants are often attacked by a diverse community of enemies, including herbivores and pathogens. Interspecific competition within phytophagous communities can be direct (e.g. interference through fighting) or indirect (e.g. using a resource deprives others of also using it) (Denno et al., 1995). Because induction and suppression of defences manifest themselves in distal tissues (Kant et al., 2008; Alba et al., 2015), it is predicted that herbivores and pathogens will also interact indirectly through the changes in the plant elicited by their feeding (Ohgushi, 2005). These interactions may lead to decreased performance, but also to facilitation: herbivores were sometimes found to benefit from the presence of conspecific or heterospecific attackers (Faeth, 1986; Harrison and Karban, 1986; Karban, 1989).

Indirect interactions in phytophagous communities via induced defences

Responses induced by one particular species can result in resistance to another (Long et al., 2007; Poelman et al., 2008a; Mouttet et al., 2013), whereas the order of herbivore arrival on the plant can influence the performance and number of herbivore species occurring later in the season (Van Zandt and Agrawal, 2004; Viswanathan et al., 2005; Erb et al., 2011). Hence, inducible plant defences can be major determinants of ecological interactions; in particular, defences depending on JA and SA appear to play important roles in determining community composition. For example, JA-deficient wild tobacco plants in the field were colonized by herbivores that normally ignore these plants (Kessler et al., 2004), and studies in which plants were sprayed with synthetic phytohormones to assess the effect of induced defences on their ecology showed that such artificial induction can decrease the abundance of herbivores when JA is applied (Thaler et al., 2001) or of pathogens when SA or SA mimics are applied (Inbar et al., 1998). However, treating tomato plants with JA increased the growth of the pathogen P. syringae, whereas induction of SA responses enhanced the performance of the beet armyworm (S. exigua) (Thaler et al., 1999). Moreover, when JA and SA responses were induced simultaneously the performance of the cabbage looper caterpillar (T. ni) increased, but not that of the thrips Frankliniella occidentalis, the spider mite T. urticae and M. sexta larvae (Thaler et al., 2002). In addition to these field studies, experiments have also been carried out in the laboratory with model organisms to reveal the mechanisms that underlie indirect interactions. Using Arabidopsis mutants impaired in the JA, ethylene or SA-pathway, de Vos et al. (2006) found that feeding by the JA-inducing caterpillar P. rapae did not, contrary to expectation, induce resistance against the necrotrrophic fungus Alternaria brassicicola. In contrast, it did reduce disease symptoms caused by P. syringae, but this effect was not (exclusively) dependent on JA, ethylene or SA. It also reduced infectiousness of the biotroph turnip crinkle virus due to an ethylene-primed SA response (de Vos et al., 2006). In addition Thaler et al. (2010) showed that P. syringae induced JA, SA and increased the activity of PIs in tomato plants and thereby reduced the growth of S. exigua caterpillars. Conversely, infection with tobacco mosaic virus induced only an SA response, causing induced susceptibility to S. exigua caterpillars and induced resistance to aphids (M. persicae). Herbivores feeding on different plant organs can also affect each other through the induction of plant responses (Soler et al., 2013). Larvae of P. rapae grew more slowly on plants that were also infested with root-feeding nematodes (Pratylenchus penetrans) and this correlated with higher foliar glucosinolate levels (Van Dam et al., 2005). Similarly, Soler et al. (2005) found that P. brassicae larvae developed more slowly on wild mustard (Brassica nigra) plants that were infested with the cabbage root fly (Delia radicum), and this in turn affected developmental rates of Cotesia glomerata, which is a parasitoid of the herbivore. Systemic signals that have been proposed to be involved in above–below-ground interactions include the phytohormones JA, ABA, ethylene, auxin and cytokinin, but for none of these has a clear role been unequivocally established. This suggests that the simultaneous induction of different defences may affect community members differently.

Indirect interactions in phytophagous communities via suppressed defences

Not only induction, but also suppression of defences can affect interactions between herbivores and pathogens. In principle, the benefits of suppression by a single herbivore species can be shared by other species in the community. Within the spider mite species T. urticae, there is intraspecific variation in the ability to induce and suppress defences (Kant et al., 2008). Some spider mites are very sensitive to the JA defences they induce in tomato plants. However, when these mites share their feeding site with other types that can suppress JA defences, their reproductive performance increases dramatically (Alba et al., 2015). In addition, suppressor mites of the species Tetranychus evansi as well as non-suppressor mites of the species T. urticae perform better when they are introduced on leaves already suppressed by T. evansi than on uninduced control leaves (Sarmento et al., 2011a,b). Similarly, whiteflies (B. tabaci) were found to suppress JA defences, and this was shown to improve the performance of spider mites on Lima bean (Zhang et al., 2009) and to disrupt the attraction of natural enemies (Zhang et al., 2009, 2013a). In turn, some parasitoids have adapted to locate hosts using SA-induced volatiles, the emission of which is not suppressed by whiteflies (Zhang et al., 2013b). Soler et al. (2012) showed that the cabbage aphid (Brevicoryne brassicae) inhibited the production of JA in cabbage (Brassica oleracea) plants, and this led to increased growth and development of the large cabbage white butterfly (P. brassicae) on plants that had been co-infested with the two species. Finally, field-grown tomato plants were frequently infested with the two-spotted spider mite T. urticae and the tomato russet mite, Aculops lycopersici; the first of these induces JA and SA defences simultaneously, whereas the second induces only SA and suppresses the JA response. Spider mites had much higher reproductive performance on plants infested with
Indirect interactions among plant defence, herbivores and predators

During recent decades, the study of interactions between plants and other organisms has developed from the investigation of relatively simple interactions between plants and herbivores to that of more complex multitrophic interactions and their importance for the structure of communities of plants and arthropods. In nature, plants are part of complex food webs in which the sum of direct and indirect interactions between organisms, either allies or enemies, determines the selection pressures on plants. Plant defences against herbivores play an important role in these interactions (Hairsson et al., 1960, Price et al., 1980, Janssen et al., 1998, Poelman et al., 2012). Plant defences comprise not only traits that interfere with herbivores directly (Walling, 2000), but also traits that operate indirectly by facilitating foraging predators and host-seeking parasitoids. These indirect plant defences often rely on the release of volatile compounds into the atmosphere, which can act as cues for prey-searching natural enemies (Dicke et al., 1990; Turlings et al., 1991).

Plant toxins ingested by herbivores may interfere with their natural enemies. Hence plant defence may actually provide a herbivore with additional protection. While sequestration is adaptive and entails storage of ingested plant toxins in specialized tissues or eggs (Duffey, 1980; Tooker and De Moraes, 2007), in principle any plant substance present anywhere in a herbivore can serve as antipredator protection. For example, tomatine incorporated in the diet of H. zeae resulted in prolonged larval development, reduced pupal eclosion and size, while it reduced adult longevity of its parasitoid Hypothenemus atacta (Campbell and Duffey, 1979). Nicotine in the diet of M. sexta decreased survival of its parasitoid Apanteles congregates (Barbosa et al., 1982), while there is evidence that plants stop producing nicotine when attacked by nicotine-tolerant herbivores (Kahl et al., 2000; Voelckel et al., 2001), possibly to facilitate parasitoids. Alternatively, sometimes predators may themselves evolve resistance to plant toxins such as nicotine (Kumar et al., 2014). In addition, glucosinolates were found to affect the performance of the second and the third trophic level (Poelman et al., 2008; Hopkins et al., 2009; Kos et al., 2012). Furthermore, Kaufman and Kennedy (1989) found that a high concentration of the methyl ketone 2-tridecanone in a particular accession of Lycopersicon hirsutum was toxic to the herbivore H. zeae but much more to the herbivore’s parasitoid Campoplexis sonorensis. Moreover, the stinkbug Podisus maculiventris reared on M. sexta caterpillars avoided prey that had been fed on tomatine and chlorogenic acid (a phenolic) in an artificial diet (Traugott and Stamp, 1997). This shows that not only growth, development and mortality but also the behaviour of natural enemies can be modulated by plant toxins ingested by their prey. There are also indications that JA-dependent plant metabolites can constrain herbivore predation. Thaler (1999) showed that treating tomato with JA increased parasitism of S. exigua by H. exigua in the field, but the parasitoids developed more slowly on these caterpillars and gained less weight than control wasps. In addition, Kaplan and Thaler (2010) observed lower predation of the caterpillar of M. sexta by the predaceous stinkbug P. maculiventris on plants overexpressing JA-related induced defences in field experiments. However, manual application of JA forces the constitutive display of defences that are normally induced, and this may result in overestimation of the magnitude of the predator response.

Another layer of complexity is added when communities harbour one or more herbivore species that can suppress plant defences (Alba et al., 2011). Suppression of defences by one species can facilitate another competing species (Sarmento et al., 2011b; Alba et al., 2015). For instance, suppression of defences by specialized strains of the two-spotted spider mite T. urticae (Kant et al., 2008) by the red spider mite T. evansi (Sarmento et al., 2011b) or by the tomato russet mite A. lycopersici (Glas et al., 2014) benefits not only these species themselves but also defence-sensitive competing species inhabiting the same leaf or plant. Hence, suppression of plant defence can indirectly mediate competition between herbivores, forcing the suppressor to adopt strategies to reduce competition with opportunistic herbivores. For instance, the spider mite T. evansi produces more web in the presence of T. urticae, and this behaviour results in elimination of the competitor (Sarmento et al., 2011b). Suppression of plant defences also reduces the emission of induced plant volatiles (Rodriguez-Saona et al., 2003; Kant et al., 2008; Zhang et al., 2009; Schwarzberg et al., 2011; Sarmento et al., 2011a), although this does not always reduce the attraction of predators (Sarmento et al., 2011a). Moreover, suppression of plant defences by spider mites can potentially backfire in the presence of predatory mites, because these prefer the eggs of prey derived from plants with suppressed JA defences to those from plants with activated JA defences (L. M. S. Ataide, University of Amsterdam, Netherlands, unpubl. res.).

Like suppression, induction also affects the performance of competitors. When plants are simultaneously attacked by more than one herbivore species, the palette of plant defences these induce together will determine their mutual interactions and those with their natural enemies. For example, cutting and trenching prevent the plant from transporting photosynthates away from, and defence compounds towards, the tissue where the herbivore is feeding (Dussourd and Denno, 1991; Delaney and Higley, 2006; Oppel et al., 2009). This allows the herbivore to exploit a nutrient-rich part of the plant without having to deal with elevated plant defences. However, if the isolated tissue is large enough, other herbivores can profit from this resource as well, without having to invest in cutting a vein or digging a trench. Similarly, density-dependent feeding efficiency is also observed with gregarious feeding (Prokopy and Roitberg, 2001). For example, gregarious aphids can create sinks in plant tissue, which are preferentially supplied with nutrients by the plant compared with parts where individual aphids feed (Larson and Whitham, 1991). Also, the adult mass of froghoppers, a predator of fecundity, peaks at intermediate juvenile group size (Wise et al., 2006). In this way, gregarious feeding
provides clear benefits because multiple herbivores can feed more efficiently than single herbivores. Interactions with the third trophic level may also shift when multiple herbivore species attack a plant simultaneously. For instance, when cabbage plants are simultaneously attacked by more than one herbivore species, the blend of volatiles released from these plants is different from that released by a singly infested plant. This new blend is less attractive to natural enemies of one of the herbivores and consequently adults of these herbivores preferred to oviposit on cabbage plants previously attacked by the other herbivore species, thus reducing the risk of parasitism of their offspring (Shiojiri et al., 2002).

Finally, induced plant defences can also mediate indirect intra- or interspecific interactions among plants, and this possibly arises from competition between plants for enemy-free space. It has long been known that non-attacked plants close to attacked plants can be warned of imminent attacks through the volatile cues released by the neighbours (Baldwin and Schultz, 1983; Bruin et al., 1992; Karban and Maron, 2002; Baldwin et al., 2006). These volatiles prime the defences of neighbouring plants such that herbivores attacking these plants will trigger the induction of defences more rapidly and usually more strongly than in non-primed plants (Engelberth et al., 2004). Since well-defended plants will deflect herbivores onto their less defended neighbour plants (McNickel and Dybzinski, 2013), the ability of plants to eavesdrop on attacked neighbour plants and be primed to an imminent herbivore attack can be considered an adaptation to increase the plant’s competitive ability.

The examples mentioned above illustrate the importance of incorporating the indirect interactions between plants, herbivores and natural enemies in the study of the evolution of plant defences. Besides the interaction between plants and their herbivores, many other organisms can benefit or suffer, either directly or indirectly, from the defensive products induced by herbivores. The net effects of plant defences on plant fitness thus depend not only on the interaction of the plant with the inducing herbivore, but also on effects on plant fitness through the interaction web associated with the plant. The true fitness effects of plant defences can therefore only be evaluated in the natural environment in which the various interacting species have evolved.

**Evolutionary consequences of defence suppression**

Defence suppression is perhaps the most striking example of a herbivore strategy that affects the performance of other herbivores living on the same host plant. There are indications that defence suppression is not always restricted to the site of the suppressor, and may act systemically throughout leaflets (Kant et al., 2008; Glas et al., 2014; Alba et al., 2015). For example, the performance of a non-suppressing strain of the spider mite *T. urticae* increased when feeding from a leaflet co-infested with a suppressor *T. urticae* strain (Kant et al., 2008) or with the suppressor species *T. evansi* (Alba et al., 2015), even when spatially separated from one another through a lanolin barrier. In addition, the performance of such non-suppressing *T. urticae* mites also increased on plants on which suppressor mites had been feeding previously but had been manually removed (Sarmento et al., 2011a). This demonstrates that the beneficial effects of defence suppression for competing herbivores are not restricted to the suppressor’s feeding site or to the moment at which the suppressor is feeding. This raises a key question: why did herbivores evolve the ability to suppress plant defences beyond their feeding site, while the alternative, i.e. the evolution of resistance to plant defences, seems an equally effective but competitively much more attractive trait for herbivores living in communities?

There is no straightforward answer to this question yet, but ecological theory does allow speculation on the adaptive benefits of systemic and long-lasting defence suppression. First of all, herbivores may simply have to work hard to keep the monopoly of their feeding site, and limit the negative effects of interspecific competition as much as possible. For example, *T. evansi* produces massive amounts of dense webbing, impenetrable for competing species (Sarmento et al., 2011b) and interferes with the reproduction of competitors (Sato et al., 2014), whereas tomato russet mites (*A. lycopersici*) may avoid competitors by feeding preferentially between the dense leaf-hair forests of tomato (van Houten et al., 2013; Glas et al., 2014). These strategies will be effective against competing herbivore species, but not against intraspecific competition. One potential benefit for herbivores of extending plant defence suppression beyond their own feeding site could be kin selection (Hamilton, 1964): when the individuals that profit from the suppressed plant defence are the suppressor’s relatives, inclusive fitness benefits for the suppressor can outweigh the costs of resource investment. Indeed, when individuals are more related to their neighbours, kin selection can theoretically increase such ‘helping’ behaviour, even when taking the negative fitness effects of stronger competition among kin into account (Mitteldorf and Wilson, 2000). However, a defence-suppressed area on a host plant is a public good (Rankin et al., 2007) when accessible to all conspecifics, and this will allow unrelated individuals to take advantage of the situation as well. These ‘cheaters’ do not have to invest resources in suppressing plant defence but do take the benefit of increased performance. In general, the evolutionary stability of public goods depends on the degree of relatedness at the local site as well as the probability of unrelated individuals dispersing to this site. A potential model system for studying defence-suppressed host plants as public goods is the defence-suppressing spider mite *T. evansi*. It usually feeds and reproduces in the same area, which increases the chance that individuals are surrounded by relatives. Indeed, high relatedness is frequently observed in *T. evansi* populations (Boubou et al., 2012).

Apart from intraspecific competition, interspecific competition can evidently also constrain the evolution of defence suppression when suppressors do not succeed in fully monopolising their feeding site (Glas et al., 2014). Herbivores may promote competing species not only through feeding, but also through oviposition. For example, *A. thaliana* leaves treated with egg extracts of the butterfly *P. brassicae* had suppressed JA-induced defence responses, resulting in increased performance of the larvae of their competitor *Spodoptera littoralis* (Bruessow et al., 2010). Interspecific competition can impose serious fitness costs on herbivores (Kaplan and Denno, 2007), and although some herbivores display behaviour (such as massive web production) associated with minimizing the
ecological costs of suppressing plant defences (Sarmiento et al., 2011b), such strategies can be costly physiological investments. Previous research suggests that web production by T. urticae spider mites shows a trade-off with reproduction (Tien et al., 2009), which would indicate a direct fitness cost of web production. In addition, like defence suppression, the web is a public good as it protects not only individual web builders but conspecifics too. Web builders could therefore also be subject to intraspecific cheaters that do not invest in web production but nevertheless benefit from its protection (Oka et al., 2009).

Given that the fitness benefits of defence suppression can vary with the ecological context, one may ask whether there is genetic variation for defence suppression within species. Kant et al. (2008) and Alba et al. (2015) obtained nearly isogenic lines from single populations of spider mites that were either suppressing or non-suppressing. Also, different populations of H. zea caterpillars were found to produce different amounts of glucose oxidase (Eichenseer et al., 1999). This suggests that genetic variation for defence suppression can be present at the species level. Hence, considering that defence suppression is potentially adaptive, what maintains this variation? First, it is likely that plants from different families differ in their molecular pathways of direct defence (Schulz, 1988). Therefore, generalist herbivores are expected to adapt to their local host plants (Agrawal, 2000), which maintains genetic variation among different herbivore populations on different host plant species. Second, defence suppression could show a trade-off with life-history traits such as oviposition and web production. In this case, the shape of the trade-off determines whether disruptive selection on defence suppression may maintain genetic variation. Third, varying ecological circumstances, such as the presence of competitors or predators, intraspecific cheaters, the relatedness of neighbours and food availability, can alter the direct fitness effects of defence suppression. Because herbivores typically cannot control such factors, it is likely that the benefits of defence suppression vary dynamically over time and space, allowing the maintenance of genetic variation within populations.

Finally, it is appealing to think of defence suppression as an adaptation of the herbivore. However, some studies suggest this may not always be the case. For example, mathematical models suggest that hosts can evolve not to mount an immune response upon infection with a pathogen if the pathogen reacts to the immune response with increased virulence (Restif, 2013). In a plant–herbivore context such ‘ mafia behaviour’ (Soler et al., 1995) would mean that herbivores increase feeding upon experiencing induced plant defence. Although empirical studies of mafia behaviour are scarce (Ponton et al., 2006), the costs of mounting an induced defence response under certain circumstances can be higher than its benefits (Fagerström et al., 1987; Herms and Mattson, 1992). For example, when exposed to herbivores, N. attenuata plants typically initiate a JA-induced accumulation of nicotine, but the accumulation of nicotine is costly and decreases the competitive ability of the plant (Baldwin et al., 1998). Hence, when larvae of the nicotine-tolerant herbivore M. sexta feed on N. attenuata, the plant responds by downregulating the accumulation of nicotine (Kahl et al., 2000). Voelckel et al. (2001) argue that this response likely reflects an adaptation of the plant to shut down an inefficient and costly defence response, because using this energy to compete with conspecifics may be more rewarding. Furthermore, when these plants attract parasitoids of M. sexta, any nicotine present in the herbivore may decrease the efficiency of this indirect defence response. Viewed this way, not mounting a direct defence response may be beneficial for plants because it prevents intoxication of their bodyguards (Fordyce, 2001; Kumar et al., 2014). Indeed, whereas continued M. sexta feeding might downregulate nicotine accumulation in N. attenuata, the emission of volatile terpenoids that attract the parasitoids is upregulated (Kahl et al., 2000). These examples show that investigating defence suppression from the plant’s perspective can provide important new insights in the power balance between the plant and the herbivore. If defence suppression was solely an adaptation of the plant, there would not be a tragedy of the commons situation involved for the herbivore. We expect, however, that both plant and herbivore play an active role in this complex phenomenon, which would call for a coevolutionary approach to understand the evolution of defence suppression.

OUTLOOK

Plant defence suppression by herbivores is a largely unexplored phenomenon, but, together with induction, it may play a profound role in the plant-mediated indirect interactions that determine community structure in the phyllosphere. The vast majority of studies on defence suppression by plant eaters has focused on plant–pathogen interactions. These studies have predominantly focused on the mechanisms of defence suppression and the counter-adaptations of plants to undo such suppression. Although there may be many similarities in the mechanisms of defence suppression and the eco-evolutionary dynamics of traits underlying suppression, we do expect fundamental differences between the interactions of plants with immobile (pathogens) and mobile (herbivores) organisms. In this review we call attention to three main themes for future research.

1. How do herbivores suppress defences? Answering this question requires the transfer of molecular biology tools from phytopathology to the field of plant–herbivore interactions, especially to identify herbivore effectors and their in planta targets. This may deliver not only genetic markers that facilitate the study of their presence in (natural) populations, but also plant breeding targets to decrease the vulnerability of crops to key pest species that suppress plant defences, such as mites, aphids and whiteflies.

2. What are the consequences of suppression for community interactions? Answering this question requires long-term laboratory, greenhouse and field studies to assess the fitness costs and benefits in simple and more complex communities, not only for the herbivores, but also for the host plants on which they live. This knowledge may also have consequences for plant resistance breeding in conjunction with biological control strategies, e.g. when plant toxins decrease the beneficial effect of natural enemies more than is gained by decreasing herbivore performance directly.

3. Which conditions cause suppression to emerge, to persist or to disappear from populations? Answering this question requires ecological and evolutionary theory to make predictions on the invasion and population dynamics of
herbivores with such traits, as well as a more detailed knowledge of the traits that allow either suppression or resistance to align these predictions with their dynamics under experimental and natural conditions.

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