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Deciphering the Role of Ethylene in Plant–Herbivore Interactions

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ABSTRACT

Most plants emit ethylene in response to herbivory by insects from many different feeding guilds. The elicitors of these ethylene emissions are thought to be microorganisms or oral secretion-specific compounds that are transferred when the attacking insect feeds. To find the receptors for these elicitors and describe the signaling cascades that are subsequently activated will be the challenge of future research. Past experiments on the function of herbivore-induced ethylene, which were biased toward the use of chemical treatments to manipulate ethylene, identified seven ethylene-dependent defense responses. In contrast, a genetic toolbox that consists of several mutants has rarely been used and to date, mutants have helped to identify only one additional ethylene-dependent defense response. Ethylene-dependent responses include the emission of specific volatile organic compounds as indirect defense, the accumulation of phenolic compounds, and proteinase inhibitor activity. Besides being ethylene regulated, these defenses depend strongly on the wound-hormone jasmonic acid (JA). That ethylene requires the concomitant induction of JA, or other signals, appears to be decisive. Rather than being the principal elicitor of defense responses, ethylene modulates the sensitivity to a second signal and its downstream responses. Given this modulator role, and the artifacts associated with the use of chemical treatments to manipulate ethylene production and perception, future advances in the study of ethylene's function in plant–herbivore interactions will likely come from the use of signaling mutants or transgenic plants. It will be exciting to see if adaptive phenotypic plasticity is largely an ethylene-mediated response.

Key words: Defense response; Ethylene emission; Herbivory; Ethephon; 1-MCP; Genetic manipulation; Mutants; VOCs

Phytohormones regulate almost all developmental processes in plants, from germination, to growth and differentiation, to their carefully timed senescence. Hormones transform external stresses into internal responses and allow a plant to adjust its phenotype to prevailing environmental conditions. Although ethylene is known to be involved in most developmental processes and has been shown to be the signal mediating the response to several external stimuli, for example, pathogens and drought, it is another phytohormone, jasmonic acid (JA), that is thought to be the main mediator of responses to herbivore attack (Halitschke and Baldwin 2005).

The observation that herbivore attack elicits an ethylene burst was made as early as 1950, when an elegant experimental procedure, the triple response

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Corresponding author; e-mail: Baldwin@ice.mpg.de of etiolated pea seedlings, was used to show that infested rose leaves produced a physiologically active ethylene release (Williamson 1950). In five subsequent decades of research, the list of plant taxa shown to emit ethylene in response to herbivore attack has grown long, but our understanding of the hormone's function in the interaction has grown only incrementally. Plants, as sessile organisms, can use ethylene as a volatile hormone to interact either with distant plant parts or with other plants in their proximity. Thus, it is surprising that research on how ethylene regulates a defense response in plant–herbivore interactions is still in its infancy.

One explanation may be the technical challenges of manipulating ethylene production, accumulation, and perception with chemical treatments. In addition to the toxic byproducts from ethephon and silverthiosulfate applications, phosphoric acid and silver, determining the right timing and concentration of these and other chemicals to manipulate ethylene signaling during complicated processes such as the wound response is clearly difficult. Ethylene and 1-methylcyclopentene (1-MCP) are gases readily diffusible through plant tissue, but their ability to provide specific control of ethylene-dependent defense responses is limited. 1-MCP has been successfully used to regulate ethylene-dependent postharvest processes of fruits and vegetables, and the physiological and biochemical responses are well known (Watkins 2006). Given that the maintenance of product quality, which involves ripening, softening, and senescence, is in some cases achieved by sequential application of 1-MCP and ethylene and depends strongly on timing (Watkins 2006), it is not surprising that the use of 1-MCP is limited when complex and faster responses during herbivory need to be simulated. Wounding of 1-MCP–treated detached fruits is known to cause unexpected responses due to feedback regulation of ethylene biosynthesis. During the wound-induced ethylene biosynthesis in *Prunus persica*, 1-MCP had no effect on 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase activity but inhibited the accumulation of its transcripts while increasing the transcript accumulation of ACC synthase (Mathooko and others 2001). Ethylene regulation during herbivory on a whole-plant level is likely to be multifaceted and thus different from the ethylene biosynthesis that occurs during developmental processes.

One example of ethylene's role during a developmental program, which implies ecological interactions, is the plant–insect mutualism pollination. Initiation of post-pollination processes, such as the regulation of floral scent and flower abscission, can be ascribed to the ethylene burst that occurs after pollination and has been successfully mimicked (Llops-Tous and others 2000; Negre and others 2003; Patterson and Bleecker 2004). Research advances have been driven by a clear definition of the traits to be observed. Such clarity with regard to response variables is lacking in studies that investigate the function of ethylene in plant–herbivore interactions. An interdisciplinary approach combining molecular, biochemical, and ecological levels of analysis, similar to that used to understand the role that volatile organic compounds (VOCs) play in plant–herbivore and tritrophic interactions (Turlings and Ton 2006), will be required to fully understand the function of ethylene in plant–herbivore interactions.

Research into ethylene's biosynthetic pathway, and the signal transduction cascades that both elicit it and are elicited by its perception, has produced some stunning results (Klee 2004; Chae and Kieber 2005), and genetically transformed ethylene-insensitive plants have recently been used to further our understanding of competitive interactions among plants (Pierik and others 2004) and of the priming that occurs in plant–pathogen interactions (De Vos and others 2006). However, these molecular advances have yet to be fully incorporated into the study of the plant defense responses elicited by herbivore attack; most research still relies heavily on the use of exogenous chemical treatments to manipulate ethylene responses. Here we review the literature that illuminates the function of ethylene in plant–herbivore interactions and predict advances that will likely result from the use of ethylene perception and biosynthesis mutants in species whose plant–herbivore interactions have been particularly well studied.

ETHYLENE EMISSION AND HERBIVORY

Elicitation of the Herbivore-induced Ethylene Emission

The fact that herbivory elicits an ethylene release has been recognized since the use of the triple response of etiolated pea seedlings to examine ethylene emanation of rose leaves that had been infected by several pathogens and the red spider mite *Tetranychus telarius* (Williamson 1950). Mite-infested tissue produced more ethylene than did healthy leaves, but the strongest emanation was caused by pathogen attack. In subsequent decades, numerous methods have been used to detect ethylene, but only two are still commonly used: either ethylene is concentrated in the headspace of infested tissues and subsequently measured by a gas chromatograph equipped with a flame-ionization detector (GC-FID), or on-line measurements of attacked plants are conducted by photo-acoustic spectroscopy (PAS), with a laser light source adjusted to the critical wavelength to excite ethylene molecules (Table 1). These techniques enable researchers to demonstrate that almost all plant taxa emit ethylene when attacked by arthropod herbivores, that the ethylene release is not due solely to the damage caused by the attacking herbivore, and that attack from herbivores of many different feeding guilds elicits a response (Table 1).

Although mechanical wounding can result in increased basal ethylene release, research using pins to simulate the feeding damage inflicted by cotton fleahoppers and basswood thrips clearly demonstrated that the insect is required for a pronounced ethylene burst (Duffey and Powell 1979; Rieske and Raffa 1995). The wound-hormone JA, which is responsible for integrating several direct and indirect plant defense responses following herbivore attack (Halitschke and Baldwin 2005), and systemin, a peptide signal that functions upstream of the oxylipin pathway leading to JA (Schilmiller and Howe 2005), have been shown to increase ethylene emissions from tomato plants above levels observed in control plants. The induced quantities were proportional to the wound-induced amounts (O'Donnell and others 1996). Hence, the herbivore-induced ethylene burst is qualitatively different from wound-induced ethylene releases; only systemin, and not JA, has been shown to elicit the release of ethylene.

The fact that ethylene is released after attack by many different feeding guilds informed early research on the mechanisms of elicitation. Early studies with piercing-sucking insects regarded the influence of ethylene to be secondary and the insect to be the vector of a microbial elicitor. R. D. Powell and co-workers, working on the microbial colonization of cotton fleahoppers, were able to separate the wound response from the fleahopper attack and to correlate the abundance of microorganisms in the herbivore with the amount of ethylene induced (Duffey and Powell 1979; Grisham and others 1987). The origin of the ethylene-induced abscission of cotton flower buds caused by insect attack was ascribed to the wounds inflicted and salivary enzymes injected, and to the microorganisms that invaded the wounds (Martin and others 1988). Studies of the ethylene emissions of onion plants, *Allium cepa*, in response to *Thrips tabaci* demonstrated that thrip extracts applied to mechanical wounds in onion plants mimicked the ethylene emission of thrip-attacked plants (Kendall and Bjostad 1990). Although whole-insect extracts were found to contain the elicitors in thrips, only the oral secretions (OS) of the beetle *Leptinotarsa decemlineata* were required to elicit the ethylene burst in attacked *Solanum tuberosum* and *Phaseolus vulgaris* plants (Kruzmane and others 2002; Steinite and others 2004). The compounds in OS, which themselves elicit the ethylene response in interactions with thrips and with beetles, are unknown.

In two well-studied plant–herbivore systems, *Manduca sexta*–*Nicotiana attenuata* and *Spodoptera exigua*–*Zea mays*, herbivore-specific defense responses that are regulated by JA, such as the emission of volatile organic compounds (VOCs) and trypsin proteinase inhibitor (TPI) activity, are elicited by fatty acid–amino acid conjugates (FACs) present in OS (Alborn and others 1997; Halitschke and others 2001). The *M. sexta*–induced ethylene emissions from *N. attenuata* plants are elicited by FACs, the same OS-derived elicitor that increases JA accumulation (C. C. von Dahl, R.A. Winz, R. Halitschke, F. Kühnemann, K. Gase and I.T. Baldwin, unpublished). However, although JA and ethylene are both OS dependent, they are regulated independently of one another. *N. attenuata* as-*lox3* plants are retarded in their JA accumulation following herbivory due to the anti-sense expression of the specific lipoxygenase (NaLOX3) that supplies fatty acid hydroperoxides to the oxylipin cascade (Halitschke and Baldwin 2003). Oral secretion–induced ethylene emissions of these as-*lox3* plants are not different from those of wild-type (WT) plants, and inverted repeat *ACO* construct (ir-*aco*) plants, which do not release ethylene after herbivore attack due to the silencing of Na*ACO* by RNAi, show an OS-induced JA burst similar to that of WT plants (C.C. von Dahl and I. T. Baldwin unpublished). This suggests that an upstream signal, which is activated by the perception of FACs, modulates the biosynthesis of these hormones. Possible candidates are mitogen-activated protein kinases (MAPKs), which regulate ethylene biosynthesis during the wound-response by altering the stability of ACC synthase (ACS) (Chae and Kieber 2005).

In contrast, the OS-specific responses of *Vigna unguiculata* and *P. vulgaris*, which include JA accumulation and the release of ethylene, were elicited by applying specific fragments of the plants' ATP synthase (called inceptins) (Schmelz and others 2006). Inceptins elicit responses in bean and cowpea but not in maize and tobacco, which suggests that the elicitors of ethylene release vary structurally from peptide fragments of plant proteins digested by

Table 1. Ethylene Emission during Herbivore Attack

Plant species	Insect species	Ethylene	Source	Emission	Detection	Reference
Gossypium hirsutum	*Pseudatomoscelis seriatus* (Het)	1.46 nmol g^{-1} FM h^{-1}	Cut bud	Accumulation	GC-FID	Duffey and Powell 1979
Vigna unguiculata	*Megalurothrips sjostedti* (Thys)	26.67 nmol g^{-1} FM h^{-1}	Cut peduncle	24 h accumulation	GC-FID	Wien and Roesing 1980
Malus domestica	*Phyllonorycter blancardella* (Lep)	0.19 nmol g^{-1} FM h^{-1}	Cut leaves	4 h accumulation	GC-FID	Kappel and others 1987
Allium cepa	*Thrips tabaci* (Thys)	0.001 nmol g^{-1} FM h^{-1}	Intact plant	24 h accumulation	GC-FID	Kendall and Bjostad 1990
Medicago sativa	*Therioaphis maculata* (Hom)	0.53 nmol g^{-1} FM h^{-1}	Cut trifoliolate	4 h accumulation	GC-FID	Dillwith and others 1991
Triticum aestivum	*Schizaphis graminum* (Hom)	1.6 nmol g^{-1} FM h^{-1}	Cut shoot	18 h accumulation	GC-FID	Anderson and Peters 1994
Hordeum vulgare	*Diuraphis noxia* (Hom)	0.006 nmol plant^{-1} h^{-1}	Intact plant	4 h accumulation	GC-FID	Miller and others 1994
(Morex)	*Schizaphis graminum* (Hom)	0.012 nmol plant^{-1} h^{-1}				
Tilia americana	*Thrips calcaratus* (Thys)	0.008 nmol g^{-1} DM h^{-1}	Intact seedling	8 h accumulation	GC-FID	Rieske and Raffa 1995
		0.009 nmol g^{-1} DM h^{-1}	Cut seedlings	(presumed)		
Nicotiana attenuata	*Manduca sexta* (Lep)	0.29 nmol plant^{-1} h^{-1}	Intact plant	Online	PAS	Kahl and others 2000
Hordeum vulgare	*Schizaphis graminum* (Hom)	0.28 nmol g^{-1} FM h^{-1}	Intact seedling	Accumulation	GC-FID	Argandoña and others 2001
	Rhopalosiphum padi (Hom)	0.21 nmol g^{-1} FM h^{-1}				
Phaseolus lunatus	*Tetranychus urticae* (Acar)	0.062 nmol plant^{-1} h^{-1}	Intact plant	Online	PAS	Arimura and others 2002
Zea mays	*Spodoptera exigua* (Lep)	0.25 nmol g^{-1} FM h^{-1}	Intact plant	1 h accumulation	GC-FID	Schmelz and others 2003
Arabidopsis thaliana	*Pieris rapea* (Lep)	0.057 nmol g^{-1} FM h^{-1}	Cut shoot	48 h accumulation	GC-FID	De Vos and others 2005
	Myzus persicae (Hom)	0.044 nmol g^{-1} FM h^{-1}				

Herbivore-induced ethylene emissions have been measured for more than 50 years, either by allowing ethylene to accumulate in the headspace of an attacked tissue and subsequently quantifying it using gas chromatography flame-ionization detection (GC-FID) or real-time photo acoustic spectroscopy (PAS) analysis with a laser source tuned so as to excite ethylene molecules. Almost all plant taxa emit ethylene after attack by a variety of herbivorous insect species. The ethylene quantities given are estimates of the published maximum values after conversion into nmol g^{-1} FM h^{-1}or nmol plant^{-1} h^{-1}, to facilitate comparisons.
Abbreviations: Lepidoptera (Lep), Thysanoptera (Thys), Heteroptera (Het), Homoptera (Hom), Acarina (Acar).

the attacking herbivores to FACs produced in the alimentary canals of herbivores. This specificity in the elicitors suggests strong selection for the ability to respond to specific attackers.

The Consequences of the Ethylene Burst for Herbivore and Plant Performance

Early research on ethylene emission during herbivory was motivated by the secondary effects such emission had on developmental processes, for example, flower bud abscission and inhibited internode elongation, which were initiated when ethylene was released after herbivore attack (Duffey and Powell 1979; Wien and Roesing 1980; Miller and others 1994). Inhibited internode and leaf blade elongation and increased flower bud abscission following herbivory were associated with the ethylene burst, which is known to mediate these phenomena independently of herbivore attack. It is, however, abundantly clear that herbivore attack results in dramatic changes in resource allocation within a plant, and the phenomena described above might well be caused by rerouting resources to the roots and thereby skirting other carbon sinks. Recently, FACs in *M. sexta* OS were found to downregulate an SNF1 kinase in *N. attenuata* via a JA-independent pathway. The suppression of the SNF1 kinase alters allocation, sending recently fixed carbon to the roots, where it is bunkered for later use during regrowth after the herbivore has left the plant (Schwachtje and others 2006). Additionally, JA-induced defense compounds are known to be costly in terms of growth and seed production, especially in herbivore-free environments, as has been demonstrated for *N. attenuata* in its natural habitat (Baldwin 1998). These herbivore-induced changes in resource allocation influence plant growth and profoundly alter herbivore performance, not only by changing the suite of secondary metabolites that function as defenses (see below), but also by changing the primary metabolites that determine the nutritional value of a plant for an herbivore.

Herbivore bioassays provide the most direct means of determining whether ethylene-mediated responses alter herbivore performance on plants. In a study that examined the performance of *Myzus persicae* on *Solanum lycopersicum* exposed to a variety of different elicitors, only MeJA and BTH treatments slowed aphid population growth, whereas aphid counts were highest on ethephon-treated tomato plants (Boughton and others 2006). Unfortunately, this study lacks an analysis of the aphid population growth in response to different ethephon concentrations. Plant growth and flower abscission showed a dose-dependent response, indicating that ethylene activity depended on concentration. This relationship is also likely to affect both the plant's response and the aphids' subsequent performance. Contrasting results were obtained for *Arabidopsis thaliana* with regard to the population growth of two phloem-feeding aphids, *Myzus persicae* (a generalist) and *Brevicoryne brassicae* (a specialist); the population of both species was the same on ethylene-insensitive *etr1* and WT plants (Mewis and others 2005). Comparing the performance of the generalist *Spodoptera littoralis* with that of the specialist *Plutella xylostella* on mutant *Arabidopsis thaliana* plants affected in ethylene signaling, *hookless 1* (*hls1-1*) and *ein2-1*, revealed that the mutants were resistant only to the generalist herbivore (Stotz and others 2000).

The treatment of an insect-resistant *Zea mays* variety with $CoCl_2$, aminoethoxyvinylglycine (AVG), or 1-MCP increased both the feeding damage inflicted by the generalist *Spodoptera frugiperda* and the herbivore's relative growth rate in comparison to untreated control plants (Harfouche and others 2006). This susceptibility was attributed to an ethylene-responsive cysteine proteinase, Mir1-CP, which was only found in the resistant variety. The insect-susceptible *Z. mays* variety was not able to accumulate the proteinase; nor did the inhibition of ethylene-signaling change herbivore resistance (Harfouche and others 2006). Thus, ethylene signaling is involved in corn's activation of resistance to *S. frugiperda*. This is in contrast to the results obtained with *Arabidopsis*: there the growth rate of the generalist *S. exigua* decreased on *etr1* plants (Mewis and others 2005; Harfouche and others 2006). A detailed analysis of the effects of ethylene on the traits that are responsible for herbivore resistance is required to understand the reasons behind these ethylene-mediated changes in herbivore performance.

Herbivore-induced Ethylene and the Defense Response of Plants

Defenses can be constitutive or inducibly expressed, and indirect or direct in their mode of action, and they are further differentiated according to their mechanism, which may be mechanical, chemical, or proteinaceous. They often target features that are common among different insect attackers, for example, the digestive or nervous system, or the mobility of the insect. Indirect defenses— for example, emitted VOCs or extrafloral nectar—

function not by targeting the attacker but by attracting parasitoids or predators of insect herbivores, and these finally kill the herbivore.

Ethylene has been shown to elicit a battery of different defensive proteins. Polyphenoloxidase (PPO) and peroxidase (POD) are enzymes that are thought to form quinines, which subsequently react with nucleophilic side chains of amino acids leading to protein cross linkage. Quinones are thought to inhibit digestion in the insect gut. Aphid infestation of barley plants rapidly elicited hydrogen peroxide followed by soluble PODs that reached maximum levels after 25 min. Additionally, aphid infestation increased ethylene emission significantly after 1 h. When plants were exposed to 20 ppm ethylene, simulating the release that occurs in aphid-infested plants, hydrogen peroxide levels increased; but after 25 min, PODs had increased to levels only half of those induced by aphids (Argandoña and others 2001). Blocking ethylene biosynthesis and perception, using amino-oxyacetic acid and 1-MCP, respectively, as well as inhibiting protein synthesis, demonstrated that increasing the POD and PPO activity of *P. vulgaris* leaves in response to wounding and to the application of OS from *L. decemlineata* depended on ethylene production and was not caused by insect-derived enzyme activity (Steinite and others 2004). In tomato plants, PPO activity showed a threefold increase after MeJA application; yet no changes were observed after ethephon treatment. Ethephon, however, increased POD activity in a dose-dependent manner (Boughton and others 2006). When ethylene was first mentioned as a possible signal mediating the wound response of tomato plants, *Proteinase inhibitor 2* (*PIN2*) transcript accumulation was shown to depend on ethylene and JA synergistically (O'Donnell and others 1996). Proteinase inhibitors are another class of enzymes interacting with the herbivore's digestive system. When a resistant variety of *Z. mays* Mp708 was analyzed using several ethylene biosynthesis blockers and 1-MCP, resistance was shown to be ethylene dependent. Ethylene production and perception had no influence on herbivore performance in the susceptible variety Tx601. The protein activity and transcript accumulation of Mir1-CP, a cysteine proteinase, were also altered by ethylene signaling. This suggests ethylene is a component of the signal transduction pathway leading to resistance in maize and is mediated by cysteine proteinases (Harfouche and others 2006).

Although many of these defensive proteins are found in all plant taxa, small-molecular-weight secondary metabolites that function as defenses tend to be highly species or family specific. For example, glucosinolates (GS) are characteristic of Brassicaceae. Several mutants of *A. thaliana* were investigated with regard to their ability to elicit GS after attack by two aphid species and one lepidopteran herbivore. The GS response to all three insects required a functional NPR1 and ETR1. Although the *etr1* mutant did not significantly influence total GS, ethylene was required for the insect-induced accumulation of aliphatic GS. As caterpillar performance was retarded on *etr1* mutants compared to wild-type Col-1 plants, ethylene may have influenced more than just the regulation of GS (Mewis and others 2005). Nicotine accumulation (characteristic for *Nicotiana spp.*) depends on the activity of putrescine *N*-methyltransferase (PMT), the first committed and regulatory step in nicotine biosynthesis. Two studies investigated the interaction of JA and ethylene in PMT transcript accumulation and subsequent nicotine accumulation in *N. sylvestris* and *N. attenuata*. The application of ethylene and the use of 1-MCP and $AgNO_3$ revealed that the MeJA-induced accumulation of PMT genes and the production of nicotine are attenuated by ethylene (Shoji and others 2000; Winz and Baldwin 2001). This explains why the increased JA accumulation in response to OS compared to wounding did not increase nicotine in OS-treated leaves. Nicotine accumulation in response to OS was higher than wound-induced amounts when the plants had been pretreated with 1-MCP (Kahl and others 2000). Furthermore, synergistic effects between JA and ethylene have been found in the phenolic and terpenoid resin formation of Douglas fir, where the MeJA-induced defense responses are amplified by ethylene (Hudgins and Franceschi 2004).

A similar synergism between JA and ethylene was observed in the elicitation of terpenoid emission in *Z. mays*. Although pretreating plants with 1-MCP did not change the level of caterpillar-induced JA accumulation, it reduced the emission of sesquiterpenes and indole, both of which are thought to function as indirect defenses. Interestingly the emission of ethylene in response to feeding *S. exigua* was also retarded. The authors argue that reduced VOC production was due to blocked perception rather than changed ethylene emission (Schmelz and others 2003). Whereas exogenous ACC also enhances the production of JA-induced (*E*)- and (*Z*)-*β*-ocimene and (*Z*)-3-hexenyl acetate in lima bean plants, the application of ethylene, in addition to MeJA, to *N. attenuata* had no synergistic effect on *cis*-*α*-bergamotene emission (Kahl and others 2000; Horiuchi and others 2001). The activity of another oxylipin-derived signal is also synergized by ethylene. (*Z*)-3-hexenol induces the emission of herbivore-induced

VOCs in exposed corn plants. Although this plant–plant signaling is enhanced if the receiver plant is exposed to ethylene, exposure to ethylene itself is not sufficient to elicit herbivore-induced VOCs in receiver plants (Ruther and Kleier 2005).

Conclusions

It is surprising how little we know about the role of ethylene in plant–herbivore interactions even after half a century of research. Ethylene signaling has been implicated in the elicitation of only eight defense responses (Figure 1). An examination of ethylene's role in plant–herbivore interactions makes it clear that studies which emphasize the role of ethylene in mediating a defense response have failed to analyze its effect on herbivore performance. On the other hand, those studies analyzing the impact of ethylene on an attacking herbivore do not analyze the secondary metabolites that are likely responsible for the changes in herbivore performance. This highlights the value of using species whose plant–herbivore interactions are particularly well studied, especially when ethylene-signaling mutants are incorporated into research programs. The fact that ethylene plays an indirect role may have contributed to the slow rate of progress. For example, the emission of particular VOCs and the accumulation of phenolics and proteinase inhibitor (PI) activity are defense responses that are ethylene mediated (Figure 1), but their elicitation is JA dependent.

A recent study of the regulation of the transcriptome during seedling growth revealed largely non-overlapping responses to seven phytohormones. Genes encoding proteins involved in signaling and transcription were specifically regulated, which is consistent with a role for hormones as triggers of particular signal-transduction cascades (Nemhauser and others 2006). This study contradicts the extensive crosstalk commonly observed among hormones. Kinases and transcription factors are likely important in the integration of signals during the regulation of protective responses against stressors (Fujita and others 2006). In herbivore–plant interactions, ethylene appears to function less as a direct elicitor and more as a modifier of signaling and downstream responses of JA. Whether such interactions are mediated by kinases or transcription factors needs to be determined.

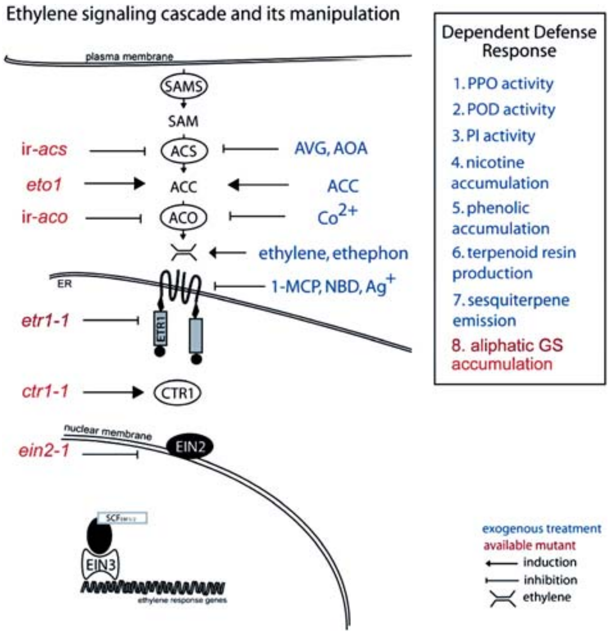

Figure 1. Summary of experimental approaches to manipulate ethylene signaling in plant-herbivore interactions. Plants modulate ethylene responses at the level of synthesis, transport, uptake, and turnover of the hormone itself, or by regulating its perception and signal transduction. Transgenic and mutant plants (marked in red) altered in many of these processes are available but their potential has not been fully utilized in the study of plant-herbivore interactions. Only one ethylene-dependent defense response (aliphatic GS accumulation) has been discovered using mutant plants. The vast majority of research has used exogenous applications of ethylene or its precursors, or various inhibitors (marked in blue), and 7 defense responses are known to be influenced by these treatments. These chemical applications (marked in blue) can have numerous non-specific side effects and their penetration into the different cell compartments is likely limited. In contrast, mutants are available through the *EIN2*-mediated responses at the nuclear membrane. The induced and inhibited steps indicated in the figure refer to the final ethylene response (transcription of ethylene responsive genes) and not necessarily to the activity of the particular step in the pathway. All 8 listed defense responses increase under the presence of ethylene. *Eto1*, *etr1-1*, *ctr1-1*, and *ein2-1* refer to plants mutated in the indicated steps, whereas ir-*acs* and ir-*aco* are inverted-repeat RNAi plants silenced in the indicated steps of the signaling cascade.

Future Directions

We predict that our understanding of ethylene's role in plant–herbivore interactions will be substantially improved once genetic tools (left panel marked red; Figure 1) are incorporated into research programs. Several mutants in ethylene biosynthesis and its downstream signaling cascade are available in *A. thaliana* and *S. lycopersicon* (Czarny and others 2006), species in which both JA-dependent defense responses and ethylene signaling are well defined.

One drawback of genetic manipulations is that when they are driven by nonspecific promoters, they are unable to manipulate ethylene signaling in a tissue-specific manner. Advances in inducible and tissue-specific promoters continue, albeit slowly, but once established, they should be included in the research on ethylene-regulated defenses. A second challenge for genetic manipulation of ethylene biosynthesis is the large gene families of ACC synthases and ACC oxidases, in which specific members mediate specific responses. In the family of tomato ethylene receptors, some members are able to compensate for the loss of other members, which complicates the interpretation of the results (Klee 2004). To avoid these problems, consensus sequences can be used to silence the expression of multiple members in a large gene family by RNAi. In contrast, specific sequence tags, frequently found in the 3'UTR, synthetic miRNAs, or antisense full-length genes, can be used to target the expression of a single family member. Although the results from mutant and transgenic plant analysis must be interpreted cautiously, they will be needed to disentangle the interplay of JA and ethylene in mediating responses to herbivore attack.

When ethylene activity requires the concomitant elicitation of JA, or of any other signal, it is likely to be influenced by numerous other adaptive responses. This might explain why exogenous applications have not been as useful in ethylene research as in the study of JA responses. Recent advances in our understanding of ethylene's role in mediating between-species interactions suggest that it plays an offensive function. The relevance of ethylene emission in competition for light was discovered using ethylene-insensitive *etr1* tobacco plants. That light signals cause the shade avoidance syndrome (SAS) is well known; what is new is that exposure to ethylene is sufficient to elicit some of these responses (Pierik and others 2004). Intraspecific competition, simulated by alterations in red/ far red light ratios, severely reduced the defensive responses of tobacco and tomato plants (Izaguirre and others 2006), suggesting that ethylene helps tailor defense responses in anticipation of competition-mediated stress. Additionally, ethylene may prime a plant, allowing it to respond more quickly to stresses it encounters, as happens when *Pieris rapae* feed on *Arabidopsis* plants that are subsequently inoculated with turnip crinkle virus (TCV). Here, increased sensitivity to virus-induced SA, caused by ethylene, changes the plant's resistance to the pathogen (De Vos and others 2006). Given that performance in a particular habitat requires the subtle tuning of numerous adaptive responses to environmental stimuli, we predict that as genetically modified or mutant plants are more commonly used to explore their performance in natural settings, ethylene's importance as a modulator of ecological responses will be revealed.

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REFERENCES

Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, and others 1997. An elicitor of plant volatiles from beet armyworm oral secretion. Science 276:945–949.

Anderson JA, Peters DC. 1994. Ethylene production from wheat seedlings infested with biotypes of *Schizaphis graminum* (Homoptera: Aphididae). Environ Entomol 23:992–998.

Argandoña VH, Chaman M, Cardemil L, Muñoz O, Zúñiga GE, and others 2001. Ethylene production and peroxidase activity in aphid-infested barley. J Chem Ecol 27:53–68.

Arimura G-I, Ozawa R, Nishioka T, Boland W, Koch T, and others 2002. Herbivore-induced volatiles induce the emission of ethylene in neighboring lima bean plants. Plant J 29:87–98.

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

Boughton AJ, Hoover K, Felton GW. 2006. Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. Entomol Exp Appl 120:175–188.

Chae HS, Kieber JJ. 2005. *Eto brute*? Role of ACS turnover in regulating ethylene biosynthesis. Trends Plant Sci 10:291–296.

Czarny JC, Grichko VP, Glick BR. 2006. Genetic modulation of ethylene biosynthesis and signaling in plants. Biotech Adv 20:672–677.

De Vos M, van Zaanen W, Koornneef A, Korzelius JP, Dicke M, and others 2006. Herbivore-induced resistance against microbial pathogens in Arabidopsis. Plant Physiol 142:352–363.

De Vos M, van Oosten VR, van Poecke RMP, van Pelt JA, Pozo MJ, and others 2005. Signal signature and transciptome changes of *Arabidopsis* during pathogen and insect attack. Mol Plant-Microbe Interact 18:923–937.

Dillwith JW, Berberet RC, Bergman DK, Neese PA, Edwards RM, and others 1991. Plant biochemistry and aphid populations: studies on the spotted alfalfa aphid, *Therioaphis maculata*. Arch Insect Biochem Physiol 17:235–251.

Duffey JE, Powell RD. 1979. Microbial induced ethylene synthesis as a possible factor of square abscission and stunting in cotton infested by cotton fleahopper. Ann Entomol Soc Am 72:599–601.

Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, K and others. 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. Curr Opin Plant Biol 9:436–442.

Grisham MP, Sterling WL, Powell RD, Morgan PW. 1987. Characterization of the induction of stress ethylene synthesis in cotton caused by the cotton fleahopper (Hemiptera: Miridae) and its microorganisms. Ann Entomol Soc Am 80:411–416.

Halitschke R, Baldwin IT. 2003. Antisense LOX expression increases herbivore performance by decreasing defense responses and inhibiting growth-related transcriptional reorganization in *Nicotiana attenuata*. Plant J 36:794–807.

Halitschke R, Baldwin IT. 2005. Jasmonates and related compounds in plant-insect interactions. J Plant Growth Regul 23:238–245.

Halitschke R, Schittko U, Pohnert G, Boland W, Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. 3. Fatty acid-amino acid conjugates in herbivore oral secretions are necessary and sufficient for herbivore-specific plant responses. Plant Physiol 125:711–717.

Harfouche AL, Shivaji R, Stocker R, Williams PW, Luthe DS. 2006. Ethylene signaling mediates a maize defense response to insect herbivory. Mol Plant-Microbe Interact 19:189–199.

Horiuchi J-I, Arimura G-I, Ozawa R, Shimoda T, Takabayash J, and others 2001. Exogenous ACC enhances volatiles production mediated by jasmonic acid in lima bean leaves. FEBS Lett 509:332–336.

Hudgins JW, Franceschi VR. 2004. Methyl jasmoante-induced ethylene production is responsible for conifer phloem defense responses and reprogramming of stem cambial zone for traumatic resin duct formation. Plant Physiol 135:2134–2149.

Izaguirre MM, Mazza CA, Biondini M, Baldwin IT, Ballaré CL. 2006. Remote sensing of future competitors: impacts on plant defenses. Proc Natl Acad Sci USA 103:7170–7174.

Kahl J, Siemens DH, Aerts RJ, Gaebler R, Kuehnemann F, and others 2000. Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. Planta 210:336–342.

Kappel F, Proctor JTA, Murr DP. 1987. Effect of spotted tentiform leafminer injury on ethylene production and ACC content in apple leaves. HortScience 22:469–471.

Kendall DM, Bjostad LB. 1990. Herbivory by *Thrips tabaci* induces greater ethylene production in intact onions than mechanical damage alone. J Chem Ecol 16:981–991.

Klee HJ. 2004. Ethylene signal transduction. Moving beyond Arabidopsis. Plant Physiol 135:660–667.

Kruzmane D, Jankevica L, Levinsh G. 2002. Effect of regurgitant from *Leptinotarsa decemlineata* on wound responses in *Solanum tuberosum* and *Phaseolus vulgaris*. Physiol Plant 115:577–584.

Llops-Tous I, Barry CS, Grierson D. 2000. Regulation of ethylene biosynthesis in response to pollination in tomato flowers. Plant Physiol 123:971–978.

Martin WR, Morgan PW, Sterling WL, Kenerley CM. 1988. Cotton fleahopper and associated microorganisms as components in the production of stress ethylene by cotton. Plant Physiol 87:280–285.

Mathooko FM, Tsunashima Y, Owino WZO, Kubo Y, Inaba A. 2001. Regulation of genes encoding ethylene biosynthetic enzymes in peach (*Prunus persica* L.) fruit by carbon dioxide and 1-methylcyclopropene. Postharvest Biol Technol 21:265–281.

Mewis I, Appel HM, Hom A, Raina R, Schultz JC. 2005. Major signaling pathways modulate Arabidopsis glucosinolate accumulation and response to both phloem-feeding and chewing insects. Plant Physiol 138:1149–4462.

Miller HL, Neese PA, Ketring DL, Dillwirth JW. 1994. Involvement of ethylene in aphid infestantion of barley. J Plant Growth Regul 13:167–171.

Negre F, Kish CM, Boatright J, Underwood B, Shibuya K, and others 2003. Regulation of methylbenzoate emission after pollination in snapdragon and petunia flowers. Plant Cell 15:2992–3006.

Nemhauser JL, Hong F, Chory J. 2006. Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. Cell 126:467–475.

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

Patterson SE, Bleecker AB. 2004. Ethylene-dependent and -independent processes associated with floral organ abscission in Arabidopsis. Plant Physiol 134:194–203.

Pierik R, Whitelam GC, Voesenek LACJ, de Kroon H, Visser EJW. 2004. Canopy studies on ethylene-insensitive tobacco identify ethylene as a novel element in blue light and plant-plant signalling. Plant J 38:310–319.

Rieske LK, Raffa KF. 1995. Ethylene emission by a deciduous tree, *Tilia americana*, in response to feeding by introduced basswood thrips, *Thrips calcaratus*. J Chem Ecol 21:187–197.

Ruther J, Kleier S. 2005. Plant–plant signaling: ethylene synergizes volatile emission in *Zea mays* induced by exposure to (*Z*)-3-hexen-1-ol. J Chem Ecol 31:2217–2222.

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

Schmelz EA, Alborn HT, Banchio E, Tumlinson JH. 2003. Quantitative relationship between induced jasmonic acid levels and volatile emission in *Zea mays* during *Spodoptera exigua* herbivory. Planta 216:665–673.

Schmelz EA, Caroll MJ, LeClere S, Phipps SM, Meredith J, and others 2006. Fragments of ATP synthase mediate plant perception of insect attack. Proc Natl Acad Sci USA 103:8894–8899.

Schwachtje J, Minchin PEH, Jahnke S, Dongen JTv, Schittko U, and others 2006. SNF1-related kinases allow plants to tolerate herbivory by allocating carbon to roots. Proc Natl Acad Sci USA 103:12935–12940.

Shoji T, Nakajima K, Hashimoto T. 2000. Ethylene suppresses jasmonate-induced gene expression in nicotine biosynthesis. Plant Cell Physiol 41:1072–1076.

Steinite I, Gailite A, Ievinsh G. 2004. Reactive oxygen and ethylene are involved in the regulation of regurgitant-induced responses in bean plants. J Plant Physiol 161:191–196.

Stotz HU, Pittendrigh BR, Kroymann J, Weniger K, Fritsche J, and others 2000. Induced plant defense responses against chewing insects. Ethylene signaling reduces resistance of Arabidopsis against Egyptian cotton worm but not Diamondback moth. Plant Physiol 124:1007–1017.

Turlings TCJ, Ton J. 2006. Exploiting scents of distress: the prospect of manipulating herbivore-induced plant odours to enhance the control of agricultural pests. Curr Opin Plant Biol 9:421–427.

Watkins CB. 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol Adv 24:389–409.

Wien HC, Roesing C. 1980. Ethylene evolution by thrips-infested cowpea provides a basis for thrips resistance screening with ethephon sprays. Nature 283:192–194.

Williamson CE. 1950. Ethylene, a metabolic product of diseased or injured plants. Phytopathology 40:205–208.

Winz RA, Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. 4. Insect-induced ethylene reduces jasmonate-induced nicotine accumulation by regulating putrescine *N*-methyltransferase transcripts. Plant Physiol 125:2189–2202.