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Plant resistance towards insect herbivores: a dynamic interaction

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Summary

Plant defences against insect herbivores can be divided into 'static' or constitutive defences, and 'active' or induced defences, although the insecticidal compounds or proteins involved are often the same. Induced defences have aspects common to all plants, whereas the accumulation of constitutive defences is species-specific. Insect herbivores activate induced defences both locally and systemically by signalling pathways involving systemin, jasmonate, oligogalacturonic acid and hydrogen peroxide. Plants also respond to insect attack by producing volatiles, which can be used to deter herbivores, to communicate between parts of the plant, or between plants, to induce defence responses. Plant volatiles are also an important component in indirect defence. Herbivorous insects have adapted to tolerate plant defences, and such adaptations can also be constitutive or induced. Insects whose plant host range is limited are more likely to show constitutive adaptation to the insecticidal compounds they will encounter, whereas insects which feed on a wide range of plant species often use induced adaptations to overcome plant defences. Both plant defence and insect adaptation involve a metabolic cost, and in a natural system most plant-insect interactions involving herbivory reach a 'stand-off' where both host and herbivore survive but develop suboptimally.

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I. Introduction

Plants and insects have coexisted for as long as 350 million years, if the earliest forms of land plants and insects are included, and have developed a series of relationships which affect the organisms at all levels, from basic biochemistry to population genetics. Although some of these relationships between the two phyla, such as pollination, are mutually beneficial, the most common interaction involves insect predation of plants, and plant defences against herbivorous insects. So common is this predator–host relationship that virtually every plant species is preyed on by at least one insect species, and, according to the coevolutionary theory of Ehrlich & Raven (1964), insect feeding on plants has been a determining factor in increasing species diversity in both herbivores and hosts (Harborne, 1988).

On the basis of this long-standing relationship, it is not surprising that the strategies employed by plants to attempt to resist or evade their insect herbivores are very diverse. Some species accumulate high levels of compounds which function as biochemical defences through their toxicity, or their physical properties; other plants do not commit resources to the accumulation of defensive compounds, but seek to minimise herbivore damage through rapid growth and development, dispersion, or choice of habitat. Even within a species, different genotypes adopt subtly different strategies for coexisting with insect pests, which can affect the partition of resources between growth and defence (Jander *et al.*, 2001). In the face of this diversity, it is perhaps more surprising that there also seems to be a defensive mechanism common to plants in general, based on the plant wounding response, and that this mechanism appears to operate even in species such as *Arabidopsis* (*Arabidopsis thaliana*), which have low levels of constitutive defence and might be assumed to evade herbivores rather than defend themselves. The induced resistance mechanism is also effective against a variety of insect herbivores on a given plant species (Thaler *et al.*, 2001). This short review will attempt to draw together a number of recent observations on the molecular bases of plant defence against insect herbivores, which have deepened our understanding of this complex interaction.

II. Accumulation of defensive compounds and induced resistance

Plant defence against insects was first envisaged in terms of compounds which the plant synthesises during the course of normal growth and development (i.e. in the absence of herbivore damage). These compounds are accumulated and stored, so that when attacked, the plant is already provided with the means to deter, or kill, the herbivore. Secondary metabolism, which involves specialised, often complex and species-specific biosynthetic pathways, was thought to provide the compounds which were accumulated, thus providing a

role for a biosynthetic function that had previously been considered wasteful. These defence mechanisms can be described as ‘static’ or constitutive, in contrast to ‘active’ or induced mechanisms in which the synthesis of defensive compounds is induced in response to insect attack (Harborne, 1988). The ‘static’–‘active’ distinction is a useful one in considering many aspects of the plant–insect interaction. A constitutive defence is often the causative factor in examples where specific plant hosts are fully resistant to attack by specific insect pests. The defence can act as a physical barrier, as in lignification or resin production, or can act as a biochemical signal perceived by the herbivore, as in deterrents of feeding or egg deposition, or can act as a toxin. The range of mechanisms of toxicity shown by different plant defensive compounds is very wide, and includes membrane disruption, inhibition of transport or signal transduction, inhibition of metabolism, and even disruption of hormonal control of developmental processes (Harborne, 1988; Bennett & Wallsgrove, 1994). Recent developments in the field of constitutive plant toxins have been ably reviewed by Wittstock & Gershenzon (2002).

On the other hand, an ‘active’ or induced defence mechanism was initially conceived in terms of the synthesis of proteins, as primary gene products, which themselves could act as toxins, or could disrupt pest metabolism (Ryan, 1978). Although this mechanism cannot come into play until the plant is attacked, it does not involve the commitment of plant resources to the synthesis of compounds which must be accumulated and stored. The view that secondary compounds are metabolic ‘dead-ends’ is not true in many cases, but with some defensive compounds, for example the alkaloid nicotine in the tobacco sp. *Nicotiana glauca* (Baldwin & Ohmmeiss, 1994), the nitrogen invested in their synthesis cannot be recovered. Induced resistance itself has a fitness cost (Baldwin, 1998; Heil & Baldwin, 2002), but this cost is exacted only if pest attack occurs, and can thus be less than that involved in constitutive defences (Simms & Fritz, 1990). ‘Active’ defence normally involves systemic induction. Not only does the defence response occur at or near the site of damage by the insect pest, but a response occurs throughout the plant, as a result of signalling molecule(s) enabling communication between different plant tissues. The systemic response may result in the production of the same defensive proteins as the local response, but differs in the kinetics of the production, and often the detailed response is different. Induced defence mechanisms are commonly involved in responses of plants to insect species where the interaction is one of partial or complete susceptibility of the host to the herbivore. The classic example of the plant wounding response, synthesis of proteinase inhibitors in leaves of potato (*Solanum tuberosum*) or tomato (*Lycopersicon esculentum*) in response to feeding by larvae of lepidopteran pest species such as tobacco hornworm (*Manduca sexta*; Ryan, 1978). The induced defence is not sufficient to make the plant fully resistant to further attack,

but results in reduced pest growth compared to plants in which proteinase inhibitor (PI) synthesis does not occur (Howe *et al.*, 1996).

Unfortunately, the useful distinction between 'static' and 'active' defence mechanisms has proven to be largely untenable when the systems are fully characterised at the molecular level. The end-products of the mechanisms, the defensive compounds themselves, are often the same in constitutive and induced defences in a given plant species. The toxic proteins produced in induced defence responses are also accumulated as constitutive defences; for example, the protein proteinase inhibitors produced as a result of the plant wounding response in potato are also accumulated as a constitutive defence in potato tubers (Garcia-Olmedo *et al.*, 1987). Similarly, tobacco species (*Nicotiana tabacum*; *Nicotiana attenuata*) accumulate proteinase inhibitors in its tissues before insect feeding, although herbivory induces the synthesis of increased levels of these defensive proteins (van Dam *et al.*, 2001). On the other hand, it has become clear that the production of defensive compounds via secondary metabolism can form part of an induced response. Expression of genes encoding enzymes involved in the biosynthesis of constitutive defensive compounds has been shown to be up-regulated by wounding; for example, synthesis of the terpenoid components of conifer (*Abies grandis* and related spp.) resins increase on wounding as a result of enhanced gene expression (Gijzen *et al.*, 1991; Bohlmann *et al.*, 1997, 1998), and insect herbivores have a similar effect (Litvak & Monson, 1998). Phytoecdysteroids are accumulated in spinach (*Spinacia oleracea*) foliage as defensive compounds, and their synthesis is up-regulated on exposure to vine weevil (*Otiorynchus sulcatus*) and concomitant tissue damage (Schmelz *et al.*, 1999); similar effects are seen in oilseed rape (*Brassica napus*), where glucosinolate content increases after insect damage by cabbage stem flea beetle (*Psylliodes chrysocephala*; Bartlett *et al.*, 1999). The synthesis of nicotine, the major alkaloid in tobacco, occurs during normal plant development, but is also induced by herbivore attack (Halitschke *et al.*, 2000). Nicotine is transported from its site of synthesis in the plant roots to aerial parts of the plant, with particular emphasis being placed on protecting reproductive tissues when leaves are damaged (Baldwin & Karb, 1995; Ohnmeiss & Baldwin, 2000), and thus this compound is both accumulated and induced. Because the biosynthetic processes involved in both 'static' and 'active' defence mechanisms are fundamentally the same, and involve expression of the same genes, the mechanisms differ only insofar as in one case gene expression occurs as a result of the normal developmental processes of the plant, whereas in the other case expression is up-regulated by a signal caused by an external stimulus.

Although the argument above makes a case for considering 'static' and 'active' defence against insect attack in plants as two sides of the same coin, current research has focussed almost entirely on induced resistance. This is not surprising, since the tools to unravel some of the signalling pathways

involved in up-regulation of gene expression in response to insect attack have become available, and the results of applying them have opened up new and unexpected areas of research. Nevertheless, it is well to remember that most, if not all, the studies of plant–insect interactions which consider changes in gene expression and causative signal pathways, are based on plants that are essentially susceptible to attack by the insect pest used. The survival of plants in the face of insect predation suggests that most interactions in nature do not result in serious plant damage, as a result of constitutive defensive strategies being employed, such as the accumulation of defensive compounds deterring or preventing feeding. Induced resistance, while of major importance in reducing the damage suffered by plants as a result of attack by insect pests, is not the causative factor in most examples of plant resistance to herbivory.

III. Signalling pathways in wound-induced resistance

1. Overview

The complexity of the responses of plants to wounding caused by insect feeding is at first sight daunting. In the model plant *Arabidopsis* changes in the steady-state levels of over 700 mRNAs were detected during defence responses in a microarray-based study (Schenk *et al.*, 2000), although not all of these changes were associated with insect predation, some being associated with pathogen-activated pathways. As a comparison, in lima bean (*Phaseolus lunatus*) only approx. 100 mRNAs were up-regulated by spider mite (*Tetranychus urticae*) infestation (Arimura *et al.*, 2000b), although a further 200 mRNAs (approx.) were up-regulated in an indirect resistance response (q.v.) by volatile signalling molecules released as a response to insect feeding. Approx. 500 mRNAs have been estimated to constitute the insect-responsive transcriptome in tobacco (Hermsmeier *et al.*, 2001). It is clear that much of the complexity of these responses is a result of changes in expression of genes which either do not encode products involved in insect resistance, or are involved in general responses to stress. For example, photosynthetic genes, which are not involved in defence, are down-regulated in tobacco in response to insect attack (Hermsmeier *et al.*, 2001), presumably to allow more resources to be allocated to producing proteins directly involved in the resistance response. Similarly, coordinated up-regulation of all defence genes, whether involved in insect resistance or not, occurs in *Arabidopsis* (Schenk *et al.*, 2000). It is true to say that although extensive lists of genes involved in plant defence and wound responses have been made (Walling, 2000), many of these genes have no known function, and only a few seem to encode products that are obviously either toxic to insects (such as proteinase inhibitors) or have the capacity to produce toxins (such as enzymes involved in secondary metabolism).

Ryan (2000) has attempted to simplify the situation by dividing the genes encoding newly synthesised proteins after wounding into three groups: antinutritional proteins or defence genes; signal pathway genes; and proteinases. This approach is helpful, and if extended a little, gives a global view of the response, in which three classes of genes are up-regulated: defence genes (including both genes encoding defensive proteins such as proteinase inhibitors, and genes encoding enzymes of secondary compound biosynthesis); signalling pathway genes (including those involved in the production of volatile compounds used as signals; q.v.); and genes involved in rerouting metabolism into the production of defensive compounds, such as proteinases involved in protein turnover.

Although the global induced resistance response to insect attack in plants is complex, a straightforward cause-and-effect analysis of the factors involved in the production of defined insecticidal compounds or proteins can still be made. This approach has been pursued with some success in recent publications by Ryan and coworkers (Ryan, 2000; Orozco-Cardenas *et al.*, 2001), which have put forward a linear description of events in the insect resistance response in tomato. In this species, the major insecticidal gene products in induced resistance are proteinase inhibitors (PIs) and polyphenol oxidase (PPO), both of which are thought to interfere with insect digestion, and thus nutrient uptake. Transgenic potato plants in which the wound-induced synthesis of PIs is suppressed by an antisense strategy down-regulating an enzyme involved in the signalling pathway, lipoxygenase (q.v.), support higher rates of development of both Colorado potato beetle (*Leptinotarsa decemlineata*) and beet armyworm (*Spodoptera exigua*) larvae compared to controls, demonstrating the importance of this mechanism of resistance (Royo *et al.*, 1999; Ortego *et al.*, 2001). The signalling pathway leading from insect wounding to production of these proteins, summarised in Fig. 1, involves four signalling molecules, which are viewed as operating in a sequential manner. The elucidation of the pathway from insect damage to production of insecticidal gene products gives an explanation for the wide-ranging global responses observed in the entire transcriptome on insect attack. The global responses which do not appear to have any direct connection with insect resistance can be accounted for in this model by the production of signalling molecules (jasmonic acid, oligogalacturonides, hydrogen peroxide) common to responses to abiotic stresses and pathogen attack, as well as the induced insect resistance response.

2. Systemin

The primary event in the signalling pathway leading to the synthesis of the defensive PI and PPO proteins in tomato is proteolytic cleavage of a precursor polypeptide, prosystemin, to release the peptide hormone systemin. This 18 amino acid

peptide was the first plant peptide hormone to be identified, and for a long time was the only peptide with a characterised role in signal transduction in plants (Ryan, 2000). The prosystemin precursor is a polypeptide of 200 amino acid residues (or 201 amino acid residues in an alternatively spliced form; Li & Howe, 2001). Prosystemin is present at low levels constitutively in leaf tissue; it lacks a signal peptide sequence or other targetting information, and is thus probably present in the cytoplasm of cells (Ryan & Pearce, 1998). On wounding, the cytoplasm is exposed to proteinases, probably as a result of mixing with contents of other cellular compartments (e.g. the vacuole), or possibly from insect saliva, and thus activation of prosystemin can occur.

Systemin is the primary signal in the wound response, as transgenic plants in which prosystemin expression is blocked by an antisense RNA strategy (McGurl *et al.*, 1992) show severe impairment in their systemic responses to wounding, and are more susceptible to attack by a lepidopteran insect herbivore (tobacco hornworm) (Orozco-Cardenas *et al.*, 1993). On the other hand, transgenic plants over-expressing prosystemin constitutively synthesised proteins that would normally be wound-inducible, to high levels (McGurl *et al.*, 1994). Systemin is mobile in the phloem of tomato plants, and thus can account for signalling in the systemic induction of resistance; it can pass across a graft junction between a transgenic rootstock overexpressing prosystemin and wild-type aerial tissue, to give high levels of constitutive proteinase inhibitor synthesis throughout the plant (McGurl *et al.*, 1994), and can be taken up through cut stems to produce a wound response when supplied as prosystemin (Dombrowski *et al.*, 1999). It is not clear whether this mobility involves transport of free peptide, or 'waves' of activation of prosystemin synthesis in vascular tissue (Jacinto *et al.*, 1999); systemin activates both the synthesis of its precursor polypeptide and of the enzymes putatively required to release the hormone, so a positive feedback system results (Ryan, 2000). Many alternative hypotheses to systemin-based signalling have been put forward, and there is some evidence that signalling pathways independent of systemin (and jasmonic acid; q.v.) do exist in tomato and other plant species to activate gene expression in unwounded tissues (O'Donnell *et al.*, 1998; Leon *et al.*, 2001). However, systemin signalling retains a central position in the wound response in tomato, and has been demonstrated to play a role in induced resistance to chewing insects.

Although the causative involvement of systemin in signal transduction in the wounding response in tomato and related species has been comprehensively established, the proteolytic processing steps in the conversion of prosystemin to systemin remain to be elucidated, as do the enzymes responsible. The whole precursor polypeptide is polar, and contains many potential protease cleavage sites; however, the cleavages which release systemin do not occur in particularly polar regions, or even at conserved sequence motifs (the N-terminal cleavage occurs between leu-ala, the C-terminal cleavage between

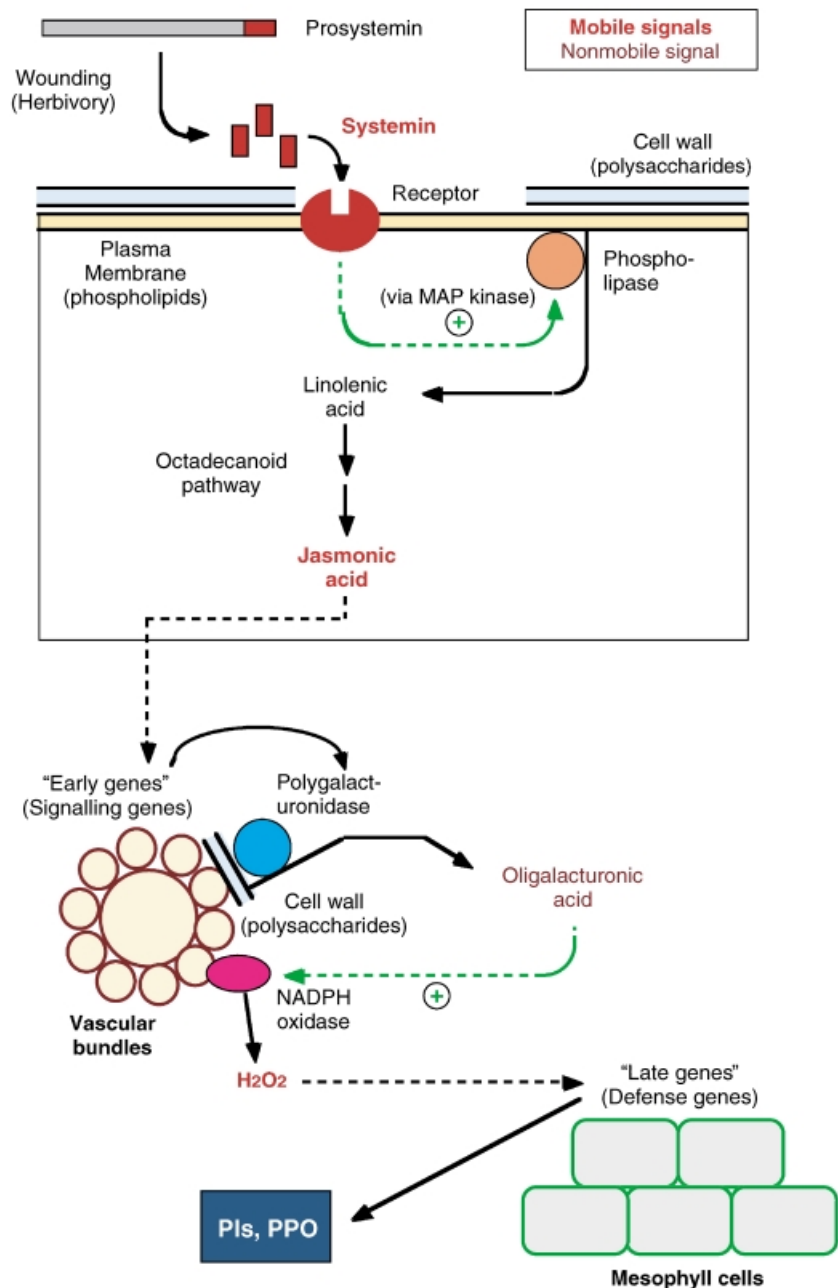


Fig. 1 Schematic diagram of the signalling pathway necessary for local and systemic synthesis of the insecticidal proteins proteinase inhibitor (PI) and polyphenol oxidase (PPO) in the wounding response in tomato. Adapted from Ryan (2000) and Orozco-Cardenas *et al.* (2001). Systemin is proposed to act as the systemic signal in this model, although evidence to suggest that jasmonate can also act systemically has been presented (Li *et al.*, 2002).

asp-asn). Whereas systemin release may be based on cleavages by sequence-specific proteinases, as is the case for animal peptide hormone processing at dibasic residues, it could also be a result of susceptibility of regions of the precursor to relatively nonspecific proteinase attack. Prosystemin is susceptible to proteolytic cleavage by proteinases present in apoplastic fluid (Dombrowski *et al.*, 1999), but these cleavages do not result in the production of systemin, and thus involvement by vacuolar or other proteinases is indicated. Proteinase genes encoding enzymes of a number of different types (cysteine and aspartic endoproteinases, and exoproteinases specific for both amino- and carboxy-termini) are a distinct category of

wound-induced genes in tomato and other plant species (Ryan, 2000). The induction of expression of these genes on wounding would seem to exclude the encoded enzymes from a role in systemin processing, but they may be present before wounding at lower levels as a result of constitutive expression. Since prosystemin synthesis is also stimulated by wounding, there is a circumstantial connection between these proteinases and systemin processing. A wound-induced serine carboxypeptidase has been localised to the vacuole (Moura *et al.*, 2001), but the kinetics of its accumulation led to the conclusion that it was concerned with protein turnover, not prosystemin processing.

Despite the central role of systemin in triggering the wounding response in tomato, peptides with sequence similarity to the hormone and its precursor are only present in a very limited range of plant species; at present this includes tomato, potato, black pepper (*Capsicum annuum*) and black nightshade (*Solanum nigrum*) (Constabel & Ryan, 1998). This range does not even include all members of the Solanaceae, because tobacco does not contain sequences similar to systemin or prosystemin. However, tobacco does contain peptides with a similar function to that of systemin, and a recent paper (Pearce *et al.*, 2001) characterises a precursor polypeptide in tobacco from which two peptides with systemin-like activity are produced by proteolytic cleavage. These peptides, and their precursor, show no apparent sequence similarity to the systemins (although the presence of at least one pair of pro-pro residues internal to the peptides is a common feature, and all the sequences contain the tripeptide pro-pro-ser). The high level of variability between closely related plant species in the sequence of not only the precursor, but also the peptide hormone, accounts for the failure to identify systemin homologues in other plant families. It is an unexpected result, based on data from animal systems, where the sequences of peptide hormones are normally well-conserved. It is possible that recognition between peptide and receptor for systemins is based on structural features other than the full amino acid sequence. If, however, recognition is based primarily on amino acid sequences, the lack of conservation of sequence in systemin-function peptides suggests that the sequences of binding regions in potential receptors for these signalling molecules must also show a high level of variability. Although it has proved possible to isolate peptide hormones

in plants using strategies based on sequence similarity, this approach has not worked for systemins outside the limited range of species given above (Ryan & Pearce, 2001).

The systemin peptide, after release from the precursor, interacts with a receptor present on the surface of plant cells. The presence of the systemin receptor has been shown by binding labelled peptide, either using isolated cell membranes or in cell culture (Meindl *et al.*, 1998; Scheer & Ryan, 1999). The receptor protein, a polypeptide of Mr 160 000, has yet to be fully characterised, but has the functional properties (dissociation constant for systemin binding approx. 10^{-10} M) that would be predicted for a similar receptor in animal systems. The receptor is assumed to be a transmembrane protein, and binding systemin causes a signal transduction event that activates a series of processes inside the cell.

3. Jasmonic acid

The signal transduction mediated by the systemin receptor results in activation of phospholipase A2, via a MAP kinase, and thus leads to the release of linolenic acid from membrane lipids. Further effects such as calcium release from vacuoles, calmodulin synthesis, and opening of ion channels in the plasma membrane (leading to its depolarization) are also stimulated by perception of the signal, and self-evidently participate in the wounding response, but are not part of the direct pathway from cause to effect in Ryan's model. Linolenic acid acts as a precursor for the synthesis of jasmonic acid, an oxylipid signalling molecule involved in stress and developmental responses in plants, via the octadecanoid pathway (Fig. 2; note that the biosynthetic pathway produces (3R,7S)-

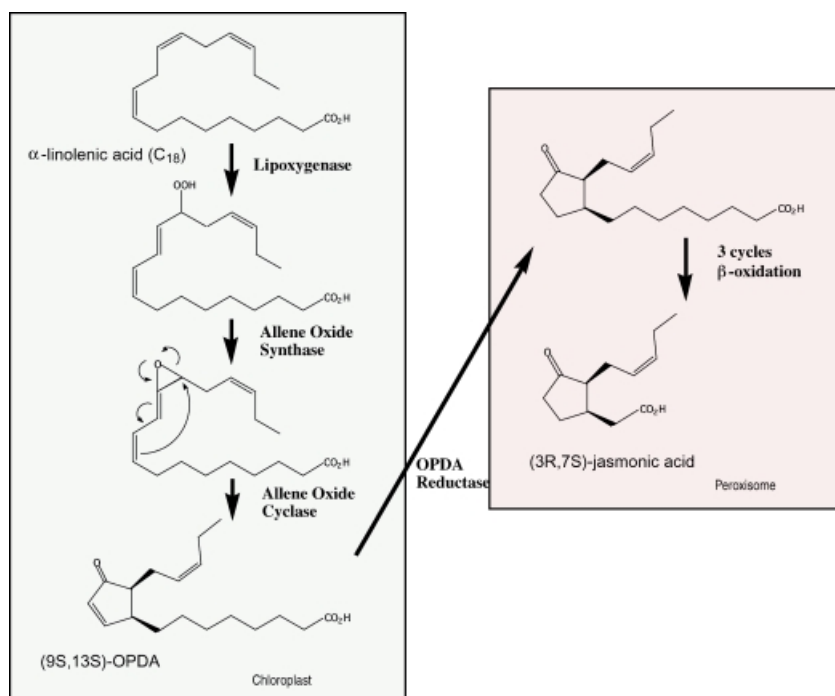


Fig. 2 The octadecanoid pathway for jasmonate biosynthesis. Jasmonate formed by this pathway can also be methylated on the carboxylic acid group by jasmonic acid carboxyl methyl transferase (using S-adenosyl methionine as the methyl group donor) to give the volatile signalling molecule methyl jasmonate.

jasmonic acid, whereas the term 'jasmonic acid' correctly refers to the mixture of epimers produced after isolation from the plant. 'Jasmonic acid' or 'jasmonate' in this review will refer to the biologically active molecule). Fatty acid-derived signal molecules in plants and the biosynthesis of jasmonates have recently been comprehensively reviewed (Schaller, 2001; Weber, 2002), and thus only selected aspects will be described here. Several complementary pieces of evidence show that jasmonic acid plays a crucial role in the defensive response to herbivores. In tomato, the *def1* mutant which does not up-regulate levels of jasmonic acid after wounding also produces lower levels of PIs, and is more susceptible to attack by lepidopteran insects (Howe *et al.*, 1996). In Arabidopsis, mutants exist which either do not produce, or are insensitive to, jasmonic acid (*fad3-2 fad7-2 fad8*; McConn *et al.*, 1997; *coi1*; Rojo *et al.*, 1998); in both cases their defensive responses to herbivorous insects are impaired.

Although activation of jasmonate biosynthesis appears to involve a kinase cascade, with complex interactions with other defence responses (Zhang & Klessig, 2001), a kinase directly responsible for the wound-induced production of jasmonic acid has been characterised. WIPK is a wound-induced protein kinase of the MAP kinase family in tobacco. It has been shown to be necessary for jasmonic acid production after wounding, and the accumulation of proteinase inhibitors, by both loss of function (suppression of expression) and gain of function (constitutive expression) assays in transgenic plants (Seo *et al.*, 1999). (Wound-induced protein kinase) WIPK has also been shown to be involved in signalling cascades which lead to the activation of omega-3 fatty acid desaturase, the enzyme which converts linoleic acid to linolenic acid, and thus could activate a pathway providing precursors for jasmonate biosynthesis (Kodama *et al.*, 2000). Although activation of phospholipase A2 was not demonstrated in these experiments, it is possible that the jasmonic acid produced in the wound response can originate from more than one source. Activation of phospholipase A2 has been observed as an early event in response to viral infection (Dhondt *et al.*, 2000). WIPK can be activated by phosphorylation by a MAP kinase designated NtMEK2(DD) in tobacco, although it is not established that this is its normal endogenous activator (Zhang & Liu, 2001).

The enzymes involved in jasmonic acid biosynthesis are generally up-regulated by wounding, or treatment with jasmonate (Mueller, 1997; Leon & Sanchez-Serrano, 1999), resulting in the signalling system having positive feedback, amplifying a small initial signal. The initial steps of the process, through to the production of (9S, 13S)-OPDA (oxophytodienoic acid), are thought to occur in the chloroplast, and possibly also in the cytoplasm. A wound induced lipoxygenase putatively catalysing the first step in jasmonate biosynthesis is targeted to the chloroplast (Heitz *et al.*, 1997), and the enzyme catalysing the synthesis of OPDA, allene oxide cyclase, is also present in chloroplasts (Ziegler *et al.*, 2000). Allene oxide synthase, which catalyses the intermediate step,

and has been shown to be the major regulatory point in the production of OPDA and jasmonate (Laudert & Weiler, 1998; Sivasankar *et al.*, 2000), also contains a chloroplast targeting sequence, although this enzyme has been shown to function in the cytoplasm (Wang *et al.*, 1999). The conversion of linolenic acid to OPDA is necessary for synthesis of defensive proteins to occur on wounding; a tomato mutant deficient in this conversion was unable to synthesise PIs and was more susceptible to insect attack (Howe *et al.*, 1996). The remaining steps of jasmonic acid biosynthesis, after the formation of OPDA, are thought to occur in peroxisomes. The initial step (conversion of the cyclopentenone ring to cyclopentanone) is catalysed by OPDA reductase (Vick & Zimmerman, 1986; Schaller & Weiler, 1997), with subsequent chain shortening of the alkane chain attached to the cyclopentane ring in jasmonic acid being effected by the β -oxidation pathway. Evidence from an Arabidopsis mutant deficient in OPDA reductase suggests that the latter part of the jasmonic acid biosynthesis pathway is not necessary for a normal wounding response to be exhibited, and that OPDA can substitute for jasmonic acid as a signalling molecule (Stintzi *et al.*, 2001). However, OPDA seems unable to substitute for jasmonate in other processes, such as control of anther and pollen development (Stintzi & Browse, 2000).

The response to wounding in plants is complicated by the first intermediate in the jasmonic acid biosynthesis pathway, 13-hydroperoxy-linolenic acid (the product of action of lipoxygenase on linolenic acid) also acting as an intermediate for the synthesis of 6-carbon hexenols and hexenals. These molecules, the so-called green leaf volatiles, play an indirect role in plant defence (q.v.), and are formed by the action of hydroperoxide lyase (Walling, 2000). Like jasmonic acid biosynthesis enzymes, synthesis of hydroperoxide lyase is up-regulated locally and systemically by wounding (Howe *et al.*, 2000).

Jasmonic acid produced locally within plant cells stimulated by systemin binding to the cell surface functions as a diffusible signalling molecule. The mobility of jasmonate as a signalling molecule is still a matter of controversy, and the literature contains contradictory data on whether the compound is only mobile locally, or can act systemically. Experiments in which the activity of the allene oxide synthase promoter was assayed in transgenic Arabidopsis led to the conclusion that neither jasmonic acid nor OPDA could lead to systemic induction of jasmonate-activated promoters, although wounding did so (Kubigsteltig *et al.*, 1999). On the other hand, exogenous jasmonic acid is mobile in the phloem (Zhang & Baldwin, 1997), and a recent paper by Li *et al.* (2002), using mutants of tomato deficient in jasmonate synthesis or in jasmonate perception, makes a convincing case for jasmonates acting as a mobile signal transmissible through graft junctions. Both prosystemin synthesis and jasmonate biosynthesis take place in vascular bundles (Jacinto *et al.*, 1997; Hause *et al.*, 2000) and there is a double feedback system in that jasmonate biosynthesis is up-regulated by

systemin, and prosystemin synthesis is up-regulated by jasmonate (Jacinto *et al.*, 1999), as well as both compounds up-regulating their own synthesis. Both molecules may therefore be able to function as systemic signals, via a mutually amplifying up-regulation spreading through vascular tissues.

Jasmonic acid is also a precursor for a volatile signalling molecule, methyl jasmonate, formed by esterification. The enzyme catalysing this reaction (jasmonic acid carboxyl methyltransferase) has been characterised (Seo *et al.*, 2001), and is itself up-regulated by wounding and jasmonate. Methyl jasmonate has received much attention as a molecule responsible for plant–plant communication. Airborne methyl jasmonate has been shown to induce proteinase inhibitor synthesis in plant leaves (Farmer & Ryan, 1990), and it has been hypothesised that a wounded plant, which is being damaged by insect herbivores, will up-regulate the synthesis of jasmonic acid and methyl jasmonate, thereby signalling to neighbouring unwounded plants to activate their defensive responses. There is abundant evidence that treatment of plants with methyl jasmonate increases resistance to a range of insect pests (Avdiushko *et al.*, 1997). Although there has been some doubt over whether the amounts of methyl jasmonate released by plants in the field is sufficient to cause significant effects in neighbouring unwounded plants, an increasing body of field-based evidence supports the hypothesis (Bruin & Dicke, 2001; Preston *et al.*, 2001). It is equally possible that methyl jasmonate may function as an airborne signal between different parts of the same plant, or between cells within tissues via the intercellular spaces (Seo *et al.*, 2001), giving further scope for roles of jasmonates in systemic signalling. A further volatile signalling molecule, ethylene, is also produced by wounding and systemin (Felix & Boller, 1995), and has been proposed as a necessary signal mediating the wound response (O'Donnell *et al.*, 1996), although more recent evidence suggests that ethylene production is regulated by jasmonate, not vice-versa (Arimura *et al.*, 2002).

4. Oligogalacturonic acid

The next step in the pathway leading to up-regulation of genes encoding insecticidal proteins in tomato is considered to be the production of oligomeric polymers of galacturonic acid (oligogalacturonic acid (OGA)), as a result of hydrolysis of polygalacturonides in the pectic component of plant cell walls. These oligogalacturonides were initially thought to be the causative signal in up-regulating proteinase inhibitor synthesis in wounded tobacco (Bishop *et al.*, 1984). Pectic fragments with a degree of polymerisation of 10–20 are most effective in producing a biological response, although fragments as small as trisaccharides are active. This hydrolysis is catalysed by polygalacturonase and pectic lyase (John *et al.*, 1997). Whereas it was initially thought that these enzymes were produced by attacking pathogens, a more recent study has identified an endogenous plant polygalacturonase encoded

by a gene whose expression is activated by wounding (Bergey *et al.*, 1999). This distinction is important, because if polygalacturonase is produced only by an attacking pathogen, oligogalacturonic acid can only participate in local responses, and cannot be involved in systemic signalling. The signal which activates expression of the wound-induced polygalacturonase gene appears to be jasmonic acid (Orozco-Cardenas & Ryan, 1999), suggesting that jasmonate is earlier in the signalling pathway than oligogalacturonic acid. This conclusion is in contradiction to an earlier study which concluded that oligogalacturonic acid caused jasmonate production (Doares *et al.*, 1995), but the earlier work used exogenously applied oligosaccharides rather than endogenously generated compounds.

Several complications are apparent when this step in the signalling process is considered. First, the plant polygalacturonase can exist as a single catalytically active subunit, or as a complex between the catalytic subunit and a regulatory (β -) subunit. The β -subunit appears to act as an inhibitor; both subunits are induced on wounding, but the kinetics of induction for the catalytic subunit are faster than for the β -subunit, resulting in an increase, then a decrease in enzyme activity over an 8-h period (Bergey *et al.*, 1999). A more fundamental problem with the putative response is that polygalacturonase expression is induced by the product of its action, oligogalacturonic acid. In the absence of any other controls, this would result in an indefinite self-amplifying synthesis of active enzyme. Possibly the β -subunit of polygalacturonase functions to prevent such a positive feedback loop being maintained; alternatively, the processes of gene expression and polygalacturonase action may be spatially separated in different cellular compartments, or in specific cell types (Bergey *et al.*, 1999). Oligogalacturonic acid is not the only oligosaccharide that can induce defence responses leading to proteinase inhibitor synthesis in tomato leaves; oligomers of β -1,4-linked glucosamine (chitosan) can also do so (Shibuya & Minami, 2001). This seems to be a distinct response from that caused by oligosaccharides derived from chitin (β -1,4-linked N-acetylglucosamine), which are active elicitors of plant defences against fungal pathogens, since the concentrations of chitosan required to produce a response are much higher than those of N-acetylchitoooligosaccharides. The structures of oligogalacturonic acid and chitosans are not very similar (see Fig. 3), and it is surprising that they produce a similar response, if that response is mediated by interaction with a common receptor. In fact, no receptors for either type of oligomer have been identified in plants, in contrast to the situation for putative receptors for chitin oligomers (Shibuya & Minami, 2001), and it may be that a relatively nonspecific interaction of the charged oligosaccharides with charged membrane lipid components takes place, rather than interaction with a specific receptor protein (Kauss *et al.*, 1989). Further events in oligogalacturonic acid-mediated signalling pathways are not well understood, but tomato leaf cell plasma membranes are depolarized by oligogalacturonides (Thain *et al.*, 1995),

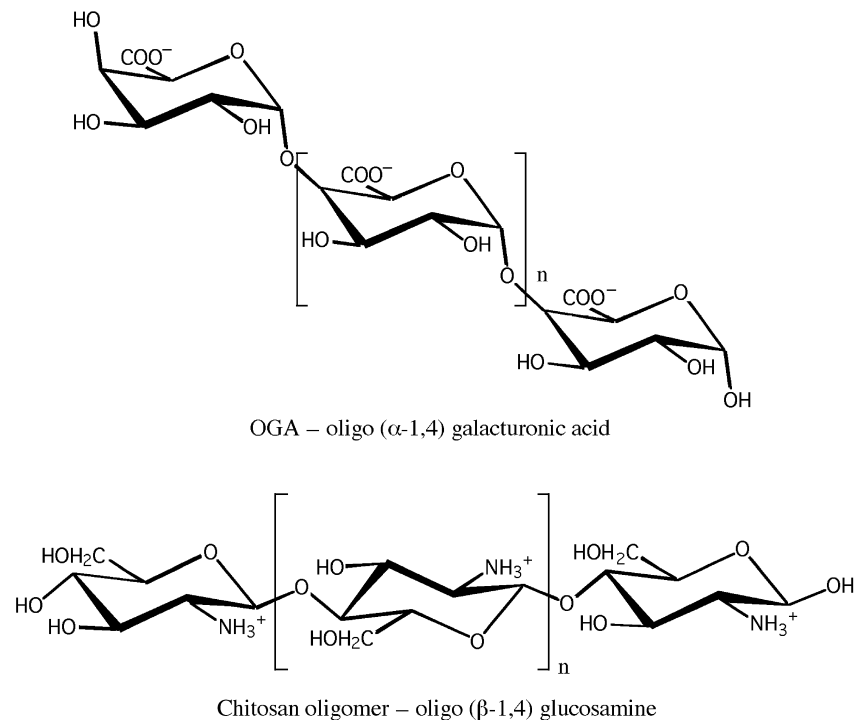


Fig. 3 Charged oligosaccharides which can induce the synthesis of proteinase inhibitors as part of the wounding response in tomato. Oligogalacturonic acid (OGA) is the 'natural' elicitor, formed by the action of polygalacturonase on plant cell wall pectins; chitosan will also act as an elicitor.

and evidence from *Arabidopsis* suggests that mobilisation of intracellular calcium, and calmodulin-related activity are involved in the response (Leon *et al.*, 1998). The membrane depolarization induced by systemin (see above) may thus reflect a local production of oligogalacturonic acid molecules.

Oligosaccharides are not mobile within the plant, and thus must act near their site of production (Baydoun & Fry, 1985). In the local response, the production of oligogalacturonic acid can take place both directly at the wounding site, as a result of pest/pathogen polygalacturonase action, and in nearby tissues in which wound-induced jasmonate synthesis has been stimulated, and the endogenous plant polygalacturonase activated. In the systemic response, evidence based on localisation of the next signalling molecule, hydrogen peroxide, suggests that oligogalacturonic acid is produced in the vascular bundle, and in cells adjacent to the vascular tissue (Orozco-Cardenas *et al.*, 2001). This is in agreement with the systemic response being mediated by a signalling molecule transmitted through the vascular system, rather than via gaseous diffusion; that is, it supports the concept that systemin rather than methyl jasmonate is the primary systemic signal within the plant.

5. Hydrogen peroxide

The involvement of reactive oxygen species in defensive responses of plants towards pathogens is well-established; infection, or the action of pathogen-derived elicitors causes an oxidative burst characterised by the production of hydrogen peroxide (Lamb & Dixon, 1997). Hydrogen peroxide is produced in plant tissues on wounding (Olson & Varner,

1993), and this response is both local and systemic (Orozco-Cardenas & Ryan, 1999). Herbivory by a chewing insect pest, corn earworm (*Helicoverpa zea*) on soya bean is known to result in the production of hydrogen peroxide in the plant as a component of induced resistance (Bi & Felton, 1995); a similar response is observed in *Arabidopsis* attacked by a plant parasitic nematode (*Heterodera glycine*; Waetzig *et al.*, 1999). The oxidative burst (and hydrogen peroxide production) can be induced by oligogalacturonic acid in soya bean cell cultures (Legendre *et al.*, 1993), and by systemin in cultured tomato cells (Stennis *et al.*, 1998). The wounding response and hydrogen peroxide generation are thus linked by a chain of causative relationships via the production of jasmonic acid and oligogalacturonic acid. Whereas high levels of hydrogen peroxide have been implicated in the induction of cell death in the hypersensitive response to pathogens (reviewed by Lamb & Dixon, 1997), the molecule can also function as a diffusible signalling molecule at lower concentrations (Alvarez *et al.*, 1998). Hydrogen peroxide can be produced by a number of routes in plant tissues, but the oxidative burst is thought to be a result of activation of a membrane-bound NADPH complex (Doke *et al.*, 1996). Activation of this enzyme by signalling mediated by oligogalacturonic acid leads to the model for defence gene induction proposed by Orozco-Cardenas *et al.* (2001) and outlined in this review.

The role of hydrogen peroxide as the final signalling molecule in the pathway leading to expression of genes encoding defensive proteins (proteinase inhibitors and polyphenol oxidase) in tomato has been demonstrated in a series of experiments in which inhibitors were used to block its

generation by the membrane-bound NADPH complex (Orozco-Cardenas *et al.*, 2001). Under these circumstances, induction of proteinase inhibitors in tomato plants exposed to systemin, jasmonate or oligogalacturonic acid was reduced by at least twofold when compared to uninhibited controls. Hydrogen peroxide accumulates in or near vascular bundles, and in intercellular spaces in leaves; the latter location is in agreement with a hypothesised role for this compound as a second messenger in stomatal closure induced by oligogalacturonic acid (Lee *et al.*, 1999). Diphenylene iodonium, an inhibitor of hydrogen peroxide production, inhibited the up-regulation of genes encoding defensive proteins, but not genes encoding proteins involved in the signalling pathway (prosystemin, jasmonate biosynthesis, polygalacturonase). Similarly, if plants were supplied with a biochemical hydrogen peroxide generation system (glucose oxidase plus glucose), up-regulation of genes encoding defensive proteins was observed, but there was no up-regulation of genes encoding proteins involved in the signalling pathway. Similar results had previously been obtained with transgenic potato plants over-expressing a fungal glucose oxidase gene, which had elevated levels of hydrogen peroxide and enhanced disease resistance (Wu *et al.*, 1995, 1997).

The final step in the process occurs when hydrogen peroxide produced near vascular bundles in tomato leaves diffuses into mesophyll cells, where it up-regulates the genes encoding the defensive proteins, which are accumulated in the vacuole in these cells. The mechanism through which the final signal transduction occurs remains to be established. Hydrogen peroxide has been shown to activate protein kinases, but it is not clear whether these are involved in the wounding response, or belong to signalling pathways leading to the production of proteins associated with disease responses (Desikan *et al.*, 1999; Chico *et al.*, 2002).

6. Crosstalk, species–species differences, and other complications

The sequential model for production of insecticidal proteins in the wounding response outlined above is useful and helpful, but represents only a small proportion of the global changes in gene expression that take place on insect wounding, and does not involve all the potential signalling molecules (and processes) which have been shown to have effects on those changes (Leon *et al.*, 2001). It is obviously only an approximation to a complex process involving multiple parallel signalling pathways, all of which contribute to the overall response. The central role of jasmonates in these processes is apparent, and has been confirmed by identifying sets of jasmonate-regulated genes in Arabidopsis (Sasaki *et al.*, 2001). Some of the multiple signalling pathways may involve novel signalling molecules (O'Donnell *et al.*, 1998), which originate from the attacking insect (Korth & Dixon, 1997; see section IV). Even in the species in which the sequential model was developed, tomato, the nature of the systemic signal is still keenly debated, and evidence suggests that more than one factor may be involved (Li *et al.*, 2002). It is beyond the scope of this review to discuss plant signalling pathways in general, but factors which influence the wounding response ('modulating signals') are relevant. The actions of several modulating signals are summarised in Fig. 4.

Several signalling molecules act as modulators of the wounding response. Abscisic acid (ABA) has been suggested to be necessary for a wounding response to occur; tomato and potato plants deficient in abscisic acid were reported to be unable to up-regulate proteinase inhibitor synthesis in response to exposure to systemin (Pena-Cortes *et al.*, 1995, 1996). ABA causes an up-regulation in jasmonic acid

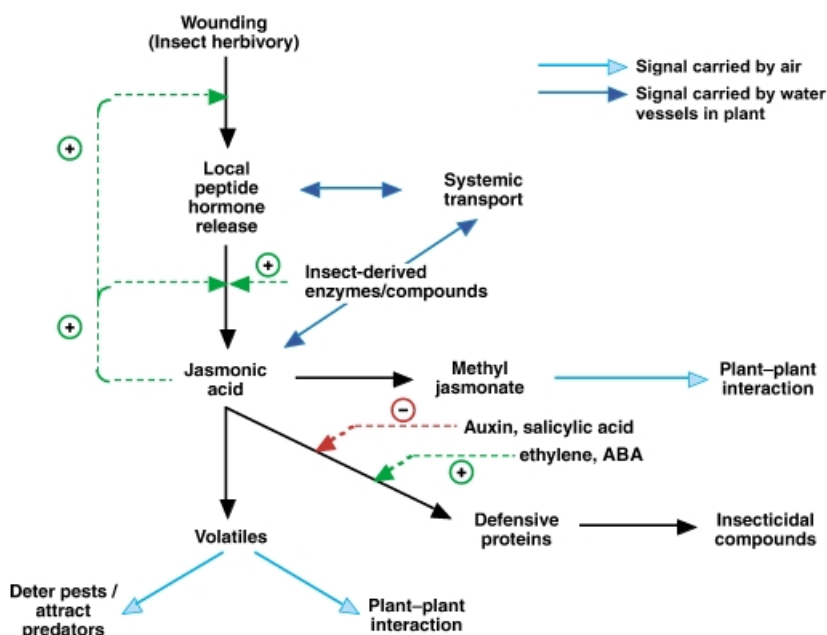


Fig. 4 Overview of the plant wounding response, and signalling molecules which can modulate it. Black solid arrows indicate a 'leads to' relation, either locally or in response to transmitted signals. Dark blue solid arrows indicate systemic signals within the plant; light blue solid arrows indicate signals transmitted by volatiles. Green dashed arrows indicate positive modulation of process, red dashed arrows indicate negative modulation of process. The solid arrow from 'jasmonic acid' to 'volatiles' indicates that jasmonic acid biosynthesis also leads to production of green leaf volatiles, and that jasmonic acid stimulates the synthesis of other volatiles such as terpenoids.

biosynthesis, but has been suggested to up-regulate the synthesis of defensive proteins through a jasmonic-acid independent pathway (Damman *et al.*, 1997). However, evidence suggests that ABA is not required for the wounding response in tomato, insofar as it only weakly induces the synthesis of the final products, proteinase inhibitors and other defence genes (Birkenmeier & Ryan, 1998). ABA appears to be required for plants to respond maximally in the wounding response (Ryan, 2000); its local synthesis at wound sites may relate to desiccation of the wounded tissues (Reymond *et al.*, 2000). Of other plant hormones, auxin is viewed as a negative modulator, and ethylene as a positive modulator, at least in tomato (Leon *et al.*, 2001). Salicylic acid, a signalling molecule involved in the development of systemic acquired resistance in response to pathogen attack (see below), acts as a negative modulator of the wounding response (Doares *et al.*, 1995; Bostock *et al.*, 2001), although there is not a simple dichotomy between the gene expression induced by insect damage and pathogens (Fidantsef *et al.*, 1999). The action of salicylic acid is one example of cross-talk between different signalling pathways. The sequential model for signalling in the wounding response, where the intermediate signalling molecules are common to many different processes, suggests that cross talk between signalling pathways should be routine. The present state of knowledge makes it more difficult to construct models which explain why the end products of signalling processes, such as synthesis of proteinase inhibitors, are specific to certain initial stimuli (e.g. wounding) and not others (e.g. pathogen infection).

Interactions between plants and insect herbivores are further complicated by differences in responses between different plant species (differences in responses to different insect herbivores are considered below). The wound response in *Arabidopsis* is based on separate signalling pathways mediated by jasmonic acid and by oligogalacturonides, which are seen as antagonistic – that is, a gene that is activated by one pathway is repressed by the other, resulting in a local response mediated by oligogalacturonides being different from a systemic response mediated by jasmonate (Leon *et al.*, 2001). However, unlike the situation in tomato, where it is accepted that the synthesis of proteinase inhibitors and polyphenol oxidase is the end point of wound-induced insect resistance, there is no clear consensus of what constitutes a similar response in *Arabidopsis*. In common with other members of the Brassicaceae (Cruciferae), *Arabidopsis* has the capacity to produce toxic glucosinolates (Wittstock & Halkier, 2002), which can be hydrolysed to the more toxic isothiocyanates and nitriles, and these are known to be toxic to a variety of herbivores (Bones & Rossiter, 1996). Although *Arabidopsis* is inherently susceptible to insect herbivory, different genotypes do show varying levels of partial resistance to generalist herbivores (Mauricio, 1998) which can be related to glucosinolate content. Defence responses to wounding in *Arabidopsis* should thus involve genes encoding enzymes involved in glucosinolate biosynthesis as an ‘end point’ of the pathway. However, most studies of the

wounding response in this species seem to have been based on a rather vaguely defined set of ‘responsive genes’ (Leon *et al.*, 2001), and thus are not really comparable to analyses of the response in tomato. Nevertheless, it is clear that wounding responses in tomato (and other Solanaceae) and *Arabidopsis* are significantly different; for example, ethylene is thought to be a positive modulator of the wounding response in tomato, but is a negative regulator of the local response in *Arabidopsis* (Stotz *et al.*, 2000), and makes the plant more susceptible to herbivory by a generalist herbivore, armyworm (*Spodoptera littoralis*). A similar effect was observed in the legume *Griffonia simplicifolia* (Zhu-Salzman *et al.*, 1998). Such differences in responses point out the high level of specificity in the interactions of plants with their insect herbivores, and warn against extrapolation of data derived from model species to other plants.

In the case of plant–insect interactions, is the use of model species an inherently flawed approach if hypotheses about the coevolution of plants and insect herbivores are to be developed and tested? Arguments can be advanced for both positive and negative answers, but neither is wholly satisfying. It is apparent that model species do not give a full picture of plant defences against herbivores, but if each specific interaction has to be considered individually, the accumulation of detail can easily obscure all other considerations. This review has tried to indicate those aspects of plant–insect interactions which can be said to be ‘general principles’, such as constitutive and induced defences in plants. The composition of the defensive compounds, and even the signalling and synthetic mechanisms involved in their production, can be expected to vary greatly between plant species. This diversity is predicted by Ehrlich and Raven’s coevolutionary hypothesis for plant–insect interactions (1964), where the driver for the diversification is insect adaptation to common defensive mechanisms (see section 7). However, the fact that great diversity in plant–insect interactions is observed in nature, does not mean this diversity cannot be superimposed on an underlying defensive system common to all plants, representing an earlier stage of evolution in the interaction. On the basis of observations that exposure to induced defences in oak (*Quercus robur*) made larvae of gypsy moth (*Lymantria dispar*) less susceptible to attack by a pathogenic nuclear polyhedrosis virus, Hunter & Schultz (1993) have argued that induced defence is a general response to tissue damage in plants, rather than an adaptive defence against herbivores. This is an extreme viewpoint, especially in view of subsequent findings that aspects of induced defence show all the characteristics of adaptive responses, but one which should not be ignored when the use of model species is considered.

IV. Insect modulation of the wounding response

Much of the above discussion has considered mechanical damage to plant tissues as equivalent to feeding by insect pests. For insects that cause widespread tissue damage, by

chewing plant tissues, or rasping surfaces, this is basically a correct view, but underestimates the plant's ability to discriminate between external damage stimuli. Results presented by Korth & Dixon (1997) established that potato plants being attacked by larvae of tobacco hornworm responded by producing proteinase inhibitor more quickly than if mechanically damaged, and also showed that the rapid induction factor was a heat stable compound present in regurgitant fluid, which would include products of the salivary glands, and possibly gut contents. The regurgitant increases jasmonic acid levels when applied to mechanical wounds on tobacco leaves (McCloud & Baldwin, 1997), suggesting that the increased response is mediated through the signalling pathway described above. The compound(s) produced by tobacco hornworm which cause this enhanced response are fatty acid conjugates (Halitschke *et al.*, 2001), similar to the compound volicitin, which induces volatile emission in maize (see below). This modulation of the wounding response is specific to insects normally feeding on tobacco; oral secretions from tobacco hornworm species (*Manduca* spp.; specialised herbivores which preferentially eat tobacco) and corn earworm (a generalist herbivore which attacks tobacco) caused a response, but oral secretions from larvae of cabbage white butterfly (*Pieris rapae*), a pest of crucifers which does not feed on tobacco, did not cause up-regulation of jasmonate levels (Schittko *et al.*, 2000). The plant is able to 'recognise' known herbivores and increase its response to attempt to deter them.

Insect salivary components do not necessarily up-regulate the wounding response. The corn earworm produces glucose oxidase in its saliva and from labial glands (Eichenseer *et al.*, 1999), and a recent report has concluded that this salivary glucose oxidase suppresses the production of nicotine, normally induced on wounding as a defensive compound, in tobacco which is attacked by these insects (Musser *et al.*, 2002). This observation appears to contradict the induction of defensive proteins (proteinase inhibitors) observed in tomato treated exogenously with glucose oxidase (Orozco-Cardenas *et al.*, 2001), where the hydrogen peroxide produced acts as a signal to induce expression of the encoding genes. The different defensive products in tobacco, proteinase inhibitors and the alkaloid nicotine, have previously been observed to show different patterns of induction on mechanical damage and insect feeding (Korth & Dixon, 1997), and oral secretions from the specialist herbivore, tobacco hornworm specifically down-regulate nicotine production while leaving other defence responses (production of volatiles) unaltered (Kahl *et al.*, 2000). It is apparent that subtle shifts in defensive responses, prompted by the herbivore, are an important factor in the plant's ability to deal with insect herbivores. For example, a study in *Arabidopsis* using microarray analysis of 150 wound-regulated genes showed that mechanical damage and feeding by larvae of cabbage white butterfly resulted in 'very different' transcript profiles (Reymond *et al.*, 2000). These differences

in responses also extend to a discrimination between different insect herbivores; for example, in tomato, feeding by lepidopteran larvae, coleopteran leaf-miners and mites resulted in different patterns of accumulation of defensive proteins (proteinase inhibitors, polyphenol oxidase, peroxidase and lipoxygenase; Stout *et al.*, 1994, 1998). These differences in responses could result from integration of the effects of multiple signalling pathways, and indicate why the complexity apparent in the wounding response has arisen.

V. Insects which evade the wounding response

This review is based on the argument that the wound response functions as a general, relatively nonspecific defence against pests which damage plant tissues, which involves the action of a relatively large set of genes, and multiple signalling molecules. Such a mechanism would not be expected to show the gene-for-gene resistance/susceptibility relationships that are characteristic of plant interactions with pathogens, and by and large this deduction is supported by experimental observation. When genetic analysis is carried out, insect resistance is often multigenic, continuous and associated with quantitative trait loci (QTLs; Stotz *et al.*, 1999; Yencho *et al.*, 2000). However, examples of specific, causal resistance genes, or genes whose induction is induced by specific pests, are known (Walling, 1999; Yencho *et al.*, 2000). These examples are associated with insect pests which have a feeding habit that minimises tissue damage, and thus are able to avoid much of the wounding response. Typically, these are homopteran species, such as aphids and whiteflies.

Most aphids and other phytophagous homopterans feed from plant vascular tissue by inserting a stylet into conductive cells. By inserting the stylet between cells, rather than puncturing them, this process can minimise cell damage, and thus avoid induction of a wounding response. A direct demonstration of this evasion of the wound response has been observed in tomato, where feeding by the aphid *Macrosiphum euphorbiae* does not induce the synthesis of proteinase inhibitors or polyphenol oxidase, the toxic proteins induced by feeding by lepidopteran larvae (Stout *et al.*, 1998). The feeding habits and gut physiology of many homopteran plant pests can be viewed as a strategy both to evade the plant wounding response, and to render the final products of the response, proteinase inhibitors and polyphenol oxidase, ineffective. Exploitation of the phloem and xylem saps as feeding sources allows the insect to exploit free amino acids as a nitrogen source, and thus inhibition of protein digestion by products of the wounding response is less likely to limit nutrient availability. Sap-sucking homopteran plant pests have been thought to lack digestive proteolysis altogether, although the occurrence of putative digestive proteinases has been reported in rice brown planthopper (*Nilaparvata lugens*; Foissac *et al.*, 2002).

Although aphids and other homopterans can be affected by 'static' plant defences in the form of accumulated secondary

defensive compounds if these are present in vascular tissues (e.g. glucosinolates in *Brassica* spp.; Chen *et al.*, 2001), or are encountered by the insect on the plant surface, or during stylet probing (Harborne, 1988), the absence of an induced resistance mechanism for these pests appears to place the plant at a disadvantage. However, while the plant wounding response is not activated by many homopteran pests, plants nevertheless respond to attack by these insects, and their responses have been found to be typical of plant responses to pathogen attack (Walling, 1999). These pathogen-induced pathways induce expression of many of the genes up-regulated during the wounding response, due to cross-talk between the signalling pathways (see above), and the involvement of reactive oxygen species, jasmonate and ethylene as common signalling molecules; the pathogen-induced pathways differ from the wounding response in the use of salicylic acid as a signalling molecule, both in local responses, and in systemic acquired resistance (Sticher *et al.*, 1997).

Application of salicylic acid does not lead to up-regulation of the synthesis of the products of the wound response, and has no effect on plant resistance to chewing insects, such as lepidopteran larvae, when effects on cotton were assayed (Bi *et al.*, 1997; Inbar *et al.*, 2001). Similarly, insect feeding does not normally cause the hypersensitive response leading to localised cell death, which is so characteristic of pathogen responses (Lamb & Dixon, 1997), although hypersensitive cell death has been observed as a response to insect egg laying by gall mites (*Contarinia* sp.; Fernandes, 1998). Because salicylic acid has a negative effect on jasmonate production in the wounding response (see above), it has the potential to interfere with the synthesis of proteinase inhibitors and polyphenol oxidase, and there is some evidence that the resistance mechanisms induced by salicylic acid actually make plants more susceptible to attack by chewing insect pests (Felton *et al.*, 1999). Analysis of plant responses is complicated by the ill-defined nature of the 'products' of the pathogen response in plants when resistance to homopteran insect pests is considered; although much evidence has been gathered on which gene products are up-regulated by insect feeding (Walling, 1999) there is no evidence that any of the chitinases, glucanases or peroxidases which are identified as defence-response proteins are toxic to aphids, or lead to the production of toxic products. The up-regulation of jasmonate levels as a response to pathogen attack introduces further problems, as increased jasmonate levels are central to the wound-induced pathway also. Aphids induce both salicylic acid- and jasmonate-responsive genes in *Arabidopsis* (Moran & Thompson, 2001), leading to the over-hasty conclusion that both wound-induced and pathogen-responsive resistance mechanisms have been activated. While it is impossible to separate the wound-induced and pathogen responsive resistance mechanisms as parts of the global plant defensive system, in which many genes are up-regulated in common in both responses, the end results of the two processes are different. When considering

the synthesis of an insecticidal end-product up-regulated by one mechanism, and not the other, there is sufficient justification for considering the pathways act separately.

The separation of resistance mechanisms mediated by the wounding response, and via pathogen responses, is exemplified by resistance to the aphid *Macrosiphum euphorbiae* in tomato. As stated above, this aphid species evades the wounding response, but it is susceptible to a resistance mechanism mediated by the gene *Mi*, which is responsible for resistance of tomato towards root-knot nematodes (*Meloidogyne incognita*; Rossi *et al.*, 1998; Vos *et al.*, 1998). The *Mi* gene encodes a leucine-rich repeat protein similar to those causally involved in resistance to fungal and bacterial pathogens (Milligan *et al.*, 1998), and resistance to both nematodes and aphids appears to involve a specific recognition of a signal molecule originating from the pathogen/pest. In agreement with this hypothesis, other aphid species are not susceptible to the resistance caused by *Mi*, and the *Mi*-mediated resistance towards *Macrosiphum euphorbiae* is specific towards certain biotypes of the pest (Goggin *et al.*, 2001), in a manner similar to the classical gene-for-gene virulence/avirulence relationships observed between plants and fungal pathogens. The insecticidal factor(s) in the resistance mediated by *Mi* is not known as yet, and there is a general lack of knowledge on molecules involved in the putative gene-for-gene signalling relationships between plants and homopteran insects. Whiteflies (*Bemisia* spp.) show complex species- and development stage-specific induction of genes in tomato (Walling, 1999), suggesting that different signals are involved in determining the specificity of the responses in the plant. Analysis of the interaction between homopteran pests and plants at the molecular level may thus pose considerable problems.

The recognition reaction mediated by receptors such as *Mi* may involve molecules derived not from the insect pest itself, but from pathogens carried by the insect acting as a vector (Stotz *et al.*, 1999). Tomato plants infested with whitefly (*Bemisia tabaci*) which was virus-free showed a negligible pathogenesis-related response compared with noninfested controls (measured by synthesis of pathogenesis-related proteins), whereas infestation with whiteflies carrying tomato mottle virus elicited a normal pathogenesis response (McKenzie *et al.*, 2002). This result provides a neat explanation of why insects can elicit a pathogen response in plants, but does not eliminate the possibility that insect-derived molecules are involved in signalling.

VI. Insect-induced emission of volatiles and tritrophic interactions

The emission of volatile molecules from plant tissue has been recognised as an important component in the interaction between plants and insects for many years, both in the attraction of pollinators and the deterrence of herbivores (Harborne, 1988; Pichersky & Gershenzon, 2002). Many of

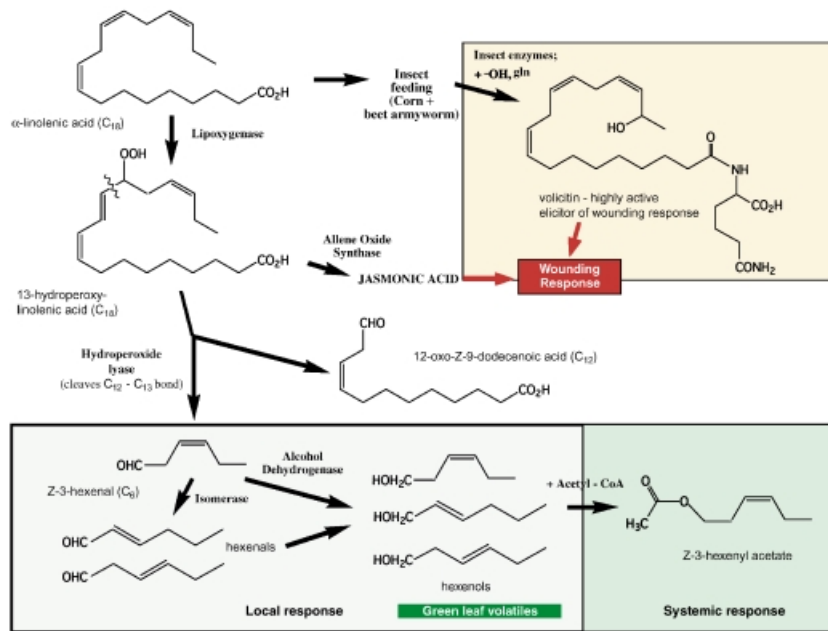


Fig. 5 Biosynthesis of green leaf volatiles from linolenic acid, via a branching reaction from the octadecanoid pathway. The biosynthetic route to volicitin, an insect inducer of defence responses, is also shown.

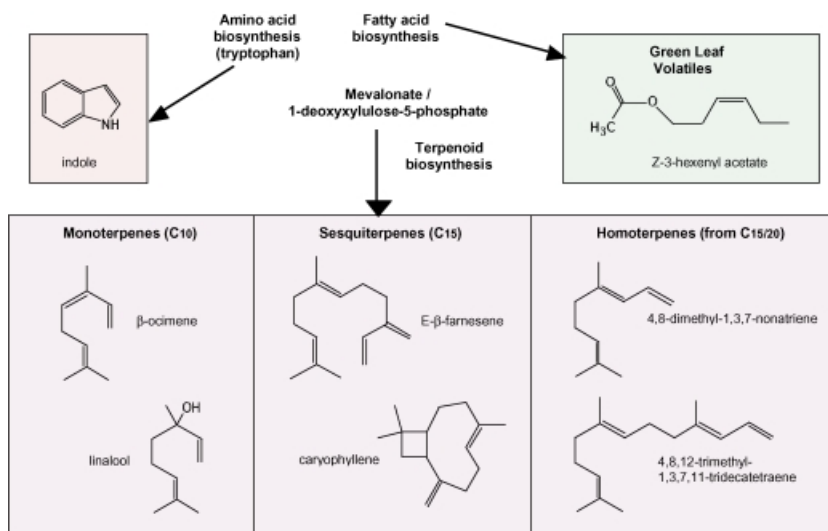


Fig. 6 Common plant volatiles synthesised in response to insect attack. These volatiles are produced both locally and systemically. Adapted from Pare & Tumlinson (1999).

these volatiles are preformed, and act in herbivore deterrence as a constitutive defence, as defined above. However, the wounding response includes the formation of volatile compounds (Pare & Tumlinson, 1997). Some of these volatiles (shown in Fig. 6) appear to be common to many different plant species, including the C_6 aldehydes, alcohols and esters referred to as 'green leaf volatiles', C_{10} and C_{15} terpenoids, and indole (Pare & Tumlinson, 1999), whereas others are products of secondary metabolism specific to particular plant species. The synthesis and release of volatiles as part of the wounding response occurs both locally and systemically (Rose *et al.*, 1996), and is activated by jasmonate (Rodríguez-Saona *et al.*, 2001), although the green leaf volatiles themselves also are able to induce defence-related genes (Bate & Rothstein,

1998), and the specific mixture of volatiles induced by jasmonate is generally different from that induced by insect feeding (q.v.; Walling, 2000). The biosynthetic routes to these compounds are various; terpenoids are synthesised through the mevalonate and 1-deoxyxylulose-5-phosphate pathways, and indole is produced via amino acid biosynthesis. Nerolidol synthase, the first enzyme on the dedicated pathway leading to C_{11} homoterpene biosynthesis, is induced by insect herbivores such as spider mite, and has been identified and characterised in cucumber, lima bean (Bouwmeester *et al.*, 1999) and maize (Degenhardt & Gershenson, 2000). As mentioned above, green leaf volatiles are formed as a branch of the jasmonate biosynthesis pathway, through the action of the enzyme hydroperoxide lyase (Fig. 5).

The volatiles emitted by plants, both preformed and induced, contribute directly to defence, and play a vital role in indirect defence strategies employed by plants (Pare & Tumlinson, 1999). As a direct defence, species-specific volatiles can have a repellent or toxic effect (for example, monoterpenes in pine; Litvak & Monson, 1998). More controversially, there is evidence that the induced green leaf and other common volatiles emitted by tobacco can deter oviposition by lepidopteran herbivores (De Moraes *et al.*, 2001; Kessler & Baldwin, 2001), although it is not clear whether this is due to the toxicity of these compounds, or the insect wishing to avoid laying eggs on a plant that is already damaged by predation. Volatiles emitted by corn after damage by lepidopteran larvae have a repellent effect on cereal aphids (Bernasconi *et al.*, 1998). Toxicity of the green leaf volatiles towards a generalist insect pest, the aphid *Myzus persicae*, was tested in transgenic potatoes in which levels of hydroperoxide lyase, the specific biosynthetic enzyme for these compounds, were reduced by an antisense strategy, leading to low levels of the compounds being present (Vancanneyt *et al.*, 2001). Aphids feeding on the transgenic plants showed increased fecundity, an indicator of improved performance, suggesting that the green leaf volatiles could have an adverse effect. Similar results have been reported when aphids were exposed directly to the compounds (Hildebrand *et al.*, 1993). However, it seems unlikely that a nonspecific herbivorous aphid like *Myzus persicae* would not be well-adapted to common compounds like the green leaf volatiles, and possibly the signalling activity of these compounds (see above) may also have led to an effect on overall plant responses. Volatiles induced by herbivory in turn induce expression of defence genes in undamaged, unconnected leaves in lima bean (Arimura *et al.*, 2000a).

The role of plant volatiles in indirect defence strategies has received much attention in recent years (Baldwin *et al.*, 2001), following observations by Turlings *et al.* (1995) and others. These studies identified a role for volatiles produced by corn and cotton plants in attracting parasitic wasps to lepidopteran larvae preying on the plants. Similar studies on *Brassica* spp. exposed to herbivory by larvae of the cabbage white butterfly had shown that plant volatiles were long-range stimuli which attracted the parasitoid *Cotesia rubecula* (Hymenoptera; a parasitic wasp) to the site of herbivory (Geervliet *et al.*, 1994). Parasitism causes paralysis in the lepidopteran larva, decreasing feeding damage, and the mortality caused by the parasite prevents pest populations building up to levels which the plant could not survive. The overall result is an increase in plant reproductive capacity (van Loon *et al.*, 2000). The interaction between plant, insect herbivore and natural enemy of insect herbivore constitutes a tritrophic interaction, where the plant-derived compound signals directly to an organism at the third trophic level. Similar observations have been made for other plant species and herbivores. For example, Arabidopsis has been shown to respond to herbivory by cabbage white butterfly larvae by producing green leaf volatiles and

terpenoids which attract a parasitoid of the pest (van Poecke *et al.*, 2001); this result emphasises that the production of green leaf and terpenoid volatiles is a common response to possibly all plant species. A response to a green leaf volatile (Z)-3-hexen-1-ol, was shown directly in the two-spotted stinkbug (*Perillus binclatus*), a predator of Colorado potato beetle (*Leptinotarsa decemlineata*), by electroantennography (Weissbecker *et al.*, 1999). As a further example, the common plant volatiles β -ocimene and *cis*-jasmone have been found to attract predators of aphids (Birkett *et al.*, 2000); the *cis*-jasmone induced persistent synthesis of the β -ocimene, which is also an effective parasitoid attractant. The plant response caused by *cis*-jasmone was persistent, and qualitatively different from that produced by methyl jasmonate. The volatile 'signals' involved in these indirect defence mechanisms are referred to as synomones by some authors, but this term is not accepted by other workers in the field.

Although some examples of indirect defence strategies mediated by plant volatiles have been well-studied, there is some debate at present over the extent to which predators and parasitoids in general actually protect the plant from its herbivores, and to what extent the plant itself directs this process. In the case of the tritrophic interaction between poplar trees (*Populus nigra*), gypsy moth (*Lymantria dispar*) larvae and the parasitoid wasp *Glyptapanteles flavicoxis*, the direct defences in the host plant induced by herbivore feeding had a deleterious effect on parasitoid development, which would greatly reduce its ability to control the pest (Havill & Raffa, 2000). An example has been described in which parasitised caterpillars show improved survival, by extending their host range to a species normally avoided (Karban & English-Loeb, 1997). On the other hand, experimental evidence for significant levels of protection, resulting in 30% increased seed production, has been shown for maize plants attacked by unparasitised and parasitised larvae of armyworm (*Spodoptera littoralis*; Hoballah & Turlings, 2001), and release of volatiles by the tobacco species *Nicotiana attenuata* was concluded to reduce herbivores per plant by 90% (Kessler & Baldwin, 2001), partly through decreased oviposition (see above) and partly through attraction of a generalist predator of insect eggs. The work of Baldwin and his group in studying the interaction between insect herbivores and a wild tobacco species (*Nicotiana attenuata*), under both laboratory and field conditions, has provided a masterly body of work on the interplay of various types of defence strategy and the fitness costs associated with them during plant development (Baldwin, 2001). This multidisciplinary approach has pointed the way to a more detailed and mature understanding of plant–insect interactions in general.

Although wounding itself can cause volatile emission, the mixture of volatiles produced differs from that induced by insect feeding, due to the presence of bioactive compounds in insect saliva and regurgitant. For example, both wounding and insect regurgitant contributed to attracting parasitoid

wasps to larvae of the diamondback moth (*Plutella xylostella*) feeding on cabbage plants (Shiojiri *et al.*, 2000), and in the stinkbug/Colorado potato beetle example given above, the predator responded strongly to 2-phenylethanol, a volatile emitted by potato specifically in response to Colorado potato beetle feeding (Weissbecker *et al.*, 1999). The identification of a compound, volicitin, in the oral secretions of beet armyworm which induced volatile synthesis in maize (Alborn *et al.*, 1997) linked insect feeding damage to plant recruitment of natural enemies of the pest. A combination of mechanical wounding and volicitin application caused volatile production similar to that caused by insect feeding.

Volicitin is a fatty acid-amino acid conjugate, produced by formation of an amide linkage between the carboxylic acid group of linolenic acid and the amino group of glutamine (Fig. 5), and 2-hydroxylation of linolenic acid; the linolenic acid is plant-derived, whereas the glutamine is provided by the insect, which also performs the chemical reactions required to produce the compound (Pare *et al.*, 1998). Volicitin has been shown to cause up-regulation of expression of genes involved in biosynthesis of both indole (Frey *et al.*, 2000) and terpene (Shen *et al.*, 2000) volatiles in maize. Similar fatty acid conjugates have been identified in oral secretions of other lepidopteran larvae (Halitschke *et al.*, 2001), and have been shown to be necessary for induced responses in tobacco. Why an insect pest should produce a compound which induces defensive responses against itself in its host remains unclear; presumably volicitin has a metabolic role within the insect, and the plant has exploited the compound for its own purposes. It has been suggested that volicitin may be a product of the gut microflora in armyworm, and that it functions as a surfactant to aid digestion (Spiteller *et al.*, 2000). Insects do not contain emulsifiers similar to bile salts in vertebrates, but do contain surfactants including phospholipids and compounds containing linolenic acid, and have been suggested to produce fatty acyl-amino acid complexes for this purpose (Deveau & Schultz, 1992; Turunen & Crailsheim, 1996). On the other hand, two corn earworm species (*Heliothis virescens* and *Helicoverpa zea*) contain enzymes in the midgut that are able to cleave the amide linkage in fatty acid-amino acid conjugates (Mori *et al.*, 2001). The two species differ in the enzymatic activity present, resulting in different levels of the elicitors in oral secretions, gut and frass (excreta). It is suggested that this difference in gut enzymes may be a causative factor in the differences between blends of volatiles emitted by plants attacked by one or other of these insect species, which has previously been shown to be used by a parasitic wasp, *Cardiochiles nigriceps*, to distinguish infestation by its host, *Heliothis virescens*, from that by *Helicoverpa zea* (De Moraes *et al.*, 1998). Mechanisms of this type explain how plants are able to produce herbivore-specific volatile mixtures that attract host-specific parasitoids or predators. There are many examples of this signalling specificity mediated by volatiles (Walling, 2000), and it is likely that more than one bio-

chemical mechanism operates to stimulate the production of herbivore-specific volatile mixtures by the plant, since aphid feeding, which minimises tissue damage and does not involve lipid digestion, can also lead to their production. For example, the parasitoid wasp *Aphidius ervi* can distinguish between plants infested by its host, the pea aphid (*Acyrtosiphon pisum*) and nonhost bean aphids (*Aphis fabae*) on the basis of emitted volatiles (Du *et al.*, 1996; Powell *et al.*, 1998). Nevertheless, the solubilisation and digestion of ingested lipids in herbivorous insects is possibly an area that would be worth further study, if the apparent contradictions in the production of volicitin and other similar elicitors by insects are to be understood.

As well as oral secretions, other insect-produced compounds may also play a role in indirect defences; for example, oviduct secretions, in combination with plant wounding, have been shown to play a similar role to volicitin in attracting an egg parasitoid of the elm leaf beetle (*Xanthogaleruca luteola*), mediated by emission of volatiles (Meiners & Hilke, 2000).

VII. Insect adaptation to plant defences

1. Insect adaptation

The success of phytophagous insects as herbivores results from their ability to successfully counteract the defensive strategies of their plant hosts. An extensive discussion of insect adaptation to plant foodstuffs lies outside the scope of this review; basic principles are very ably reviewed by Harborne (1988).

In the same way that plant defence mechanisms were formally divided into 'static' or constitutive and 'active' or induced, insect mechanisms for dealing with plant defensive compounds can also be divided formally into constitutive and induced responses, with the proviso that the two categories overlap to a large extent, as with plant defence mechanisms. A further distinction may be made in insect feeding habits, where species are divided into generalist herbivores, which are able to survive on a wide range of host species (although in a particular location they may preferentially consume a single species), and specialist herbivores, which are only able to survive on a limited range of host species, or, in extreme cases, only a single host species. It is this adaptation to specific host species which has been hypothesised to drive species divergence in phytophagous insects, and although experimental evidence for such a process has been lacking, an emerging consensus is in support of the concept (Berlocher & Feder, 2002). A specialist herbivore can adopt constitutive detoxification mechanisms for dealing with plant defensive compounds, since it is bound to encounter them when feeding from its chosen plant hosts. For example, when the flea beetles *Phyllotreta nemorum* and *Phyllotreta cruciferae*, specialist feeders on cruciferous plant species containing glucosinolates, were allowed to feed on transgenic Arabidopsis plants

expressing glucosinolates at four times the normal level, no deleterious effects compared to controls were observed (Nielsen *et al.*, 2001). On the other hand, *Arabidopsis* plants engineered to accumulate dhurrin, a cyanogenic glycoside normally produced in sorghum, were resistant to *Phyllotreta nemorum*, showing that the insect's detoxification mechanisms were specific to the secondary metabolites it normally encountered (Tattersall *et al.*, 2001). Even in specialist herbivores there is considerable evidence for a metabolic cost involved in constitutive detoxification (e.g. furanocoumarin detoxification in parsnip webworm, *Depressaria pastinacella*; Berenbaum & Zangerl, 1994). Specialist herbivores with constitutive adaptations, like plants with constitutive defences, also show induced up-regulation of the 'constitutive' detoxification mechanisms. Tobacco hornworm constitutively expresses the cytochrome P-450 enzymes needed to detoxify nicotine, the constitutive defence compound produced by tobacco (Snyder *et al.*, 1994), but the amounts of these enzymes are increased by the presence of nicotine in the diet (Snyder *et al.*, 1993). A major advantage gained by specialist herbivores is the ability to sequester plant secondary compounds as a defence against their own predators (Dobler, 2001), which can simply be stored, or metabolised to insect-specific compounds. Generalist herbivores trade off their ability to be effective herbivores of a wider range of plant species against less efficient mechanisms for dealing with specific insecticidal compounds, having to rely on induced responses (Bernays & Chapman, 2000). Nevertheless, they have the capacity to deal with many insecticidal compounds under suitable circumstances; for example, desert locusts (*Schistocerca gregaria*) can feed on crucifer species with very high glucosinolate contents if allowed to adapt to this food source (Mainguet *et al.*, 2000). A comparison of specialist and generalist insects feeding on *Hypericum perforatum* concluded that specialists needed less adaptation to deal with hypericin, the phototoxin accumulated as a plant defence, and were able to detoxify the compound more effectively (Guillet *et al.*, 2000).

The main types of detoxification enzymes used by insects are cytochrome P-450 monooxygenases (Feyereisen, 1999) and glutathione S-transferases (GSTs; Yu, 1996). These enzymes have been studied extensively in connection with the ability of many insect pests to detoxify insecticides, but their role in detoxifying plant secondary compounds has become well-established. Cytochrome P-450 enzymes are induced by isoquinoline alkaloids encountered in a natural host, saguaro cactus, in the fruit fly (*Drosophila melanogaster*; Danielson *et al.*, 1998), and by xanthotoxin in the generalist herbivore corn earworm (Li *et al.*, 2000). The availability of the complete sequence of the *Drosophila melanogaster* genome will enable a systematic study of its detoxifying enzymes, both P-450s and GSTs, to be made (Wilson, 2001). This insect may prove a useful model for studying how herbivorous insects exploit the resources of their genome to overcome plant defences, although each plant-insect interaction has its own

species-specific aspects. In contrast to the situation in plants, one aspect of insect adaptation responses that has received comparatively little attention is the signalling mechanism(s) linking ingestion of the toxin and induction of gene expression; *Drosophila melanogaster* may also prove a good model for elucidating the pathways involved.

The responses of insect herbivores to insecticidal proteins in the plant wounding response have also been studied in some detail. Polyphenol oxidase has been identified as insecticidal on the basis of conjugation of phenolics to proteins, decreasing its digestibility (Felton *et al.*, 1992), and is systemically induced on wounding in potato (Thipyapong *et al.*, 1995) and tomato (Constabel *et al.*, 1995). Phenolic acids have been shown to induce oxidative stress in herbivorous lepidopteran larvae (Summers & Felton, 1994). However, recent results have identified mechanisms by which lepidopteran larvae can overcome the effects of dietary oxidised phenolics by maintaining reducing conditions in the gut (Barbehenn *et al.*, 2001). A study in which transgenic tobacco lines over- and under-producing phenols were tested for resistance to larvae of corn earworm did not provide evidence for these compounds having any causal role in insect resistance, and instead led to the conclusion that foliar phenolics could have beneficial antioxidant effects for insects (Johnson & Felton, 2001). Coleopteran and lepidopteran herbivorous insects are also able to adapt to dietary proteinase inhibitors (Jongsma & Bolter, 1997) by the production of inhibitor-insensitive proteinases (Bolter & Jongsma, 1995; Broadway, 1995; Jongsma *et al.*, 1995; Broadway, 1996), which are selected from a repertoire of digestive enzymes available in the insect genome (Bown *et al.*, 1997). Larvae of a generalist lepidopteran herbivore, *Helicoverpa armigera*, were shown to be adapted to the inhibitors present in their host plant, but not to inhibitors from non-host plants (Harsulkar *et al.*, 1999).

In a natural ecosystem, there must be a balance between plants and herbivores. Although an adapted insect may be able to complete its life cycle on a plant which produces defensive compounds, the plant defensive strategy must be sufficient to prevent the insect eliminating its host, that is, the plant must also be able to complete its life cycle. This review has tried to indicate how many factors are involved in determining the success of insect herbivory, and plant defence, but the net result is often a partial success for both parties. The balance of a natural ecosystem is a different situation from that in agriculture, where uncontrolled insect pests can lead to very high levels of crop damage and loss. The high density of suitable host plants in intensive agriculture means that insect adaptation to defence responses by the crop, even if only partially successful, can lead to very rapid increases in the pest population. Some crop pests are specialist herbivores, originally of the wild progenitors of agricultural crop species, and thus have constitutive adaptations to crop defences, but many serious insect pests, such as corn earworm, are generalist herbivores. The ability of these insects to detoxify a variety

of insecticidal compounds or proteins, particularly those encountered as a common plant response to damage, such as proteinase inhibitors, makes them pests of many different crops. Endogenous mechanisms of plant defence are thus likely to have only a limited success in insect control in agricultural contexts, although spraying with methyl jasmonate has been put forward as a method of inducing defence responses against pests in the field (Baldwin, 1996).

2. Exploitation of plant defence mechanisms in agriculture

Pest control in modern agriculture has undergone something of a revolution, and is increasingly moving away from reliance on exogenously applied pesticides, towards more 'environmentally friendly' methods. This move has been brought about partly by consumer pressure, partly by economic considerations (cost of spraying for pesticides being a significant factor) and partly by an increasing level of resistance to common pesticides in many agricultural insect pests. Exploitation of plant defences is already a component of integrated pest management (IPM) programmes, which seek to minimise insect damage through a combination of crop rotation to prevent build-up of pest populations, natural biological control (exploiting predators and parasitoids of pests), endogenous resistance in the crop, whether partial or almost complete, and a minimal level of pesticide application only when necessary. Breeding programmes to exploit naturally occurring resistance genes in the gene pool available to a crop species have met with some success; for example, resistance genes to rice brown planthopper have been incorporated into improved rice varieties from pre-existing germplasm (Khush & Brar, 1991). However, the limitations to this technique are also shown by this example, as resistance is only effective against specific biotypes of the pest, and can be broken easily by introduction of a different biotype. Pest adaptation is equally a problem when endogenous resistance is used to defend plants against herbivores as it is when pesticides are used, with the strength of the selection pressure being a determining factor. This may be why many examples of plant resistance to pests in natural systems prove to depend on more than one mechanism, with the resistance produced by any one mechanism being partial. The partial resistance can be viewed as a balance between the deleterious effects on the pest of adapting to the plant's defensive mechanisms being balanced by the selection pressure to do so.

Although, as argued above, endogenous resistance in many crops is not adequate to deal with heavy pest infestations in an agricultural environment, it should be exploited in agriculture. On the basis of increased knowledge of defence mechanisms, particularly induced responses, there is clearly scope for enhancing endogenous defence against herbivores in crops, without introducing genetic material from outside the available gene pool, by conventional breeding strategies. The

level of partial resistance against pest species achieved using inherent genetic resources may well be sufficient to give adequate protection to a crop under a suitable IPM programme, especially with an increased contribution from predators and parasitoids of the pest. However, the work of Halkier's group (Nielsen *et al.*, 2001), showing that increased levels of endogenous defence compounds (glucosinolates) did not give resistance to specialised herbivores in *Arabidopsis*, clearly points out the limitations of this approach. The strategy of reliance of the available gene pool in a crop for protection against herbivores cannot give complete protection, and is therefore dependent on two factors: first, that farmers are willing to tolerate a level of pest damage that is below a certain threshold, determined by the IPM programme; and second, that consumers are willing to tolerate produce that has been subjected to that level of pest damage. If a good economic case is made, the requirements of the farmer can be met; whether the requirements of the consumer can be met is another matter.

The introduction of foreign genes conferring resistance to insect herbivores into crop plants has been, in the case of Bt toxins, the major success in applying plant genetic engineering technology to agriculture. Bt toxins are highly active and specific, and give a very high level of protection in a crop against specific pest species, although resistance to other pest species may be low or nonexistent. The limited range of effectiveness of these toxins makes it necessary to continue to protect the crop against herbivores, often by continuing to use exogenous pesticides, albeit at reduced application rates. In deploying these insect-resistant transgenic plants, comprehensive IPM-style resistance management plans have been found essential to prevent pest resistance to the toxin developing (Feldman & Stone, 1997). Given that such programmes are already in place, they could be exploited to give increased control of nontarget herbivores, with enhanced endogenous defence playing an important role. The limitations of relying on the available gene pool in enhancing endogenous defence, discussed above, can be ignored in this case, since the use of transgenic plants is already assumed. There is thus the possibility of exploiting the whole range of plant defence mechanisms, by transferring the necessary genes from one species to another, in addition to exploiting bacterial toxins. Where suitable Bt toxins are not available for control of specific pests, for example, in targeting homopteran insects, plant defence mechanisms could even be the preferred option for pest-resistant transgenics. Transfer of endogenous defence mechanisms from one plant species to another has been achieved many times since the first report of a proteinase inhibitor from one plant species (cowpea; *Vigna unguiculata*) protecting transgenic plants of another (tobacco) against an insect herbivore (Hilder *et al.*, 1987). This strategy has yet to be applied in a commercial agricultural setting, mainly because the levels of protection achieved are usually not considered sufficient to be commercially viable. Engineering a multimechanistic resistance, which could exploit induced

and indirect defences as well as a constitutive direct defence, and which could incorporate highly effective and specific insecticides such as Bt toxin as well as a range of partial resistance mechanisms (Maqbool *et al.*, 2001), remains for the most part a possibility for the future.

VIII. Conclusions

1. The basic response of plants to herbivory is the wounding response, leading to the activation of defence genes and the production of insecticidal compounds (including proteins).
2. Many plant species also accumulate insecticidal compounds and proteins as a 'static' or constitutive defence against insect herbivores. In a given species, insecticidal products used in induced and constitutive defences may be the same.
3. The production of defensive compounds by plants carries a metabolic penalty, and the balance between induced and constitutive defences can be altered by both genotype and environment.
4. The wounding response is both local and systemic; the local and systemic responses may differ both qualitatively and quantitatively.
5. The wounding response is different to the response to pathogens, and systemic acquired resistance, although some genes are activated by both pathways.
6. The wounding response involves multiple signalling pathways and signal molecules, but jasmonate plays a central role in mediating anything other than very local responses.
7. The wounding response is made species-specific to the plant host both by the presence of genes responsible for the production of specific secondary compounds, and by the production of potentially insecticidal proteins which differ from species to species.
8. In species (and/or tissues) which accumulate secondary compounds as a constitutive defence the wounding response becomes a secondary defence, which is used to deter herbivores adapted to the accumulated compound(s).
9. The wounding response can be modulated by chemicals produced by the insect, or by the action of insect enzymes, to adapt the response to specific herbivore species.
10. Production of certain volatiles (green leaf volatiles, terpenoids) is a common aspect of the wounding response in many plant species.
11. Species-specific responses by the host, and modulation by the herbivore, make the overall volatile production specific to a given plant–insect interaction.
12. Although volatile production may have a deterrent effect, a common aim in these plant responses is to recruit natural enemies of the pest (predators, parasites) as an indirect defence mechanism.

13. Insects which feed on the contents of vascular tissue evade the wounding response to a greater or lesser degree by avoiding extensive tissue damage, but may instead activate the pathogen response mechanism, and produce defensive responses through that route.
14. Plants are able to use volatile signalling molecules to communicate between individuals, so that defence responses to insect herbivores can be induced in unattacked plants.
15. Insect adaptations to overcome the effects of plant defences can also be considered as constitutive and induced. Insect species which have a limited host range tend to rely more on constitutive adaptation mechanisms, which can be optimised for the chosen host(s). Generalist insect herbivores rely more on induced mechanisms to deal with a range of insecticidal compounds and proteins.
16. Adaptations to plant defence mechanisms carry a cost to the insect, which results in retardation of growth and development.

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