Chapter 1
Direct Defenses in Plants and Their Induction by Wounding and Insect Herbivores

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Resistance factors for direct plant defense against herbivorous insects comprise plant traits that negatively affect insect preference (host plant selection, oviposition, feeding behavior) or performance (growth rate, development, reproductive success) resulting in increased plant fitness in a hostile environment. Such traits include morphological features for physical defense, like thorns, spines, and trichomes, epicuticular wax films and wax crystals, tissue toughness, as well as secretory structures and conduits for latices or resins. They also include compounds for chemical defense, like secondary metabolites, digestibility reducing proteins, and antinutritive enzymes. All these traits may be expressed constitutively as preformed resistance factors, or they may be inducible and deployed only after attack by insect herbivores. The induction of defensive traits is not restricted to the site of attack but extends to non-infested healthy parts of the plants. The systemic nature of plant responses to herbivore attack necessitates a long-distance signaling system capable of generating, transporting, and interpreting alarm signals produced at the plant–herbivore interface. Much of the research on the signaling events triggered by herbivory has focused on tomato and other solanaceous plants. In this model system, the peptide systemin acts at or near the wound site to amplify the production of jasmonic acid. Jasmonic acid or its metabolites serve as phloem-mobile long-distance signals, and induce the expression of defense genes in distal parts of the plant. In this chapter, we will provide an overview of physical and chemical defense traits, and review the signaling mechanisms that account for their inducible expression after insect attack.

1.1 Introduction

Plants, flowering plants in particular, exhibit a tremendous diversity in size and shape, ranging from just a few millimeters in the tiny duckweeds to almost 100

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meters in giant eucalyptus trees. Some may complete their life cycle in a few weeks, while others live thousands of years. The amazing diversity results from the adaptation to different, oftentimes hostile environments, as exemplified by the early evolution of land plants. The colonization of land by plants, dating back some 480 million years according to fossil records (Kenrick and Crane 1997), marks the beginning of an evolutionary success story, with flowering plants now occupying every habitat on Earth except the regions surrounding the poles, the highest mountaintops, and the deepest oceans (Soltis and Soltis 2004). The colonization of land was a major event in the history of plant life, and at the same time, paved the way for the explosive evolution of terrestrial ecosystems. Despite the vulnerability of plants as sessile organisms to adverse biotic and abiotic conditions, they actually dominate over much of the land surface. This apparent success of flowering plants relies on the evolved ability to persist in unfavorable and variable environments by virtue of effective resistance systems that are based on a combination of physical, chemical, and developmental features (Schoonhoven et al. 2005). It was recognized by Stahl in 1888 that the great diversity of mechanical and chemical 'means of protection of plants were acquired in their struggle for existence within the animal world' leading to the conclusion that 'the animal world [. . .] deeply influenced not only their morphology but also their chemistry' (Stahl 1888; Fraenkel 1959). Hence, not only thorns and spines as morphological resistance traits, but also the bewildering variety of plant secondary chemicals attest to the selective pressure exerted by phytophagous animals (Fraenkel 1959; Ehrlich and Raven 1964).

It was later discovered that induced expression of resistance traits increases plant fitness in environments that harbor a variety of plant parasites. The inducibility of plant resistance was first reported for fungal and bacterial pathogens in the early 1900s (Karban and Kuc 1999) and, much later, inducible defenses were shown to exist also against insect herbivores. In their seminal paper of 1972, Green and Ryan demonstrated that tomato and potato plants accumulate inhibitors of trypsin and chymotrypsin-like serine proteinases throughout their aerial tissues, as a direct consequence of insect-mediated damage or mechanical wounding (Green and Ryan 1972). Proteinase inhibitors are present constitutively in high concentrations in plant storage organs, and a possible function as protective agents against insects was discussed at that time (Lipke et al. 1954; Applebaum and Konijn 1966). Green and Ryan suggested that the expression of proteinase inhibitors may be regulated in leaves to make the plant less palatable and perhaps lethal to invading insects. The accumulation of proteinase inhibitors in aerial tissues was proposed to constitute an inducible defense system, directly affecting the performance of leaf-consuming insects by starving them of nutrients, thus resulting in enhanced plant resistance against herbivory (Green and Ryan 1972). It is now clear that the nutritional quality of the foliage is an important determinant of herbivore growth and development (Painter 1936; Berenbaum 1995; Schoonhoven et al. 2005) and anti-nutritional defense as part of the plant's arsenal for induced resistance is well accepted (Rhoades and Cates 1976; Felton 2005).

Thirty-five years of research following the initial discovery by Ryan and coworkers established plant resistance against insect herbivores as a highly dynamic process. In addition to the proteinase inhibitors, many more inducible factors have been identified which contribute to direct defense and which have the potential to enhance host plant fitness after herbivore attack. These are aspects that will be introduced in this chapter to provide the background for a more detailed discussion of the defensive role of individual proteins in the subsequent, more focused chapters of this volume. Another aspect of induced resistance that has fascinated researchers since the seminal Green-and-Ryan-paper is the systemic nature of the response: defense proteins accumulate not only at the site of wounding but also systemically in unwounded tissues of the infested plant. Obviously, a signal must be generated locally as a consequence of insect feeding which is then propagated throughout the plant, and able to induce the expression of defense proteins at distant sites (Green and Ryan 1972; Ryan and Moura 2002). Our current understanding of systemic wound signaling for direct defense will also be summarized here.

1.2 Inducible Resistance Factors for Direct Defense

Since the initial observation of proteinase inhibitor accumulation in wounded tomato and potato plants, inducibility by herbivory has been shown for a large number of other potential resistance factors (Walling 2000; Gatehouse 2002). In the light of recent studies analyzing induced responses at the level of the entire transcriptome, we now begin to appreciate the full breadth and highly dynamic nature of plant-insect interactions. Numerous studies have shown that herbivory causes large-scale changes in gene expression (Cheong et al. 2002; Delessert et al. 2004; Reymond et al. 2004; Smith et al. 2004; Voelckel and Baldwin 2004; Zhu-Salzman et al. 2004; De Vos et al. 2005; Schmidt et al. 2005; Ralph et al. 2006b; Thompson and Goggin 2006; Broekgaarden et al. 2007). In hybrid poplar, for example, it is estimated that 11% of the transcriptome is differentially regulated by insect feeding (Ralph et al. 2006a). However, inducibility of a certain gene or enzyme per se is not sufficient evidence for a function in plant defense. Whereas the potential contribution of a given trait to plant resistance can be readily tested in a laboratory setting by comparing herbivore preference and/or performance on plants that differ in the trait of interest, a role in plant defense implies that expression of the resistance trait is associated with a gain in plant fitness; such associations must ultimately be demonstrated in field experiments that simulate ‘real world’ conditions (Karban and Myers 1989).

A further requisite for the evolution of inducible defense systems is heritable variation in the degree of inducibility (Karban and Myers 1989; Agrawal 1999). Genetic variation has frequently been observed in natural populations, e.g., for physical (trichome density) or chemical (glucosinolate content) resistance characters in *Arabidopsis*, and both traits are associated with fitness costs (Mauricio 1998). For induced resistance traits, however, a fitness benefit has been demonstrated in only a few cases. One example is radish plants that were induced to accumulate higher levels of glucosinolates and to produce trichomes at increased density. Compared to control plants, these induced plants exhibited both increased resistance to herbivory and increased seed mass (a correlate of lifetime fitness). This experiment confirmed a role in direct defense for trichomes and glucosinolates as inducible physical and chemical resistance factors, respectively (Agrawal 1998, 1999). Likewise, in *Nicotiana attenuata*, the induced production of nicotine as a chemical resistance factor was associated with metabolic costs, but provided a fitness benefit when plants were under attack by herbivores (Baldwin 1998; see also Steppuhn and Baldwin this volume). Although these findings should not be generalized and a defensive role should not be assumed for all plant responses to wounding and herbivory, the prevalence of inducible resistance traits in present day plant-herbivore systems implies that such responses are likely the result of natural selection imposed by insect herbivores during evolution.

Any plant trait that interferes with host plant selection, oviposition, or feeding of an insect herbivore is a potential resistance factor and may further contribute to plant defense. Most prominent among these traits are morphological features and the chemical composition of the plant, both of which have long been recognized as constitutive resistance characters (Stahl 1888; Fraenkel 1959), and were also the focus of initial studies on inducible resistance to insect herbivores. In this chapter, we provide a brief overview of inducible factors that lead to enhanced resistance through direct effects on insect preference or performance. The traditional distinction between plant defense traits that are either morphological or chemical is used throughout this volume (see also Table 1 on page 2 in the Introduction). It is important to realize, however, that this classification is often arbitrary because any morphological feature is the manifestation of a genetically regulated biochemical process and, therefore, also chemical at its very basis.

1.2.1 Morphological Features for Physical Defense

Insect herbivores from all feeding guilds must make contact with the plant surface in order to establish themselves on the host plant. It is therefore not surprising that physical and chemical features of the plant surface are important determinants of resistance. Epicuticular wax films and crystals cover the cuticle of most vascular plants. In addition to their important role in desiccation tolerance, they also increase slipperiness, which impedes the ability of many non-specialized insects to populate leaf surfaces. The physical properties of the wax layer as well as its chemical composition are important factors of preformed resistance (see Müller this volume). Whereas induced changes in wax production and surface chemistry have been observed, evidence for a role of the cuticle and epicuticular waxes in induced resistance is still scant. Wax biosynthesis and composition are known to vary during plant development, and the physico-chemical properties of the cuticle respond to changes in season and temperature (Müller this volume). Considering the ingenuity of plants in dealing with their offenders, it would thus be surprising if regulated production of wax on the leaf surface were not adopted to influence the outcome of plant-insect interactions.

Other components of the plant surface that serve a role in constitutive defense include thorns and spines directed against mammalian herbivores, and hairs (trichomes) which are effective against insects (Myers and Bazely 1991; Schoonhoven et al. 2005). Non-glandular trichomes may serve as structural resistance factors preventing small insects from contacting the leaf surface or limiting their movement. Morphological and chemical resistance factors are combined in glandular trichomes. Glands produce substances which may repel insect herbivores or deter them from feeding (antixenosis), or immobilize them on the leaf surface. Quite interestingly, trichome density in some plant species increases in response to insect feeding, and therefore constitutes an inducible resistance trait. The defensive role of trichomes is discussed in more detail by Dalin et al. (this volume).

Leaf toughness is an important physical factor for plant resistance, as it affects the penetration of plant tissues by mouthparts of piercing-sucking insects, and also increases mandibular wear in biting-chewing herbivores (Schoonhoven et al. 2005). Leaf toughness is frequently correlated with insect resistance and is a good predictor of herbivory rates (Bergvinson et al. 1995; Coley and Barone 1996; Howlett et al. 2001). Although leaf toughness is typically regarded as a physical character, this trait exemplifies the general difficulty in drawing clear distinctions between physical and chemical resistance factors. Cell wall reinforcement for enhanced leaf toughness results from the deposition of ‘chemicals’, including macromolecules such as lignin, cellulose, suberin, and callose, small organic molecules (e.g., phenolics), and even inorganic silica particles (Schoonhoven et al. 2005). Enhanced synthesis and/or deposition of these chemicals after wounding leads to induced physical resistance (McNaughton and Tarrants 1983; Bernards this volume; Ginzberg this volume).

Another anatomical defense found in plants of diverse phyolgenetic origin is a network of canals such as lacticifers (latex-containing living cells) or resin ducts (resin-filled intercellular spaces) that store latex or resins under internal pressure. When the canal system is severed, the contents are exuded and may entrap or even poison the herbivore. Out of more than 50 plant families for which such defense systems have been described, the well-studied milkweeds (genus *Asclepias* in the family Asclepiadacea) may serve as an example. Milkweed latices coagulate upon exposure to air and immobilize small insect larvae. As an additional chemical resistance factor, the latex may contain large amounts of toxic cardenolides (Dussourd and Hoyle 2000; Agrawal 2004). Fascinatingly, many specialist herbivores that feed on milkweed or other latex-producing plants employ feeding strategies that block the flow of latex to intended feeding sites. Such feeding behavior has evolved independently in several phylogenetic lineages, and can be viewed as a counteradaptation of herbivores to circumvent latex-based plant defenses (Carroll and Hoffman 1980; Dussourd and Eisner 1987; Dussourd and Denno 1994).

A widely appreciated and well-established form of anatomical protection are the resin-based defenses in conifers (Berryman 1972). The resin, which is a mixture of monoterpenes, sesquiterpenes, and diterpene resin acids, accumulates in resin ducts and related secretory structures. Stem-boring bark beetles and other insects that breach the resin duct system are expelled (‘pitched out’) from the bore hole by resin flow. Upon exposure to air, the highly volatile monoterpene fraction evaporates, leaving the insects trapped in the solidifying resin acids and the wound site sealed (Phillips and Croteau 1999; Trapp and Croteau 2001). Although this complex resin-based defense system in conifers is preformed, it is further induced in response to wounding. Among the inducible components of the system are terpene biosynthesis (Bohlmann this volume) and the formation of new resin ducts (Krokene et al. this volume).

Finally, the wound healing process itself can be considered as a wound-induced anatomical trait for enhanced resistance. Efficient sealing of the wound is important to prevent water loss and opportunistic infections by bacterial and fungal pathogens at the site of tissue damage. Wound closure may involve extensive cell division and formation of wound callus (e.g., Guariguata and Gilbert 1996). In the case of plants with resin- and latex-based defenses, coagulation of the exudates may efficiently seal the wound site. More generally, a sealing cell layer is formed by infusion of antimicrobial and water-impermeable substances, including lignin and suberin (Rittinger et al. 1987). This may be followed by the induction of cell division and the formation of a periderm as a protective tissue that is impermeable to water and resistant to pathogens. Wound periderm formation and its potential contribution to plant defense are discussed in greater detail by Ginzberg (this volume).

1.2.2 Metabolites and Enzymes for Chemical Defense

Plant chemicals that play a role in direct defense impair herbivore performance by one of two general mechanisms: these chemicals may reduce the nutritional value of plant food, or they may act as feeding deterrents or toxins. There has been considerable debate as to which of these two strategies is more important for host plant selection and insect resistance. An important part of this debate concerns the extent to which variation in the levels of primary and secondary metabolites has evolved as a plant defense (Berenbaum, 1995). Plant primary metabolism, which is shared with insects and other living organisms, provides carbohydrates, amino acids, and lipids as essential nutrients for the insect. Food quality is largely determined by the availability of these nutrients, and its importance for longevity, size, fecundity, and death rates in herbivorous insects has been recognized early on by Painter (1936). In addition, more than 100,000 plant compounds (i.e., secondary metabolites) have been identified with no apparent role in primary metabolism, and many of these have been regarded as expendable metabolic waste products. While many secondary metabolites are in fact expendable for primary metabolism, it is now widely accepted that they serve important ecological functions in the interaction of plants with their biotic and abiotic environment.

According to the paradigm put forward by Fraenkel in his seminal paper in 1959, secondary metabolites in a given plant species may act both as repellents for generalist (polyphagous) insects and as attractants for specialist (monophagous) insects, and may thus be largely responsible for host range restriction (Fraenkel 1959). In addition to these allelochemical functions, secondary metabolites also act in multiple ways as toxins, feeding deterrents, as digestibility reducers or antinutritives, as precursors for physical defense, and as volatiles in indirect defense (Bennett and Wallsgrove 1994; Karban and Baldwin 1997). Despite their diversity in structure, activity, and distribution in the plant kingdom, all secondary compounds are derived from universally available intermediates of primary metabolism, including sugar phosphates (erythrose 4-phosphate), acetyl-coenzyme A, and amino acids, and are conveniently classified according to their biosynthetic pathways as phenolics, terpenoids, and alkaloids. Each of these classes of compounds and their role in induced resistance are the focus of subsequent chapters of this volume. While the importance of secondary metabolites in plant defense remained undisputed for decades following Fraenkel's landmark paper (Fraenkel 1959), the realization that some secondary metabolites (e.g., tannins and phenolics) exert anti-nutritive activity brought greater attention to the idea that food quality, nutritional value, and variation in primary metabolism may have evolved as a plant defense (Feeny 1970; Rhoades and Cates 1976; Berenbaum 1995). The relevance of nutritional quality as a resistance trait was further supported by Ryan and coworkers (Green and Ryan 1972) who showed that induced expression of serine proteinase inhibitors contributes to plant defense by interfering with the insect's digestive processes, thus limiting the availability of essential amino acids.

Following the landmark study of Green and Ryan, many workers reported that the overall chemical composition of the plant is greatly influenced by developmental and environmental parameters, including herbivory. Induced changes in plant chemistry involve the biosynthesis of a wide variety of secondary metabolites, including phenolics, terpenoids, alkaloids, cyanogenic glucosides, and glucosinolates (Karban and Baldwin 1997; Constabel 1999; Chapters 8–10, this volume). It was further shown that the induction of anti-nutritional proteins is not limited to serine proteinase inhibitors, but includes inhibitors of other classes of proteases, oxidative enzymes, amino acid-metabolizing enzymes, and lectins (Constabel 1999; Felton 2005). Such examples of protein-based defenses are further discussed in Chapters 11–14 of this volume.

1.2.3 Metabolic Reconfiguration to Shift from a Growth- to a Defense-Oriented State

The numerous anatomical and chemical changes associated with induced resistance require massive reprogramming of gene expression. For the quantitative analysis of large-scale changes in gene expression, novel techniques have been developed in recent years. Most notable among these approaches are microarray technologies for the identification of differentially expressed transcripts, and even more recently, techniques for high-throughput proteomic analysis (Kessler and Baldwin 2002; Kuhn and Schaller 2004; Giri et al. 2006; Lippert et al. 2007). With the advent of these techniques, it is now possible to obtain a relatively unbiased account of the plant's response to herbivory. Many of the genes required for the expression of known resistance traits were in fact shown to be upregulated during plant-insect interaction. Consistent with the activation of structural defenses, genes of general phenylpropanoid metabolism and monolignol biosynthesis, lignin polymerization, and cell wall fortification are induced by wounding or herbivory in hybrid poplar (Smith et al. 2004; Lawrence et al. 2006; Major and Constabel 2006; Ralph et al. 2006a), Sitka spruce (Ralph et al. 2006b), and *Arabidopsis* (Cheong et al. 2002; Delessert et al. 2004; Reymond et al. 2004). Likewise, the activation of chemical defenses is accompanied by the induction of genes involved in secondary metabolism, including phenolics, polyamine, and alkaloid biosynthesis in *N. attenuata* (Voelckel and Baldwin 2004; Schmidt et al. 2005; Giri et al. 2006), the genes for the formation of phenolics and terpenes in spruce and poplar (Ralph et al. 2006a, b), and phenolic metabolism and glucosinolate biosynthesis in *Arabidopsis* (Cheong et al. 2002; Reymond et al. 2004). These studies also confirmed the activation of genes for antidigestive and antinutritional defenses (e.g., proteinase inhibitors, oxidative enzymes, lectins), and for the signaling of the resistance response (e.g., jasmonic acid and ethylene biosynthesis, transcription factors). Notably, however, these genes represent only a fraction of the total wound-induced changes in gene activity. The insect-responsive transcriptome was estimated to comprise 10% of all transcripts, suggesting that massive reprogramming of gene expression is required to bring about a shift from growth-oriented to defense-oriented plant metabolism (Hui et al. 2003; Ralph et al. 2006a). The latter state involves the activation of genes for general stress responses (oxidative stress, dehydration stress, heat-shock proteins), protein turnover (e.g., proteases), and transport processes (e.g., aquaporins, lipid transfer proteins, ABC transporters, sugar and peptide transporters), as well as modulation of primary metabolism (carbohydrate and lipid metabolism, nitrogen assimilation), and downregulation of photosynthesis and chloroplast function. These changes in gene expression may reflect the herbivore-induced reallocation of resources from primary processes to defense (Voelckel and Baldwin 2004; Ralph et al. 2006b).

Efficient mobilization of plant resources is likely to facilitate the expression of costly resistance traits, including the accumulation of defense proteins, the synthesis of secondary metabolites, and the formation of structural defenses. On the other hand, mobilization of resources may also contribute to plant tolerance of herbivory. Unlike resistance, tolerance does not affect herbivore preference or performance, but rather allows the host plant to minimize the fitness consequences of tissue loss. Tolerance and resistance are therefore viewed as alternative and complementary strategies for plant defense against insect herbivores (Karban and Myers 1989; Mauricio 2000; Weis and Franks 2006). Whereas tolerance is still not well-understood at the molecular level, it may include the mobilization of leaf carbon and nitrogen that is threatened by herbivory, and temporary storage of these resources for later regrowth. The induction of protein turnover, lipid and carbohydrate metabolism, and transport functions observed in microarray studies (see above) may thus be equally relevant for both tolerance and induced expression of resistance traits. Temporary storage of resources occurs in organs that are less susceptible to herbivory, e.g., the root system. Indeed, a change in sink-source relations was observed in *N. attenuata* after simulated herbivore attack, resulting in increased allocation of sugars to roots and enhanced tolerance (Schwachtje et al. 2006). Likewise, the induction of vegetative storage proteins frequently observed in response to wounding (Staswick 1994; Christopher et al. 2004; Reymond et al. 2004; Major and Constabel 2006) may allow the plant to buffer mobilized resources for later use in re-growth. Remarkably, such a role as interim storage or temporary protein depot had already been suggested for proteinase inhibitor I in tomato and potato plants, the first protein shown to be systemically induced by herbivory (Ryan and Huisman 1969; Green and Ryan 1972).

1.3 Systemic Signaling for Induced Direct Defense

An important feature of many wound-induced direct defense responses is their occurrence in undamaged tissues located far from the site of wounding. Wound-inducible serine proteinase inhibitors (PIs) represent one of the best examples of a systemically induced defense response. In tomato plants, *PI* genes are expressed in distal leaves within 1–2 hrs after insect attack or mechanical wounding (Ryan 2000; Strassner et al. 2002). The rapid and systemic nature of this response is analogous to vertebrate immune responses in which endocrine signals are delivered to target tissues via the circulatory system (Bergey et al. 1996). However, because plants lack mobile defender cells, systemic signals must be transmitted long distances via mechanisms that are specific to plants (Malone 1996; León et al. 2001; Schilmiller and Howe 2005). Ryan's pioneering work on systemic wound signaling inspired generations of plant biologists to investigate the underlying mechanisms of this fascinating response.

The widespread occurrence of systemic defense responses in the plant kingdom implies the existence of common mechanisms to generate, transport, and perceive alarm signals that are generated at the site of tissue damage. Wound-inducible PIs in tomato and other solanaceous plants have been widely used as a model system in which to study the molecular mechanism of systemic wound signaling. Green and Ryan (1972) proposed that chemical signals produced at the wound site travel through the plant and activate PI expression in undamaged leaves. Identification of these signaling compounds was facilitated by a simple bioassay in which test solutions (e.g., containing an elicitor) are supplied to tomato seedlings through the cut stem, followed by measurement of PI accumulation in the leaves. Extensive use of this assay led to the discovery of several distinct classes of PI-inducing compounds, including cell-wall-derived oligogalacturonides (OGAs), systemin, jasmonic acid (JA), and hydrogen peroxide (Ryan 2000; Gatehouse 2002). Physical signals (e.g., hydraulic forces and electrical signals) generated by tissue damage have also been implicated in the systemic signaling process (Wildon et al. 1992; Malone 1996). Currently, a major challenge is to determine how these diverse signals interact with one another to promote intercellular communication across long distances.

Farmer and Ryan (1992) established the current paradigm that extracellular signals such as OGAs and systemin (so-called primary wound signals), generated in response to wounding, trigger the intracellular production of JA via the octadecanoid pathway and that JA, in turn, activates the expression of defensive genes. Wound-induced production of OGAs is catalyzed by a family of polygalacturonases (PGs) that are expressed in various plant tissues (Bergey et al. 1999). OGAs are relatively immobile in the plant vascular system and thus are thought to act as local mediators. However, because PG activity is induced systemically in response to wounding, OGAs could also amplify defense responses in undamaged leaves (Ryan 2000). OGA-mediated signal transduction may result from direct physical effects of these compounds on the plasma membrane or may involve specific receptors (Navazio et al. 2002).

Systemin was the first bioactive peptide discovered in plants (Pearce et al. 1991). This 18-amino-acid peptide is derived from proteolytic cleavage of a larger precursor protein, prosystemin. When used in the tomato seedling bioassay, systemin is $>10,000$-fold more active than OGAs in inducing PI expression. Several lines of evidence indicate that systemin serves a key role in induced defense responses in tomato. For example, transgenic plants expressing an antisense *prosystemin* (*Prosys*) cDNA are deficient in wound-induced systemic expression of PIs and, as a consequence, are more susceptible to insect herbivores (McGurl et al. 1992; Orozco-Cárdenas et al. 1993). Overexpression of prosystemin from a *35S::Prosys* transgene constitutively activates PI expression in the absence of wounding, thereby conferring enhanced resistance to herbivores (McGurl et al. 1994; Li et al. 2002; Chen et al. 2005). Forward genetic analysis has shown that genes required for systemin-mediated signaling are essential for wound-induced expression of *PI* and other defense-related genes (Howe and Ryan 1999; Howe 2004). Thus, wounding and systemin activate defense genes through a common signaling pathway.

Transcriptional activation of defense genes in response to systemin requires the biosynthesis and subsequent action of JA (Farmer and Ryan 1992; Howe, 2004). The systemin signaling pathway is initiated upon binding of the peptide to a 160-kDa plasma membrane-bound receptor (SR160) that was identified as a member of the leucine-rich repeat (LRR) receptor-like kinase family of proteins (Scheer and Ryan 1999, 2002). Binding of systemin to the cell surface is associated with several rapid signaling events, including increased cytosolic Ca^{2+} levels, membrane depolarization, and activation of a MAP kinase cascade (Felix and Boller 1995; Stratmann and Ryan 1997; Moyen et al. 1998; Schaller and Oecking 1999). The precise mechanism by which systemin activates JA synthesis remains to be determined. There is evidence indicating that a systemin-regulated phospholipase A_2 activity in tomato leaves releases linolenic acid, a JA precursor, from lipids in the plasma membrane (Farmer and Ryan 1992; Narváez-Vásquez et al. 1999). Alternatively, the role of a chloroplast-localized phospholipase A_1 in JA biosynthesis (Ishiguro et al. 2001) raises the possibility that systemin perception at the plasma membrane is coupled to the activation of a similar lipase in the chloroplast. JA synthesized in response to systemin, OGAs, and wounding acts in concert with ethylene (O'Donnell et al. 1996) and hydrogen peroxide (Orozco-Cárdenas et al. 2001; Sagi et al. 2004) to positively regulate the expression of downstream target genes.

1.3.1 Genetic Analysis of the Wound Response Pathway in Tomato

Genetic analysis provides a powerful approach to identify components of the systemic wound response pathway. The robust nature of wound-induced PI expression in tomato, together with facile assays for PIs and other biochemical markers (e.g., polyphenol oxidase) of the response, has been exploited for this purpose. Forward genetic screens identified mutants that are defective in PI expression in response to mechanical wounding or treatment with methyl-JA (MeJA) (Howe 2004; Li et al. 2004). Additional screens have been conducted to identify mutations that suppress the inductive effects of the *35S::Prosys* transgene (Howe and Ryan 1999). These screens have yielded numerous mutants that are deficient in wound-induced systemic expression of defensive genes. That most of these mutants display altered resistance to arthropod herbivores and various pathogens demonstrates the importance of induced responses to plant protection (Howe 2004).

Map-based cloning and candidate gene approaches were used to identify genes defined by forward genetic analysis. The *spr2* and *acx1* mutants, which were generated by ethylmethane sulfonate mutagenesis, are defective in genes required for JA biosynthesis. *Spr2* encodes a plastidic ω-3 fatty acid desaturase that converts linoleic acid to the JA precursor linolenic acid (Li et al. 2003). *ACX1* encodes a peroxisomal acyl-CoA oxidase that catalyzes the first step in the β-oxidation stage of JA synthesis (Li et al. 2005). The *jasmonate insensitive1* (*jai1*) mutant harbors a deletion in the tomato ortholog of the *Arabidopsis Coronatine insensitive1* (*Coi1*) gene (Li et al. 2004). *Coi1* encodes an F-box protein that is essential for expression of jasmonate-responsive genes, including many wound-responsive genes involved in anti-insect defense (Xie et al. 1998). Reverse genetic strategies identified several additional wound response mutants of tomato. For example, transgenic lines specifically engineered for defects in JA biosynthesis (Stenzel et al. 2003), ethylene synthesis (O'Donnell et al. 1996), ABA signaling (Carrera and Prat 1998), and ROS production (Sagi et al. 2004) are impaired in wound-induced systemic PI expression and other defense responses.

1.3.2 Jasmonate Performs a Key Role in Systemic Wound Signaling

Despite significant progress in the identification of genes that regulate systemic defense responses, relatively little is known about the specific role of these components in the long-distance signaling pathway. In theory, genes required for the systemic response could play a role in production of the mobile signal, translocation of the signal from damaged to undamaged leaves, signal perception by target cells in distal leaves, or subsequent signaling steps leading to expression of target genes. Classical grafting techniques provide a powerful approach to determine whether a particular mutant is defective in the production of the systemic (i.e., graft-transmissible) wound signal or the recognition of that signal in responding leaves (Li et al. 2002). Reciprocal grafting experiments performed with the

JA-insensitive *jai1* mutant showed that jasmonate perception (i.e., COI1) is essential for recognition of the mobile signal in distal responding leaves (Fig. 1.1Ad). These studies also suggest that the mobile signal is produced in the absence of COI1 (Fig. 1.1Ac). Experiments conducted with JA biosynthetic mutants (e.g., *acx1*) showed that production of the graft-transmissible signal depends on JA biosynthesis in wounded tissues (Fig. 1.1Bc). The ability of JA-deficient scions to express PIs in response to a signal emanating from wild-type rootstock leaves further indicated that de novo JA synthesis is likely not necessary for recognition of the mobile signal in the responding leaves (Fig. 1.1Bd). Based on these collective studies, it was proposed that JA (or a JA derivative) is a critical component of the systemic signal (Schilmiller and Howe 2005). These findings are also consistent with DNA microarray studies showing that local and systemic tissues undergo distinct signaling events (Strassner et al. 2002).

The plant vascular system is involved in long-distance trafficking of a wide range of signaling compounds (Lucas and Lee 2004). Recent studies provide direct evidence that jasmonates are transported in the phloem (Fig. 1.2). For example, several JA biosynthetic enzymes are located in the companion cell-sieve element complex of the vascular bundle (Hause et al. 2003; Wasternack 2007). This observation is supported by the occurrence of JA in phloem bundles from *Plantago major* (Hause et al. 2003) and the preferential accumulation of jasmonates in the tomato leaf midrib (Stenzel et al. 2003). The hypothesis that the systemic signal is translocated in the phloem is further supported by the fact that wound-induced systemic responses are strongly enhanced by the strength of vascular connections between wounded and responding leaves (Davis et al. 1991; Schittko and Baldwin 2003). The rate of movement of the endogenous signal in tomato plants is estimated between 1 and 5 cm/hr (Schilmiller and Howe 2005). The ability of the phloem to transport small molecules at rates up to 40 cm/hr (Fisher 1990) could readily accommodate such a signal. Because systemic PI expression is mediated by a signal traveling within the plant rather than a signal diffusing through the atmosphere (Farmer and Ryan 1992), it is unlikely that volatile MeJA released at the wound site is a causal factor for systemic PI expression in tomato.

The idea that JA (or a JA derivative) functions as a mobile wound signal implies that JA synthesized in damaged leaves is transported to distal undamaged leaves. In tomato and other dicots, however, systemic increases in JA levels in response to mechanical damage are generally very low (i.e., $<10\%$ of that in damaged leaves) or not significant (Strassner et al. 2002). In those cases where systemic increases in JA levels have been reported, it was not determined whether accumulation of the signal results from de novo synthesis in undamaged leaves or JA transport from wounded source leaves. Grafting experiments (see above) support the latter possibility, as does the phloem mobility and systemic signaling activity of exogenous JA (Farmer and Ryan 1992; Zhang and Baldwin 1997). Low levels of wound-induced JA in systemic leaves may reflect sequestration of the signal in specific cell types of the vasculature. An alternative, though not mutually exclusive, possibility is that the phloem-mobile pool of JA is rapidly metabolized to another bioactive derivative. JA derivatives produced by methylation, glycosylation, hydroxylation, sulfonation, amino acid

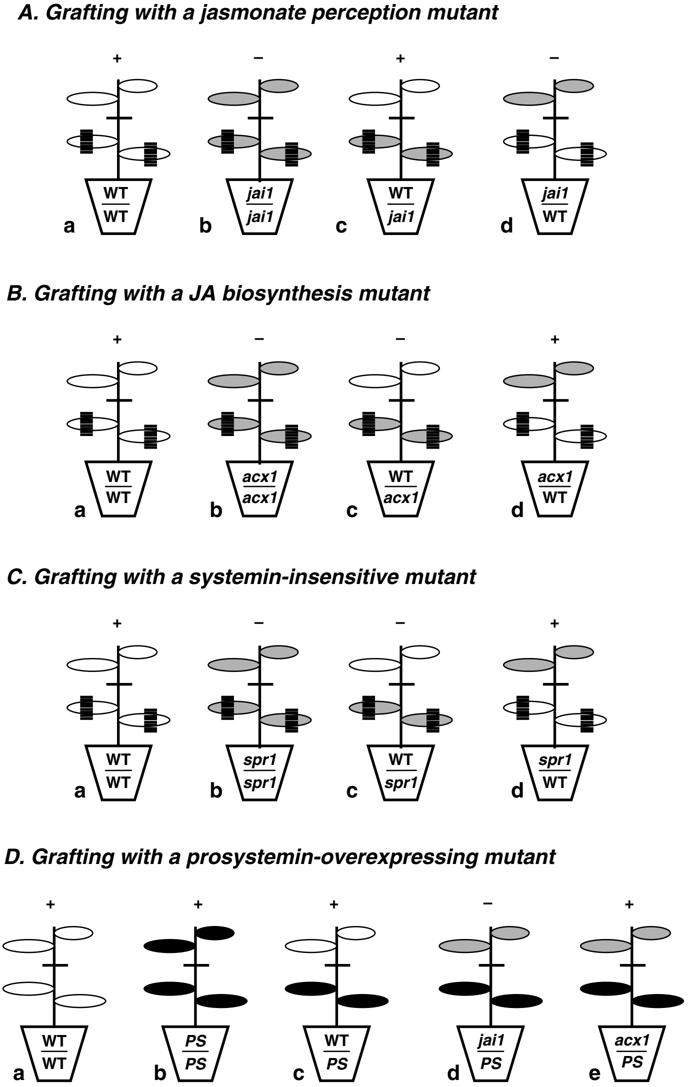

Fig. 1.1 Schematic diagram of grafting experiments used to study the role of JA in systemic wound signaling in tomato. Scions and rootstocks of the indicated genotype were joined at the graft junction (horizontal bar). For experiments shown in A, B, and C, rootstock leaves were wounded (hatched mark) and PI gene expression in the undamaged scion leaves was measured 8 hrs later. (**A**) The *jai1* mutant was used to investigate the role of jasmonate perception in systemic wound signaling. (**B**) The *acx1* mutant was used to study the role of JA synthesis in systemic wound signaling. (**C**) The *spr1* mutant was used to study the role of systemin perception in systemic signaling. For experiments depicted in panel D, no wounds were inflicted because the *35S::Prosys* (PS) transgenic line constitutively produces a systemic signal. '+' and '−' denote the expression or lack of expression, respectively, of PIs in undamaged scion leaves. Unfilled ovals correspond to wild-type (WT) leaves. Gray-shaded ovals depict leaves on mutants (*jai1, acx1, or spr1*) that are defective in systemic wound signaling. Black ovals depict leaves on the *35S::Prosys* transgenic line

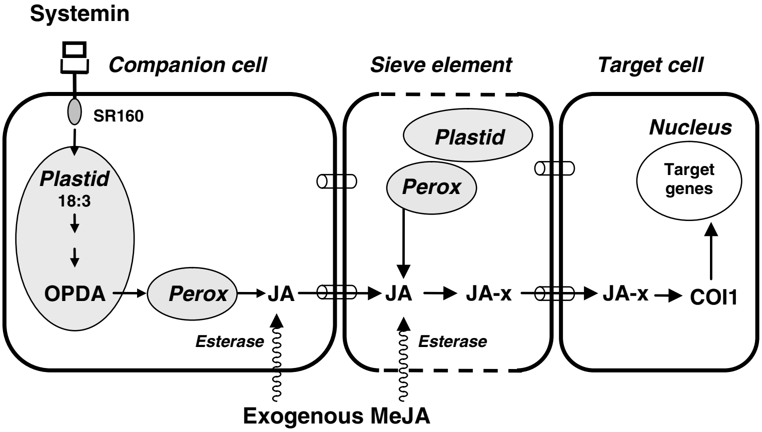

Fig. 1.2 Schematic model showing the role of JA in systemic wound signaling. Chloroplastic (Plastid) and peroxisomal (Perox) JA biosynthetic enzymes are located in vascular bundles of the leaf. Binding of systemin to its receptor (SR160) activates JA accumulation. JA synthesis in tomato leaves is also activated by systemin-independent pathways (not shown; Lee and Howe 2003). JA produced in the companion cell-sieve element complex is transported in the phloem via plasmodesmata connections between cells. JA, or a covalently modified form of JA (JA-x; such as JA-Ile), activates target gene expression in distal undamaged leaves through COI1. Esterases may convert exogenous MeJA to JA upon diffusion of MeJA across membranes

conjugation, and decarboxylation have been described (Wasternack 2007). One or more of these modifications could conceivably alter the transport, stability, or interaction of JA with target molecules (Fig. 1.2).

MeJA and certain jasmonoyl-amino acid conjugates (e.g., JA-Ile) are potent elicitors of defense gene expression (Wasternack et al. 1998). The dependence of MeJA- and JA-Ile-induced responses on COI1 indicates that both compounds are candidates for signals in the systemic wound response. Analysis of mutants that fail to produce MeJA or JA-Ile provides a powerful approach to test this hypothesis. Conversion of JA to MeJA is mediated by JA carboxyl methyltransferase (JMT), whereas conversion of JA to JA-Ile is catalyzed by the ATP-dependent adenylate-forming enzyme JAR1 (Seo et al. 2001; Staswick and Tiryaki 2004). Although the effect of loss of *JMT* function on wound-induced defense responses is not known, it is firmly established that JAR1-mediated production of JA-Ile plays a critical role in numerous jasmonate-signaled processes (Staswick and Tiryaki 2004). Moreover, recent studies have shown that JAR1 homologs in *N. attenuata* are required for wound-induced defense responses to insect attack (Kang et al. 2006). JA-Ile's key role in induced defense raises the possibility that biological responses previously attributed to JA/MeJA are in fact mediated by JA-Ile or other amino acid conjugates of JA. Consistent with this notion, physical interaction between COI1 and repressors of jasmonate-dependent gene expression, which results in proteasome-dependent degradation of the repressor proteins, was recently shown to be promoted by JA-Ile but not by JA or MeJA (Thines et al. 2007). The potency of exogenous MeJA as an elicitor of gene expression may reflect its ability to readily penetrate cellular membranes (Fig. 1.2). Once inside the cell, MeJA is likely converted to JA by specific or non-specific esterases (Stuhlfelder et al. 2004), followed by conversion to JA-Ile by JAR1 (Staswick and Tiryaki 2004). The use of *jar* mutants in grafting experiments, together with direct measurement of JA-Ile levels in phloem exudates and wounded tissues, promises to provide additional insight into the role of this bioactive conjugate in the wound signaling pathway.

1.3.3 Amplification of the Jasmonate Signal by Systemin

Activation of PI expression by systemin requires the synthesis and subsequent action of JA (Schilmiller and Howe 2005; Wasternack 2007). In the context of long-distance wound signaling, this role for systemin can be reconciled with the above-mentioned grafting studies if it is postulated that systemin activates JA synthesis at or near the site of tissue damage (Li et al. 2002; Ryan and Moura 2002). This model is consistent with grafting studies showing that a *35S::Prosys* transgenic rootstock constitutively generates a systemic signal that activates PI expression in wild-type scion leaves (Fig. 1.1Dc) (McGurl et al. 1994). Recognition of the *35S::Prosys*-derived signal in scion leaves is blocked by *jai1* but not by mutations such as *acx1* that disrupt JA biosynthesis (Fig. 1.1Dd-e) (Li et al. 2002). These findings suggest that *35S::Prosys*-expressing tissues constitutively synthesize JA, which is then mobilized to scion leaves where it initiates COI1-dependent responses in target cells. This model is consistent with the observation that *35S::Prosys* plants accumulate increased JA levels in the absence of wounding (Chen et al. 2006). Activation of PI expression in JA-deficient scions (Fig. 1.1De) indicates that the long-distance signal produced by *35S::Prosys* rootstocks is likely not systemin, but rather a signal that activates PI expression in the absence of de novo JA synthesis.

A role for systemin in localized JA production is also in agreement with results obtained from analysis of the systemin-insensitive mutant *spr1* (Howe and Ryan 1999; Lee and Howe 2003). *spr1* mutants express *PI* genes in response to elicitation by OGA and JA, but not in response to systemin and prosystemin. *Spr1* is presumably required for a signaling step that links systemin perception at the plasma membrane to activation of JA synthesis in the chloroplast. Interestingly, systemic PI expression in *spr1* plants is impaired much more than the local response (Lee and Howe 2003). This phenotype is very similar to that of *Prosys* antisense plants (Orozco-Cárdenas et al. 1993), and provides evidence that (pro)systemin functions mainly in the long-distance response. Grafting experiments provided evidence that *Spr1* function (i.e., systemin perception) is involved primarily in the generation of the systemic signal in wounded leaves and is not required for recognition of the signal in undamaged responding leaves (Fig. 1.1C). The most straightforward interpretation of these results is that (pro)systemin acts at or near the wound site to amplify JA accumulation and the strength of the systemic response.

It thus appears that numerous signals, including JA, systemin, and H_2O_2, interact through a positive feedback loop to propagate the long-distance signal via the phloem (Ryan 2000; Ryan and Moura 2002; Schilmiller and Howe 2005; Wasternack 2007). Future work is needed to understand how these signals interact with one another to promote the systemic wound response, and to determine which of these signals are functionally conserved in other plant species. The absence of *Prosys* gene homologs outside the Solanaceae suggests that systemin may have evolved in a narrow range of plants, perhaps as a mechanism to amplify systemic defense responses to insect attack (Howe 2004). The notion that systemin function is rapidly evolving is supported by recent studies indicating that a systemin homolog in *Solanum nigrum* is not involved in wound-induced direct defense responses (Schmidt and Baldwin 2006). Jasmonate-based signaling, on the other hand, appears to play a central role in regulating responses to biotic stress in all plants. Increasing evidence indicates that the role of jasmonates in promoting systemic defense may be more general than previously realized (Truman et al. 2007). These collective findings validate Ryan's original concept that chemical alarm signals produced at the plant-pest interface mediate systemic immunity to biotic stress.

1.4 Perspectives

Since the initial discovery by Ryan and coworkers of digestibility-reducing proteinase inhibitors as an inducible defense in the Solanaceae 35 years ago, inducible mechanisms for direct defense against insect herbivores have been identified throughout the plant kingdom, from unicellular green alga (Hessen and van Donk 1993; van Donk and Hessen 1993; Lampert et al. 1994) to trees (Bohlmann this volume). A plethora of inducible morphological and chemical resistance factors have been identified that reduce the availability of nutrients (e.g., incorporation of silica as structural reinforcement, antinutritive secondary metabolites and proteins), or are outright toxic to the herbivore (e.g., secondary metabolites including terpenoids, phenolics, and alkaloids). Numerous microarray studies aimed at analyzing global changes in gene expression after herbivory have confirmed the induced expression of many defensive genes. Moreover, the massive reprogramming of gene expression observed in these studies suggests that herbivory results in a shift from growth-oriented to defense-oriented plant metabolism (Hui et al. 2003; Ralph et al. 2006a, b). The number of herbivore-induced genes appears to greatly exceed the requirements for known resistance traits, suggesting that additional components of induced defense reamin to be discovered. Indeed, in addition to interfering directly with herbivore behavior or physiology, plants may use 'scorched earth' or 'escape strategies' as complementary defense measures. Valuable C and N resources are mobilized in organs threatened by herbivory, and are either used for the synthesis of resistance factors, or stored out of reach of the herbivore. Presumably, the resulting nutrient-deprived plant organs will poorly support the growth and development of attacking herbivores. Re-allocation of mobilized resources to temporary storage proteins (vegetative storage proteins and proteinase inhibitors) and/or underground storage organs (bulbs and tubers) supports later re-growth, and may allow plants to escape herbivory in time. Indeed, enhanced carbon allocation to roots in response to herbivory was recently observed in *N. attenuata*, resulting in delayed senescence and a prolonged reproductive phase. Sucrose transport to roots was found to be controlled by SNRK1, a protein kinase that was rapidly downregulated in leaves after attack by *Manduca sexta* (Schwachtje et al. 2006). Such ‘civilian defenses’ (Karban and Baldwin 1997) leading to enhanced tolerance of herbivory are still poorly understood at the molecular level and will be an important field for future research.

Tremendous progress has also been made with respect to the signaling events that lead to the systemic expression of defensive traits in response to herbivory. This includes the discovery of systemin as the first peptide with hormone-like activity in plants, which is now thought to act in the vicinity of the wound site to amplify the production of a long-distance signal in the vasculature. Although the systemic signal molecule remains to be identified, recent evidence suggests that JA or a JA metabolite – possibly JA-Ile – may act as a phloem-mobile signal. The perception of jasmonates and activation of defense genes in target tissues was shown to depend on COI1, which is part of an E_3 ubiquitin ligase (SCF^{COI1}) that was predicted to tag a repressor of JA signaling for degradation by the ubiquitin-proteasome pathway. Several members of the JAZ (Jasmonate ZIM domain) family of proteins have recently been identified as targets of SCF^{COI1} in tomato and *Arabidopsis*. At least two JAZ proteins are known to act as negative regulators of jasmonate-dependent transcription, and the COI1/JAZ1 complex was suggested to be the site of JA-Ile perception (Chini et al. 2007; Thines et al. 2007). Despite these exciting findings, there are still important questions to be resolved with respect to systemic wound signaling. This is particularly true for the early events in signal transduction that couple tissue damage to the activation of the octadecanoid pathway for JA production. Most notably, the events following systemin perception at the cell surface and the subsequent release of polyunsaturated fatty acids for oxylipin biosynthesis in chloroplasts remain to be elucidated. The tomato *spr1* mutant is impaired in this process and the identification of the genetic defect in *spr1* may turn out to be an important step in this direction.

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References

Agrawal AA (1998) Induced responses to herbivory and increased plant performance. Science 279:1201–1202

Agrawal AA (1999). Induced plant defense: evolution of induction and adaptive phenotypic plasticity. In: Agrawal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens and herbivores: biochemistry ecology and agriculture. APS Press, Minnesota, pp 251–268

Agrawal AA (2004) Resistance and susceptibility of milkweed: competition, root herbivory, and plant genetic variation. Ecology 85:2118–2133

Applebaum SW, Konijn AM (1966) The presence of a *tribolium*-protease inhibitor in wheat. J Insect Physiol 12:665–669

Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc Natl Acad Sci USA 95:8113–8118

Bennett RN, Wallsgrove RM (1994) Secondary metabolites in plant defence mechanisms. New Phytol 127:617–633

Berenbaum MR (1995) Turnabout is fair play: secondary roles for primary compounds. J Chem Ecol 21:925–940

Bergey DR, Howe GA, Ryan CA (1996) Polypeptide signaling for plant defensive genes exhibits analogies to defense signaling in animals. Proc Natl Acad Sci USA 93:12053–12058

Bergey DR, Orozco-Cardenas M, de Moura DS, Ryan CA (1999) A wound- and systemin- inducible polygalacturonase in tomato leaves. Proc Natl Acad Sci USA 96:1756–1760

Bergvinson DJ, Hamilton RI, Arnason JT (1995) Leaf profile of maize resistance factors to european corn borer, *Ostrinia nubilalis.* J Chem Ecol 21:343–354

Berryman AA (1972) Resistance of conifers to invasion by bark beetle-fungus associations. BioScience 22:598–602

Broekgaarden C, Poelman E, Steenhuis G, Voorrips R, Dicke M, Vosman B (2007) Genotypic variation in genome-wide transcription profiles induced by insect feeding: *brassica oleracea* – *Pieris rapae* interactions. BMC Genomics 8:239

Carrera E, Prat S (1998) Expression of the *Arabidopsis* abi1-1 mutant allele inhibits proteinase inhibitor wound-induction in tomato. Plant J 15:765–771

Carroll CR, Hoffman CA (1980) Chemical feeding deterrent mobilized in response to insect herbivory and counteradaptation by *Epilachna tredecimnotata.* Science 209:414–416

Chen H, Jones AD, Howe GA (2006) Constitutive activation of the jasmonate signaling pathway enhances the production of secondary metabolites in tomato. FEBS Lett 580:2540–2546

Chen H, Wilkerson CG, Kuchar JA, Phinney BS, Howe GA (2005) Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. Proc Natl Acad Sci USA 102:19237–19242

Cheong YH, Chang H-S, Gupta R, Wang X, Zhu T, Luan S (2002) Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis.* Plant Physiol 129:661–677

Chini A, Fonseca S, Fernández G, Adie B, Chico JM, Lorenzo O, García-Casado G, López-Vidriero I, Lozano FM, Ponce MR, Micol JL, Solano R (2007) The JAZ family of repressors is the missing link in jasmonate signalling. Nature 448:666–671

Christopher ME, Miranda M, Major IT, Constabel CP (2004) Gene expression profiling of systemically wound-induced defenses in hybrid poplar. Planta 219:936–947

Coley PD, Barone JA (1996) Herbivory and plant defenses in tropical forests. Annu Rev Ecol Syst 27:305–335

Constabel CP (1999) A survey of herbivory-inducible defensive proteins and phytochemicals. In: Agrawal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens and herbivores. Biochemistry, ecology, and agriculture. APS Press, St. Paul, Minesota, pp 137–166

Davis JM, Gordon MP, Smit BA (1991) Assimilate movement dictates remote sites of wound-induced gene expression in poplar leaves. Proc Natl Acad Sci USA 88:2393–2396

De Vos M, Van Oosten VR, Van Poecke RMP, Van Pelt JA, Pozo MJ, Mueller MJ, Buchala AJ, Metraux JP, Van Loon LC, Dicke M et al (2005) Signal signature and transcriptome changes of *Arabidopsis* during pathogen and insect attack. Mol Plant Microbe Int 18:923–937

Delessert C, Wilson IW, Van der Straeten D, Dennis ES, Dolferus R (2004) Spatial and temporal analysis of the local response to wounding in *Arabidopsis* leaves. Plant Mol Biol 55:165–181

Dussourd DE, Denno RF (1994) Host-range of generalist caterpillars – trenching permits feeding on plants with secretory canals. Ecology 75:69–78

Dussourd DE, Eisner T (1987) Vein-cutting behavior: insect counterploy to the latex defense of plants. Science 237:898–901

Dussourd DE, Hoyle AM (2000) Poisoned plusiines: toxicity of milkweed latex and cardenolides to some generalist caterpillars. Chemoecology 10:11–16

Ehrlich PR, Raven PH (1964) Butterflies and plants: a study in coevolution. Ecology 18:586–608

Farmer EE, Ryan CA (1992) Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell 4:129–134

Feeny P (1970) Seasonal change in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51:565–581

Felix G, Boller T (1995) Systemin induces rapid ion fluxes and ethylene biosynthesis in *Lycopersicon peruvianum* cells. Plant J 7:381–389

Felton GW (2005) Indigestion is a plant's best defense. Proc Natl Acad Sci USA 102:18771–18772

Fisher DB (1990) Measurement of phloem transport rates by an indicator-dilution technique. Plant Physiol Biochem 94:455–462

Fraenkel GS (1959) The raison d'etre of secondary plant substances: these odd chemicals arose as a means of protecting plants from insects and now guide insects to food. Science 129:1466–1470

Gatehouse JA (2002) Plant resistance towards insect herbivores: a dynamic interaction. New Phytol 156:145–169

Giri AP, Wunsche H, Mitra S, Zavala JA, Muck A, Svatos A, Baldwin IT (2006) Molecular interactions between the specialist herbivore *Manduca sexta* (*Lepidoptera, Sphingidae*) and its natural host *Nicotiana attenuata*. VII. Changes in the plant's proteome. Plant Physiol 142:1621–1641

Green TR, Ryan CA (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defense mechanism against insects. Science 175:776–777

Guariguata MR, Gilbert GS (1996) Interspecific variation in rates of trunk wound closure in a panamanian lowland forest. Biotropica 28:23–29

Hause B, Hause G, Kutter C, Miersch O, Wasternack C (2003) Enzymes of jasmonate biosynthesis occur in tomato sieve elements. Plant Cell Physiol 44:643–648

Hessen DO, van Donk E (1993) Morphological changes in *Scenedesmus* induced by substances released from *Daphnia*. Arch Hydrobiol 127:129–140

Howe GA (2004) Jasmonates as signals in the wound response. J Plant Growth Regul 23:223–237

Howe GA, Ryan CA (1999) Suppressors of systemin signaling identify genes in the tomato wound response pathway. Genetics 153:1411–1421

Howlett BG, Clarke AR, Madden JL (2001) The influence of leaf age on the oviposition preference of *Chrysophtharta bimaculata* (Olivier) and the establishment of neonates. Agric For Entomol 3:121–127

Hui D, Iqbal J, Lehmann K, Gase K, Saluz HP, Baldwin IT (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (*Lepidoptera, Sphingidae*) and its natural host *Nicotiana attenuata*: V. Microarray analysis and further charaterization of large-scale changes in herbivore-induced mRNAs. Plant Physiol 131:1877–1893

Ishiguro S, Kawai-Oda A, Nishida I, Okada K (2001) The *defective in anther dehiscence1* gene encodes a novel phospholipase a1 catalyzing the initial step of jasmonic acid biosynthesis, which synchronizes pollen maturation, anther dehiscence, and flower opening in *Arabidopsis*. Plant Cell 13:2191–2209

Kang J-H, Wang L, Giri A, Baldwin IT (2006) Silencing threonine deaminase and JAR4 in N*icotiana attenuata* impairs jasmonic acid-isoleucine-mediated defenses against *Manduca sexta.* Plant Cell 18:3303–3320

Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago

Karban R, Myers JH (1989) Induced plant responses to herbivory. Ann Rev Ecol Sys 20:331–348

Karban R, Kuc J (1999). Induced resistance against pathogens and herbivores: an overview. In: Agrawal AA, Tuzun S, Bent E (eds) Induced plant defenses against pathogens and herbivores. Biochemistry, ecology, and agriculture, APS Press, St. Paul, Minnesota, pp 1–16

Kenrick P, Crane PR (1997) The origin and early evolution of plants on land. Nature 389:33–39

Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. Ann Rev Plant Biol 53:299–328

Kuhn E, Schaller A (2004). DNA microarrays: methodology, data evaluation, and application in the analysis of plant defense signaling. In: Setlow JK (ed) Genetic engineering, principles and methods. Kluwer Academic, Plenum, pp 49–84

Lampert W, Rothhaupt KO, von Elert E (1994) Chemical induction of colony formation in a green alga (*Scenedesmus acutus*) by grazers (*Daphnia*). Limnol Oceanogr 39:1543–1550

Lawrence SD, Dervinis C, Novak N, Davis JM (2006) Wound and insect herbivory responsive genes in poplar. Biotechnol Lett 28:1493–1501

Lee GI, Howe GA (2003) The tomato mutant *spr1* is defective in systemin perception and the production of a systemic wound signal for defense gene expression. Plant J 33:567–576

León J, Rojo E, Sánchez-Serrano JJ (2001) Wound signalling in plants. J Exp Bot 52:1–9

Li L, Li C, Lee GI, Howe GA (2002) Distinct roles for jasmonate synthesis and action in the systemic wound response of tomato. Proc Natl Acad Sci USA 99:6416–6421

Li C, Liu G, Xu C, Lee GI, Bauer P, Ling HQ, Ganal MW, Howe GA (2003) The tomato *suppressor of prosystemin-mediated responses2* gene encodes a fatty acid desaturase required for the biosynthesis of jasmonic acid and the production of a systemic wound signal for defense gene expression. Plant Cell 15:1646–1661

Li CY, Schilmiller AL, Liu GH, Lee GI, Jayanty S, Sageman C, Vrebalov J, Giovannoni JJ, Yagi K, Kobayashi Y et al (2005) Role of beta-oxidation in jasmonate biosynthesis and systemic wound signaling in tomato. Plant Cell 17:971–986

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA (2004) The tomato homolog of coronatine-insensitive1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell 16:126–143

Lipke H, Fraenkel GS, Liener IE (1954) Effect of soybean inhibitors on growth of *Tribolium confusum*. J Agr Food Chem 2:410–414

Lippert D, Chowrira S, Ralph SG, Zhuang J, Aeschliman D, Ritland C, Ritland K, Bohlmann J (2007) Conifer defense against insects: proteome analysis of sitka spruce (*Picea sitchensis*) bark induced by mechanical wounding or feeding by white pine weevils (*Pissodes strobi*). Proteomics 7:248–270

Lucas WJ, Lee JY (2004) Plant cell biology – plasmodesmata as a supracellular control network in plants. Nat Rev Mol Cell Bio 5:712–726

Major IT, Constabel CP (2006) Molecular analysis of poplar defense against herbivory: comparison of wound- and insect elicitor-induced gene expression. New Phytol 172:617–635

Malone M (1996). Rapid, long-distance signal transmission in higher plants. Advances in botanical research. Academic Press, San Diego, pp 163–228

Mauricio R (1998) Costs of resistance to natural enemies in field populations of the annual plant *Arabidopsis thaliana.* Am Nat 151:20–28

Mauricio R (2000) Natural selection and the joint evolution of tolerance and resistance as plant defenses. Evol Ecol 14:491–507

McGurl B, Orozco-Cardenas M, Pearce G, Ryan CA (1994) Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. Proc Natl Acad Sci USA 91:9799–9802

McGurl B, Pearce G, Orozco-Cardenas M, Ryan CA (1992) Structure, expression and antisense inhibition of the systemin precursor gene. Science 255:1570–1573

McNaughton SJ, Tarrants JL (1983) Grass leaf silicification: natural selection for an inducible defense against herbivores. Proc Natl Acad Sci USA 80:790–791

Moyen C, Hammond-Kosack KE, Jones J, Knight MR, Johannes E (1998) Systemin triggers an increase of cytoplasmic calcium in tomato mesophyll cells: Ca^{2+} mobilization from intra- and extracellular compartments. Plant Cell Environ 21:1101–1111

Myers JH, Bazely D (1991). Thorns, spines, prickles, and hairs: are they stimulated by herbivory and do they deter herbivores? In: Tallamy DW Raupp MJ (eds) Phytochemical induction by herbivores. John Wiley & Sons, New York, pp 325–344

Narváez-Vásquez J, Florin-Christensen J, Ryan CA (1999) Positional specificity of a phospholipase a activity induced by wounding, systemin, and oligosaccharide elicitors in tomato leaves. Plant Cell 11:2249–2260

Navazio L, Moscatiello R, Bellincampi D, Baldan B, Meggio F, Brini M, Bowler C, Mariani P (2002) The role of calcium in oligogalacturonide-activated signalling in soybean cells. Planta 215:596–605

O'Donnell PJ, Calvert C, Atzorn R, Wasternack C, Leyser HMO, Bowles DJ (1996) Ethylene as a signal mediating the wound response of tomato plants. Science 274:1914–1917

Orozco-Cárdenas M, McGurl B, Ryan CA (1993) Expression of an antisense prosystemin gene in tomato plants reduces the resistance toward *Manduca sexta* larvae. Proc Natl Acad Sci USA 90:8273–8276

Orozco-Cárdenas ML, Narváez-Vasquez J, Ryan CA (2001) Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell 13:179–191

Painter RH (1936) The food of insects and its relation to resistance of plants to insect attack. Am Nat 70:547–566

Pearce G, Strydom D, Johnson S, Ryan CA (1991) A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. Science 253:895–898

Phillips MA, Croteau RB (1999) Resin-based defenses in conifers. Trends Plant Sci 4:184–190

Ralph S, Oddy C, Cooper D, Yueh H, Jancsik S, Kolosova N, Philippe RN, Aeschliman D, White R, Huber D et al. (2006a) Genomics of hybrid poplar (*Populus trichocarpa x deltoides*) interacting with forest tent caterpillars (*Malacosoma disstria*): normalized and full-length cDNA libraries, expressed sequence tags, and a cDNA microarray for the study of insect-induced defences in poplar. Mol Ecol 15:1275–1297

Ralph SG, Yueh H, Friedmann M, Aeschliman D, Zeznik JA, Nelson CC, Butterfield YSN, Kirkpatrick R, Liu J, Jones SJM et al (2006b) Conifer defence against insects: microarray gene expression profiling of sitka spruce (*Picea sitchensis*) induced by mechanical wounding or feeding by spruce budworms (*Choristoneura occidentalis*) or white pine weevils (P*issodes strobi*) reveals large-scale changes of the host transcriptome. Plant Cell Environ 29:1545–1570

Reymond P, Bodenhausen N, Van Poecke RM, Krishnamurthy V, Dicke M, Farmer EE (2004) A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 16:3132–3147

Rhoades DF, Cates RG (1976). Towards a general theory of plant antiherbivore chemistry. In: Wallace JW Mansell RL (eds) Biochemical interaction between plants and insects. Plenum Press, New York, pp 168–213

Rittinger PA, Biggs AR, Peirson DR (1987) Histochemistry of lignin and suberin deposition in boundary layers formed after wounding in various plant species and organs. Can J Bot 65: 1886–1892

Ryan CA (2000) The systemin signaling pathway: differential activation of plant defensive genes. Biochim Biophys Acta 1477:112–121

Ryan CA, Huisman W (1969) The regulation of synthesis and storage of chymotrypsin inhibitor I in leaves of potato and tomato plants. Plant Physiol 45:484–489

Ryan CA, Moura DS (2002) Systemic wound signaling in plants: a new perception. Proc Natl Acad Sci USA 99:6519–6520

Sagi M, Davydov O, Orazova S, Yesbergenova Z, Ophir R, Stratmann JW, Fluhr R (2004) Plant respiratory burst oxidase homologs impinge on wound responsiveness and development in *Lycopersicon esculentum*. Plant Cell 16:616–628

Schaller A, Oecking C (1999) Modulation of plasma membrane H^+-ATPase activity differentially activates wound and pathogen defense responses in tomato plants. Plant Cell 11: 263–272

Scheer JM, Ryan CA (1999) A 160 kda systemin receptor on the cell surface of *Lycopersicon peruvianum* suspension cultured cells: kinetic analyses, induction by methyl jasmonate and photoaffinity labeling. Plant Cell 11:1525–1535

Scheer JM, Ryan CA (2002) The systemin receptor SR160 from *Lycopersicon peruvianum* is a member of the LRR receptor kinase family. Proc Natl Acad Sci USA 99:9585–9590

Schilmiller AL, Howe GA (2005) Systemic signaling in the wound response. Curr Opin Plant Biol 8:369–377

Schittko U, Baldwin IT (2003) Constraints to herbivore-induced systemic responses: bidirectional signaling along orthostichies in *Nicotiana attenuata*. J Chem Ecol 29:763–770

Schmidt S, Baldwin IT (2006) Systemin in *Solanum nigrum*. The tomato-homologous polypeptide does not mediate direct defense responses. Plant Physiol Biochem 142:1751–1758

Schmidt DD, Voelckel C, Hartl M, Schmidt S, Baldwin IT (2005) Specificity in ecological interactions. Attack from the same lepidopteran herbivore results in species-specific transcriptional responses in two solanaceous host plants. Plant Physiol 138:1763–1773

Schoonhoven LM, Van Loon JJA, Dicke M (2005) Insect-plant biology, Oxford University Press, Oxford

Schwachtje J, Minchin PEH, Jahnke S, van Dongen JT, Schittko U, Baldwin IT (2006) SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. Proc Natl Acad Sci USA 103:12935–12940

Seo HS, Song JT, Cheong J-J, Lee J-H, Lee Y-W, Hwang I, Lee JS, Choi YD (2001) Jasmonic acid carboxyl methyltransferase: a key enzyme for jasmonate-regulated plant responses. Proc Natl Acad Sci USA 98:4788–4793

Smith CM, Rodriguez-Buey M, Karlsson J, Campbell MM (2004) The response of the poplar transcriptome to wounding and subsequent infection by a viral pathogen. New Phytologist 164:123–136

Soltis PS, Soltis DE (2004) The origin and diversification of angiosperms. Am J Bot 91:1614–1626

Stahl E (1888) Pflanzen und Schnecken: Biologische Studie über die Schutzmittel der Pflanzen gegen Schneckenfrass. Jena Z Naturwiss 22:557–684

Staswick PE (1994) Storage proteins of vegetative plant tissue. Ann Rev Plant Physiol Plant Mol Biol 45:303–322

Staswick PE, Tiryaki I (2004) The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. Plant Cell 16:2117–2127

Stenzel I, Hause B, Maucher H, Pitzschke A, Miersch O, Ziegler J, Ryan CA, Wasternack C (2003) Allene oxide cyclase dependence of the wound response and vascular bundle-specific generation of jasmonates in tomato – amplification in wound signalling. Plant J 33:577–589

Strassner J, Schaller F, Frick UB, Howe GA, Weiler EW, Amrhein NA, Macheroux P, Schaller A (2002) Characterization and cdna-microarray expression analysis of 12-oxophytodienoate reductases reveals differential roles for octadecanoid biosynthesis in the local versus the systemic wound response. Plant J 32:585–601

Stratmann JW, Ryan CA (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 USA 94:11085–11089

Stuhlfelder C, Mueller MJ, Warzecha H (2004) Cloning and expression of a tomato cDNA encoding a methyl jasmonate cleaving esterase. Eur J Biochem 271:2976–2983

Thines B, Katsir L, Melotto M, Niu Y, Mandaokar A, Liu G, Nomura K, He SY, Howe GA, Browse J (2007) JAZ repressor proteins are targets of the $\mathrm{SCF}^{\mathrm{COI1}}$ complex during jasmonate signalling. Nature 448:661–665

Thompson GA, Goggin FL (2006) Transcriptomics and functional genomics of plant defence induction by phloem-feeding insects. J Exp Bot 57:755–766

Trapp S, Croteau R (2001) Defensive resin biosynthesis in conifers. Ann Rev Plant Physiol Plant Mol Biol 52:689–724

Truman W, Bennettt MH, Kubigsteltig I, Turnbull C, Grant M (2007) *Arabidopsis* systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. Proc Natl Acad Sci USA 104:1075–1080 van Donk E, Hessen DO (1993) Grazing resistance in nutrient-stressed phytoplankton. Oecologia 93:508–511

Voelckel C, Baldwin IT (2004) Herbivore-induced plant vaccination. Part II. Array-studies reveal the transience of herbivore-specific transcriptional imprints and a distinct imprint from stress combinations. Plant J 38:650–663

Walling LL (2000) The myriad plant responses to herbivores. J Plant Growth Regul 19:195–216

Wasternack C (2007) Jasmonates: an update on biosynthesis, signal transduction and action in plant stress response, growth and development. Ann Bot, 1–17

Wasternack C, Ortel B, Miersch O, Kramell R, Beale M, Greulich F, Feussner I, Hause B, Krumm T, Boland W et al (1998) Diversity in octadecanoid-induced gene expression of tomato. J Plant Physiol 152:345–352

Weis AE, Franks SJ (2006) Herbivory tolerance and coevolution: an alternative to the arms race? New Phytol 170:423–425

Wildon DC, Thain JF, Minchin PEH, Gubb IR, Reilly AJ, Skipper YD, Doherty HM, O'Donnell PJ, Bowles DJ (1992) Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. Nature 360:362–65

Xie D-X, Feys BF, James S, Nieto-Rostro M, Turner JG (1998) *Coi1*: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science 280:1091–1094

Zhang ZP, Baldwin IT (1997) Transport of [2-C-14] jasmonic acid from leaves to roots mimics wound-induced changes in endogenous jasmonic acid pools in *Nicotiana sylvestris*. Planta 203:436–441

Zhu-Salzman K, Salzman RA, Ahn JE, Koiwa H (2004) Transcriptional regulation of sorghum defense determinants against a phloem-feeding aphid. Plant Physiol 134:420–431