

# Signals Regulating Multiple Responses to Wounding and Herbivores

Guy L. de Bruxelles and Michael R. Roberts\*

Department of Biological Sciences, IENS, Lancaster University, Lancaster, LA1 4YQ, U.K.

\*Author for correspondence. Tel: +44 1524 593525. Fax: +44 1524 834854. e-mail: m.r.roberts@lancaster.ac.uk

**ABSTRACT:** Damage inflicted by herbivore feeding necessitates multiple defence strategies in plants. The wound site must be sealed and defence responses mounted against the herbivore itself and invading opportunistic pathogens. These defences are controlled both in time and space by highly complex regulatory networks which themselves are modulated by interactions with other signalling pathways. In this review, we describe the signalling events which occur in individual wounded leaves, in systemic unwounded regions of the plant and between the plant and other organisms, and attempt to place these events in the context of a co-ordinated system. Key signals which are discussed include ion fluxes, active oxygen species, protein phosphorylation cascades, the plant hormones jasmonic acid, ethylene, abscisic acid and salicylic acid, peptide signals, glycans, volatile chemicals and physical signals such as hydraulic and electrical signals. Themes which emerge upon consideration of the published data are that glycans and peptide elicitors are likely primary triggers of wound-induced defence responses and that they function through the action of jasmonic acid, a central mediator of defence gene expression, whose effect is modulated by ethylene. In the field, wound signalling pathways are significantly impacted upon by other stress response pathways, including pathogen responses which often operate through potentially antagonistic signals such as salicylic acid. However, gross generalisations are not possible since some wound and pathogen responses operate through common jasmonate- and ethylene-dependent pathways. Understanding the ways in which local and systemic wound signalling pathways are co-ordinated individually and in the context of the plants wider environment is a key challenge in the application of this science to crop protection strategies.

**Key Words:** signalling; wounding; herbivory; pathogen responses; tritrophic interactions.

## I. INTRODUCTION

Plants can sustain physical damage by many means, perhaps the most common being herbivore feeding. Since microbial pathogens may also attack at sites of wounding, plants have evolved complex systems of defence against herbivores and pathogens. Many of these responses are inducible and are activated specifically as a result of wounding. The wound response has been the subject of much study over the past three decades, during which time it has been adopted as an important model system for the investigation of long-range signalling in plants. This research activity follows the discovery by Ryan and co-workers in the early 1970s that defensive proteins accumulated not only in mechanically wounded leaves of tomato and potato plants, but also in distant unwounded leaves (Green and Ryan, 1972).

The proteins detected in this study were proteinase inhibitors which were subsequently found to inhibit the digestive proteases of foraging insect herbivores (reviewed by Ryan, 1990). Proteinase inhibitors are also found as wound-inducible genes in diverse plant species, from grasses to trees, and are commonly used as marker genes for the wound response. Since these proteins have a primary role in defence against chewing herbivores, mechanical wounding of plant tissues is often used as a means to perform controlled molecular and physiological analyses of the signals and responses important to inducible defence.

It is now clear that tissue damage initiates a series of inter-related inducible responses throughout the wounded plant. These include localised events which seal the

wound site and guard against opportunistic pathogen invasion, the production of defensive proteins and secondary metabolites in the damaged leaf and at distant sites, through to the production of volatile signals involved in communication with other organisms. The signalling events which mediate these different responses are of interest to plant scientists not only because they are intrinsically biologically interesting, but because they are important from an agricultural perspective. An understanding of the interplay between the different defence pathways targeted towards different pests and pathogens is essential for the development of optimised crop protection strategies. The complexity of the events involved in plant defence is hinted at from analysis of the genome of *Arabidopsis thaliana*. It is estimated that 2005 genes (11.5% of the total number of genes) are involved in defence, whilst a further 1855 (10.4%) genes are involved in signalling (The *Arabidopsis* Genome Initiative, 2000).

Over recent years, model systems for wound signalling have been developed in several plant species and a number of common principles have emerged. However, it is also apparent that different plants have adopted different defence strategies, and that common signals are used to different ends in different plants. In addition, there is an increasing collection of data illustrating significant interplay between the pathways mediating wound responses and those controlling responses to microbial pathogens. In this review we attempt to summarise the key signalling events occurring in a wounded plant, compare signalling in response to mechanical wounding and insect herbivory and consider wound responses in the broader context of overall plant defence.

## **II. AN OVERVIEW OF THE WOUND RESPONSE AND WOUND SIGNALLING**

### **A. Defence**

Within the immediate vicinity of a wound site, whether caused mechanically or by herbivore feeding, one must think of several distinct populations of cells. First, there will be a layer of irreparably damaged cells, which despite being unable to mount a pro-active response themselves will nevertheless release many different molecules which could act either as elicitors of responses in neighbouring intact cells or as defensive toxins.

Bordering the damaged cells will be a layer of intact cells that are likely to be under severe stress and which may also generate wound signals. Further away from the wound site will be healthy, undamaged cells which may be the targets of the signals generated by the cells around the wound site. Lastly, we know that aerial tissues away from the wounded leaf also perceive and respond to these signals.

Because there are a large number of opportunistic plant pathogens which are able to gain entry to plant tissues through wound sites, one of the primary roles of the intact cells surrounding areas of damage is to form a physical barrier to restrict pathogen invasion. Amongst the processes involved are the strengthening of the cell wall and the physical isolation of cells from their neighbours. Cell wall strengthening is achieved in part via rapid oxidative cross-linking of existing cell wall proteins which takes place minutes after wounding (Bradley *et al.*, 1992). Later, the *de novo* synthesis of additional hydroxyproline-rich cell wall glycoproteins that have a structural role, such as extensins, is commonly observed (reviewed by Showalter, 1993). Other localised wound responses include the production of phenolic-derived polymers such as lignin and suberin which are involved in sealing the wound site against both infection and water loss. The cells immediately around the wound site also synthesise callose, a  $\beta$ -1,3-glucan polymer (e.g. Thomson *et al.*, 1995), a process which appears to involve a calcium-induced change in cellulose synthase activity to callose synthase activity. Callose formation is particularly pronounced in the plasmodesmatal pores, and results in a reduction in plasmodesmatal aperture and hence reduced intercellular movement of macromolecules and virus particles.

Chemical defences against invading pathogens and herbivores are also produced in response to wounding, both in the wounded leaf and at systemic sites. These chemical defences can be separated into two major categories - secondary metabolites and proteins. Although secondary metabolites with defensive functions may be present constitutively, the majority are induced by

wounding and/or pathogens. These include terpenoids, alkaloids, and phenolics and are collectively known as phytoalexins. Phytoalexins and lignin and suberin monomers are all produced via the phenylpropanoid biosynthetic pathway. Following wounding, increased flux into this pathway is facilitated by the rapid, co-ordinated induction of several key phenylpropanoid biosynthetic genes, such as phenylalanine ammonia-lyase and chalcone synthase (Hahlbrock and Scheel, 1989; Dixon and Paiva, 1995). Wound-inducible proteins with direct defensive properties include protease inhibitors,  $\alpha$ -amylase inhibitors, polyphenol oxidase and lectins, all of which have proposed roles as insect anti-feedants. Basic isoforms of the anti-microbial pathogenesis-related (PR) protein family, such as chitinases and  $\beta$ -1,3-glucanases, are also wound-induced. For more extensive surveys of wound-induced genes, see Zhou and Thornburg (1999) and Constabel (1999).

## B. Signalling

There are also many other wound-inducible genes which do not have direct defensive roles. These are involved in the generation and perception of signals regulating the response to wounding. Of course, the inducibility of such genes implies earlier signals which regulate them and constitutively present receptors for these primary signals. The investigation of the complex temporal and spatial array of signalling events in wounded plants will form the central focus of this review. The earliest known events detected in wounded leaves include ion fluxes across the plasma membrane, changes in cytoplasmic calcium concentration, the generation of active oxygen species and changes in protein phosphorylation patterns. These phenomena appear to be conserved in all plants tested, and are all associated with intracellular signal generation in many other plant and animal systems. These early events occur in the first few minutes following damage, and are probably not directly responsible for inducing defence gene expression. Instead, as a mass of data now shows, defence gene expression is mediated primarily through the synthesis and action of the phytohormone jasmonic acid (JA). Other hormones with important roles in regulating wound gene expression are ethylene and abscisic acid (ABA). The synthesis of JA and ethylene is well

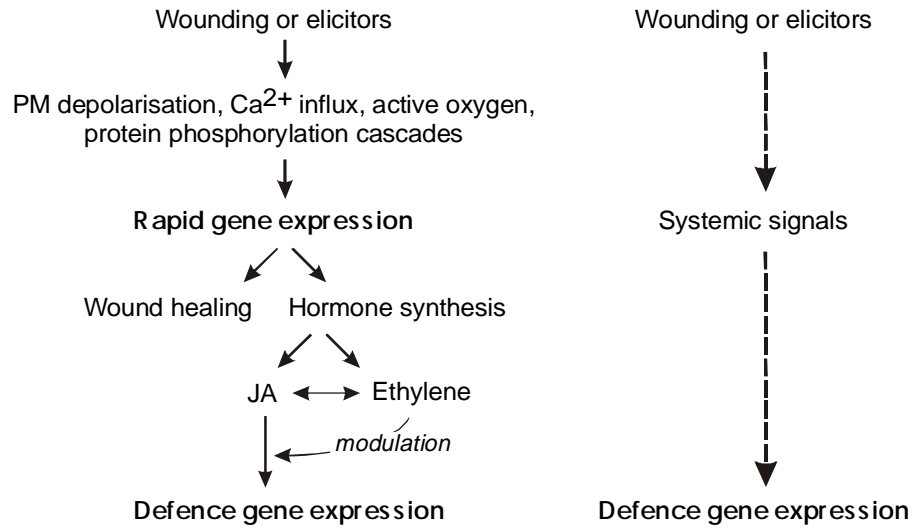
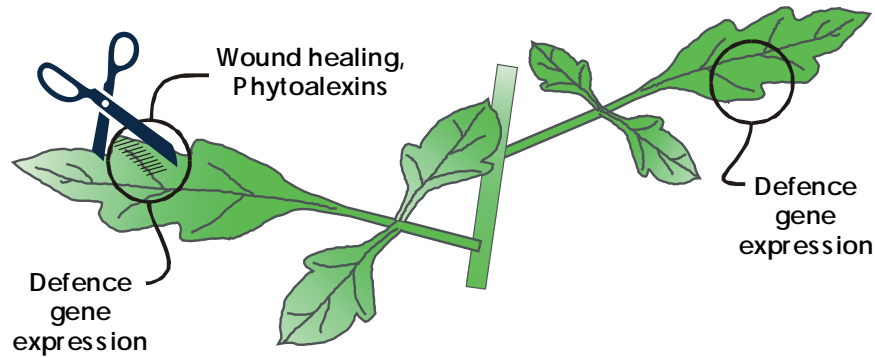
characterised, with many of the genes encoding their biosynthetic enzymes being up-regulated within 30-60 minutes of wounding, leading to peaks in hormone synthesis in wounded leaves at 1-2 hours. Other elicitors of wound responses have also been identified, the most important of which include cell wall glycans such as oligogalacturonides (OGAs), and, in solanaceous plants at least, a peptide hormone called systemin. These elicitors of wound responses may either be primary signals released upon cellular damage, or may function to amplify the response in the wounded leaf. In addition, they may also perform a key role in systemic signalling. Proposed mechanisms for the transmission of signals to unwounded sites include electrical activity, the active transport of elicitors in the phloem and the passive transport of elicitors via hydraulic mass flow in the xylem. Finally, wounding inflicted by insect herbivory also results in signalling beyond the plant itself to mediate an indirect form of defence. Plants under attack from herbivores produce characteristic blends of volatile compounds that serve to attract predators and parasitoids of those herbivores.

In the following sections, we will provide an assessment of the nature and significance of these different wound- and herbivore-induced signals, a summary of which is presented in Fig. 1. This will be based on experiments conducted mainly in model systems such as tomato and *Arabidopsis*, but also in many other plants, where the key events appear to be conserved.

## III. EARLY SIGNALLING EVENTS

### A. Ion fluxes

One of the most rapid events following tissue damage or the application of elicitors of wound (or indeed pathogen) responses, is a series of ion fluxes at the plasma membrane. These ion fluxes have been known for some time, and data has accumulated suggesting that they play a causal role in defence gene activation. For



**FIGURE 1.** A generalised scheme for the wound response. Wounding leads to the activation of gene expression in both local and systemic leaves. Overall gene expression profiles are different between the wounded and unwounded leaves however. The summary of signalling events shown occurring in the wounded leaf is the best available model based on combined data from many different systems. Whether the signalling events occurring in response to the systemic signal are the same as those in the wounded leaf is not clear.

practical reasons, most work has used the application of elicitors to suspension cell cultures to monitor ion fluxes (e.g. Mathieu *et al.*, 1991; Messiaen and van Cutsem, 1994; Felix and Boller, 1995), but some studies have used intact roots (Hush *et al.*, 1992) or intact or semi-intact leaf tissue (e.g. Thain *et al.*, 1990; Moyen and Johannes, 1996). These and other studies show that in common with many pathogen derived elicitors, wounding, glycans and the tomato systemin peptide all cause a rapid depolarisation of the plasma membrane electrical potential. This depolarisation event is associated with an efflux of  $K^+$  ions, a concomitant influx of protons, and an alkalinisation of the extracellular medium.

Chemical agents which disrupt these ion fluxes have been demonstrated to affect subsequent defence gene expression. The fungal toxin fusaric acid, for example, which activates the plasma membrane  $H^+$ -ATPase and hyperpolarises the membrane, is a potent inhibitor of wound, glycan and systemin-induced gene expression (Doherty and Bowles, 1990; Messiaen and van Cutsem, 1994; Schaller and Oecking, 1999). Conversely, it was demonstrated by Schaller and Oecking (1999) that wound-responsive genes were activated following applications of inhibitors of the  $H^+$ -ATPase which mimic plasma membrane depolarisation in the

absence of any other stimulus. Interestingly, whilst H<sup>+</sup>-ATPase activation by fusicoccin inhibits wound gene expression, it induces pathogen-related gene expression (Schaller and Oeking 1999; Roberts and Bowles, 1999). On this basis, Schaller and Oeking (1999) suggested that H<sup>+</sup>-ATPase activity may regulate a switch between wound and pathogen responses. It is difficult to see how this might operate in the plant, however, because in fact, both wound and pathogen elicitors provoke very similar membrane depolarisation events.

One feature of the ion fluxes occurring in the wound response which has maintained general interest is the fact that mechanical damage evokes electrical activity transmissible over long distances. Initially, it was thought by many that this might represent the causative mobile signal in the induction of gene expression at systemic sites. The current understanding of the role of electrical activity in systemic signalling will be discussed below.

## B. Calcium signalling

Another ion which has been implicated in wound signalling is the calcium ion, a ubiquitous and important second messenger in eukaryotes. Several studies have shown rapid increases in cytoplasmic Ca<sup>2+</sup> concentrations following wounding (e.g. Knight *et al.*, 1993) or elicitor application (e.g. Mathieu *et al.*, 1991; Messiaen *et al.*, 1993; Messiaen and van Cutsem, 1994; Chandra *et al.*, 1997; Moyon *et al.*, 1998). Calcium channel blockers, calcium ionophores and agents which mobilise intracellular Ca<sup>2+</sup> stores have been shown to modulate elicitor-induced gene expression, suggesting a causal role in signalling (Messiaen and van Cutsem, 1994; Léon *et al.*, 1998). Evidence supporting a role for Ca<sup>2+</sup> in wound signalling has come from the identification of a range of inducible genes encoding Ca<sup>2+</sup>-regulated proteins. Calmodulin is a primary target for Ca<sup>2+</sup> in many signalling systems, and touch and wound-induced calmodulin genes have been identified in several laboratories (Braam, 1992; Vian *et al.*, 1996; Stankovic and Davies, 1997; Bergey *et al.*, 1999a). Other wound-responsive proteins involved in signal transduction include a calmodulin domain protein kinase (CDPK; Yoon *et al.*, 1999) and a calcineurin B homologue (Kudla *et al.*, 1999), a subunit of the Ca<sup>2+</sup>/calmodulin-dependent protein phosphatase 2B. Many calmodulin-binding proteins are known in

plants (reviewed by Snedden and Fromm, 1998), but as yet there is little data linking wound-induced Ca<sup>2+</sup> signals with such proteins.

Several pharmacological approaches have been used to demonstrate a requirement for calcium in wound-induced gene expression. Messiaen and van Cutsem, (1994) showed that Ca<sup>2+</sup> channel blockers eliminated the induction of PAL enzyme activity and PAL gene expression in response to OGAs in carrot cells. Experiments with pharmacological agents in *Arabidopsis* suggest that Ca<sup>2+</sup> release from intracellular stores and calmodulin may be involved in the differential regulation of separate JA-dependent and JA-independent pathways (Léon *et al.*, 1998). In these experiments with *Arabidopsis*, the inferred role of Ca<sup>2+</sup> is downstream of JA biosynthesis and perception. In the case of systemin-induced cytoplasmic Ca<sup>2+</sup> increases in tomato cells, both intracellular and extracellular sources of Ca<sup>2+</sup> appear to be involved. Since JA was unable to elicit Ca<sup>2+</sup> flux, Moyon *et al.*, (1998) suggest that Ca<sup>2+</sup> signalling is upstream of JA signalling. Calcium could therefore play multiple roles in wound signalling. In comparison with studies on pathogen elicitor-induced Ca<sup>2+</sup> signalling, the role and nature of Ca<sup>2+</sup> fluxes in the wound response has been little investigated.

## C. Active oxygen species

The production of active oxygen species, including the superoxide radical (O<sub>2</sub><sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), is an early event in the response of plants to wounding and many other biotic and abiotic stresses. As for Ca<sup>2+</sup> signalling, however, the nature and function of the oxidative burst in response to wounding or herbivory is far less understood than is its role during pathogen responses. Systems where the generation of active oxygen has been described include insect feeding in soybean (Bi *et al.*, 1995), wounding, OGAs and systemin in tomato leaves (Orozco-Cardenas and Ryan, 1999) and wounding of *Zinnia* stems (Olson and Varner, 1993), winter squash mesocarp

(Watanabe and Sakai, 1998) and maize leaves (Guan and Scandalios, 2000).

The analyses of active oxygen described in the existing literature suggest that two major phases of production occur during a wound response. The first is a very rapid burst of  $O_2^-$  and  $H_2O_2$  which is maximal in the first minutes following wounding of winter squash mesocarp (Watanabe and Sakai, 1998) or OGA application to tobacco cells (Chandra *et al.*, 1997). A similar rapid oxidative burst, evidenced by cross-linking of cell wall proteins, occurs within a few minutes of wounding bean seedlings (Bradley *et al.*, 1992). A second, more sustained elevation in  $H_2O_2$  then occurs over a period of hours (Orozco-Cardenas and Ryan, 1999). Experiments using enzyme activity assays and inhibitors suggest that the major source of active oxygen is the superoxide-generating plasma membrane-bound NADPH oxidase complex (Bi *et al.*, 1995; Watanabe and Sakai, 1998; Orozco-Cardenas and Ryan, 1999). Wound-induced  $H_2O_2$  is then produced from superoxide via the action of superoxide dismutase (Watanabe and Sakai, 1998). Interestingly, plants possess homologues of the mammalian gp91<sup>phox</sup> subunit of the neutrophil respiratory burst oxidase, but with an additional N-terminal domain containing  $Ca^{2+}$ -binding EF-hand motifs (Keller *et al.*, 1998). This suggests a possible link between wound-induced  $Ca^{2+}$  fluxes and active oxygen production.

In contrast to the very rapid primary phase of active oxygen production,  $H_2O_2$  production over a period of hours was detected in tomato and several other species tested (Orozco-Cardenas and Ryan, 1999). This second phase of  $H_2O_2$  production correlates with wound-induced polygalacturonase activity, and at least in tomato, is JA-dependent (Orozco-Cardenas and Ryan, 1999). The authors suggest that the regulated activity of polygalacturonase may release endogenous OGAs, which then act as elicitors of  $H_2O_2$  in local and systemic tissues. This scenario would place  $H_2O_2$  some way down the chain of events linking wounding with defence gene expression, whereas the first phase oxidative burst may be analogous to the early oxidative burst elicited by pathogens which is important in pathogen defence signalling.

Several functions for active oxygen have been suggested. These include cross-linking of cell wall components (Bradley *et al.*, 1992), and direct anti-insect activity (Bi *et al.*, 1995). Since  $H_2O_2$  is able to potentiate pathogen responses (for review see Lamb and Dixon, 1997), Orozco-Cardenas and

Ryan, (1999) suggested that it may also prime defences against potential pathogen invasion at wound sites.  $H_2O_2$  may have an even more direct role in signalling. Elegant studies using reconstituted signalling systems with  $H_2O_2$ -responsive reporter genes in *Arabidopsis* protoplasts, showed that  $H_2O_2$  activates specific MAP kinase cascades, which in turn regulate defence gene expression (Kovtun *et al.*, 2000). Although wound-induced genes were not tested in this study, it is of interest that the two *Arabidopsis* MAPK proteins activated in this system are orthologs of the wound-induced tobacco MAP kinases "WIPK" and "SIPK." These two protein kinases are implicated in early wound signalling, as we shall now see.

#### D. Protein phosphorylation

Reversible phosphorylation is a ubiquitous mechanism for the regulation of protein activity, and one which is employed commonly in signal transduction pathways, including wounding (e.g. Rojo *et al.*, 1998). Several protein kinases have been identified which are induced by wounding, either in terms of increased protein activity or increased gene expression, including a member of the abscisic acid-induced protein kinase family (Lee *et al.*, 1998) and a glycogen synthase kinase 3 homologue (Jonak *et al.*, 2000). Functions for these kinases are currently unknown. However, the protein kinases which have been most rigorously studied are members of the mitogen activated protein kinase (MAPK) family. MAP kinases are named after their animal counterparts, which regulate signalling in response to factors that regulate cell division and stress responses. Equivalent protein kinase activities induced by wounding were first identified in tobacco both biochemically (Usami *et al.*, 1995), and through the cloning of a cDNA representing a MAPK homologue whose mRNA accumulates in response to wounding and pathogen infection (Seo *et al.*, 1995). Similar MAPK activities that are also induced very rapidly - within a few minutes of wounding - have subsequently been identified in *Arabidopsis*, alfalfa and tomato (Mizoguchi

*et al.*, 1996; Bogre *et al.*, 1997; Stratmann and Ryan, 1997). It has also been reported that rapid MAPK activation also occurs in systemic leaves of wounded tobacco and tomato (Seo *et al.*, 1995; Stratmann and Ryan, 1997). The tomato MAPK activity was also induced by treatment of plants with OGAs or systemin. With the exception of the tomato MAPK, the genes for all these MAPK activities have been cloned. Interestingly, all show transient increases in transcript levels following wounding, whereas total protein levels remain constant. Stimulus-induced transcription of MAPK genes is uncommon, but in this case may represent a mechanism to maintain protein levels following turnover of activated kinase, since enzyme activation precedes the increases in transcript levels.

Attempts to uncover the role of MAPK activity in plant defence responses has largely focused on tobacco, where in addition to the wound-induced MAPK ("WIPK") originally identified by Seo *et al.* (1995), a second wound-induced MAPK activity has also been characterised. This second MAPK was originally identified as a salicylic acid-induced MAPK, termed "SIPK," (Zhang and Klessig, 1997) and was assumed to be involved primarily in plant pathogen responses. However, it has subsequently been shown to be responsible for the major wound-induced MAPK activity, with WIPK activation showing similar kinetics but at a quantitatively lower level (Zhang and Klessig, 1998). Both kinases are activated by multiple stresses (Kumar and Klessig, 2000), making definitive genetic or biochemical investigations of their function difficult. Despite these problems, studies from Ohashi's group on WIPK suggest that this MAPK does indeed play a key role in wound signalling. Transgenic tobacco plants in which WIPK was co-suppressed failed to accumulate JA and proteinase inhibitors in response to wounding (Seo *et al.*, 1995). Intriguingly, they instead accumulated salicylic acid (SA) and expressed acidic PR genes, events characteristic not of a wound response but of a pathogen response. This finding raises interesting questions regarding possible cross-talk between wound and pathogen signalling pathways, an issue which will be discussed in more detail in a later section of this review. More recently, the same laboratory showed that over-expression of the WIPK gene lead to increased constitutive levels of WIPK activity, which correlated with increased levels of JA and proteinase inhibitors in unwounded plants (Seo *et al.*, 1999). Unfortunately, whether

the activity of SIPK was altered in the transgenics with modified WIPK was not addressed in either case. In tomato, a MAPK activity similar to that induced by wounding is also induced by ultraviolet radiation. This treatment does not induce a wound response in itself, but it does increase the systemic expression of Pin genes in response to a subsequent wound (Stratmann *et al.*, 2000a). Whether the UV-induced activity is identical to the wound-induced activity, or is perhaps able to potentiate its activation in response to wounding, is unknown.

Several groups have proposed models in which wound-induced MAP kinases activate phospholipase A<sub>2</sub>, releasing linolenic acid from the plasma membrane which then acts as a substrate for JA biosynthesis (e.g. Narváez-Vásquez *et al.*, 1999). Interestingly, linolenic acid is a potent inhibitor of a protein phosphatase 2C activity that is responsible for MAPK pathway inactivation (Baudouin *et al.*, 1999). These observations suggest a potential mechanism by which the activation of the JA biosynthetic pathway could function in positive feedback regulation of wound-induced MAPK activity. These propositions remain to be tested experimentally, however, and we await the identification of the first *bona fide* substrate for a wound-induced MAP kinase. Knowledge of such targets, as well as the mechanisms by which MAPK cascades are activated, are essential before we can gain a fuller understanding of the roles of these signalling pathways in wounding and other stresses.

#### IV. ELICITORS OF DEFENCE GENE EXPRESSION

The early signalling events described above usually occur in the first few minutes after wounding. They are likely to trigger a number of responses, including cell wall fortification at the wound site, alterations in metabolism and the generation of signals, including systemic signals, which in turn regulate defence gene expression. A number of plant hormones are known which can either induce defence genes directly, or are required for the full defence response. In

addition, several groups of molecules which are not generally considered as classical plant hormones have also been identified as elicitors of defence genes. The biology of these various hormone and elicitor systems will be considered below before we attempt to integrate them into the scheme of events which constitutes the wound response.

### A. Jasmonic acid

Jasmonic acid was originally identified as a potential signal in wounding when its volatile derivative, methyl jasmonate (MeJA), was identified as a potent inducer of proteinase inhibitor (Pin) genes in tomato (Farmer and Ryan, 1990). Experiments at that time showed that leaves of tomato plants even expressed Pin genes when placed next to sagebrush plants (*Artemisia tridentata*), which produce high levels of MeJA in their leaves. However, subsequent studies showed that whilst wounded tomato plants do not synthesise enough MeJA to elicit Pin gene expression in neighbouring plants, local application of JA produces a signal which is transmitted to systemic sites within the same plant (Farmer *et al.*, 1992). Around the same time, JA was shown to accumulate endogenously in wounded leaves (Creelman *et al.*, 1992). As a combined result of these observations, JA is now accepted to be a key intracellular signal in mediating responses to insect attack, wounding and elicitors such as OGAs and systemin.

The biosynthesis of JA, which has been reviewed recently (Creelman and Mullet, 1997; León and Sánchez-Serrano, 1999), is catalysed by a number of regulated enzymes present in several different sub-cellular compartments, and is collectively known as the octadecanoid pathway. JA biosynthesis begins when lipid precursors are released from cellular membranes, most probably the plasma membrane or chloroplast membranes. The actual membrane(s) involved, and the mechanism of lipolysis in wounded tissues has yet to be proven. As mentioned above, wound-induced PLA<sub>2</sub> (Narváez-Vásquez *et al.*, 1999) is a strong candidate, and recently, phospholipase D (PLD) has also been shown to be essential for JA synthesis and JA responses in *Arabidopsis* (Wang *et al.*, 2000). PLD may produce substrates for PLA<sub>2</sub>, or directly activate lipoxygenases involved in JA synthesis. The next steps in JA biosynthesis most probably occur in the chloroplast. Here, lipoxygenases

convert linolenic acid into lipid hydroperoxides which are converted into a key intermediate, 12-oxo-phytodienoic acid (OPDA) via the action of allene oxide synthase (AOS) and allene oxide cyclase (AOC). OPDA is then reduced in the cytoplasm before undergoing three rounds of  $\beta$ -oxidation in the peroxisome to produce JA. Finally, it is clear that in addition to methyl jasmonate, many JA conjugates are formed, including amino acid conjugates which are bioactive (Krumm *et al.*, 1995; Wasternack *et al.*, 1998). Significantly, many of the genes encoding JA biosynthetic enzymes are themselves induced by wounding and often also by JA, providing wound-response feedback systems for control of JA levels in the plant (reviewed by León and Sánchez-Serrano, 1999). Kinetic analyses suggest that wound-induced JA synthesis is biphasic, with an initial ethylene-independent phase followed by a stronger ethylene-dependent peak in synthesis (O'Donnell *et al.*, 1996; Laudert and Weiler, 1998).

Perhaps one of the more commonly overlooked features of JA signalling in wounded plants is the fact that while it accumulates to high levels in wounded leaves, much smaller increases are observed in systemic leaves (Laudert and Weiler, 1998; Bowles, 1998; Rojo *et al.*, 1999). Whilst JA is absolutely required for many systemic wound responses, the question of whether it functions in the systemic leaves, or acts only in the wounded leaf to mediate systemic signalling is a matter for debate in the literature (Bowles 1998; Ryan, 2000). The site of biosynthesis and the site of action of JA appears to be critical for an appropriate response to occur. Exogenous application of JA and MeJA to plants has been shown to induce defence gene expression (e.g. Farmer and Ryan, 1990; Farmer *et al.*, 1992) and is sufficient to provide resistance against certain insects (e.g. Thaler *et al.*, 1996; McConn *et al.*, 1997). Despite this, manipulation of JA biosynthesis in transgenic plants has not produced the predicted results. In one experiment, over-expression of chloroplast AOS significantly increased the concentration of JA in transgenic plants, but this did not lead to Pin gene expression (Harms *et al.*, 1995). Over-expression of

cytoplasmic AOS caused accumulation of OPDA, but not JA (Wang *et al.*, 1999). However, in both studies, wounding caused greater accumulation of JA in the transgenic plants than in the wild type, and the induction of defence genes was enhanced.

Several key experiments in JA signalling have been possible because of the availability of well-characterised JA-deficient and JA-insensitive mutants of tomato and *Arabidopsis*. These include the tomato mutant JL5, originally isolated because of its inability to mount a systemic defence response (Lightner *et al.*, 1993) and subsequently found to be defective in the conversion of lipid hydroperoxides into OPDA (Howe *et al.*, 1996). This mutant was later renamed *defenceless-1 (def-1)* as it is much more susceptible to herbivory by tobacco hornworm caterpillars than the wild-type (Howe *et al.*, 1996). This experiment suggests that JA is essential for insect defence, a conclusion which was substantiated by a similar experiment in which JA-deficient *Arabidopsis* plants were found to be highly susceptible to attack by fungal gnat larvae (McConn *et al.*, 1997). Interestingly, JA application was able to restore resistance to fungal gnat larvae, but only a sub-set of wound-induced genes were expressed in response to exogenous JA (McConn *et al.*, 1997). The existence of both JA-dependent and JA-independent wound signalling pathways in *Arabidopsis* was also highlighted by Titarenko *et al.*, (1997). Similarly, JA-independent wound-induced genes of tomato are also known (O'Donnell *et al.*, 1998). Since JA alone is able to elicit resistance against multiple insect pests, without activating expression of all wound-induced genes, it seems possible that JA is mainly responsible for the regulation of genes encoding proteins with defensive functions. Other, JA-independent wound-induced genes may play roles which are dispensable for defence in undamaged, elicitor-treated plants, such as wound healing.

Cloning of the *COII* gene, mutations in which disrupt JA perception in *Arabidopsis*, recently revealed the mechanism of JA signalling at the molecular level (Xie *et al.*, 1998). *COII* encodes an F-box protein, a class of protein which forms a part of a multi-protein complex involved in targeted proteolysis (Xie *et al.*, 1998). Current data suggest that JA-responsive genes are suppressed by negative regulatory proteins which can be specifically degraded following JA perception in a *COII*-dependent manner. Inducible gene expression systems based on the relief of suppression, rather than on a direct positive induction mechanism, may

be common in plants. For example, auxin signalling also functions via targeted proteolysis of negative regulators via a protein complex homologous to that involved in the JA response (Leysner, 1998).

Interestingly, many rapidly induced wound-responsive genes are also expressed following treatment with cycloheximide, an inhibitor of protein translation (e.g. see Liu *et al.*, 1993; Rojo *et al.*, 1998; Suzuki *et al.*, 1998; Fujimoto *et al.*, 2000; Durrant *et al.*, 2000). This suggests the existence of inhibitors of gene expression whose rapid turnover means that they can be removed by blocking *de novo* translation with cycloheximide. This turnover may be regulated by protein complexes of the kind implicated in JA signalling. It must be recognised that these early genes are not themselves induced by JA, however, so they are not likely to be regulated by the actual *COII*-containing complex. Additionally, the expression of many JA-dependent defence genes activated later in the wound response is generally blocked by cycloheximide, indicating that new protein factors need to be synthesised to drive their transcription. Perhaps it is these factors which are the primary targets of JA signalling?

## B. Ethylene

The hormone ethylene is involved in plant development and is produced in response to many stresses (Kende, 1993). Wound-induced ethylene is produced as a result of the activation of 1-aminocyclopropane-1-carboxylate synthase (ACS) and 1-aminocyclopropane-1-carboxylate oxidase (ACO), the two dedicated ethylene biosynthetic enzymes. There are many reports that genes encoding specific isoforms of these enzymes are themselves wound-inducible. The genes encoding basic isoforms of PR proteins are generally induced by both ethylene application and wounding, which suggests that ethylene may be the mechanism for their activation by wounding. In addition to its direct affect on gene expression, the other major role of ethylene appears to be to co-ordinate gene expression along with JA. In

tomato, JA-mediated Pin gene expression is ethylene-dependent (O'Donnell et al., 1996). Furthermore, ethylene appears to regulate JA biosynthesis in tomato (O'Donnell et al., 1996) and *Arabidopsis* (Laudert and Weiler, 1998). This cooperation between ethylene and JA is also apparent in several pathogen-resistance mechanisms (reviewed by Pieterse and van Loon, 1999). Conversely, ethylene negatively regulates the expression of a set of JA-responsive genes in wounded *Arabidopsis* leaves (Rojo et al., 1999). Similarly, ethylene negatively regulates wound-induced lectin genes in *Griffonia* (Zhu-Salzman et al., 1998). In both cases, whilst suppressed in wounded leaves, these genes are expressed in systemic leaves which do not synthesize ethylene. In contrast to the suggestions that ethylene modulates JA responses in wounded *Arabidopsis* leaves, (Laudert and Weiler, 1998; Rojo et al., 1999), recent cDNA microarray analysis of 150 *Arabidopsis* defence-related genes indicated that there were no differences in local wound-induced gene expression in an ethylene-insensitive mutant compared to wild-type, (Reymond et al., 2000). The reason for this striking difference in results is not clear, but may be related to different wounding or sampling procedures.

The effects of ethylene on herbivore defence have been investigated in *Arabidopsis*, with intriguing results (Stotz et al., 2000). *Arabidopsis* mutants blocked in ethylene signalling were no different from wild-type when challenged with a generalist herbivore, the diamondback moth, which induces wound-response genes when feeding on wild-type plants. However, the same ethylene-insensitive mutants were actually more resistant than wild-type to a specialist herbivore, the Egyptian cotton worm (Stotz et al., 2000). One interpretation of this result stems from the observation that ethylene suppresses the transcription of *Arabidopsis* systemic wound genes (Rojo et al., 1999). This in turn implies that ectopic expression of these genes may occur in wounded leaves in the ethylene-insensitive mutant plants providing greater protection against Egyptian cotton worm, but not the diamondback moth. These data highlight the fact that different defences are required to provide protection against different herbivores and that interactions between different signalling pathways provide a potential mechanism for regulating those different responses. A further example of such interplay is found in tobacco, where elevated levels of wound ethylene induced by

tobacco hornworm feeding, suppress the JA-dependent synthesis of nicotine, a major protectant involved in insect defence (Kahl et al., 2000). Initially, it might appear that the herbivore has hijacked the negative regulatory effect of ethylene for its own benefit. However, the situation is complicated by the fact that the tobacco horn worm is actually nicotine-tolerant and accumulates nicotine in order to deter parasitoids. In this specific case then, it is the JA-dependent nicotine response which has been hijacked for the herbivore's benefit. Kahl et al., (2000) suggest that the repression of nicotine synthesis by ethylene is a plant defence mechanism which makes the caterpillars more susceptible to parasitoids by reducing their nicotine content. If this is indeed the case, then this example provides an excellent illustration of the co-evolution of plant-herbivore interactions.

Components of the ethylene signalling pathway have been well-studied (reviewed by Johnson and Ecker, 1998). The molecular mechanisms of ethylene-mediated defence gene expression are also beginning to be unraveled. The promoters of a number of ethylene-induced defence genes contain a sequence element termed the ethylene response element (ERE), or "GCC-box." These include members of the basic PR gene family. The ERE is bound by a class ethylene-inducible transcription factors termed ERE binding proteins (EREBPs), or ethylene response factors (ERFs) (Ohme-Takagi and Shinshi, 1995). Several members of this class of transcription factor are themselves rapidly wound-induced in tobacco in an ethylene- and JA-independent manner and their subsequent activation in response to ethylene is required for defence gene transcription (Suzuki et al., 1998).

### C. Glycans

The glycan chitosan, which is not found in plants but is a component of fungal cell walls, and fragments from the plant cell wall polymer, pectin, have long been known to induce Pin gene expression in tomato (for discussion, see Bowles, 1998). In fact in tomato, OGAs induce many responses in

common with wounding. Their application to the plant results in a rapid depolarisation of the plasma membrane and influx of calcium into the cytoplasm (Thain *et al.*, 1990; Mathieu *et al.*, 1991, Messiaen and van Cutsem, 1994), the generation of active oxygen (Orozco-Cardenas and Ryan, 1999), and the induction of JA (Doares *et al.*, 1995a) and ethylene (O'Donnell *et al.*, 1996) biosynthesis. Furthermore, like wounding, OGAs induce Pin gene expression in tomato in a JA-dependent manner (Doares *et al.*, 1995a). In *Arabidopsis*, OGAs are able to induce a class of wound-induced genes expressed predominantly in the local, wounded leaf (Rojo *et al.*, 1999). These genes are JA-independent in the *Arabidopsis* wound response, whereas a second class of genes are expressed predominantly in the systemic leaves in response to a JA-dependent pathway (Titarenko *et al.*, 1997; Rojo *et al.*, 1999). Furthermore, not only can OGAs induce the local wound response genes, it was shown that the application of OGAs can block the ability of exogenous JA to induce expression of the systemic class of genes (Rojo *et al.*, 1999). Since JA accumulates to high levels in wounded leaves, these observations led to the suggestion that in wounded *Arabidopsis* leaves, OGAs released following damage contribute to the induction of locally expressed genes, but repress the class of systemic wound response genes that would otherwise be induced in response to JA (Rojo *et al.*, 1999). This scenario clearly differs from that in other plants such as tomato where OGAs induce both local and systemic gene expression.

In the experiments discussed above, it was also demonstrated that suppression of the JA response pathway by OGAs is ethylene-dependent, and that in fact ethylene alone is sufficient for suppression of the JA pathway in local leaves (Rojo *et al.*, 1999). This finding leaves open the question of whether OGAs are active signals *in planta*, or whether a more direct wound-induced ethylene burst may account for the differential regulation of the JA pathway in local and systemic leaves. However, ethylene is not required for the induction of local wound gene expression in *Arabidopsis* (Reymond *et al.*, 2000), so OGAs remain prime candidates. Until recently, it was unclear whether bioactive OGAs are indeed produced during a wound response, and therefore whether they have a real *in vivo* role. Many microbial pathogens produce pectin-degrading enzymes capable of releasing OGAs which probably function in pathogen response signalling. Now though, a plant

polygalacturonase activity has been identified that is regulated by wounding in tomato and several other species, including *Arabidopsis* (Bergey *et al.*, 1999b; Orozco-Cardenas and Ryan 1999). This opens the way for a more directed approach to address the role of OGAs in wounding and insect defence.

#### D. ABA

The plant hormone abscisic acid (ABA) is best known for its role in mediating the response to water stress, due to its involvement in the regulation of both stomatal closure and dehydration-induced gene expression. There is also evidence of a role for ABA in mediating the wound response, although the exact nature of this role remains unclear. Early work showed that ABA application can directly induce PinII gene expression locally and systemically in potato and to a lesser extent in tomato and tobacco (Peña-Cortés *et al.*, 1989). Analysis of ABA-deficient mutants of potato and tomato provided further evidence for a requirement for ABA in the wound-induction of Pin genes (Peña-Cortés *et al.*, 1989; 1991) and wound-induced ABA accumulation was demonstrated (Peña-Cortés *et al.*, 1991). This evidence along with other data supporting a direct role for ABA in mediating the wound response has been reviewed by Peña-Cortés *et al.*, (1995). However, recent evidence suggests a more indirect role for ABA in mediating the wound response than previously thought. Birkenmeier and Ryan, (1998) found that exogenous ABA was able to induce PinII expression in tomato to a much lesser extent than either wounding or JA application and showed that endogenous ABA levels only increase significantly as a result of wounding in tissue surrounding the wound site. These observations led the authors to suggest that ABA is necessary for the maintenance of a normal state of physiology rather than it being a *bona fide* wound signalling molecule. Additionally, ABA is also likely to play a role in mediating the induction of dehydration-responsive genes that occur locally following wounding. More recently, cDNA microarray analysis carried out by

Reymond *et al.*, (2000) suggested that in *Arabidopsis* at least, dehydration may play a wider role in directly controlling wound gene induction. Many of the wound-induced genes identified in this study were also induced by dehydration, and the overall profile of wound-induced gene expression was more similar to that induced by dehydration than by insect feeding (Reymond *et al.*, 2000). It is likely that the extent of tissue damage incurred by the plant will determine the extent to which dehydration and ABA influence gene expression during a wound response.

### **E. A model for signalling in the wound response**

The key questions still outstanding in this area are what is the primary stimulus of the early signalling events in the wound response and in what order do the elicitors of defence gene expression act in the plant? By viewing the available data in terms of the ability of the different elicitors to provoke the rapid signalling responses characteristic of wounding, we can confirm previous conclusions (Fig. 1) and begin to suggest new answers to these questions. Table 1 shows a summary of the ability of different wound and elicitor treatments to stimulate the different signalling events discussed in this review. What is immediately apparent is that JA is unable to elicit those rapid signalling processes which are observed in wounded tissues. This corroborates previous conclusions of a role for JA as a downstream target of the early signalling events where it functions as an intracellular signal for defence gene induction. OGAs are the only elicitors able to mimic all aspects of signalling in the wounded leaf. Since OGAs are also the only elicitor known to induce local gene expression in wounded *Arabidopsis* (Rojo *et al.*, 1999), it is possible that they represent the primary signal which initiates the wound response. In addition to the wound-induced polygalacturonase activity described by Orozco-Cardenas and Ryan (1999), an analysis of the recently completed *Arabidopsis* genome sequence shows that there are many genes encoding proteins with predicted functions in pectin degradation. Perhaps some of these enzymes are rapidly activated at sites of wounding, releasing OGAs which trigger the wound response. The systemin peptide from tomato, which has been proposed as a systemic signal (see below), elicits most of the wound-associated signalling events,

though not the rapid oxidative burst. Interestingly, in terms of signal generation, the response to systemin is more closely related to the local rather than the systemic wound response. Perhaps peptide and glycan signals released following wounding may act to amplify the wound signal as well as being required to generate the systemic signal. The possible mechanisms for transmission of information from damaged tissues to systemic sites are considered below.

## **V. THE SEARCH FOR THE SYSTEMIC SIGNAL**

One of the most fascinating and elusive aspects of the wound response, is the ability of a signal generated at the site of wounding to be transmitted to distant parts of the plant. Over the years, a number of molecules have been suggested as potential transmissible wound signals, along with electrical and hydraulic signals. For example, both JA and OGAs were proposed as systemic signals when they were identified as elicitors of Pin gene expression in tomato. In each case, subsequent studies have shown that they are either immobile in the plant or not sufficient to explain observed systemic responses. There is, however, convincing data that unidentified chemical elicitors may be transported over long distances in the xylem via hydraulic surges caused by damage to the vasculature. Electrical activity has been known for many years to be associated with wounding in plants and the behaviour of long range electrical signals has been the subject of much research, with no definitive role yet established. The true signal or signals are not yet known, but we will try and address some of the key issues in systemic signalling below.

### **A. Systemic signals and their route of transmission**

Firstly, it is clear that whatever the nature of the signal, it is carried by the vasculature. Systemic signalling absolutely requires vascular connections and the distribution and amplitude of systemic gene expression is related to how direct the vascular connections

**TABLE 1****The Ability of Wound and Elicitor Treatments to Stimulate Signalling Events Associated with the Wound Response.**

SIGNALLING EVENTS INITIATED	TREATMENT				
	Wounding: local leaf	Wounding: systemic leaf	OGA application	JA application	Systemin application
Plasma membrane depolarisation	✓	✓	✓	x	✓
Rapid Ca <sup>2+</sup> influx	✓	? <sup>1</sup>	✓	x	✓
Rapid oxidative burst (O <sub>2</sub> <sup>-</sup> )	✓	x	✓	x	x
Late oxidative burst (H <sub>2</sub> O <sub>2</sub> )	✓	✓	✓	✓ <sup>3</sup>	✓
Protein kinase activation	✓	✓	✓	x	✓
JA biosynthesis	✓	+/- <sup>2</sup>	✓	✓	✓
Ethylene biosynthesis	✓	x	✓	+/- <sup>4</sup>	✓

<sup>1</sup> Not reported.

<sup>2</sup> Different reports indicate either no increase or limited increases in systemic JA levels.

<sup>3</sup> May be mediated via OGAs (Orozco-Cardenas and Ryan, 1999).

<sup>4</sup> JA induces ethylene in tomato (O'Donnell *et al.*, 1996), but not in *Arabidopsis* (Rojo *et al.*, 1999).

are between the wound site and the site where gene expression is measured, either in response to insect feeding (Jones *et al.*, 1993), heat wounding (Rhodes *et al.*, 1999) or mechanical wounding (Orians *et al.*, 2000). Some reports have suggested that the systemic signal is carried in the phloem. The tomato systemin peptide (which will be discussed in detail in the following section), for example, appears to be mobile in the phloem on the basis of analyses of radiolabelled peptide transport and phloem loading inhibitors (Narváez-Vásquez *et al.*, 1994). An electrical signal associated with Pin gene expression in tomato was also carried by the phloem (Rhodes *et al.*, 1996). However, this result highlights the variety of different abiotic stresses which can induce systemic expression of typical wound responsive genes. The electrical signal carried in the phloem characterised by Rhodes and co-workers (1996) was generated by heat wounding. Other studies have shown that the mechanisms of local and systemic Pin gene expression in response to mechanical (crushing) damage and heat damage are quite distinct (e.g. Herde *et al.*, 1995; 1998).

Early studies on transmissible electrical activity induced by wounding had lead to the suggestion that this electrical activity *per se* may be the systemic signal responsible for gene expression. The work of Wildon *et al.*, (1992) showed that the

systemic signal generated by mechanical damage was not carried by the phloem. Furthermore, the systemic accumulation of proteinase inhibitors correlated with an electrical signal with the characteristics of an action potential (Wildon *et al.*, 1992). Importantly, it has been found that applied electrical signals which generate action potentials are able also to induce Pin gene expression (Herde *et al.*, 1995; Stankovic and Davies, 1996). These electrical signals generally consist of a wave of membrane depolarisation, such as that described earlier, which perpetuates from cell to cell. There are in fact two different types of systemic electrical signal - a fast action potential which occurs on mechanical wounding or after external electrical stimulation and a slower, variation potential, which is likely generated by transient variations in hydraulic tension in the xylem (e.g. Malone and Stankovic, 1991; Herde *et al.*, 1995; 1998; Stankovic and Davies, 1997; 1998). How these different electrical signals relate to the wound-induced systemic signal is not really understood. They may themselves function as primary signals, or they may simply be a secondary consequence of the passage of

chemical and/or hydraulic signals in the xylem.

Hydraulic signals produced as a release of xylem tension at sites of damage are also candidates to carry the systemic signal. These hydraulic signals are readily measurable throughout the plant following relatively minor mechanical wounding and even insect feeding (Alarcon and Malone, 1994). Detailed studies by Malone and co-workers have shown that hydraulic signals correlate with wound-induced gene expression. However, the hydraulic signal alone is not sufficient for full Pin gene expression, since Pin genes are not strongly induced by cutting the stem with a sharp blade. The hydraulic signal most probably represents the transport in the xylem by mass-flow of elicitor molecules released from damaged cells at the wound site (Malone *et al.*, 1994a; 1994b). Malone and Alarcon (1995) substantiated these predictions by showing that systemic wound signalling could be blocked simply by manipulating xylem pressure via increased environmental humidity, and that the systemic signal could pass through heat-killed tissue. Finally, the signal responsible for wound-induced tomato MAPK activation in systemic leaves must also be xylem-transmissible, since it was also unaffected by stem-girdling (Stratmann and Ryan, 1997).

## **B. Systemin — a peptide signal implicated in systemic signalling**

Of all the chemical systemic signals proposed over the years, that which has remained the most widely accepted candidate is a small polypeptide. Systemin is an 18 amino acid peptide first identified as a potent proteinase inhibitor-inducing factor from tomato leaves (Pearce *et al.*, 1991). Since then, a mass of data has been presented which support the proposition that systemin is a mobile, systemic wound signal (reviewed by Ryan, 1998; 2000). Although related peptides have been identified in other solanaceous species (Constabel *et al.*, 1998), it is as yet unclear whether systemin represents a general peptide-based wound signalling mechanism, or one which has arisen in a specific plant family. Nor has definitive proof been gained that systemin is indeed the mobile signal. What is clear, however, is that in tomato, systemin is central to the expression of wound-induced genes and is required for systemic signalling.

Systemin is expressed in the plant in the C-terminal region of a much larger (200 amino acid)

precursor, known as prosystemin (McGurl *et al.*, 1992). The prosystemin gene is expressed primarily in the vascular regions of the aerial parts of the plant and is itself wound-inducible (Jacinto *et al.*, 1997). Two key experiments highlight the importance of systemin in wound signalling in tomato. Firstly, transgenic plants in which prosystemin gene expression is suppressed via antisense RNA expression, exhibit significantly reduced systemic Pin gene expression in response to wounding (McGurl *et al.*, 1992) and reduced resistance towards tobacco hornworm larvae (Orozco-Cardenas *et al.*, 1993). Secondly, transgenic plants over-expressing prosystemin under the control of the constitutive CaMV 35S promoter show high levels of Pin gene expression throughout the plant (McGurl *et al.*, 1994). These plants also constitutively express many other systemic wound response proteins, which has permitted the molecular identification of many of these (Berger *et al.*, 1996). What is interesting about these plants is that although it is the full prosystemin precursor which is expressed, wounding is not necessary for gene expression (McGurl *et al.*, 1994). Furthermore, recent experiments with recombinant prosystemin have shown that the full length precursor is as biologically active as the 18 amino acid systemin peptide when applied to tomato plants, and no evidence for proteolytic cleavage to release systemin could be found (Dombrowski *et al.*, 1999). Perhaps the key difference lies in the mobility of the 18 amino acid systemin peptide compared to the prosystemin precursor.

When wild-type tomato shoots were grafted onto prosystemin over-expressing transgenic root stocks, the wild type scions expressed Pin genes, demonstrating the ability of the prosystemin expressing tissues to produce a systemic signal in the absence of wounding (McGurl *et al.*, 1994). This signal is not necessarily systemin itself, but interestingly, it is unlikely that the signal in these experiments could be explained by the xylem mass-flow model. An interesting reciprocal experiment that might also be very illuminating would be to test the ability of prosystemin antisense scions to express Pin genes when grafted onto prosystemin over-

expressing root stocks. This would determine whether gene expression required production of systemin in systemic leaves, or whether a systemin-generated signal from the wounded leaf is sufficient. Grafts between JA mutants and systemin transgenics might also be useful in this context. New tomato mutants deficient in the ability to respond to systemin recently isolated by Howe and Ryan (1999) should prove useful in further defining the role of this peptide in systemic wound signalling.

The effects of systemin on tomato cells and tissues have been studied extensively, as described in the preceding sections, and recent data is emerging regarding its mode of perception by the cell. Detailed experiments with synthetic systemin analogues containing amino acid substitutions or deletions previously demonstrated that the entire peptide is required for full biological activity and that the C-terminal four residues are the most important in inducing wound gene expression (Pearce *et al.*, 1993). This conclusion was supported by results with recombinant prosystemin derivatives (Dombrowski *et al.*, 1999). While the C-terminal region of systemin is required for its activity, the N-terminal region appears to be essential for the interaction with its receptor, as judged by activity assays with different regions of the systemin peptide and radiolabelled systemin-receptor binding assays (Meindl *et al.*, 1998). A systemin receptor has also been identified by the Ryan laboratory (Scheer and Ryan, 1999). This receptor is inhibited by suramin, an inhibitor of animal cytokine and growth factor receptors (Stratmann *et al.*, 2000b), which may suggest some structural and functional similarity with this family of proteins.

Thus, there are several possible systemic wound signals, and more than one route for their long distance transmission. Indeed, different systemic signals may turn out to be involved in different aspects of the wound response. A further route for systemic signal transmission which has not yet been considered is not through the plant at all, but through the air. As we have already seen, the role of JA in wound signalling was first suggested by the identification of its volatile derivative methyl jasmonate as an inducer of Pin genes (Farmer and Ryan, 1990), though it is no longer considered a likely systemic signal for the induction of defensive gene expression. Indeed, at this time there is still no convincing evidence for the systemic signal required for defence gene

expression being airborne in model systems like tomato and *Arabidopsis*. However, volatile signals are well known to play other roles in plant defence against herbivores which we will now discuss.

## VI VOLATILE PLANT SIGNALS

Plant responses to herbivory can be loosely classified in terms of direct and indirect defences. Direct defences can be both constitutive, comprising structural barriers to potential attackers and the storage of toxic compounds, or inducible, such as the synthesis and activation of defensive proteins and metabolites. So far we have only considered the signals involved in the regulation of inducible direct defences in the plant. The signals responsible for indirect defence are also now becoming more widely investigated. Indirect defences involve the synthesis and release of numerous volatile compounds which serve the dual function of acting as both SOS signals between plants and as attractants of predators and parasitoids, which are natural enemies of attacking herbivores. Termed tritrophic interactions, these indirect defences involve complex interactions between plants, herbivores and their natural enemies, and have been the subject of many recent reviews (Paré *et al.*, 1999; Paré and Tumlinson, 1999; Sabelis *et al.*, 1999; Stotz *et al.*, 1999; Agrawal, 2000; Paiva, 2000; Walling, 2000). In some instances, they may even involve further levels of interaction involving predators at higher trophic levels or insect pathogens.

Tritrophic interactions appear quite common in nature, and there are several examples of these interactions being exploited in agricultural systems as a form of pest management. However, the mechanisms governing these interactions remain poorly understood. The release of volatile signals following herbivore attack may have both beneficial and detrimental consequences for the plant. The main benefits are that the arrival of predators and parasitoids may serve to reduce herbivore numbers (although the effects of parasitism are slow), and that volatiles may also act as warning signals to

other herbivores that a plant's defences have been activated. However, as a natural consequence of the coevolution of tritrophic interactions, the release of volatiles following herbivore feeding may also have the detrimental effect of signalling the plant's presence to other herbivores. Although the role that plant volatiles play in indirect defence has mainly been studied in tritrophic interactions involving plants, herbivores and their natural enemies, there is also evidence of a role for plant volatiles in interplant protection against pathogens (Shul'ayev *et al.*, 1997).

### **A. Herbivory-induced biosynthesis and release of volatiles**

The leaves of healthy, undamaged plants constitutively synthesise a number of volatile compounds, which are both constantly released at low levels and stored in specialised storage organs located on the leaves. These include monoterpenes, sesquiterpenes and aromatics, and 'green-leaf volatiles' which are synthesised via the lipoxygenase pathway (reviewed by Paré *et al.*, 1999). Herbivore attack has been characterised as involving several different phases of increased volatile release, including significant changes to the blend of volatiles emitted. Firstly, the level of terpene and 'green-leaf volatile' emission increases, either through their release from damaged storage organs or by their rapid synthesis from stored precursors. A second, slower phase of volatile release has been shown to involve the synthesis of a new set of plant volatiles involving at least four biosynthetic pathways (Paré *et al.*, 1999; Paiva, 2000; Agrawal, 2000). The synthesis of these herbivore-induced volatile compounds has been shown to be light-dependent (Paré and Tumlinson, 1997a; 1997b Halitschke *et al.*, 2000; Maeda *et al.*, 2000), with herbivore-specific blends of volatiles being released several hours after the initial herbivore attack. Like the induction of direct defence responses resulting from mechanical wounding, herbivore-induced volatile synthesis and release is triggered both locally at the site of damage and systemically, throughout the undamaged parts of the plant (Dicke and Dijkman, 1992; Turlings and Tumlinson, 1992; Röse *et al.*, 1996; Paré and Tumlinson, 1998). The nature of the herbivore-induced systemic signal responsible for triggering volatile release is not yet known. However, experiments have shown that it clearly

differs from those mediating the systemic response to mechanical wounding. Although the application of elicitors of the wound-response does result in increased volatile synthesis, the blend released differs from those identified following insect feeding (Dicke *et al.*, 1999; Ozawa *et al.*, 2000).

### **B. Functions of herbivore-induced plant volatiles.**

The observed complexity and variation between different species-specific volatile blends strongly suggests that interaction between plants and natural enemies of herbivores has co-evolved over many thousands of years. Laboratory-based studies have shown that it is volatiles released by the attacked plant that act as attractants to predators and parasitoids rather than those released by the herbivores themselves (Dicke, 1994; De Moraes *et al.*, 1998). There have been many documented examples of predators and parasitoids being attracted to herbivore infested plants (e.g. Tumlinson *et al.*, 1993; Turlings *et al.*, 1995; Takabayashi and Dicke, 1996; Dicke *et al.*, 1998; Rao *et al.*, 1999; Guerrieri *et al.*, 1999; Mayland *et al.*, 2000). Less is known about how induced plant volatiles function under far more complex field conditions where many competing plant and insect species coexist in an environment filled with different odours (see review by Sabelis *et al.*, 1999). Both plant and herbivore species affect the composition of induced volatiles, and it is becoming clear that both predators and parasitoids are able to differentiate between various blends of herbivore-induced volatiles to an amazing degree. This has recently been demonstrated by De Moraes *et al.*, (1998), who showed that attacks on three important crop species by two closely related herbivore species resulted in distinct volatile-emission profiles in each case. Using these olfactory cues, the parasitic wasp, *Cardiochilles nigriceps*, was able to differentiate between them to locate its host herbivore.

In addition to mediating tritrophic interactions, herbivore-induced volatiles are also able to induce direct plant defences in neighbouring leaves. For example, research

into gene expression during lima bean/spider mite interactions showed that the volatiles induced by spider mites differ from those induced by mechanical wounding, and that only the herbivore-induced volatiles triggered expression of all the defence genes studied (Arimura *et al.*, 2000a). Furthermore, cDNA microarray analysis of genes expressed in the same system showed that exposure of leaves to herbivore-induced volatiles leads to major changes in gene expression, including the up-regulation of large number of defence-related genes (Arimura *et al.*, 2000b). Interestingly, it was found that many more genes are activated by the volatiles in undamaged leaves than are activated in the infested leaves.

Herbivore-induced volatiles may also act as direct defences themselves. Bernasconi *et al.*, (1998), showed that aphids were repelled by the odour of wounded maize plants treated with caterpillar regurgitant, suggesting that the volatiles released during this interaction serve to act as an airborne warning to other potential herbivores. Hence, these blends of herbivore-induced volatile chemicals are at once able to act as signals within the plant, as signals between the plant and other organisms, and as direct defence molecules.

### C. Insect elicitors of volatile biosynthesis.

Herbivore-induced volatiles clearly differ in their blend, composition and effect in comparison with those emanating from mechanically wounded plants. For example, Bouwmeester *et al.*, (1999), found that lima bean sesquiterpene synthase, an enzyme involved in volatile biosynthesis, was up-regulated by two-spotted mite feeding but not by mechanical wounding. Similarly, Halitschke *et al.*, (2000), reported that the release of three volatile compounds, (E)-beta-ocimene, cis-alpha-bergamotene and linalool, were induced by caterpillar feeding and larval oral secretions, but not by wounding. Other differences between blend of volatiles released following either herbivore or mechanical wounding treatments have also been reported (Paré and Tumlinson, 1997b; Alborn *et al.*, 1997; De Moraes *et al.*, 1998). Additionally, although wound-induced volatiles can affect insect behaviour, the duration of these changes is significantly shorter than those induced by herbivore feeding (van Loon *et al.*, 2000; Weissbecker *et al.*, 2000).

These differences in volatile biosynthesis between mechanically wounded plants and those exposed to herbivory are now known to be due to the presence of elicitors in the saliva and gut regurgitants of insects (reviewed by Felton and Eichenseer, 1999 and Paré and Tumlinson, 1999). In several cases, the application of saliva or gut regurgitants to wounded tissue resulted in the emission of an identical volatile profile to that seen following attack by the insect itself (Boland *et al.*, 1992; Alborn *et al.*, 1997; 2000; Halitschke *et al.*, 2000). Analysis of the saliva from the herbivore *Spodoptera exigua* has led to the isolation and characterisation of one such elicitor, *N*-(17-hydroxylinolenoyl)-L-glutamine, named volicitin (Alborn *et al.*, 1997). Purified volicitin applied to maize plants induced a volatile blend that attracted *Coesia marginiventris*, the same species of parasitic wasp normally attracted by *Spodoptera exigua* feeding. Subsequent studies have shown that the caterpillar synthesises volicitin through the addition of a hydroxyl group and a glutamine to plant-derived linolenic acid (Paré *et al.*, 1998) and the authors suggest that volicitin has an as yet unknown essential function in the caterpillar's life cycle (Paré and Tumlinson, 1999). It is clear that the host plant is able to recognise and respond to volicitin by triggering the synthesis of defensive volatiles, although the signalling mechanism that mediates these events remains unclear. Recently, a sesquiterpene cyclase gene, *Stc1*, has been cloned from maize and shown to be induced by *Spodoptera exigua* feeding, the insect's saliva and by volicitin (Shen *et al.*, 2000). Knockout analysis has identified *Stc1* as being involved in the synthesis of the volatile compound sesquiterpene naphthalene. Another volicitin-responsive gene from maize, *Igl*, encoding an enzyme thought to be involved in indole biosynthesis, has also recently been identified (Frey *et al.*, 2000). Interestingly, expression of both genes is activated by mechanical damage as well as by volicitin and feeding. The only other volatile elicitor from insect oral secretions identified so far is a  $\beta$ -glucosidase from the caterpillar, *Pieris brassicae* (Mattiacci *et al.*, 1995).  $\beta$ -glucosidase was shown to be able to

induce the same volatile blend when applied to cabbage leaves as that obtained following feeding by the caterpillar itself, with both treatments resulting in the attraction of parasitic wasps. However, these experiments were carried out using gut regurgitants from *Pieris brassicae* which, as discussed by Felton and Eichenseer in their 1999 review, might contain compounds not normally present in the insect's saliva during feeding. In contrast to this view, it has recently been suggested that gut bacteria from herbivores might be involved in the biosynthesis of elicitors of plant volatiles (Spiteller *et al.*, 2000).

#### **D. Role of JA and compounds of the octadecanoid pathway in volatile induction.**

The involvement of the octadecanoid pathway in the synthesis of 'green leaf volatiles' and the central role of its end product, JA, in mediating the response to mechanical wounding has led to the suggestion that JA may also be a key regulator of volatile biosynthesis in plants. Research undertaken so far does point to an important role for this hormone in herbivore-induced volatile biosynthesis, but indicates that in at least some plant/herbivore interactions, other essential signalling molecules are also required. The role that JA plays during herbivory-triggered volatile induction clearly involves separate signalling pathways to those involved in its induction of direct defences in the wound response. The application of JA to plants results in volatile blends that far more closely resemble those seen following herbivore feeding than following mechanical wounding. Supporting these observations, Arimura *et al.*, (2000a) observed differences in the expression pattern of defence genes in lima bean leaves following exposure to volatiles induced either by two-spotted spider mite feeding or mechanical wounding. Wound-induced volatiles activated expression of only one of six defence genes tested, whereas the spider mite-induced volatiles induced all six, including a lipoxygenase (LOX) involved in JA biosynthesis. The authors suggest that LOX induction is followed by JA biosynthesis and the subsequent induction of JA-mediated defence genes. Lima bean leaves thus appear able to differentiate between herbivore-induced volatiles and those produced following mechanical damage, with

interplant induction of direct defence responses only occurring as a result of herbivore attack.

The signals regulating volatile synthesis are necessarily complex. In lima bean, both early and late intermediates of the octadecanoid pathway are able to induce volatile biosynthesis, but different intermediates induce different classes of volatile (Koch *et al.*, 1999). Early intermediates of JA biosynthesis, including linolenic acid and OPDA, induced the biosynthesis of homoterpenes, whereas JA did not, but instead induced the synthesis of sesquiterpenes. Ozawa *et al.*, (2000), reported that when exogenous JA was applied to detached lima bean leaves, a blend of volatiles was emitted that were both qualitatively and quantitatively similar to those emitted following feeding by two species of caterpillar, *Spodoptera exigua* and *Mythimma seperata*. However, the blend of volatiles produced from the same plant species during feeding by the two-spotted spider mite, *Tetranychus urticae*, exhibited several differences from that elicited by JA application, and could only be mimicked by either methyl salicylate (MeSA) application or by JA followed by MeSA. The authors suggest that herbivore-specific volatile induction pathways exist in lima bean and that the response to caterpillars is mediated via a JA-dependent pathway whereas the response to the two-spotted spider mite is co-regulated by both JA and SA-dependent pathways (Ozawa *et al.*, 2000). Another possibility is that volatile induction in response to the two spotted spider mite is solely under the control of a SA-dependent pathway, as MeSA treatment alone was sufficient to exactly mimic the volatile blend measured during mite feeding. The complexity of herbivore-induced volatile signalling was further highlighted in a study by Dicke *et al.*, (1999), who observed that despite the differences described above, the blend of volatiles released by lima bean leaves exposed to exogenous JA was able to attract specialist predators of the two-spotted spider mite. However, when these predators were given the choice between JA-induced volatiles and those induced by spider mites,

they showed a clear preference for volatile blends emitted from mite infested plants.

In a field experiment, application of exogenous JA to tomato plants induced both direct and indirect defences against the caterpillar species *Spodoptera exigua* (Thaler, 1999a). Induction of direct defences was evidenced by an increase in the activity of known octadecanoid pathway-induced defence proteins and by slower caterpillar development rates on JA-treated plants compared to controls. Caterpillars located either on or near JA-treated plants also had a significantly higher level of parasitism by the endoparasitic wasp, *Hyposoter exiguae*, than did caterpillars on untreated plants. This observation implicates JA in the regulation of both direct and indirect defence signalling. However, although these results suggest a potential role for JA as a biocontrol agent, it remains to be shown whether endogenous JA is the key regulator of indirect defence responses in the naturally occurring tritrophic interaction between tomato, *S. exigua* and *H. exiguae*. Experiments using this system in conjunction with available defence signalling mutants including JA- and SA-deficient and insensitive mutants could provide further insight into the significance of these signalling molecules in indirect defence responses.

## VII. MECHANICAL WOUNDING MIMICS SOME, BUT NOT ALL RESPONSES TO INSECT FEEDING

Mechanical damage clearly induces the expression of many genes that function in defence against chewing insects and other herbivores. Consequently, wounding is often used as an experimental procedure to investigate plant defence responses against herbivory. However, whilst a major result of herbivory is wounding and many wound-induced genes are induced by herbivory, the effects of insect feeding on a plant are more complex.

As with plant-pathogen interactions, different insect-host interactions have evolved in nature, such that some insects have specific hosts, whilst others are more generalist pests. Specialists in particular often evolve mechanisms to overcome or suppress host defences. For example, a major defence of members of the tobacco family is the synthesis of nicotine, which is toxic to most insects. The tobacco hornworm, however, is nicotine-tolerant and actually accumulates the toxin in its body as a

deterrent to parasitoids. Hence, it is not always possible to correlate an apparent defence response with resistance. Other classes of insect pests do not induce typical wound responses at all. Examples include aphids and whitefly, which are sap suckers rather than herbivores and which induce responses more typical of pathogen resistance (Walling, 2000).

In the preceding discussion of direct and indirect defences elicited by mechanical or herbivore-imposed wounding, there are numerous examples of differences in the signalling pathways and responses activated by these stresses. The differences between volatile emissions specific to individual herbivores and mechanical damage, although remarkable, are not altogether surprising viewed in the context of their function in attracting predators and parasitoids of specific herbivores. Less well catalogued are examples of differences in direct defence against herbivory in comparison with wounding alone. However, the expression of defence genes in potato was reported to be accelerated following tobacco hornworm caterpillar feeding relative to mechanical wounding (Korth and Dixon, 1997). Similarly, JA biosynthesis is amplified by the same herbivore relative to wounding alone in tobacco (McCloud and Baldwin, 1997). As with the elicitation of volatile production, in both cases factors in the saliva of the feeding caterpillars were identified as the agents responsible for the difference in the responses.

cDNA microarrays are currently providing a powerful resource to investigate large-scale changes in gene expression following different plant treatments, and to compare profiles of gene expression between treatments. Microarray analysis has recently been used to investigate the roles of different defence signalling molecules such as JA, ethylene and salicylic acid (SA) in *Arabidopsis*, for example (Schenk *et al.*, 2000). More relevant to wound signalling, Reymond *et al.*, (2000), recently examined the expression of around 150 defence-related genes from *Arabidopsis* in response to wounding and insect feeding. Of the genes identified as induced by mechanical wounding, expression of half of these was

JA-dependent, corroborating the earlier, more limited observations of Titarenko *et al.*, (1997). Comparisons were also made between mechanical wounding, dehydration and insect feeding, with the unpredicted result that the profile of genes induced by wounding was more similar to that induced by dehydration than insect feeding, though there was significant overlap in the wound and insect-induced profiles (Reymond *et al.*, 2000). Only one of the 150 genes examined was induced by feeding but not by wounding. These results serve to highlight the fact that wounding is merely one aspect of herbivory, and that the perception of insect-derived molecules influences all aspects of the plants response to insect feeding. This realisation will no doubt shape the immediate future of studies of plant defence against wounding and herbivory.

### **VIII. CROSS TALK BETWEEN DEFENCE SIGNALLING PATHWAYS**

Over recent years, the study of plant responses to wounding has undergone an interesting and exciting change in direction. Until fairly recently, wounding and plant defence against herbivores have been studied separately from the response to pathogens. With the growing recognition that in the field plants are rarely challenged by a solitary pest or pathogen and with increasing collaboration between plant physiologists, entomologists and ecologists, research into plant defence has moved away from the traditional pairwise approach towards analysing what is taking place in plants faced simultaneously with multiple attackers. For a long time, research into the wound response has used mechanical wounding as a model system. This research has focused primarily on elucidating the signalling mechanisms regulating the wound response with particular emphasis given to understanding the systemic induction of wound-responsive genes. Much of this research has been laboratory based and carried out under strictly controlled experimental conditions, using young plants exposed to a variety of mechanical wounding techniques. However, in light of recent studies it is becoming clear that the changes induced by mechanical damage are not necessarily an accurate reflection of what is occurring in plants exposed to insect feeding. This awareness, together with the rapid development of powerful new molecular and genetic tools has seen a move in the research

emphasis towards a broader study of plant defence mechanisms under more realistic conditions.

### **A. Responses to pathogens**

One of the consequences of wounding and herbivory is that plants are exposed to infection by pathogenic micro-organisms at sites of tissue damage. Indeed, plants in the field are under threat of attack by potential pathogens at all times. Despite this, the great majority of microbial challenges to plants result in incompatible interactions and do not lead to disease. This is because plants possess either effective constitutive or inducible defence mechanisms or else is a result of the attacked plant being unable to support the life cycle of a particular micro-organism (Hammond-Kosack and Jones 1996). There are two recognised types of incompatible interaction. The most common is termed non-host resistance, and occurs when an entire plant species demonstrates resistance to a specific pathogen (Heath, 2000). This form of disease resistance is displayed by most plant species against the majority of potentially pathogenic species. The second category, host resistance, also involves an incompatible reaction but arises when a particular plant genotype from within a susceptible species displays specific resistance to a particular pathogen species or pathovar (Heath, 2000). Plant defence against pathogens has been the subject of numerous recent informative reviews (e.g. Glazebrook, 1999; Heath, 2000; Stahl and Bishop, 2000).

Plants possess a wide array of defence mechanisms with which they are able to respond to pathogen invasion. Although most plant species possess some form of constitutive anti-microbial defence, e.g. impenetrable epidermal layers or preformed secondary metabolites and proteins with anti-microbial activities, the majority of defence responses appear to be inducible. These can be triggered by a number of specific and non-specific elicitors either originating from the microbial invader or from damage to the host plant cell walls. In cases of host resistance, incompatible interactions are rapidly

triggered through a mechanism known as gene-for-gene resistance (reviewed by Hammond-Kosack and Jones, 1996; Jones and Jones, 1997; Ronald, 1998; Ellis *et al.*, 2000). Gene-for-gene resistance involves an interaction between a specific plant resistance gene product and a specific pathogen derived avirulence gene product leading to the switching on of plant defences including hypersensitive cell death in the tissue occupied by the invading pathogen. Non-specific elicitors can induce plant defences in both compatible and incompatible interactions and include phytoalexins, extracellular microbial compounds, pectic enzymes and fatty acids (Lyon and Newton, 1999). Recent reports indicate that both specific and non-specific elicitors activate similar defence pathways and it has been proposed that it is the more rapid induction of these responses during incompatible interactions that result in successful resistance to the invading pathogen (Somssich and Hahlbrock, 1998; Dong, 1998; Romeis *et al.*, 1999; Durrant *et al.*, 2000; Heath, 2000).

Defence responses associated with pathogen invasion are similar in many respects to defence responses activated by wounding. Cell wall cross-linking and lignification occurs to strengthen the barrier to pathogens and phytoalexin and antimicrobial (PR) protein synthesis is initiated. Recent gene expression profiling data from tomato suggests that the majority of genes induced early in a gene-for-gene resistance response are also rapidly induced by wounding (Durrant *et al.*, 2000). Many of the early signalling events which follow pathogen elicitor recognition also bear a surprising overall resemblance to those induced by wounding. Pathogen responses involve similar changes in plasma membrane ion flux, including  $\text{Ca}^{2+}$  influx, the generation of active oxygen species and the activation of similar protein phosphorylation cascades. Useful recent reviews of the process of the recognition of microbial elicitors and early signalling events triggering plant defence responses include Blumwald *et al.*, (1998), Ebel and Mithöfer, (1998) and Grant and Mansfield, (1999). Of particular note is the recent finding that nitric oxide, NO, which is an important signal in animal systems, has a key role in plant pathogen responses too (reviewed by Klessig *et al.*, 2000). NO functions alongside the production of  $\text{H}_2\text{O}_2$  in the oxidative burst to orchestrate the hypersensitive response and the expression of defence genes, and also appears to act synergistically with SA in the development of resistance (Durner *et al.*, 1998;

Delledonne *et al.*, 1998). Later events involved in the development of pathogen resistance differ substantially from wound responses, and are divided into responses in the infected leaf, which give rise to local acquired resistance (LAR) and responses occurring throughout the rest of the plant, known as systemic acquired resistance (SAR). Systemic resistance involves the induction of pathogen-related defence genes in response to, as yet unidentified, systemic signal(s) originating from the site of infection (reviewed by van Loon, 1997; Glazebrook, 1999; Hammerschmidt, 1999; Shetty and Kumar, 1999). Several different pathways of systemic resistance in plants have now been identified and it is clear that these involve complex and potentially interacting signalling pathways.

## B. Systemic pathogen resistance

The best characterised of the systemic pathogen responses is that of systemic acquired resistance (SAR), which results in resistance against a broad range of pathogens and involves the induction of a distinct set of PR proteins (Ryals *et al.*, 1996). Salicylic acid (SA), a product of the phenylpropanoid pathway, has been shown to be essential for the induction of SAR (Gaffney *et al.*, 1993; Vernooij *et al.*, 1994, Pallas *et al.*, 1996; reviewed by Ryals *et al.*, 1996) and acts through the action of the signalling protein NPR1 to induce PR genes (Cao *et al.*, 1994; 1997). The role of SA in the defence response of plants has been recently reviewed by Dempsey *et al.*, (1999) and Hammerschmidt and Smith-Becker, (1999). Although the requirement for SA in mediating SAR has been clearly demonstrated in several experimental systems, recent evidence suggests that SA-independent pathways of SAR activation also exist. For example, the over-expression of pathogen-responsive isoforms of soybean calmodulin in transgenic tobacco elicited broad spectrum resistance against pathogens and SAR-associated gene expression in an SA-independent manner (Heo *et al.*, 1999). Similarly, Dong and Beer, (2000) demonstrated that riboflavin could induce

pathogen resistance and SAR-associated PR gene expression in both wild-type and SA-deficient *Arabidopsis* and tobacco. As with SA-dependent SAR responses, SA-independent riboflavin-induced resistance and PR gene expression required the action of NPR1.

In addition to SAR, another inducible form of systemic resistance to pathogens has been identified and characterised. Induced Systemic Resistance (ISR) as it is known, is stimulated by rhizosphere-associated growth promoting bacteria and has been shown to result in resistance to a number of pathogens in aerial parts of the plant (Pieterse *et al.*, 1996; 1998; Knoester *et al.*, 1998; reviewed by Pieterse and van Loon, 1999). Unlike SAR, ISR is SA-independent and does not involve the induction of SAR-associated PR proteins. ISR is mediated instead through the action of two plant hormones normally associated with the wound response - jasmonic acid and ethylene. Surprisingly, like SAR, ISR requires the action of the NPR1 protein (Pieterse *et al.*, 1998). It is not yet understood how the two pathways diverge downstream of NPR1 action to produce distinct resistance responses. In addition to their involvement in mediating ISR, JA and ethylene have also been implicated in resistance to several fungal pathogens (Epple *et al.*, 1997; Knoester *et al.*, 1998; Staswick *et al.*, 1998; Thomma *et al.*, 1998; Vijayan *et al.*, 1998; Kozlowski *et al.*, 1999) and the induction of non-SAR associated pathogen-responsive genes (Epple *et al.*, 1995; Penninckx *et al.*, 1996;1998; García-Ponce and Rocha-Sosa, 2000). The application of exogenous JA has also been reported to increase resistance to certain fungal pathogens (Kozlowski *et al.*, 1999; Thomma *et al.*, 2000). However, it must be remembered that the ability of exogenously applied hormones to induce pathogen-related genes is not necessarily an indication of an *in vivo* role in pathogen-response signalling. For example, although ethylene gas is able to induce a set of TMV-induced genes in tobacco leaves, the expression of these same genes in response to TMV infection does not require ethylene (Guo *et al.*, 2000).

The complexity of the response to pathogens was also highlighted recently by Kachroo *et al.*, (2000) in a study of resistance to turnip crinkle virus (TCV) in *Arabidopsis*. They showed that resistance to TCV involves a novel SA-dependent but NPR1-independent signalling pathway, indicating that more than one functional SA-regulated defence pathway exists in *Arabidopsis*.

One pathway requires the NPR1 protein and confers resistance to fungal and bacterial pathogens whilst a second NPR1-independent pathway induces resistance to viruses. This novel resistance response is also independent of the action of JA and ethylene. Support for the existence of an SA-dependent NPR1-independent disease response pathway has come from a recent analysis of double mutants in *Arabidopsis*. Clarke *et al.*, (2000), reported that introduction of the *npr1* mutation into the SA-accumulating *cpr5* mutant background only partially reduced the *cpr5* mutant's normal constitutive disease resistance.

In addition to SA and JA/ethylene mediated systemic resistance, recent evidence suggests that additional novel signalling pathways might also be involved in inducing disease resistance. Guo *et al.*, (2000) have described the induction of several SA- and ethylene-independent tobacco genes locally and systemically following inoculation by TMV. Similarly, *C. fulvum* resistance in tomato has recently been demonstrated to be independent of the action of both SA and ethylene (Brading *et al.*, 2000). However, an alternative explanation for these latter observations is that resistance to *C. fulvum* is mediated independently by two concurrent resistance pathways, one mediated by SA and one by JA/ethylene, such that loss of both pathways would be required for resistance to break down (Brading *et al.*, 2000).

### C. Crosstalk between defence response signalling pathways

It is clear that plant defence involves multiple signalling pathways with no one systemic response inducing resistance to all potential pathogens. Although different systemic responses have been shown to induce resistance to different groups of pathogens, it is clear that there are overlapping subsets of pathogens against whom resistance is induced by more than one defence pathway. It is probable that under field conditions, several systemic defence pathways can be concomitantly activated, inducing resistance to a broader range of potential pathogens than would be possible

through the action of any one systemic response alone. The different systemic signalling pathways identified so far are complex and share some common intermediates both with each other and with intermediates of wound response signalling pathways, indicating that there is the potential for crosstalk between the different pathways.

In the field, not only are plants faced with having to defend themselves against pathogens and herbivores, but they must reconcile the fact that these attacks may often occur concurrently, with opportunistic pathogens circumventing the plants natural physical defences by gaining entry to the plants internal tissues via sites of wound damage. Many insects are well known as vectors for viral pathogens for example. The mechanisms by which plants activate different defence response pathways in response to multiple and divergent attackers and the potential for crosstalk between the different pathways has been the topic of much recent discussion (Bostock, 1999; Maleck and Dietrich, 1999; Stout and Bostock, 1999; Cosgrove *et al.*, 2000; Paul *et al.*, 2000; Hatcher and Paul, 2000). The role of SA in pathogen resistance has long been recognised and the recent observations that JA and ethylene also are involved in pathogen resistance suggests a central and complex role for these hormones in regulating the plants wide array of defence responses (reviewed by Dong, 1998; Reymond and Farmer, 1998). The co-operation of JA and ethylene in certain systemic pathogen resistance mechanisms and in wound and herbivore signalling is particularly noteworthy in this regard.

Much of the initial research into the question of cross-talk between pathways focused on the relationship between SA and the octadecanoid pathway. SA can inhibit JA biosynthesis (Peña-Cortés *et al.*, 1993) and JA perception (Doares *et al.*, 1995b), and it has been hypothesised that the pathways of SA-mediated SAR and the JA-mediated wound response might therefore be mutually antagonistic. Although only a proportion of wound-inducible genes are regulated by the action of JA (Titarenko *et al.*, 1997; Rojo *et al.*, 1998; Reymond *et al.*, 2000), studies of JA-insensitive and JA biosynthetic mutants have demonstrated that jasmonates are essential for protection against insect attack (Howe *et al.*, 1996; McConn *et al.*, 1997). Similarly, application of exogenous JA or MeJA has been shown to induce resistance against a range of herbivores under both laboratory and field conditions (Thaler *et al.*, 1996; Baldwin, 1998; Thaler, 1999A; Thaler, 1999B;

Thaler *et al.*, 1999; Omer *et al.*, 2000). The suggestion therefore arises that these JA-dependent responses may be attenuated in plants in which SA levels have been increased as a result of pathogen attack. At the same time, the involvement of JA in responses to certain pathogens considerably widens the potential for interaction between defence signalling pathways and raises intriguing questions about the effects of these interactions on plant defence against multiple attackers. Although there is now strong evidence for mutual antagonism between SA and JA signalling pathways in some plant-herbivore/ plant-pathogen interactions, there is also increasing evidence to the contrary, suggesting that the interactions between different defence responses are more complex than at first envisaged.

SA and related phenolic acids were originally shown to inhibit the wound-induction of Pin genes in tomato (Doherty *et al.*, 1988; Peña-Cortés *et al.*, 1993; Doares *et al.*, 1995b). The exact mechanism for this inhibition of JA responses remains unclear and potentially involves multiple sites of action. Peña-Cortés *et al.*, (1993) were able to overcome SA inhibition of wound-induced Pin gene expression in dark-incubated plants by the application of exogenous JA, suggesting that SA blocks wound-induced JA biosynthesis. In contrast, a subsequent study found that SA was able to inhibit exogenous JA-induced Pin gene expression, indicating in this case that the site of SA inhibition is downstream of JA synthesis (Doares *et al.*, 1995b). There have been several further reports of antagonism of JA-inducible responses by SA. Baldwin *et al.*, (1997) observed decreases in JA-dependent nicotine accumulation in *Nicotiana sylvestris* following the application methyl salicylate. In a follow-up study, Preston *et al.*, (1999) observed that in TMV infected tobacco plants, wounding resulted in reduced levels of nicotine compared to uninfected controls, which the authors suggest is due to antagonism of wound-induced JA-dependent signalling by the increased endogenous SA levels resulting from TMV infection. The recent observation that the *Arabidopsis eds4* mutant, which has reduced endogenous SA levels and increased susceptibility to

pathogens, displayed enhanced JA-dependent responses provides genetic evidence for SA's antagonism of JA-signalling (Gupta *et al.*, 2000). Conversely, there is also evidence for the inhibition of SA accumulation and signalling by JA. Wounded tobacco plants expressing the rice RGP1 gene were shown to accumulate SA and express acidic PR genes instead of displaying the usual wild-type JA-mediated responses (Sano *et al.*, 1994). The application of MeJA to these transgenics abolished this SA accumulation (Sano *et al.*, 1996). Mutual antagonism between SA and JA in defence signalling was also reported by Niki *et al.*, (1998) who showed that in tobacco leaf discs both wound and MeJA-dependent induction of basic PR genes could be significantly reduced by the application of SA and that SA-dependent acidic PR gene induction could be inhibited by treatment with MeJA. Taken together, these observations suggest that similar to SA's antagonism of JA, JA interacts negatively with the SA response in two ways, by inhibiting both SA accumulation and action.

In addition to measuring changes in gene expression and protein synthesis which result from antagonism between SA and JA, several recent studies have provided compelling evidence that *in vivo* resistance to herbivores and pathogens is also affected by these interactions. In comparison with wild-type, transgenic tobacco plants with suppressed levels of phenylalanine ammonia-lyase (PAL) had lower SA levels, a reduced SAR response and decreased pathogen resistance following inoculation with TMV (Maher *et al.*, 1994; Felton *et al.*, 1999). Conversely, PAL over-expressors challenged with TMV displayed enhanced SA levels, an increased SAR response and improved pathogen resistance (Pallas *et al.*, 1996). In the PAL suppressed plants, pre-induction of systemic defence responses by a period of insect feeding resulted in higher endogenous JA levels and increased resistance to subsequent feeding by *Heliothis virescens* than in wild-type (Felton *et al.*, 1999). Following a similar pre-induction, PAL over-expressors had lower than wild-type levels of both endogenous JA and resistance to insect feeding. These observations provide strong evidence for crosstalk between SA and JA signalling pathways and of an antagonistic role for SA in modulating the JA-dependent response to herbivores.

A series of combined laboratory and field based experiments on tomato provide further evidence for crosstalk between SA and JA mediated

defence responses and the potential for compromised pest resistance following simultaneous induction of multiple defence pathways. Fidantsef *et al.*, (1999) showed that benzothiadiazole-7-carbothioic acid (BTH), an SA mimic used commercially as a crop protectant, induced expression of the PR gene *P4*, but not *PINII*, whereas JA induced *PINII* and not *P4* expression. Application of the two elicitors simultaneously reduced expression of both marker genes indicating a bi-directional negative interaction between the two signalling pathways. Furthermore, in greenhouse based experiments it was shown that application of BTH to tomato leaves increased resistance to the bacterial pathogen *P. syringae* and concurrently reduced the plants resistance to grazing by the herbivore *Helicoverpa zea* (Stout *et al.*, 1999). Taking this analysis into the field Thaler *et al.*, (1999) showed that the application of BTH to tomato plants enhanced resistance to *P. syringae* and decreased resistance to grazing by the herbivore *Spodoptera exigua* compared to that seen in untreated plants. Application of JA produced the opposite effect by enhancing resistance to *S. exigua* and reducing resistance to *P. syringae* infection. When the two elicitors were applied simultaneously resistance to either attacker was reduced compared to that observed following their separate application, indicating that crosstalk resulting in mutual inhibition was occurring between the two response pathways.

These experiments also demonstrate the need for caution in interpreting results from elicitor based experiments. The authors observed clear differences in patterns of gene expression and inducible resistance to *P. syringae* and *H. zea* between experiments employing chemical elicitors and those using the organisms themselves to elicit defence responses. For example, whereas BTH application simultaneously increased resistance to *P. syringae* and decreased it to *H. zea*, prior exposure to either *P. syringae* or *H. zea* subsequently increased the plant's resistance to both attackers (Stout *et al.*, 1999). *P. syringae*-induced resistance to *H. zea* may well be due to its induction of *PINII* and other wound-responsive proteins being mediated via an SA-independent pathway

(Pautot *et al.*, 1991; Fidantsef *et al.*, 1999; Stout *et al.*, 1999). These observations suggest that in tomato a complex network of defence signalling pathways exist and that in addition to antagonism, the concurrent activation of overlapping resistance pathways involving more than one defence elicitor is possible. It remains unclear whether *P. syringae* mediated induction of *PINII* is JA-dependent and it would be interesting to see whether *P. syringae* and other pathogens induce *PINII* expression in JA signalling mutants.

The studies described above provide strong evidence of mutual antagonism between SA and JA-dependent defence signalling pathways. However, there is also increasing evidence that SA and JA-dependent pathways can act synergistically under some conditions, suggesting that the regulation of defence signalling is extremely complex and finely tuned. van Wees *et al.*, (2000) demonstrated that in *Arabidopsis*, SA-dependent SAR and JA- and ethylene-dependent ISR could be coinduced with no evidence for crosstalk between the two pathways. They reported that coinduction led to greater resistance against the pathogen *P. syringae* than that obtained when either resistance pathway was induced on its own. Supporting these observations, another study found no evidence of antagonism between SA and JA when both were applied to *Arabidopsis*. The combined treatment afforded similar levels of protection against fungal pathogens to those observed following treatment by each elicitor separately (Thomma *et al.*, 1998). Several genes have been shown to be synergistically induced by SA and JA (Xu *et al.*, 1994; Schweizer *et al.*, 1997), and many other defence- and signalling-related genes have been identified as being responsive to both JA and SA (reviewed by Reymond and Farmer, 1998). Recently, Schenk *et al.*, (2000) used cDNA microarray technology to analyse expression levels of over 2000 putative defence genes from *Arabidopsis* following treatment with a number of elicitors, including MeJA and SA. They identified 192 that were induced by SA and 168 by MeJA, including 55 that appeared to be responsive to both elicitors. Further analysis using combinations of elicitors compared against single treatments should reveal interesting data about the extent of mutual antagonism that is occurring between SA and JA mediated defence responses.

It is clear from this conflicting evidence regarding defence signalling that plant defence can no longer be considered in terms of separate

wound/herbivore and pathogen response pathways and that the overall picture indicates a complex system of interacting and overlapping networks of responses. Despite the enormous range and variety of potential attackers, induction of the majority of plant defences appears to be activated through the individual or combined action of a small number of low molecular weight signalling molecules. Available evidence suggests that although the different resistance pathways induce resistance to different types of pathogens and herbivores, there is the potential for overlaps between these groups. There are many areas of defence signalling which remain poorly understood. What is the nature of the relationship between SA- and JA-mediated responses and how is it possible that SA can apparently both antagonise and enhance JA-dependent responses within the same tissue? Little is understood about the mechanisms controlling ISR nor of the role that JA and ethylene play in mediating this resistance. Similarly, it is not yet known how JA responses are differentially regulated with plant cells - the mechanisms controlling the response to wounding and pathogens clearly differ from those regulating ISR. Presumably, the spatial and temporal separation of the sites of biosynthesis and action of different signals during attack by pests and pathogens in the field is an important determinant of how and whether signalling pathways interact. This separation may occur at the whole plant level, the cellular level, or even the subcellular level. Addressing such issues in combination with large-scale analysis of gene expression patterns will be necessary to clarify when and where signalling cross-talk can be expected.

## IX. SUMMARY

Plants respond to wounding by initiating an array of different defences which are regulated by a complex network of inter- and intracellular signalling pathways. As more data emerge, it is becoming impossible to view wound signalling in terms of a small number of linear pathways, and it is currently difficult to produce an accurate generalisation of wound signalling events.

However, some common elements do emerge. Core signalling events initiated immediately following tissue damage include ion fluxes across the plasma membrane, an oxidative burst and the activation of protein phosphorylation cascades. Key among the later signals produced is jasmonic acid, which has a central role in mediating expression of direct defence genes and in volatile signal production. Ethylene is also an important signal, which acts as a modulator of JA responses as well as inducing defence responses in its own right. Volatile compounds are important signals for indirect defences against herbivores and are also potential elicitors of direct defences in unchallenged plant tissues. Other molecules that may be released upon tissue damage to act as elicitors of the wound response in both challenged and systemic leaves include cell wall degradation products and peptide hormones. Finally, when considering herbivore-induced wounding, one must also consider the signalling pathways involved in the perception of herbivore-derived elicitors of plant defence responses. Perhaps the most elusive signal whose identity remains to be proven is the systemically transmitted signal. An important contribution to this and other areas of wound signalling would be the identification of new mutants impaired in specific aspects of the wound response, perhaps along with a characterisation of wound responses in existing mutants of key signalling proteins in pathogen responses.

Many wound response signals are either shared with or appear to be antagonistic to those known to be important in pathogen responses. This issue is particularly important from an applied perspective, where it is desirable to identify agents that might act as effective crop protectants. There are also clear differences between species, illustrated by the opposing impact of ethylene on JA-dependent defences in *Arabidopsis* and tomato. This suggests that no one model system for wound signalling is sufficient, and it might be especially important to develop a model for cereal crops. Equally, we may need to move away from the concept of separate wound and pathogen signalling pathways altogether, and think more in terms of pathways controlled by different hormones and genes, such as the different responses regulated by salicylic acid and/or *NPR1* and by JA and ethylene. While recent work has attempted to address the question of cross-talk and antagonism between herbivore and pathogen resistance, the answers are equivocal. Future studies need to be addressed at the whole

organism level, rather than using exogenous applications of elicitors such as JA and SA where the natural temporal and spatial separation of events which inevitably occur during real plant-pathogen and plant-herbivore interactions are lost.

## REFERENCES

- Agrawal, A. A. 2000. Mechanisms, ecological consequences and agricultural implications of tri-trophic interactions. *Curr. Opin. Plant Biol.* **3**:329-335.
- Alarcon, J. J. and Malone, M. 1994. Substantial hydraulic signals are triggered by leaf-biting insects in tomato. *J. Exp. Bot.* **45**:953-957.
- Alborn, H. T., Turlings, T. C. J., Jones, T. H., Stenhagen, G., Loughrin, J. H., and Tumlinson, J. H. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* **276**:945-949.
- Alborn, H. T., Jones, T. H., Stenhagen, G. S., and Tumlinson, J. H. 2000. Identification and synthesis of volicitin and related components from beet armyworm oral secretions. *J. Chem. Ecol.* **26**:203-220.
- Arimura, G., Ozawa, R., Shimoda, T., Nishioka, T., Boland, W., and Takabayashi, J. 2000a. Herbivory-induced volatiles elicit defence genes in lima bean leaves. *Nature* **406**:512-515.
- Arimura, G., Tashiro, K., Kuhara, S., Nishioka, T., Ozawa, R., and Takabayashi, J. 2000b. Gene responses in bean leaves induced by herbivory and by herbivore-induced volatiles. *Biochem. Biophys. Res. Commun.* **277**:305-310.
- Baldwin, I. T., Zhang, Z. P., Diab, N., Ohnmeiss, T. E., McCloud, E. S., Lynds, G. Y., and Schmelz, E. A. 1997. Quantification, correlations and manipulations of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*. *Planta* **201**:397-404.
- Baldwin, I. T. 1998. Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl. Acad. Sci. U. S. A.* **95**:8113-8118.
- Baudouin, E., Meskiene, I., and Hirt, H. 1999. Unsaturated fatty acids inhibit MP2C, a protein phosphatase 2C involved in the wound-induced MAP kinase pathway regulation. *Plant J.* **20**:343-348.
- Bergey, D. R., Hoi, G. A., and Ryan, C. A. 1996. Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. *Proc. Natl. Acad. Sci. U. S. A.* **93**:12053-12058.
- Bergey, D. R. and Ryan, C. A. 1999a. Wound- and systemin-inducible calmodulin gene expression in tomato leaves. *Plant Mol. Biol.* **40**:815-823.

- Bergey, D. R., Orozco-Cardenas, M., de Moura, D. S., and Ryan, C. A. 1999b. A wound- and systemin-inducible polygalacturonase in tomato leaves. *Proc. Natl. Acad. Sci. U. S. A.* **96**:1756-1760.
- Bernasconi, M. L., Turlings, T. C. J., Ambrosetti, L., Bassetti, P., and Dorn, S. 1998. Herbivore-induced emissions of maize volatiles repel the corn leaf aphid, *Rhopalosiphum maidis*. *Entomol. Exp. Appl.* **87**:133-142.
- Bi, J. L. and Felton, G. W. 1995. Foliar oxidative stress and insect herbivory - primary compounds, secondary metabolites, and reactive oxygen species as components of induced resistance. *J. Chem. Ecol.* **21**:1511-1530.
- Birkenmeier, G. F. and Ryan, C. A. 1998. Wound signaling in tomato plants - Evidence that ABA is not a primary signal for defense gene activation. *Plant Physiol.* **117**:687-693.
- Blumwald, E., Aharon, G. S., and Lam, B. C. H. 1998. Early signal transduction pathways in plant-pathogen interactions. *Trends Plant Sci.* **3**:342-346.
- Bogre, L., Ligterink, W., Meskiene, I., Barker, P. J., Heberle-Bors, E., Huskisson, N. S., and Hirt, H. 1997. Wounding induces the rapid and transient activation of a specific MAP kinase pathway. *Plant Cell* **9**:75-83.
- Boland, W., Feng, Z., Donath, J., and Gabler, A. 1992. Are acyclic C-11 and C-16 homoterpenes plant volatiles indicating herbivory. *Naturwissenschaften* **79**:368-371.
- Bostock, R. M. 1999. Signal conflicts and synergies in induced resistance to multiple attackers. *Physiol. Mol. Plant Pathol.* **55**:99-109.
- Bouwmeester, H. J., Verstappen, F. W. A., Posthumus, M. A., and Dicke, M. 1999. Spider mite-induced (3S)-(E)-nerolidol synthase activity in cucumber and lima bean. The first dedicated step in acyclic C11-homoterpene biosynthesis. *Plant Physiol.* **121**:173-180.
- Bowles, D. 1998. Signal transduction in the wound response of tomato plants. *Phil. Trans. R. Soc. Lond. B* **353**:1495-1510.
- Braam, J. 1992. Regulation of expression of calmodulin and calmodulin-related genes by environmental stimuli in plants. *Cell Calcium* **13**:457-463.
- Brading, P. A., Hammond-Kosack, K. E., Parr, A., and Jones, J. D. G. 2000. Salicylic acid is not required for Cf-2- and Cf-9-dependent resistance of tomato to *Cladosporium fulvum*. *Plant J.* **23**:305-318.
- Bradley, D. J., Kjellbom, P., and Lamb, C. J. 1992. Elicitor-induced and wound-induced oxidative cross-linking of a proline-rich plant-cell wall protein - a novel, rapid defense response. *Cell* **70**:21-30.
- Cao, H., Bowling, S. A., Gordon, A. S., and Dong, X. N. 1994. Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* **6**:1583-1592.
- Cao, H., Glazebrook, J., Clarke, J. D., Volko, S., and Dong, X. N. 1997. The *Arabidopsis NPR1* gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. *Cell* **88**:57-63.
- Chandra, S., Stennis, M., and Low, P. S. 1997. Measurement of Ca<sup>2+</sup> fluxes during elicitation of the oxidative burst in aequorin-transformed tobacco cells. *J. Biol. Chem.* **272**:28274-28280.
- Clarke, J. D., Volko, S. M., Ledford, H., Ausubel, F. M., and Dong, X. N. 2000. Roles of salicylic acid, jasmonic acid, and ethylene in CPR-induced resistance in *Arabidopsis*. *Plant Cell* **12**:2175-2190.
- Constabel, C. P., Yip, L., and Ryan, C. A. 1998. Prosystemin from potato, black nightshade, and bell pepper: primary structure and biological activity of predicted systemin polypeptides. *Plant Mol. Biol.* **36**:55-62.
- Constabel, C. P. 1999. A survey of herbivory-inducible defensive proteins and phytochemicals. **In:** *Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.* pp. 137-166. Agrawal, A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Cosgrove, D. J., Gilroy, S., Kao, T., Ma, H., and Schultz, J. C. 2000. Meeting report: Plant signalling 2000. Cross talk among geneticists, physiologists, and ecologists. *Plant Physiol.* **124**:499-505.
- Creelman, R. A., Tierney, M. L., and Mullet, J. E. 1992. Jasmonic acid methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene-expression. *Proc. Natl. Acad. Sci. U. S. A.* **89**:4938-4941.
- Creelman, R. A. and Mullet, J. E. 1997. Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**:355-381.
- De Moraes, C. M., Lewis, W. J., Pare, P. W., Alborn, H. T., and Tumlinson, J. H. 1998. Herbivore-infested plants selectively attract parasitoids. *Nature* **393**:570-573.
- Delledonne, M., Xia, Y. J., Dixon, R. A., and Lamb, C. 1998. Nitric oxide functions as a signal in plant disease resistance. *Nature* **394**:585-588.
- Dempsey, D. A., Shah, J., and Klessig, D. F. 1999. Salicylic acid and disease resistance in plants. *Critical Reviews in Plant Sciences* **18**:547-575.
- Dicke, M. and Dijkman, H. 1992. Induced defense in detached uninfested plant leaves - effects on behavior of herbivores and their predators. *Oecologia* **91**:554-560.
- Dicke, M. 1994. Local and systemic production of volatile herbivore-induced terpenoids - their role in plant-carnivore mutualism. *J. Plant Physiol.* **143**:465-472.
- Dicke, M., Takabayashi, J., Posthumus, M. A., Schutte, C., and Krips, O. E. 1998. Plant-phytoseiid interactions mediated by herbivore-induced plant volatiles: variation in production of cues and in responses of predatory mites. *Exp. Appl. Acarol.* **22**:311-333.
- Dicke, M., Gols, R., Ludeking, D., and Posthumus, M. A. 1999. Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *J. Chem. Ecol.* **25**:1907-1922.
- Dixon, R. A. and Paiva, N. L. 1995. Stress-induced phenylpropanoid metabolism. *Plant Cell* **7**:1085-1097.
- Doares, S. H., Syrovets, T., Weiler, E. W., and Ryan, C. A. 1995a. Oligogalacturonides and chitosan activate plant defensive genes through the

- octadecanoid pathway. *Proc. Natl. Acad. Sci. U. S. A.* **92**:4095-4098.
- Doares, S. H., Narváez-Vásquez, J., Conconi, A., and Ryan, C. A. 1995b. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiol.* **108**:1741-1746.
- Doherty, H. M., Selvendran, R. R., and Bowles, D. J. 1988. The wound response of tomato plants can be inhibited by aspirin and related hydroxybenzoic acids. *Physiol. Mol. Plant Pathol.* **33**:377-384.
- Doherty, H. M. and Bowles, D. J. 1990. The role of pH and ion-transport in oligosaccharide-induced proteinase-inhibitor accumulation in tomato plants. *Plant Cell Env.* **13**:851-855.
- Dombrowski, J. E., Pearce, G., and Ryan, C. A. 1999. Proteinase inhibitor-inducing activity of the prohormone prosystemin resides exclusively in the C-terminal systemin domain. *Proc. Natl. Acad. Sci. U. S. A.* **96**:12947-12952.
- Dong, X. N. 1998. SA, JA, ethylene, and disease resistance in plants. *Curr. Opin. Plant Biol.* **1**:316-323.
- Dong, H. and Beer, S. V. 2000. Riboflavin induces disease resistance in plants by activating a novel signal transduction pathway. *Phytopathology* **90**:801-811.
- Durner, J., Wendehenne, D., and Klessig, D. F. 1998. Defense gene induction in tobacco by nitric oxide, cyclic GMP and cyclic ADP ribose. *Proc. Natl. Acad. Sci. U. S. A.* **95**:10328-10333.
- Durrant, W. E., Rowland, O., Piedras, P., Hammond-Kosack, K. E., and Jones, J. D. G. 2000. cDNA-AFLP reveals a striking overlap in race-specific resistance and wound response gene expression profiles. *Plant Cell* **12**:963-977.
- Ebel, J. and Mithöfer, A. 1998. Early events in the elicitation of plant defence. *Planta* **206**:335-348.
- Ellis, J., Dodds, P., and Pryor, T. 2000. The generation of plant disease resistance gene specificities. *Trends Plant Sci.* **5**:373-379.
- Epple, P., Apel, K., and Bohlmann, H. 1995. An *Arabidopsis thaliana* thionin gene is inducible via a signal transduction pathway different from that for pathogenesis related proteins. *Plant Physiol.* **109**:813-820.
- Epple, P., Apel, K., and Bohlmann, H. 1997. Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*. *Plant Cell* **9**:509-520.
- Farmer, E. E. and Ryan, C. A. 1990. Interplant communication - airborne methyl jasmonate induces synthesis of proteinase-inhibitors in plant leaves. *Proc. Natl. Acad. Sci. U. S. A.* **87**:7713-7716.
- Farmer, E. E., Johnson, R. R., and Ryan, C. A. 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. *Plant Physiol.* **98**:995-1002.
- Felix, G. and Boller, T. 1995. Systemin induces rapid ion fluxes and ethylene biosynthesis in *Lycopersicon peruvianum* cells. *Plant J.* **7**:381-389.
- Felton, G. W., Korth, K. L., Bi, J. L., Wesley, S. V., Huhman, D. V., Mathews, M. C., Murphy, J. B., Lamb, C., and Dixon, R. A. 1999. Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. *Curr. Biol.* **9**:317-320.
- Felton, G. W. and Eichenseer, H. 1999. Herbivore saliva and its effects on plant defense against herbivores and pathogens. In: *Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.* pp. 19-36. Agrawal, A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Fidantsef, A. L., Stout, M. J., Thaler, J. S., Duffey, S. S., and Bostock, R. M. 1999. Signal interactions in pathogen and insect attack: expression of lipoxygenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato, *Lycopersicon esculentum*. *Physiol. Mol. Plant Pathol.* **54**:97-114.
- Frey, M., Stettner, C., Pare, P. W., Schmelz, E. A., Tumlinson, J. H., and Gierl, A. 2000. An herbivore elicitor activates the gene for indole emission in maize. *Proc. Natl. Acad. Sci. U. S. A.* **97**:14801-14806.
- Fujimoto, S. Y., Ohta, M., Usui, A., Shinshi, H., and Ohme-Takagi, M. 2000. *Arabidopsis* ethylene-responsive element binding factors act as transcriptional activators or repressors of GCC box-mediated gene expression. *Plant Cell* **12**:393-404.
- Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* **261**:754-756.
- Garcia-Ponce, B. and Rocha-Sosa, M. 2000. The octadecanoid pathway is required for pathogen-induced multi-functional acetyl-CoA carboxylase accumulation in common bean (*Phaseolus vulgaris* L.). *Plant Science* **157**:181-190.
- Glazebrook, J. 1999. Genes controlling expression of defense responses in *Arabidopsis*. *Curr. Opin. Plant Biol.* **2**:280-286.
- Grant, M. and Mansfield, J. 1999. Early events in host-pathogen interactions. *Curr. Opin. Plant Biol.* **2**:312-319.
- Green, T. R. and Ryan, C. A. 1972. Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. *Science* **175**:776-777.
- Guan, L. M. and Scandalios, J. G. 2000. Hydrogen peroxide-mediated catalase gene expression in response to wounding. *Free Radic. Biol. Med.* **28**:1182-1190.
- Guerrieri, E., Poppy, G. M., Powell, W., Tremblay, E., and Pennacchio, F. 1999. Induction and systemic release of herbivore-induced plant volatiles mediating in-flight orientation of *Aphidius ervi*. *J. Chem. Ecol.* **25**:1247-1261.
- Guo, A. L., Salih, G., and Klessig, D. F. 2000. Activation of a diverse set of genes during the tobacco resistance response to TMV is independent of salicylic acid; induction of a subset is also ethylene independent. *Plant J.* **21**:409-418.

- Gupta, V., Willits, M. G., and Glazebrook, J. 2000. *Arabidopsis thaliana* EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: Evidence for inhibition of jasmonic acid signaling by SA. *Mol. Plant Microbe Interact.* **13**:503-511.
- Hahlbrock, K. and Schell, D. 1989. Physiology and molecular-biology of phenylpropanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **40**:347-369.
- Halitschke, R., Kessler, A., Kahl, J., Lorenz, A., and Baldwin, I. T. 2000. Ecophysiological comparison of direct and indirect defenses in *Nicotiana attenuata*. *Oecologia* **124**:408-417.
- Hammerschmidt, R. 1999. Induced disease resistance: how do induced plants stop pathogens? *Physiol. Mol. Plant Pathol.* **55**:77-84.
- Hammerschmidt, R. and Smith-Becker, J. A. 1999. The role of salicylic acid in plant defense. In: *Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.* pp. 37-54. Agrawal, A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Hammond-Kosack, K. E. and Jones, J. D. G. 1996. Resistance gene-dependent plant defense responses. *Plant Cell* **8**:1773-1791.
- Harms, K., Atzorn, R., Brash, A., Kühn, H., Wasternack, C., Willmitzer, L., and Peña-Cortés, H. 1995. Expression of a flax allene oxide synthase cDNA leads to increased endogenous jasmonic acid (JA) levels in transgenic potato plants but not to a corresponding activation of JA-responding genes. *Plant Cell* **7**:1645-1654.
- Hatcher, P. E. and Paul, N. D. 2000. On integrating molecular and ecological studies of plant resistance: variety of mechanisms and breadth of antagonists. *Journal of Ecology* **88**:702-706.
- Heath, M. C. 2000. Nonhost resistance and nonspecific plant defenses. *Curr. Opin. Plant Biol.* **3**:315-319.
- Heo, W. D., Lee, S. H., Kim, M. C., Kim, J. C., Chung, W. S., Chun, H. J., Lee, K. J., Park, C. Y., Park, H. C., Choi, J. Y., and Cho, M. J. 1999. Involvement of specific calmodulin isoforms in salicylic acid-independent activation of plant disease resistance responses. *Proc. Natl. Acad. Sci. U. S. A.* **96**:766-771.
- Herde, O., Fuss, H., Peña-Cortés, H., and Fisahn, J. 1995. Proteinase inhibitor II gene expression induced by electrical stimulation and control of photosynthetic activity in tomato plants. *Plant Cell Physiol.* **36**:737-742.
- Herde, O., Peña-Cortés, H., Willmitzer, L., and Fisahn, J. 1998. Time-resolved analysis of signals involved in systemic induction of Pin2 gene expression. *Botanica Acta* **111** :383-389.
- Howe, G. A., Lightner, J., Browse, J., and Ryan, C. A. 1996. An octadecanoid pathway mutant (JL5) of tomato is compromised in signaling for defense against insect attack. *Plant Cell* **8**:2067-2077.
- Howe, G. A. and Ryan, C. A. 1999. Suppressors of systemin signaling identify genes in the tomato wound response pathway. *Genetics* **153**:1411-1421.
- Hush, J. M., Newman, I. A., and Overall, R. L. 1992. Utilization of the vibrating probe and ion-selective microelectrode techniques to investigate electrophysiological responses to wounding in pea roots. *J. Exp. Bot.* **43**:1251-1257.
- Jacinto, T., McGurl, B., Franceschi, V., Delano-Freier, J., and Ryan, C. A. 1997. Tomato prosystemin promoter confers wound-inducible, vascular bundle-specific expression of the  $\beta$ -glucuronidase gene in transgenic tomato plants. *Planta* **203**:406-412.
- Johnson, P. R. and Ecker, J. R. 1998. The ethylene gas signal transduction pathway: A molecular perspective. *Annu. Rev. Genet.* **32**:227-254.
- Jonak, C., Beisteiner, D., Beyerly, J., and Hirt, H. 2000. Wound-induced expression and activation of WIG, a novel glycogen synthase kinase 3. *Plant Cell* **12**:1467-1475.
- Jones, C. G., Hopper, R. F., Coleman, J. S., and Krischik, V. A. 1993. Control of systemically induced herbivore resistance by plant vascular architecture. *Oecologia* **93**:452-456.
- Jones, D. A. and Jones, J. D. G. 1997. The role of leucine-rich repeat proteins in plant defences. *Advances in Botanical Research* **24**:89-167.
- Kachroo, P., Yoshioka, K., Shah, J., Dooner, H. K., and Klessig, D. F. 2000. Resistance to turnip crinkle virus in *Arabidopsis* is regulated by two host genes and is salicylic acid dependent but *NPR1*, ethylene, and jasmonate independent. *Plant Cell* **12**:677-690.
- Kahl, J., Siemens, D. H., Aerts, R. J., Gabler, R., Kuhnemann, F., Preston, C. A., and Baldwin, I. T. 2000. Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta* **210**:336-342.
- Keller, T., Damude, H. G., Werner, D., Doerner, P., Dixon, R. A., and Lamb, C. 1998. A plant homolog of the neutrophil NADPH oxidase gp91<sup>phox</sup> subunit gene encodes a plasma membrane protein with Ca<sup>2+</sup> binding motifs. *Plant Cell* **10**:255-266.
- Kende, H. 1993. Ethylene biosynthesis. *Annu. Rev. Plant Physiol.* **44**:283-307.
- Klessig, D. F., Durner, J., Noad, R., Navarre, D. A., Wendehenne, D., Kumar, D., Zhou, J. M., Shah, J., Zhang, S. Q., Kachroo, P., Trifa, Y., Pontier, D., Lam, E., and Silva, H. 2000. Nitric oxide and salicylic acid signaling in plant defense. *Proc. Natl. Acad. Sci. U. S. A.* **97**:8849-8852.
- Knight, M. R., Read, N. D., Campbell, A. K., and Trewavas, A. J. 1993. Imaging calcium dynamics in living plants using semisynthetic recombinant aequorins. *J. Cell Biol.* **121**:83-90.
- Knoester, M., van Loon, L. C., van den Heuvel, J., Hennig, J., Bol, J. F., and Linthorst, H. J. M. 1998. Ethylene-insensitive tobacco lacks nonhost resistance against soil-borne fungi. *Proc. Natl. Acad. Sci. U. S. A.* **95**:1933-1937.
- Koch, T., Krumm, T., Jung, V., Engelberth, J., and Boland, W. 1999. Differential induction of plant volatile biosynthesis in the lima bean by early and late intermediates of the octadecanoid signaling pathway. *Plant Physiol.* **121**:153-162.
- Korth, K. L. and Dixon, R. A. 1997. Evidence for chewing insect-specific molecular events

- distinct from a general wound response in leaves. *Plant Physiol.* **115**:1299-1305.
- Kovtun, Y., Chiu, W. L., Tena, G., and Sheen, J. 2000. Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. *Proc. Natl. Acad. Sci. U. S. A.* **97**:2940-2945.
- Kozlowski, G., Buchala, A., and Metraux, J. P. 1999. Methyl jasmonate protects Norway spruce [*Picea abies* (L.) Karst.] seedlings against *Pythium ultimum* Trow. *Physiol. Mol. Plant Pathol.* **55**:53-58.
- Krumm, T., Bandemer, K., and Boland, W. 1995. Induction of volatile biosynthesis in the Lima bean (*Phaseolus lunatus*) by leucine and isoleucine conjugates of 1-oxo- and 1-hydroxyindan-4-carboxylic acid: Evidence for amino acid conjugates of jasmonic acid as intermediates in the octadecanoid signalling pathway. *FEBS Lett.* **377**:523-529.
- Kudla, J., Xu, Q., Harter, K., Gruissem, W., and Luan, S. 1999. Genes for calcineurin B-like proteins in *Arabidopsis* are differentially regulated by stress signals. *Proc. Natl. Acad. Sci. U. S. A.* **96**:4718-4723.
- Kumar, D. and Klessig, D. F. 2000. Differential induction of tobacco MAP kinases by the defense signals nitric oxide, salicylic acid, ethylene, and jasmonic acid. *Mol. Plant Microbe Interact.* **13**:347-351.
- Lamb, C. and Dixon, R. A. 1997. The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**:251-275.
- Laudert, D. and Weiler, E. W. 1998. Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling. *Plant J.* **15**:675-684.
- Lee, S. H., Lee, M. H., Chung, W. I., and Liu, J. R. 1998. WAPK, a Ser/Thr protein kinase gene of *Nicotiana tabacum*, is uniquely regulated by wounding, abscisic acid and methyl jasmonate. *Mol. Gen. Genet.* **259**:516-522.
- Léon, J., Rojo, E., Titarenko, E., and Sánchez-Serrano, J. J. 1998. Jasmonic acid-dependent and -independent wound signal transduction pathways are differentially regulated by Ca<sup>2+</sup>/calmodulin in *Arabidopsis thaliana*. *Mol. Gen. Genet.* **258**:412-419.
- Léon, J. and Sánchez-Serrano, J. J. 1999. Molecular biology of jasmonic acid biosynthesis in plants. *Plant Physiol. Biochem.* **37**:373-380.
- Leyser, O. 1998. Auxin signalling: Protein stability as a versatile control target. *Curr. Biol.* **8**:R305-R307.
- Lightner, J., Pearce, G., Ryan, C. A., and Browse, J. 1993. Isolation of signaling mutants of tomato (*Lycopersicon esculentum*). *Mol. Gen. Genet.* **241**:595-601.
- Liu, D. R., Li, N., Dubes, S., Kalinski, A., Herman, E., and Mattoo, A. K. 1993. Molecular characterization of a rapidly and transiently wound-induced soybean (*Glycine max* L.) gene encoding 1-aminocyclopropane-1-carboxylate synthase. *Plant Cell Physiol.* **34**:1151-1157.
- Lyon, G. D. and Newton, A. C. 1999. Implementation of elicitor mediated induced resistance in agriculture. In: *Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.* pp. 299-318. Agrawal, A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Maeda, T., Takabayashi, J., Yano, S., and Takafuji, A. 2000. Effects of light on the tritrophic interaction between kidney bean plants, two-spotted spider mites and predatory mites, *Amblyseius womersleyi* (Acari : Phytoseiidae). *Exp. Appl. Acarol.* **24**:415-425.
- Maher, E. A., Bate, N. J., NI, W. T., Elkind, Y., Dixon, R. A., and Lamb, C. J. 1994. Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. *Proc. Natl. Acad. Sci. U. S. A.* **91**:7802-7806.
- Maleck, K. and Dietrich, R. A. 1999. Defense on multiple fronts: How do plants cope with diverse enemies? *Trends Plant Sci.* **4**:215-219.
- Malone, M. and Stankovic, B. 1991. Surface-potentials and hydraulic signals in wheat leaves following localized wounding by heat. *Plant Cell Env.* **14**:431-436.
- Malone, M., Palumbo, L., Boari, F., Monteleone, M., and Jones, H. G. 1994a. The relationship between wound-induced proteinase inhibitors and hydraulic signals in tomato seedlings. *Plant Cell Env.* **17**:81-87.
- Malone, M., Alarcon, J. J., and Palumbo, L. 1994b. An hydraulic interpretation of rapid, long-distance wound signaling in the tomato. *Planta* **193**:181-185.
- Malone, M. and Alarcon, J. J. 1995. Only xylem-borne factors can account for systemic wound signaling in the tomato plant. *Planta* **196**:740-746.
- Mathieu, Y., Kurkdjian, A., Xia, H., Guern, J., Koller, A., Spiro, M. D., O'Neill, M., Albersheim, P., and Darvill, A. 1991. Membrane responses induced by oligogalacturonides in suspension-cultured tobacco cells. *Plant J.* **1**:333-343.
- Mattiacci, L., Dicke, M., and Posthumus, M. A. 1995.  $\beta$ -glucosidase: an elicitor of herbivore-induced plant odor that attracts host-searching parasitic wasps. *Proc. Natl. Acad. Sci. U. S. A.* **92**:2036-2040.
- Mayland, H., Margolies, D. C., and Charlton, R. E. 2000. Local and distant prey-related cues influence when an acarine predator leaves a prey patch. *Entomol. Exp. Appl.* **96**:245-252.
- McCloud, E. S. and Baldwin, I. T. 1997. Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*. *Planta* **203**:430-435.
- McConn, M., Creelman, R. A., Bell, E., Mullet, J. E., and Browse, J. 1997. Jasmonate is essential for insect defense in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* **94**:5473-5477.
- McGurl, B., Pearce, G., Orozco-Cardenas, M., and Ryan, C. A. 1992. Structure, expression, and antisense inhibition of the systemin precursor gene. *Science* **255**:1570-1573.
- McGurl, B., Orozco-Cardenas, M., Pearce, G., and Ryan, C. A. 1994. Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively

- induces proteinase inhibitor synthesis. *Proc. Natl. Acad. Sci. U. S. A.* **91**:9799-9802.
- Meindl, T., Boller, T., and Felix, G. 1998. The plant wound hormone systemin binds with the N-terminal part to its receptor but needs the C-terminal part to activate it. *Plant Cell* **10**:1561-1570.
- Messiaen, J., Read, N. D., van Cutsem, P., and Trewavas, A. J. 1993. Cell wall oligogalacturonides increase cytosolic free calcium in carrot protoplasts. *J. Cell Sci.* **104**:365-371.
- Messiaen, J. and van Cutsem, P. 1994. Pectic signal transduction in carrot cells - membrane, cytosolic and nuclear responses induced by oligogalacturonides. *Plant Cell Physiol.* **35**:677-689.
- Mizoguchi, T., Irie, K., Hirayama, T., Hayashida, N., Yamaguchi-Shinozaki, K., Matsumoto, K., and Shinozaki, K. 1996. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* **93**:765-769.
- Moyen, C. and Johannes, E. 1996. Systemin transiently depolarizes the tomato mesophyll cell membrane and antagonizes fusicoccin-induced extracellular acidification of mesophyll tissues. *Plant Cell Env.* **19**:464-470.
- Moyen, C., Hammond-Kosack, K. E., Jones, J., Knight, M. R., and Johannes, E. 1998. Systemin triggers an increase of cytoplasmic calcium in tomato mesophyll cells: Ca<sup>2+</sup> mobilization from intra- and extracellular compartments. *Plant Cell Env.* **21**:1101-1111.
- Narváez-Vásquez, J., Orozco-Cardenas, M. L., and Ryan, C. A. 1994. A sulfhydryl reagent modulates systemic signaling for wound-induced and systemin-induced proteinase inhibitor synthesis. *Plant Physiol.* **105**:725-730.
- Narváez-Vásquez, J., Florin-Christensen, J., and Ryan, C. A. 1999. Positional specificity of a phospholipase A activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. *Plant Cell* **11**:2249-2260.
- Niki, T., Mitsuhara, I., Seo, S., Ohtsubo, N., and Ohashi, Y. 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. *Plant Cell Physiol.* **39**:500-507.
- O'Donnell, P. J., Calvert, C., Atzorn, R., Wasternack, C., Leyser, H. M. O., and Bowles, D. J. 1996. Ethylene as a signal mediating the wound response of tomato plants. *Science* **274**:1914-1917.
- O'Donnell, P. J., Truesdale, M. R., Calvert, C. M., Dorans, A., Roberts, M. R., and Bowles, D. J. 1998. A novel tomato gene that rapidly responds to wound- and pathogen-related signals. *Plant J.* **14**:137-142.
- Ohme-Takagi, M. and Shinshi, H. 1995. Ethylene-inducible DNA binding proteins that interact with an ethylene-responsive element. *Plant Cell* **7**:173-182.
- Olson, P. D. and Varner, J. E. 1993. Hydrogen peroxide and lignification. *Plant J.* **4**:887-892.
- Omer, A. D., Thaler, J. S., Granett, J., and Karban, R. 2000. Jasmonic acid induced resistance in grapevines to a root and leaf feeder. *Journal of Economic Entomology* **93**:840-845.
- Orians, C. M., Pomerleau, J., and Ricco, R. 2000. Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. *J. Chem. Ecol.* **26** :471-485.
- Orozco-Cardenas, M., McGurl, B., and Ryan, C. A. 1993. Expression of an antisense prosystemin gene in tomato plants reduces resistance towards *Manduca sexta* larvae. *Proc. Natl. Acad. Sci. U. S. A.* **90**:8273-8276.
- Orozco-Cardenas, M. and Ryan, C. A. 1999. Hydrogen peroxide is generated systemically in plant leaves by wounding and systemin via the octadecanoid pathway. *Proc. Natl. Acad. Sci. U. S. A.* **96**:6553-6557.
- Ozawa, R., Arimura, G., Takabayashi, J., Shimoda, T., and Nishioka, T. 2000. Involvement of jasmonate- and salicylate-related signaling pathways for the production of specific herbivore-induced volatiles in plants. *Plant Cell Physiol.* **41**:391-398.
- Paiva, N. L. 2000. An introduction to the biosynthesis of chemicals used in plant-microbe communication. *J. Plant Growth Reg.* **19**:131-143.
- Pallas, J. A., Paiva, N. L., Lamb, C., and Dixon, R. A. 1996. Tobacco plants epigenetically suppressed in phenylalanine ammonia-lyase expression do not develop systemic acquired resistance in response to infection by tobacco mosaic virus. *Plant J.* **10**:281-293.
- Paré, P. W. and Tumlinson, J. H. 1997a. Induced synthesis of plant volatiles. *Nature* **385**:30-31.
- Paré, P. W. and Tumlinson, J. H. 1997b. *De novo* biosynthesis of volatiles induced by insect herbivory in cotton plants. *Plant Physiol.* **114**:1161-1167.
- Paré, P. W. and Tumlinson, J. H. 1998. Cotton volatiles synthesized and released distal to the site of insect damage. *Phytochem.* **47**:521-526.
- Paré, P. W., Alborn, H. T., and Tumlinson, J. H. 1998. Concerted biosynthesis of an insect elicitor of plant volatiles. *Proc. Natl. Acad. Sci. U. S. A.* **95**:13971-13975.
- Paré, P. W., Lewis, W. J., and Tumlinson, J. H. 1999. Induced plant volatiles: Biochemistry and effects on parasitoids. **In:** *Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.* pp. 167-180. Agrawal, A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Paré, P. W. and Tumlinson, J. H. 1999. Plant volatiles as a defense against insect herbivores. *Plant Physiol.* **121**:325-331.
- Paul, N. D., Hatcher, P. E., and Taylor, J. E. 2000. Coping with multiple enemies: an integration of molecular and ecological perspectives. *Trends Plant Sci.* **5**:220-225.
- Pautot, V., Holzer, F. M., and Walling, L. L. 1991. Differential expression of tomato proteinase inhibitor-I and inhibitor-II genes during

- bacterial pathogen invasion and wounding. *Mol. Plant Microbe Interact.* **4**:284-292.
- Pearce, G., Strydom, D., Johnson, S., and Ryan, C. A. 1991. A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science* **253**:895-898.
- Pearce, G., Johnson, S., and Ryan, C. A. 1993. Structure-activity of deleted and substituted systemin, an 18-amino acid polypeptide inducer of plant defensive genes. *J. Biol. Chem.* **268**:212-216.
- Peña-Cortés, H., Sánchez-Serrano, J. J., Mertens, R., Willmitzer, L., and Prat, S. 1989. Abscisic-acid is involved in the wound-induced expression of the proteinase inhibitor-II gene in potato and tomato. *Proc. Natl. Acad. Sci. U. S. A.* **86**:9851-9855.
- Peña-Cortés, H., Willmitzer, L., and Sánchez-Serrano, J. J. 1991. Abscisic acid mediates wound induction but not developmental-specific expression of the proteinase inhibitor-II gene family. *Plant Cell* **3**:963-972.
- Peña-Cortés, H., Albrecht, T., Prat, S., Weiler, E. W., and Willmitzer, L. 1993. Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta* **191**:123-128.
- Peña-Cortés, H., Fisahn, J., and Willmitzer, L. 1995. Signals involved in wound-induced proteinase inhibitor II gene expression in tomato and potato plants. *Proc. Natl. Acad. Sci. U. S. A.* **92**:4106-4113.
- Penninckx, I. A. M. A., Eggermont, K., Terras, F. R. G., Thomma, B. P. H. J., De Samblanx, G. W., Buchala, A., Metraux, J. P., Manners, J. M., and Broekaert, W. F. 1996. Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. *Plant Cell* **8**:2309-2323.
- Penninckx, I. A. M. A., Thomma, B. P. H. J., Buchala, A., Metraux, J. P., and Broekaert, W. F. 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. *Plant Cell* **10**:2103-2113.
- Pieterse, C. M. J., van Wees, S. C. M., Hoffland, E., van Pelt, J. A., and van Loon, L. C. 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* **8**:1225-1237.
- Pieterse, C. M. J., van Wees, S. C. M., van Pelt, J. A., Knoester, M., Laan, R., Gerrits, N., Weisbeek, P. J., and van Loon, L. C. 1998. A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell* **10**:1571-1580.
- Pieterse, C. M. J. and van Loon, L. C. 1999. Salicylic acid-independent plant defence pathways. *Trends Plant Sci.* **4**:52-58.
- Preston, C. A., Lewandowski, C., Enyedi, A. J., and Baldwin, I. T. 1999. Tobacco mosaic virus inoculation inhibits wound-induced jasmonic acid-mediated responses within but not between plants. *Planta* **209**:87-95.
- Rao, A., Vinson, S. B., Gilstrap, F. E., and Michels, G. J. 1999. Response of an aphid parasitoid, *Aphelinus asychis* to its host, plant, host-plant complex, and to malathion. *Entomol. Exp. Appl.* **91**:449-456.
- Reymond, P. and Farmer, E. E. 1998. Jasmonate and salicylate as global signals for defense gene expression. *Curr. Opin. Plant Biol.* **1**:404-411.
- Reymond, P., Weber, H., Damond, M., and Farmer, E. E. 2000. Differential gene expression in response to mechanical wounding and insect feeding in *Arabidopsis*. *Plant Cell* **12**:707-719.
- Rhodes, J. D., Thain, J. F., and Wildon, D. C. 1996. The pathway for systemic electrical signal conduction in the wounded tomato plant. *Planta* **200**:50-57.
- Rhodes, J. D., Thain, J. F., and Wildon, D. C. 1999. Evidence for physically distinct systemic signalling pathways in the wounded tomato plant. *Ann. Bot.* **84**:109-116.
- Roberts, M. R. and Bowles, D. J. 1999. Fusicoccin, 14-3-3 proteins, and defense responses in tomato plants. *Plant Physiol* **119**:1243-1250.
- Rojo, E., Titarenko, E., León, J., Berger, S., Vancanneyt, G., and Sánchez-Serrano, J. J. 1998. Reversible protein phosphorylation regulates jasmonic acid-dependent and -independent wound signal transduction pathways in *Arabidopsis thaliana*. *Plant J.* **13**:153-165.
- Rojo, E., León, J., and Sánchez-Serrano, J. J. 1999. Cross-talk between wound signalling pathways determines local versus systemic gene expression in *Arabidopsis thaliana*. *Plant J.* **20**:135-142.
- Romeis, T., Piedras, P., Zhang, S. Q., Klessig, D. F., Hirt, H., and Jones, J. D. G. 1999. Rapid Avr9- and Cf-9-dependent activation of MAP kinases in tobacco cell cultures and leaves: Convergence of resistance gene, elicitor, wound, and salicylate responses. *Plant Cell* **11**:273-287.
- Ronald, P. C. 1998. Resistance gene evolution. *Curr. Opin. Plant Biol.* **1**:294-298.
- Röse, U. S. R., Manukian, A., Heath, R. R., and Tumlinson, J. H. 1996. Volatile semiochemicals released from undamaged cotton leaves - A systemic response of living plants to caterpillar damage. *Plant Physiol.* **111**:487-495.
- Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H. Y., and Hunt, M. D. 1996. Systemic acquired resistance. *Plant Cell* **8**:1809-1819.
- Ryan, C. A. 1990. Proteinase inhibitors in plants: genes for improving defenses against insects and pathogens. *Annu. Rev. Phytopathol.* **28**:425-449.
- Ryan, C. A. and Pearce, G. 1998. Systemin: A polypeptide signal for plant defensive genes. *Annu. Rev. Cell Dev. Biol.* **14**:1-17.
- Ryan, C. A. 2000. The systemin signalling pathway: differential activation of plant defensive genes. *Biochim. Biophys. Acta* **1477**:112-121.
- Sabelis, M., Janssen, A., Pallini, A., Venzon, M., Bruin, J., Drukker, B., and Scutareanu, P. 1999. Behavioral responses of predatory and herbivory arthropods to induced plant volatiles: From evolutionary ecology to agricultural applications. In: *Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.* pp. 269-296. Agrawal,

- A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Sano, H., Seo, S., Orudjev, E., Youssefian, S., Ishizuka, K., and Ohashi, Y. 1994. Expression of the gene for a small GTP-binding protein in transgenic tobacco elevates endogenous cytokinin levels, abnormally induces salicylic acid in response to wounding, and increases resistance to tobacco mosaic virus infection. *Proc. Natl. Acad. Sci. U. S. A.* **91**:10556-10560.
- Sano, H., Seo, S., Koizumi, N., Niki, T., Iwamura, H., and Ohashi, Y. 1996. Regulation by cytokinins of endogenous levels of jasmonic and salicylic acids in mechanically wounded tobacco plants. *Plant Cell Physiol.* **37**:762-769.
- Schaller, A. and Oecking, C. 1999. Modulation of plasma membrane H<sup>+</sup>-ATPase activity differentially activates wound and pathogen defense responses in tomato plants. *Plant Cell* **11**:263-272.
- Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S. C., and Manners, J. M. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proc. Natl. Acad. Sci. U. S. A.* **97**:11655-11660.
- Schweizer, P., Buchala, A., Silverman, P., Seskar, M., Raskin, I., and Metraux, J. P. 1997. Jasmonate-inducible genes are activated in rice by pathogen attack without a concomitant increase in endogenous jasmonic acid levels. *Plant Physiol.* **114**:79-88.
- Seo, S., Okamoto, N., Seto, H., Ishizuka, K., Sano, H., and Ohashi, Y. 1995. Tobacco map kinase - a possible mediator in wound signal-transduction pathways. *Science* **270**:1988-1992.
- Seo, S., Sano, H., and Ohashi, Y. 1999. Jasmonate-based wound signal transduction requires activation of WIPK, a tobacco mitogen-activated protein kinase. *Plant Cell* **11**:289-298.
- Shen, B. Z., Zheng, Z. W., and Dooner, H. K. 2000. A maize sesquiterpene cyclase gene induced by insect herbivory and volicitin: Characterization of wild-type and mutant alleles. *Proc. Natl. Acad. Sci. U. S. A.* **97**:14807-14812.
- Shetty, H. S. and Kumar, V. U. 1999. Signaling in plants during induction of resistance against pathogens. *Current Science* **76**:640-646.
- Showalter, A. M. 1993. Structure and function of plant cell wall proteins. *Plant Cell* **5**:9-23.
- Shulaev, V., Silverman, P., and Raskin, I. 1997. Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature* **386**:738-738.
- Snedden, W. A. and Fromm, H. 1998. Calmodulin, calmodulin-related proteins and plant responses to the environment. *Trends Plant Sci.* **3**:299-304.
- Somssich, I. E. and Hahlbrock, K. 1998. Pathogen defence in plants - a paradigm of biological complexity. *Trends Plant Sci.* **3**:86-90.
- Spiteller, D., Dettner, K., and Boland, W. 2000. Gut bacteria may be involved in interactions between plants, herbivores and their predators: Microbial biosynthesis of N-acylglutamine surfactants as elicitors of plant volatiles. *Biol. Chem.* **381**:755-762.
- Stahl, E. A. and Bishop, J. G. 2000. Plant-pathogen arms races at the molecular level. *Curr. Opin. Plant Biol.* **3**:299-304.
- Stankovic, B. and Davies, E. 1996. Both action potentials and variation potentials induce proteinase inhibitor gene expression in tomato. *FEBS Lett.* **390**:275-279.
- Stankovic, B. and Davies, E. 1997. Intercellular communication in plants: electrical stimulation of proteinase inhibitor gene expression in tomato. *Planta* **202**:402-406.
- Stankovic, B. and Davies, E. 1998. The wound response in tomato involves rapid growth and electrical responses, systemically up-regulated transcription of proteinase inhibitor and calmodulin and down-regulated translation. *Plant Cell Physiol.* **39**:268-274.
- Staswick, P. E., Yuen, G. Y., and Lehman, C. C. 1998. Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare*. *Plant J.* **15**:747-754.
- Stotz, H. U., Kroymann, J., and Mitchell-Olds, T. 1999. Plant-insect interactions. *Curr. Opin. Plant Biol.* **2**:268-272.
- Stotz, H. U., Pittendrigh, B. R., Kroymann, J., Weniger, K., Fritsche, J., Bauke, A., and Mitchell-Olds, T. 2000. Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of *Arabidopsis* against Egyptian cotton worm but not diamondback moth. *Plant Physiol.* **124**:1007-1017.
- Stout, M. J., Fidantsef, A. L., Duffey, S. S., and Bostock, R. M. 1999. Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. *Physiol. Mol. Plant Pathol.* **54**:115-130.
- Stout, M. J. and Bostock, R. M. 1999. Specificity of induced responses to arthropods and pathogens. **In: Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.** pp. 183-210. Agrawal, A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Stratmann, J. W. and Ryan, C. A. 1997. Myelin basic protein kinase activity in tomato leaves is induced systemically by wounding and increases in response to systemin and oligosaccharide elicitors. *Proc. Natl. Acad. Sci. U. S. A.* **94**:11085-11089.
- Stratmann, J. W., Stelmach, B. A., Weller, E. W., and Ryan, C. A. 2000a. UVB/UVA radiation activates a 48 kDa myelin basic protein kinase and potentiates wound signaling in tomato leaves. *Photochem. Photobiol.* **71**:116-123.
- Stratmann, J., Scheer, J., and Ryan, C. A. 2000b. Suramin inhibits initiation of defense signaling by systemin, chitosan, and a  $\beta$ -glucan elicitor in suspension-cultured *Lycopersicon peruvianum* cells. *Proc. Natl. Acad. Sci. U. S. A.* **97**:8862-8867.
- Suzuki, K., Suzuki, N., Ohme-Takagi, M., and Shinshi, H. 1998. Immediate early induction of mRNAs for ethylene-responsive transcription

- factors in tobacco leaf strips after cutting. *Plant J.* **15**:657-665.
- Takabayashi, J. and Dicke, M. 1996. Plant-carnivore mutualism through herbivore-induced carnivore attractants. *Trends Plant Sci.* **1**:109-113.
- Thain, J. F., Doherty, H. M., Bowles, D. J., and Wildon, D. C. 1990. Oligosaccharides that induce proteinase inhibitor activity in tomato plants cause depolarization of tomato leaf cells. *Plant Cell Env.* **13**:569-574.
- Thaler, J. S. 1999a. Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* **399**:686-688.
- Thaler, J. S. 1999b. Jasmonic acid mediated interactions between plants, herbivores, parasitoids, and pathogens: A review of field experiments in tomato. In: *Induced plant defences against pathogens and herbivores. Biochemistry, ecology and agriculture.* pp. 319-334. Agrawal, A. A., Tuzun, S., and Bent, E. Ed., APS Press, St Paul, Minnesota.
- Thaler, J. S., Stout, M. J., Karban, R., and Duffey, S. S. 1996. Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. *J. Chem. Ecol.* **22**:1767-1781.
- Thaler, J. S., Fidantsef, A. L., Duffey, S. S., and Bostock, R. M. 1999. Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance. *J. Chem. Ecol.* **25**:1597-1609.
- The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **408**:796-815.
- Thomma, B. P. H. J., Eggermont, K., Penninckx, I. A. M. A., Mauch-Mani, B., Vogelsang, R., Cammue, B. P. A., and Broekaert, W. F. 1998. Separate jasmonate-dependent and salicylate-dependent defense response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc. Natl. Acad. Sci. U. S. A.* **95**:15107-15111.
- Thomma, B. P. H. J., Eggermont, K., Broekaert, W. F., and Cammue, B. P. A. 2000. Disease development of several fungi on *Arabidopsis* can be reduced by treatment with methyl jasmonate. *Plant Physiol. Biochem.* **38**:421-427.
- Thomson, N., Evert, R. F., and Kelman, A. 1995. Wound-healing in whole potato tubers - a cytochemical, fluorescence, and ultrastructural analysis of cut and bruise wounds. *Can. J. Bot.* **73**:1436-1450.
- Titarenko, E., Rojo, E., Léon, J., and Sánchez-Serrano, J. J. 1997. Jasmonic acid-dependent and -independent signaling pathways control wound-induced gene activation in *Arabidopsis thaliana*. *Plant Physiol.* **115**:817-826.
- Tumlinson, J. H., Lewis, W. J., and Vet, L. E. M. 1993. How parasitic wasps find their hosts. *Sci. Am.* **268**:100-106.
- Turlings, T. C. J. and Tumlinson, J. H. 1992. Systemic release of chemical signals by herbivore-injured corn. *Proc. Natl. Acad. Sci. U. S. A.* **89**:8399-8402.
- Turlings, T. C. J., Loughrin, J. H., McCall, P. J., Röse, U. S. R., Lewis, W. J., and Tumlinson, J. H. 1995. How caterpillar-damaged plants protect themselves by attracting parasitic wasps. *Proc. Natl. Acad. Sci. U. S. A.* **92**:4169-4174.
- Usami, S., Banno, H., Ito, Y., Nishihama, R., and Machida, Y. 1995. Cutting activates a 46-kilodalton protein kinase in plants. *Proc. Natl. Acad. Sci. U. S. A.* **92**:8660-8664.
- van Loon, L. C. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. *European Journal of Plant Pathology* **103**:753-765.
- van Loon, J. J. A., de Vos, E. W., and Dicke, M. 2000. Orientation behaviour of the predatory hemipteran *Perillus bioculatus* to plant and prey odours. *Entomol. Exp. Appl.* **96**:51-58.
- van Wees, S. C. M., de Swart, E. A. M., van Pelt, J. A., van Loon, L. C., and Pieterse, C. M. J. 2000. Enhancement of induced disease resistance by simultaneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U. S. A.* **97**:8711-8716.
- Vernooij, B., Friedrich, L., Morse, A., Reist, R., Kolditz-Jawhar, R., Ward, E., Uknes, S., Kessmann, H., and Ryals, J. 1994. Salicylic acid is not the translocated signal responsible for inducing systemic acquired resistance but is required in signal transduction. *Plant Cell* **6**:959-965.
- Vian, A., Henry-Vian, C., Schantz, R., Ledoigt, G., Frachisse, J. M., Desbiez, M. O., and Julien, J. L. 1996. Is membrane potential involved in calmodulin gene expression after external stimulation in plants? *FEBS Lett.* **380**:93-96.
- Vijayan, P., Shockey, J., Levesque, C. A., Cook, R. J., and Browse, J. 1998. A role for jasmonate in pathogen defense of *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.* **95**:7209-7214.
- Walling, L. L. 2000. The myriad plant responses to herbivores. *J. Plant Growth Reg.* **19**:195-216.
- Wang, C., Zien, C. A., Afithile, M., Welti, R., Hildebrand, D. F., and Wang, X. 2000. Involvement of phospholipase D in wound-induced accumulation of jasmonic acid in *Arabidopsis*. *Plant Cell* **12**:2237-2246.
- Wang, C. X., Avdiushko, S., and Hildebrand, D. F. 1999. Overexpression of a cytoplasm-localized allene oxide synthase promotes the wound-induced accumulation of jasmonic acid in transgenic tobacco. *Plant Mol. Biol.* **40**:783-793.
- Wasternack, C., Ortel, B., Miersch, O., Kramell, R., Beale, M., Greulich, F., Feussner, I., Hause, B., Krumm, T., Boland, W., and Parthier, B. 1998. Diversity in octadecanoid-induced gene expression of tomato. *J. Plant Physiol.* **152**:345-352.
- Watanabe, T. and Sakai, S. 1998. Effects of active oxygen species and methyl jasmonate on expression of the gene for a wound-inducible 1-aminocyclopropane-1-carboxylate synthase in winter squash (*Cucurbita maxima*). *Planta* **206**:570-576.
- Weissbecker, B., van Loon, J. J. A., Posthumus, M. A., Bouwmeester, H. J., and Dicke, M. 2000. Identification of volatile potato sesquiterpenoids

- and their olfactory detection by the two-spotted stinkbug *Perillus bioculatus*. *J. Chem. Ecol.* **26**:1433-1445.
- Wilton, D. C., Thain, J. F., Minchin, P. E. H., Gubb, I. R., Reilly, A. J., Skipper, Y. D., Doherty, H. M., O'Donnell, P. J., and Bowles, D. J. 1992. Electrical signaling and systemic proteinase inhibitor induction in the wounded plant. *Nature* **360**:62-65.
- Xie, D. X., Feys, B. F., James, S., Nieto-Rostro, M., and Turner, J. G. 1998. *COII*: An *Arabidopsis* gene required for jasmonate-regulated defense and fertility. *Science* **280**:1091-1094.
- Xu, Y., Chang, P. F. L., Liu, D., Narasimhan, M. L., Raghothama, K. G., Hasegawa, P. M., and Bressan, R. A. 1994. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell* **6**:1077-1085.
- Yoon, G. M., Cho, H. S., Ha, H. J., Liu, J. R., and Lee, H. S. P. 1999. Characterization of NtCDPK1, a calcium-dependent protein kinase gene in *Nicotiana tabacum*, and the activity of its encoded protein. *Plant Mol. Biol.* **39**:991-1001.
- Zhang, S. Q. and Klessig, D. F. 1997. Salicylic acid activates a 48-kD MAP kinase in tobacco. *Plant Cell* **9**:809-824.
- Zhang, S. Q. and Klessig, D. F. 1998. The tobacco wounding-activated mitogen-activated protein kinase is encoded by SIPK. *Proc. Natl. Acad. Sci. U. S. A.* **95**:7225-7230.
- Zhou, L. and Thornburg, R. 1999. Wound-inducible genes in plants. **In**: *Inducible Gene Expression in Plants*. pp. 127-167. Reynolds, P. H. S. Ed., CABI Publishing, Wallingford, UK.
- Zhu-Salzman, K., Salzman, R. A., Koiwa, H., Murdock, L. L., Bressan, R. A., and Hasegawa, P. M. 1998. Ethylene negatively regulates local expression of plant defense lectin genes. *Physiol. Plant.* **104**:365-372.