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Signal interactions in induced resistance to pathogens and insect herbivores

Richard M. Bostock[1,3], Richard Karban[2], Jennifer S. Thaler[4], Philip D. Weyman[1] and David Gilchrist[1,3]
[1]*Department of Plant Pathology,* [2]*Department of Entomology,* [3]*The Center for Engineering Plants for Resistance Against Pathogens (CEPRAP), University of California, Davis, CA 95616-8680, USA (Phone: +1 530 7520308; Fax: +1 530 7525674; E-mail: rmbostock@ucdavis.edu);* [4]*Department of Botany, University of Toronto, Toronto M5S 3B2, Canada*

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Abstract

Plants are often simultaneously challenged by pathogens and insects capable of triggering an array of responses that may be beneficial or detrimental to the plant. The efficacy of resistance mechanisms can be strongly influenced by the mix of signals generated by biotic stress as well as abiotic stress such as drought, nutrient limitation or high soil salinity. An understanding of their biochemical nature, and knowledge of the specificity and compatibility of the signaling systems that regulate the expression of inducible responses could optimize the utilization of these responses in crop protection. Signaling conflicts and synergies occur during a plant's response to pathogens and insect herbivores, and much of the research on defense signaling has focused on salicylate- and jasmonate-mediated responses. We will review our results using tomato (*Lycopersicon esculentum*) in greenhouse and field studies that illustrate a trade-off between salicylate- and jasmonate-mediated signaling, and discuss research on strategies to minimize the trade-off that can occur following the application of chemical elicitors of resistance. In addition, there is evidence of another signaling system that mediates endogenous levels of ceramide in the plant. This signal is associated with programmed cell death and protection of tomato against the fungal pathogen *Alternaria alternata* f. sp. *lycopersici*.

Introduction

Plants resist pathogens and insect herbivores by complex defenses that are poorly understood. These defenses are strongly influenced by the mix of signals generated by external biotic and abiotic stresses. It seems intuitive that we need a solid, fundamental knowledge of their genetic and biochemical nature, and of the specificity and compatibility of the signaling networks, to utilize natural defenses optimally in agriculture. Some studies reveal synergies among signals generated during infection to induce host defense and resistance (Bostock, 1999; Graham and Graham, 1994; Hammerschmidt, 1993; Xu et al., 1994). However, other studies point to a potential increased vulnerability when plants contend with different types of attackers that engage different response pathways (Felton et al., 1999; Thaler et al., 1999). There is also substantial evidence that abiotic stresses, such as drought, nutrient limitation, or high soil salinity, predispose plants to disease and predation through systemic modification of host physiology that severely compromises resistance (Abeles et al., 1992; O'Donnell et al., 1996). As all of the candidate defense-related signal molecules can strongly influence multiple processes in plant growth and development, it is not unexpected that these signals can interact in ways that can influence their ability to affect plant responses to pests (Abeles et al., 1992; Bostock, 1999; Hamberg and Gardner, 1992; Raskin, 1992; Staswick, 1992). The potential for signaling conflicts and unproductive interactions should provoke caution with respect to the use

of so-called 'safe' chemistry targeted to engage host resistance mechanisms.

The current understanding of the biochemical and molecular basis for resistance and susceptibility of plants to pests has advanced as a result of studies that typically have used genetically well-defined, single plant–pest interactions (Parker et al., 2000). What emerges from these studies is an understanding that the dominant signaling pathways involved and the cellular responses to these signals are complex and highly regulated, that similarity, even identity, as well as dissimilarity exists in the specific genes and gene products induced by different stresses, and that positive and negative cross-talk occurs among pathways regulating host gene expression and response. Consideration of the mechanistic basis for the short-circuiting of resistance pathways could suggest strategies to avoid futile signal combinations, and thereby help optimize inducible resistance for pest management and foster its judicious use in agriculture. It follows that effective utilization of host resistance, and particularly chemically- and biologically-induced resistance, could be enhanced by an integrated approach to the study of plant performance to the multiple stresses that occur in the field.

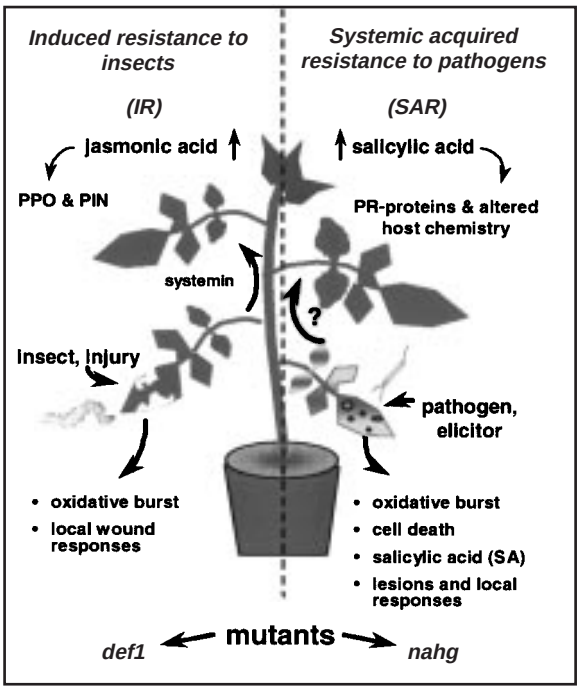

Figure 1. Comparison of general features of IR and SAR, with particular reference to their occurrence in tomato.

Comparison of SA- and JA-mediated induced resistance

The important roles of salicylic acid (SA) and jasmonic acid (JA) in defense signaling have become known largely through studies of systemic acquired resistance (SAR) to pathogens (in the case of SA), and through studies of wound/herbivore induced resistance (IR) to insects (in the case of JA). We have used SAR and IR as convenient terminology to discriminate the two contexts of study, but recognize that this distinction is somewhat arbitrary and alternative terminology is often used. Figure 1 illustrates some of the key features of both response pathways, and calls attention to the powerful role that plant mutants have played in discerning the critical involvement of each signal (e.g., *def*1 (Howe et al., 1996); *nahG* (Gaffney et al., 1993)). Although much of this work has been achieved with agronomic species, such as tobacco and cucumber as models, the more recent studies in Arabidopsis with the many mutants now available in both signal generation and perception are fast revealing critical elements within the transduction paths (Delaney, 2000; Howe and Ryan, 1999).

Pathogens and insects, in a general sense, can elicit many of the same responses in plant foliage, albeit the temporal and spatial expression in relation to the onset of attack may differ widely depending on the attacking agent (Agrawal, 2000; Hatcher, 1995; Karban and Baldwin, 1997). Although these shared features enticed early speculation of a common signal transduction pathway governing resistance responses to both insects and pathogens, more intensive work in this area has made it clear that plant hosts respond differently to different attackers to engage different response pathways. Subsequent investigations suggested that there might be a dichotomy in signaling governing responses to pathogens and insects, but this notion too has turned out to be simplistic and was not borne out by further research (Fidantsef et al., 1999). Nonetheless, our studies and those from other groups have shown that negative interactions can occur between the SA and JA pathways in plants, with consequences for host resistance against attackers. We also now know that the SA- and JA-response pathways are not mutually exclusive in certain stress contexts (e.g., infection by *Pseudomonas* spp.), and that additional cues such as ethylene can function as synergists or enhancers of

the action of the other signals (Xu et al., 1994). This raises intriguing questions about differential regulation of SA and JA synthesis and perception in the plant, and their degree of cross-talk with other signaling pathways.

Signaling cross-talk and evidence for trade-offs in resistance in greenhouse and field studies with tomato

That cross-talk can occur among signaling pathways is evident from much of the early work on phytohormone regulation of plant disease resistance, wherein exogenous application of 'classical' hormones in various combinations to plant tissues were shown to modify disease phenotypes (i.e., susceptible to resistant, and vice versa (Bostock and Stermer, 1989)). Since phytohormones and other cellular signals capable of modifying host resistance and susceptibility also govern highly regulated processes seemingly unrelated to defense, it would seem that cross-talk would be expected to occur. Genoud and Métraux (1999) nicely summarized many of the positive and negative interactions that have been reported for endogenous and exogenous (e.g., light) signals, and drew upon the analogy that these interactions might behave as a Boolean network containing logic gates and circuits, as has been proposed for modeling other complex biological networks. Specific research that helped frame some of our experimental studies derives from the demonstration of inverse metabolic responses to wounding and pathogens or their elicitors (Bostock and Stermer, 1989; Choi et al., 1992; 1994), and the early report by Doherty et al. (1988) and subsequently by others (Pena-Cortes et al., 1993) showing salicylate inhibition of wound responsive, JA-regulated genes. These studies suggested to us some important, and perhaps obvious, questions. Since salicylates inhibit JA-responsive gene expression, would SAR-induced plants show an increased vulnerability to insects and perhaps other attackers? Do chemical inducers of resistance to one type of pest influence resistance to another type of pest?

About six years ago, we embarked upon a collaborative effort to address issues of cross-talk in signaling using tomato *(Lycopersicon esculentum)* challenged with various pathogens and insects, a system that provides a good model for studies of systemic signaling. An experimental format developed by Sean Duffey and his colleagues was adopted, in which both

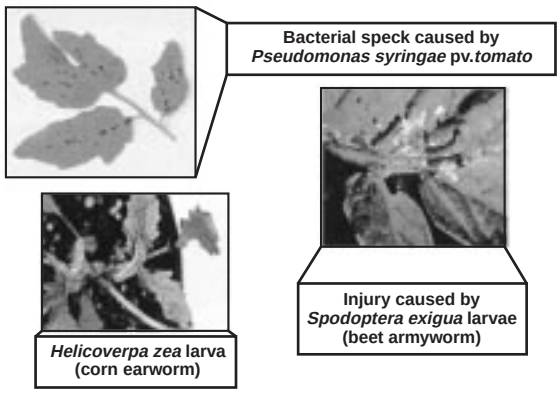

Figure 2. Principal biological agents used in this study.

local and systemic responses could be monitored and foliar resistance to various kinds of attackers could be assessed conveniently (Stout et al., 1994). In most of our greenhouse experiments, the terminal leaflets of the third and fourth leaves of four-week-old tomato plants were challenged to provide the 'inducing' stimulus. After an appropriate period the subterminal leaflets of the same leaf or leaflets of different leaves were inoculated with pathogens or exposed to insects to assess resistance. In most of these experiments, we used *Pseudomonas syringae* pv. *tomato (Pst)*, causal agent of bacterial speck disease, and larvae of the noctuids, *Helicoverpa zea* (corn earworm) or *Spodoptera exigua* (beet armyworm), in various combinations (Figure 2). Additional pathogenic agents (e.g., *Phytophthora infestans)* and insects (e.g., aphids, cabbage looper) also were examined. Changes in foliar biochemistry and gene expression were monitored at the time of challenge, focusing on various hallmark indicators to assess the degree of SA- and JA-response coupling. Chemical inducers of resistance, specifically a synthetic mimic of SA action, benzothiadiazole (BTH; Gorlach et al., 1996), and the octadecanoids JA or methyl jasmonate, were tested as well to compare host responses induced by the elicitors with those induced by the biotic agents. Most of this work has now been published (Fidantsef et al., 1999; Stout et al., 1998; 1999; Thaler et al., 1999). Highlights, including some unpublished results, are summarized here.

In experiments with tomato foliage we found that the type of herbivory by insects of different feeding guilds determined the nature of the induced responses. Chewing damage caused during feeding by larvae of the noctuids *S. exigua* or *H. zea* strongly induced

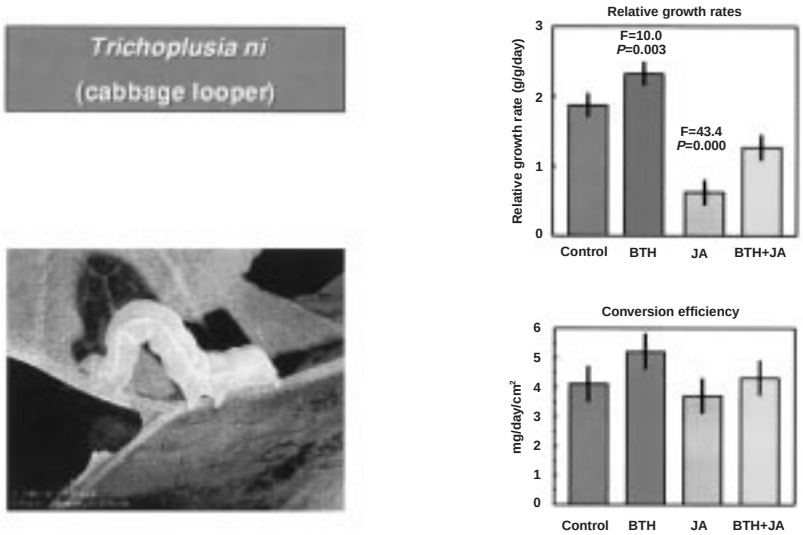

Figure 3. Performance of cabbage looper larvae on chemically-induced foliage of tomoto.

proteinase inhibitors and polyphenol oxidase activity, and artificial wounding or application of JA to the plants could mimic this effect. Feeding damage resulted in the foliage throughout the plant becoming less palatable to larvae of these same species upon subsequent challenge, as reflected in reduced relative growth rates and survival of larvae on the protected leaves. Similarly, application of JA, but not SA or BTH, strongly 'protected' tomato plants against noctuid larvae, which showed much reduced relative growth rates on the treated, induced leaves. In contrast, aphid feeding damage by either of two species of aphid (*Macrosiphum euphorbia* and *Myzus persicae*) induced host responses that were similar to those observed with pathogens or SA/BTH treatment, i.e. the aphids were potent inducers of PR proteins but did not elicit proteinase inhibitors (Fidantsef et al., 1999; Stout et al., 1999).

Pst strongly induced the set of responses typically associated with SAR and SA signaling, such as PR-protein expression both locally and systemically, and induced resistance to subsequent *Pst* infection as manifested by reduced lesion number and size in the systemically protected leaves. Likewise, treatment of tomato leaves with BTH (1.2 mM) induced resistance to *Pst*. However, *Pst* also strongly induced proteinase inhibitors and the responses typically associated with octadecanoid treatment or wounding by noctuid larvae. Larvae allowed to feed on foliage from the *Pst*-induced plants performed significantly less well than on control leaves.

The results with *Pst* are surprising in the light of the experiments with chemically-induced plants. Noctuid larvae performed better on BTH-treated plants expressing SAR (for example see Figure 3), and simultaneous treatment of leaves with BTH and JA partially offset the JA-induced resistance. BTH also inhibited the expression of JA-mediated responses such as polyphenoloxidase and proteinase inhibitor induction. Thus, although SA and BTH can inhibit JA-mediated resistance and increase the vulnerability of tomato foliage to herbivory by certain insects, *Pst* infection in some way allows for the concurrent expression of both the SA and JA signaling pathways. How this occurs is unresolved. It is possible that bacterial infection enlists spatially distinct cell types in which SA and JA signal generation and perception are segregated, with a degree of segregation and site-specificity that could not be expected when these chemicals are applied exogenously to the foliage. The bacteria might also generate additional signals that nullify any SA counteraction of JA-signaling.

In addition to extensive greenhouse studies, we also demonstrated that a trade-off in resistance signaling could occur under field conditions. In artificially-infested tomato plants growing in the field during the summer in Davis, CA and maintained using standard agricultural practices, stimulation of SAR with BTH attenuated the JA-induced expression of polyphenol oxidase, and compromised host plant resistance to larvae of *S. exigua* (Thaler et al., 1999). Conversely, treatment of plants with JA, at concentrations that

induce resistance to insects, reduced PR protein gene expression induced by BTH, and partially reversed the protective effect of BTH against bacterial speck disease. Thus, the trade-off may operate in both directions, although the basis for the latter effect in field-grown plants is unclear and was not observed consistently under greenhouse conditions.

From studies conducted in the greenhouse and field with a variety of challenge organisms we find there is reciprocity in induced resistance to certain insects and pathogens, with *Pst* inducing resistance to itself and to noctuid larvae, and, to some extent, vice versa. However, SAR induction by chemical treatment may carry a potential risk by increasing the vulnerability of plants to certain insects. Is the trade-off observed when BTH-treated tomatoes are challenged with other types of insects? Recent experiments indicate that mites may perform slightly better on tomatoes expressing chemically-induced SAR, but performance of thrips and *Manduca sexta* larvae appear to be unaffected on SAR-expressing plants relative to control plants (unpublished results).

Can adjustment of the timing of application and the concentrations of BTH and JA avoid the trade-off? To some extent yes. Our experiments indicate that reducing the concentrations of BTH and JA to levels that still afford protection when applied singly and staggering the application of the elicitors over a 48 h period, rather than applying them simultaneously, avoided the trade-off (unpublished results). However, the trade-off was observed regardless of concentration whenever the chemicals were applied at the same time.

Ceramide: another component in systemic signaling and defense?

JA and SA are not the only signals we have investigated in tomato in the context of plant–pathogen interactions. Cell death is known to be induced in tomato by AAL-toxins and fumonisins. The AAL-toxins and fumonisins are a group of chemically related phytotoxins produced by *Alternaria alternata* f. sp. *lycopersici* and *Fusarium moniliforme*, respectively. These compounds, which bear a structural relationship to the sphingoid base, sphingosine, also are widespread mycotoxins with important health implications (Figure 4; Gilchrist et al., 1995). They have potent pathologic effects in animals ranging from neoplasms to renal, neural, and hepatic necrosis. *A. alternata* f. sp. *lycopersici* causes the Alternaria stem canker disease

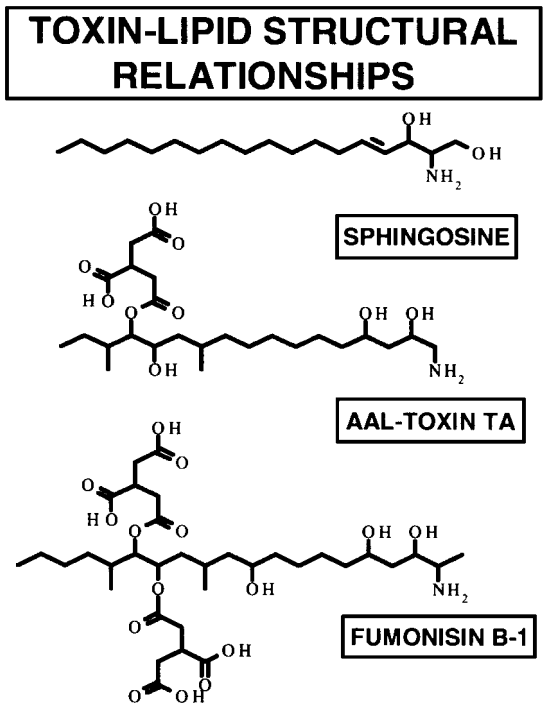

Figure 4. Chemical structures of sphingosine and sphingosine-analog mycotoxin.

in tomatoes, while *F. moniliforme* causes pink ear rot of maize and is associated with post-harvest contamination of many different food staples. AAL-toxin is a host-selective pathotoxin having specific toxicity to tomato lines containing the recessive *asc* gene, which is also the host range determinant of susceptibility to the pathogen (Gilchrist and Grogan, 1976). Tomato lines homozygous for the recessive Asc allele are extremely sensitive to a family of AAL-toxins and the structurally-related fumonisins. Several years ago we demonstrated that both toxins also induce programmed cell death (apoptosis) in tomato plants (Wang et al., 1996a), as they do in animal cells (Wang et al., 1996b).

In plants and animals, AAL-toxin and fumonisin have been shown to be potent inhibitors of ceramide synthase, a step that catalyzes the conversion of sphinganine to ceramide (Figure 5; Merrill et al., 1993). Ceramide and other sphingolipids are now acknowledged to play important regulatory roles in cellular homeostasis, whereby the intracellular levels of ceramide provide a sort of 'biostat' for the cell to govern cell growth, viability and differentiation (Hannun, 1996), and changes in ceramide levels can evoke profound physiological responses (Gomez-Munoz, 1998;

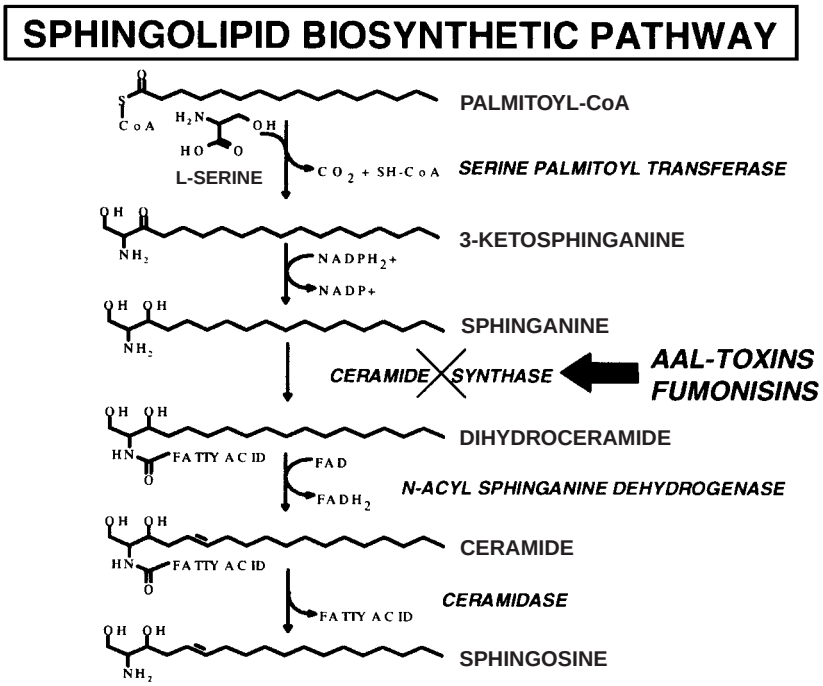

Figure 5. Ceramide biosynthetic pathway and site of action of sphingosine-analog mycotoxins.

Haimovitz-Friedman et al., 1997). Sphingoid bases also are mediators of signal transduction leading to apoptosis in animals. In light of the importance of ceramide and related sphingolipids as second messengers, and the structural similarity of the toxins to sphingosine, the connection between alterations in sphingolipid metabolism in tomato plants and toxin-induced cell death was examined.

Significant inhibition of ceramide synthase in microsomal preparations of tomato occurred at 20 nM with an I_{50} in the range 35–40 nM for AAL-toxin TA and fumonisin FB1. Although the specific perturbations in physiological processes required for cell death following toxin exposure are complex (Gilchrist et al., 1992; Moore et al., 1999), the impact on ceramide metabolism in the plant cell is dramatic. Time course analyses of sensitive leaf tissue (*asc/asc*) following toxin exposure showed that the inhibition in ceramide synthase activity, with the expected perturbations in the pool sizes of sphinganine (the substrate) and ceramide (the product), preceeded the onset of cell death. These changes were not observed in the resistant, *Asc/Asc* line, which is insensitive to the toxins.

Since the TA-toxin and FB1 inhibit ceramide synthesis with a concomitant drop in ceramide levels in the cell, we reasoned that exogenous application of ceramides might compensate for the drop in ceramide levels in toxin-treated tissue, and thereby interfere with the effects of toxin on cellular responses. Such effects have been reported in mammalian cell cultures where a series of synthetic, soluble and bio-active ceramide esters have been used successfully to manipulate ceramide-responsive activities (Gomez-Munoz, 1998). Indeed, pretreatment of tomato explants with each of several of these soluble ceramide analogs, known to mimic the biological effects of ceramide in mammalian systems, prevented toxin-induced cell death in tomato. In contrast, the chemically similar but biologically inactive dihydroceramide did not prevent toxin-induced cell death. Remarkably, exposure of the roots of tomato explants in a synthetic growth medium to the ceramide analogs resulted in systemic protection of the leaves and stems against disease caused by a toxin-producing isolate of *A. alternata* f. sp. *lycopersici.* In contrast, inoculated explants pretreated with dihydroceramide succumbed to disease as expected. Thus, ceramides protect against death caused by the sphingosine analog mycotoxin and against disease caused by a pathogen that deploys the toxin as a virulence factor.

Treatment of the explants, which were exposed to the synthetic ceramides via their roots, accumulated as much as three times the amount of ceramide in their leaves compared to the untreated controls. The synthetic ceramides used in our study are not converted *in planta* to the naturally occurring ceramide. Since the majority of a radiolabelled-ceramide analog presented to the plant using the same treatment format remains in the roots, our results suggest the intriguing possibility that a systemic second messenger triggers an increase in endogenous ceramide pools throughout the plant. The implication here is that ceramide-mediated signaling represents another pathway for regulating disease resistance both locally and systemically in the plant.

Conclusions

Cross-talk in defense signaling has emerged as an important and lively field in plant science research. With the mutants and chemical elicitors now available to manipulate responses in Arabidopsis, tomato and other species, and the keen interest in the topic by many research groups worldwide, progress should be rapid in defining points of synergy and of vulnerability in the pathways that govern plant responses to external stresses. Mutants in signal generation and perception are providing focus to the search for the critical elements in defense signal transduction (Delaney, 2000; Felton and Korth, 2000; Felton et al., 1999). The notion that there are distinct and opposing response pathways for defense against pathogens and insect herbivores has now been shown to be overly simplistic, and there appear to be multiple response pathways invoked depending on the specific stress context (Bostock, 1999; Thomma et al., 1998; Vijayan et al., 1998). For example, even the trade-off that was observed between the SA and JA defense pathways in chemically-induced plants was not observed when *Pst* was used as an inducing agent (also see the study by van Wees et al. (2000) illustrating complementary effects of JA-dependent ISR and SA-dependent SAR in Arabidopsis). There is also a growing body of evidence for defense signaling that appears to be independent of SA and JA (Cameron, 2000; Guo et al., 2000; Nawrath and Metraux, 1999; Titarenko et al., 1997).

We anticipate that an understanding of the circuits that govern plant responses to biotic and abiotic stresses will reveal novel strategies for pest management, with significant benefits to crop production (Bray, 1993; Chapin, 1991). Critical to success in this endeavor will be the recruitment and participation of the diverse biological, physiological and ecological expertise in the plant and pest sciences, coupled with the innovations that are emerging from the molecular and genomic sciences. It is anticipated that the application of functional genomic and proteomic approaches in the analysis of the signal effectors and responses will provide a comprehensive view of the key players in the process. The more 'global' perspective achieved through such powerful approaches to the study of stress-related plant gene expression and metabolism will undoubtedly alter current views on the placement of defense signaling within the larger context of the regulation of signaling networks that control cellular homeostasis and viability. It will be important to integrate this information with realistic field studies, where multiple stressors can operate simultaneously, to rigorously assess the efficacy of induced resistance in plant health.

Acknowledgements

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