The Plant Cell, Vol. 22: 3193–3205, October 2010, www.plantcell.org © 2010 American Society of Plant Biologists

RESEARCH ARTICLES

Arachidonic Acid: An Evolutionarily Conserved Signaling Molecule Modulates Plant Stress Signaling Networks [C][W]

Tatyana Savchenko,[a] Justin W. Walley,[a,b] E. Wassim Chehab,[a,1] Yanmei Xiao,[a] Roy Kaspi,[a] Matthew F. Pye,[c] Maged E. Mohamed,[d,2] Colin M. Lazarus,[d] Richard M. Bostock,[c] and Katayoon Dehesh[a,3]

[a] Department of Plant Biology, University of California, Davis, California 95616
[b] Department of Biological Sciences, University of California, San Diego, California 92093-0380
[c] Department of Plant Pathology, University of California, Davis, California 95616
[d] School of Biological Sciences, University of Bristol, Bristol BS8 1UG, United Kingdom

Fatty acid structure affects cellular activities through changes in membrane lipid composition and the generation of a diversity of bioactive derivatives. Eicosapolyenoic acids are released into plants upon infection by oomycete pathogens, suggesting they may elicit plant defenses. We exploited transgenic *Arabidopsis thaliana* plants (designated EP) producing eicosadienoic, eicosatrienoic, and arachidonic acid (AA), aimed at mimicking pathogen release of these compounds. We also examined their effect on biotic stress resistance by challenging EP plants with fungal, oomycete, and bacterial pathogens and an insect pest. EP plants exhibited enhanced resistance to all biotic challenges, except they were more susceptible to bacteria than the wild type. Levels of jasmonic acid (JA) were elevated and levels of salicylic acid (SA) were reduced in EP plants. Altered expression of JA and SA pathway genes in EP plants shows that eicosapolyenoic acids effectively modulate stress-responsive transcriptional networks. Exogenous application of various fatty acids to wild-type and JA-deficient mutants confirmed AA as the signaling molecule. Moreover, AA treatment elicited heightened expression of general stress-responsive genes. Importantly, tomato (*Solanum lycopersicum*) leaves treated with AA exhibited reduced susceptibility to *Botrytis cinerea* infection, confirming AA signaling in other plants. These studies support the role of AA, an ancient metazoan signaling molecule, in eliciting plant stress and defense signaling networks.

INTRODUCTION

In animals and plants, fatty acids (FAs) are key molecules that participate in various biological processes. Structural properties of FAs, such as their chain length and their degree of desaturation, largely determine the nature of these processes. In unicellular organisms, such as *Escherichia coli* and yeast, FAs have been shown to regulate gene transcription (Black et al., 2000). In mammals, the expression of many genes is modulated positively or negatively by FAs through changes in rate of transcription or posttranscriptional modifications (Duplus et al., 2000; Huang et al., 2004; Pegorier et al., 2004). In plants, FAs and/or their derived metabolites are also recognized as signaling molecules central to various biological processes.

Exogenous and endogenous unsaturated FAs and FA metabolites can significantly alter plant gene expression and metabolism to influence the outcome of plant–microbe and plant–herbivore interactions (Upchurch, 2008). Mounting evidence from studies with *Arabidopsis thaliana* plants defective in stearoyl-acyl carrier protein-desaturase, the enzyme responsible for conversion of stearic acid (18:0) to oleic acid (18:1 Δ^9), has established that the levels of 18:1 are a critical regulator of salicylic acid (SA)- and jasmonic acid (JA)-mediated defense signaling in the plant (A. Kachroo et al., 2003; P. Kachroo et al., 2003, 2005; Chandra-Shekara et al., 2007; Venugopal et al., 2009). Specifically, reduction of the 18:1 levels leads to constitutive expression of *PR* genes and enhanced resistance to *Hyaloperonospora arabidopsidis* (formerly *Peronospora parasitica*), in concert with reduced expression of a subset of JA-dependent response genes and decreased resistance to *Botrytis cinerea* (Kachroo et al., 2001). An important role for linoleic acid (LA; 18:2 $\Delta^{9,12}$) in conidiation, development, and aflatoxin synthesis has been described in the interaction between toxigenic *Aspergillus flavus* and its hosts, with apparent reciprocity in the ability of the plant and pathogen to influence oxylipin profiles during the interaction (Brodhagen et al., 2008).

Plants also respond to exogenous treatment with eicosapolyenoic acids and to pathogens containing them during infection. Specifically, eicosapentaenoic acid (EPA; 20:5 $\Delta^{5,8,11,14,17}$) and arachidonic acid (AA; 20:4 $\Delta^{5,8,11,14}$) are potent elicitors of

[1] Current address: Biochemistry and Cell Biology, Rice University, Houston, TX 77005-1892.
[2] Current address: School of Pharmacy, University of Zagazig, Zagazig 44519, Egypt.
[3] Address correspondence to kdehesh@ucdavis.edu.
The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantcell.org) is: Katayoon Dehesh (kdehesh@ucdavis.edu).
[C]Some figures in this article are displayed in color online but in black and white in the print edition.
[W]Online version contains Web-only data.
www.plantcell.org/cgi/doi/10.1105/tpc.110.073858

programmed cell death and defense responses in Solanaceous plants (Bostock et al., 1981; Knight et al., 2001; Garcia-Pineda et al., 2004) and are reported to induce resistance to viruses in potato (*Solanum tuberosum*) and tobacco (*Nicotiana tabacum*) (Rozhnova et al., 2003; Ozeretskovskaya et al., 2004). Treatment of potato with AA and EPA results in the coordinate activation of defense-related responses (Bostock et al., 1986) and a dramatic and immediate shift in terpenoid metabolism, redirecting the pathway from higher terpenoids (i.e., steroid glycoalkaloids) to sesquiterpenoid phytoalexins (Tjamos and Kuć, 1982; Choi et al., 1992, 1994). FAs not commonly found in plants, such as EPA and AA that are abundant in lipids of *Phytophthora* species and related oomycetes, are released into plant tissue from spores during early stages of infection (Ricker and Bostock, 1992). Metabolic studies in potato have shown that AA is relatively stable compared with naturally occurring LA (18:2 $\Delta^{9,12}$), with a portion of the AA oxidized to hydroxy acids and other products and a larger portion rapidly incorporated into phospho- and glycero-lipids (Preisig and Kuć, 1988). A critical structural feature for their elicitor activity in plants is *cis*-unsaturation at the Δ^5-position, and evidence supports the participation of plant lipoxygenases in this activity (Bostock et al., 1981, 1992; Preisig and Kuć, 1985). Based on these observations, it has been proposed that eicosapolyenoic acids have transorganismal signaling activity (Rozhnova et al., 2003; Ozeretskovskaya et al., 2004).

Plants and animals possess parallel, analogous FA signaling systems. Perhaps the best example are the parallel roles played by FA oxidation products oxylipins (plants) and eicosanoids (animals) in mediating stress signaling cascades. Plant oxylipins, also referred to as octadecanoids, are the oxidation products of LA or linolenic acid (ALA; 18:3 $\Delta^{9,12,15}$), and a major product of this pathway is JA (Farmer et al., 1998; Blee, 2002). JA and its derivatives can modulate a wide range of biological processes in plants, among them plant resistance to insects and pathogens (Gardner, 1995; Blee, 2002). Animal lipid mediators, eicosanoids, include prostaglandins, leukotrienes, lipoxins, and other related compounds derived primarily from AA (Schultz, 2002). Similar to oxylipins, eicosanoids play multiple biological roles in animals, including regulating wound responses, inflammation, and immune responses (Magnan and Vervloet, 1999; van Ryn et al., 2000). The structures and biosynthesis of JA and its precursors and derivatives are analogous to those of animal eicosanoids. The parallels between these molecules is further reinforced by their functional similarities, as they are synthesized and released for immediate and local, as well as systemic, responses to stresses (Straus and Glass, 2001; Blee, 2002).

Most plants do not contain eicosapolyenoic acids. However, because of the abundance of these FAs in oomycete pathogens and their potential signaling activity in plants when delivered by external challenge, we questioned whether endogenous production of eicosapolyenoic acids in plants also would modulate plant defense responses. To address this question, we examined the resistance of transgenic lines that produce eicosapolyenoic acids, mainly eicosadienoic acid (EDA; 20:2 $\Delta^{11,14}$), eicosatrienoic acid (ETrA; 20:3 $\Delta^{11,14,17}$), and AA, to a wide range of biotic challengers. The outcome of these studies, together with data obtained from external application of various FAs, advance the notion that as in animal systems, AA in plants can function as a signaling molecule that not only triggers FA-mediated defense responses, but also elicits general stress signaling networks.

RESULTS

EDA, ETrA, and AA Are the Dominant Eicosapolyenoic Acids in EP Leaves

Two different strategies were employed to generate independent transgenic *Arabidopsis* lines producing eicosapolyenoic acids via the Δ^8-desaturation pathway. In one approach, an eicosapolyenoic acid–producing transgenic line (designated as EP1) was generated by sequential introduction of three sets of constitutively expressed genes (C18-Δ^9-elongase, Δ^8-desaturase, and Δ^5-desaturase) (Qi et al., 2004). In the second approach, line EP2 was generated by a single transformation step using a binary vector containing these same genes in tandem (described in Methods). In the Δ^8-desaturation pathway, LA and ALA are first elongated by a C18-Δ^9-elongase to EDA (20:2 $\Delta^{11,14}$) and ETrA (20:3 $\Delta^{11,14,17}$), respectively. A Δ^8-desaturase introduces a double bond at the Δ^8 position of the carbon chain to produce dihomo-γ-linolenic acid (20:3 $\Delta^{8,11,14}$) and eicosatetraenoic acid (20:4 $\Delta^{8,11,14,17}$), the substrates used by a Δ^5-desaturase to produce AA and EPA. These FAs esterified predominantly to phosphatidylcholine cause no visible effect on plant morphology (Fraser et al., 2004). Monitoring EP plants at all developmental stages established that these plants are phenotypically indistinguishable from the wild type. Analysis of FA composition of leaves determined that EP plants contain easily detectable levels of eicosapolyenoic acids, predominantly EDA, ETrA, and AA (Table 1).

Table 1. EP Plants Contain Eicosapolyenoic Acids, Mainly EDA, EtrA, and AA

FA mol % of Total	Wild Type	EP1	EP2
16:0	14.50 ± 0.403	13.99 ± 0.285	12.61 ± 0.053
16:1Δ^7	2.02 ± 0.169	2.28 ± 0.156	2.43 ± 0.112
16:2$\Delta^{7,10}$	0.61 ± 0.063	0.63 ± 0.168	0.68 ± 0.023
16:3$\Delta^{7,10,13}$	15.11 ± 0.186	15.82 ± 0.545	16.34 ± 0.069
18:0	1.41 ± 0.243	1.15 ± 0.136	2.07 ± 0.029
18:1Δ^9	1.33 ± 0.192	1.04 ± 0.017	0.78 ± 0.086
18:2$\Delta^{9,12}$	14.18 ± 0.139	12.89 ± 0.476	10.33 ± 0.144
18:3$\Delta^{9,12,15}$	50.72 ± 0.705	49.79 ± 0.232	40.38 ± 0.066
20:0	0.12 ± 0.015	0.09 ± 0.012	0.12 ± 0.015
20:2$\Delta^{11,14}$ (EDA)		0.77 ± 0.057	6.20 ± 0.043
20:3$\Delta^{11,14,17}$ (ETrA)		0.95 ± 0.055	7.34 ± 0.053
20:3$\Delta^{8,11,14}$ (DGLA)		0.02 ± 0.01	0.16 ± 0.081
20:4$\Delta^{8,11,14,17}$ (ETA)		0.16 ± 0.039	0.24 ± 0.052
20:4$\Delta^{5,8,11,14}$ (AA)		0.42 ± 0.067	0.25 ± 0.016
20:5$\Delta^{5,8,11,14,17}$ (EPA)		0.01 ± 0.006	0.08 ± 0.017
Total C20 PUFA		2.33	14.27

FA analyses were performed on leaf tissue and each value is the mean ± SD of six samples. DGLA, dihomo-γ-linolenic acid; ETA, eicosatetraenoic acid

EP Plants Are Differentially Resistant to Biotic Stresses

In response to a change in quantity or type of fatty acid composition of membrane lipids, plants display a number of alterations in their defenses against stresses. To examine whether endogenous production of eicosapolyenoic acids would alter plant defense responses, we exposed EP and wild-type plants to a range of biotic challengers, including a piercing-sucking insect (aphid [*Myzus persicae*]), two isolates of the fungal pathogen *B. cinerea* (Grape and B05.10), an oomycete pathogen (*Phytophthora capsici*), and a bacterial pathogen (*Pseudomonas syringae* pv *tomato* DC3000 [*Pst*]). EP1 and EP2 plants responded similarly to the biotic stresses examined. These data indicated that EP plants are significantly more resistant to all biotic challengers, except *Pst* (Figures 1A to 1E; see Supplemental Figures 1A and 1B online). Specifically, dual choice assays showed that aphid nymph deposition is significantly lower on EP than on wild-type plants (Figure 1A). Furthermore, infection of leaves by two isolates of *B. cinerea* resulted in a significantly smaller lesion size on EP than wild-type leaves (Figures 1B; see Supplemental Figure 1A online). EP plants also displayed enhanced resistance to *P. capsici*, as reflected by the number of *P. capsici* sporangia and by the amount of pathogen DNA present on these plants compared with the corresponding wild type (Figures 1C and 1D). By contrast, EP plants are notably more susceptible to *Pst* infection (Figure 1E; see Supplemental Figure 1B online). These data collectively demonstrate that the in vivo perturbation of FA composition of membrane lipids significantly and differentially altered the tolerance of EP plants to these biotic challengers.

The Levels of JA and SA Metabolites and Their Corresponding Transcripts Are Altered in EP Plants

The lifestyle of a plant pathogen can influence which defense signal transduction pathway is activated. In *Arabidopsis*, the SA signaling pathway predominantly mediates the response to biotrophic pathogens (McDowell and Dangl, 2000), while JA-dependent defense responses are more closely associated with response to infection with necrotrophic pathogens that acquire nutrients from dead or dying host cells (Glazebrook, 2005). In addition to necrotrophic pathogens, the JA signaling pathway is also important for the activation of defense cascades in response to wounding and herbivores, including piercing-sucking insects (Staswick et al., 1998; Halim et al., 2006; Chehab et al., 2008).

To determine whether changes in the levels of SA and JA underlie the observed altered tolerance of EP plants to the biotic challengers examined, we measured the levels of these metabolites in EP and wild-type plants. These data demonstrate that JA levels, specifically in wounded EP1 and EP2 plants, are approximately twofold higher than their corresponding wild-type levels (Figure 2A; see Supplemental Figure 2A online). The similarity between elevated levels of JA in response to wounding in EP1 and EP2 plants is despite the ~20% reduction in the levels of 18:3, the primary FA precursor for the JA biosynthetic pathway, in EP2 compared with the wild-type and EP1 plants (Table 1). These elevated levels of JA are consistent with the enhanced resistance of EP plants to aphids, *B. cinerea*, and *P. capsici* (Figures 1A to 1D; see Supplemental Figure 1A online). In contrast with JA levels, there is an ~40% reduction in the SA levels in unwounded plants with a concomitant enhanced susceptibility of EP plants to *Pst* (Figures 1E and 3A; see Supplemental Figures 1B and 4A online).

To address the molecular basis of the altered JA and SA levels, we examined the relative expression levels of JA and SA pathway genes via real-time quantitative PCR. Specifically, we examined relative expression levels of a subset of JA biosynthetic and JA-inducible genes (Figures 2B and 2C; see Supplemental Figure 2B online). Among the biosynthetic genes, we focused on genes encoding a phospholipase A (*DONGLE* [*DGL*]), six *LIPOXYGENASEs* (*LOX1-6*), and *ALLENE OXIDE SYNTHASE* (*AOS*). *DGL* is a member of the *PLA_1-I* family that has galactolipase as well as phospholipase A_1 (PLA_1) enzymatic activity and is known to be required for the production of basal and wound-induced levels of JA (Hyun et al., 2008). The activity of DGL leads to the release of unesterified FAs that are then oxygenated by LOXs and converted to their corresponding hydroperoxides. These hydroperoxides are substrates for the several competing oxylipin branch pathway genes, among them AOS, the major control point in the JA biosynthetic pathway (Feussner and Wasternack, 2002). We also examined the expression levels of *VEGETATIVE STORAGE PROTEIN2* (*VSP2*), a JA-inducible marker gene (Lorenzo and Solano, 2005). These data collectively demonstrate that, with the exception of *LOX1* and *LOX4*, which are not significantly altered, the expression levels of all the other genes examined are significantly higher in EP than the corresponding wild-type plants (Figure 2C; see Supplemental Figure 2B online). Thus, these data support the notion that even minor perturbation of FA composition of membrane lipids leads to modulation of transcriptional networks underlying JA production, thereby enhancing plant resistance to a range of biotic stresses.

We also examined the relative expression levels of the *HYDROPEROXIDE LYASE* (*HPL*), the main gene in the HPL branch of the oxylipin pathway responsible for production of aldehydes and oxoacids (Chehab et al., 2008). As shown (see Supplemental Figure 3 online), the expression of *HPL* is also notably enhanced in wounded EP1 compared with the wild-type plants. We were unable to measure the levels of HPL-derived product since the *Arabidopsis* accession Columbia-0 is a natural loss-of-function mutant in *HPL* and thus C_6-aldehydes, although it produces the *HPL* truncated transcript (Duan et al., 2005; Chehab et al., 2008). This finding shows the broad regulatory impact of perturbation of fatty acid composition of membrane lipids in modulating the JA and HPL oxylipin transcriptional networks.

To determine if there is a correlation between SA metabolite levels and the expression of SA pathway transcripts, we analyzed the relative expression of SA biosynthetic and SA-inducible genes by real-time quantitative PCR (Figures 3B and 3C; see Supplemental Figure 4B online). For these analyses, we selected genes required for the biosynthesis and transport of SA, *ENHANCED DISEASE SUSCEPTIBILITY5* (*EDS5*), *PHYTOALEXIN DEFICIENT4* (*PAD4*), and *ISOCHORISMATE SYNTHASE1* (*ICS1*). *EDS5* (previously named *sid1*) encodes a protein with sequence similarity to the multidrug and toxin extrusion family of transporter proteins, suggested to transport SA or its precursor out of the plastid after synthesis (Nawrath et al., 2002). *PAD4* encodes a lipase/esterase-like protein that is involved in a positive

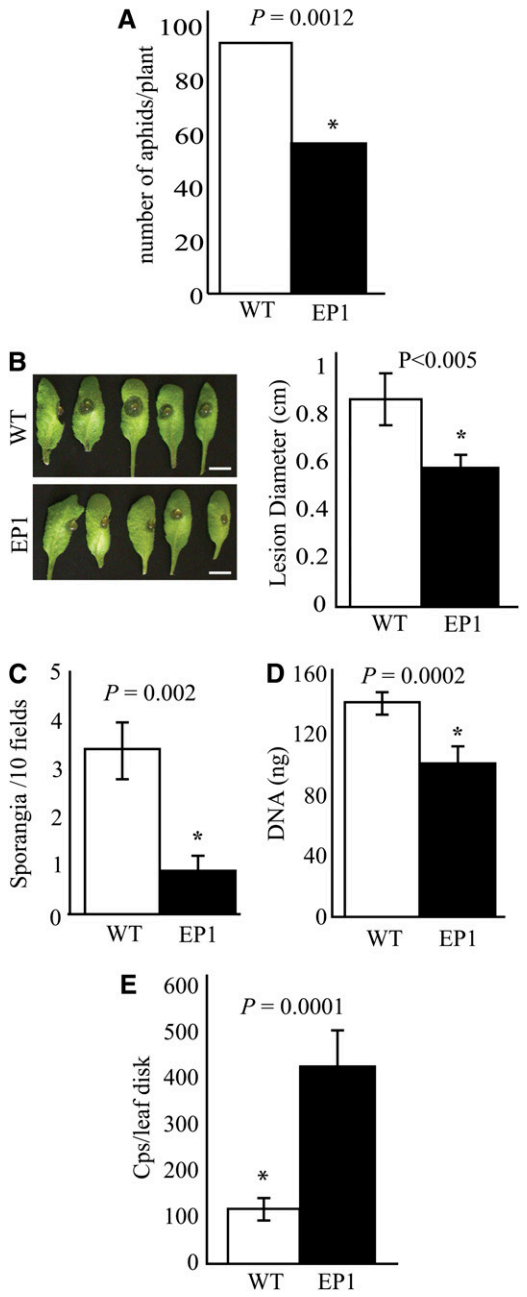

Figure 1. EP Plants Exhibit Altered Tolerance to a Range of Biotic Challengers.

(A) Choice bioassays performed on pairs of wild-type and EP plants that were caged. A single female aphid (*M. persicae*) was released in each cage. The initial nymph deposition preference was determined within 2 d of aphid release, in four independent experiments. Bar graphs represent the actual numbers of aphids. One-tailed binomial tests were used to determine significance (P = 0.0012).

(B) Visual symptoms 3 d after spot inoculation with conidia of *B. cinerea* (grape isolate). Lesion size determined 3 d after inoculation. Data are means of 48 independent biological replicates ± SE. Asterisks denote a significant difference from the wild type (P < 0.005) as determined by *t* tests.

regulatory loop that increases SA levels (Jirage et al., 1999), and *ICS1* encodes the enzyme for SA synthesis (Wildermuth et al., 2001). To examine the SA-inducible genes, we focused on *NONEXPRESSOR OF PR GENE1* (*NPR1*) (Cao et al., 1994), the transcription factor *WRKY70* as a key regulator of JA-SA cross-talk (Li et al., 2004), and *PATHOGENESIS-RELATED1* (*PR1*), a marker for intact SA signaling (Shah, 2003). These data show that the basal expression levels of all the genes, except that of *EDS5*, are significantly lower in EP compared with wild-type plants (Figure 3C; see Supplemental Figure 4B online). The reduction in the expression levels of these genes is consistent with a reduced level of SA and enhanced susceptibility of EP plants to *Pst* (Figures 1E, 3A, and 3C; see Supplemental Figures 1B and 4 online).

AA-Mediated Modulation of Expression of JA and SA Pathway Genes and Metabolites

To identify the FA responsible for the modulation of JA and SA pathway gene transcripts and their respective metabolites, we exogenously treated wild-type leaves with the FA substrates of the oxylipin pathway, namely, LA and ALA, as well as the predominant 20-carbon-long FAs absent in the wild type but present in EP plants, namely, EDA, ETrA, and AA. These experiments established AA as the only FA capable of increasing basal levels of JA (Figure 4A; see Supplemental Figure 5A online). Furthermore, we demonstrated that the effect of AA on JA production is concentration dependent and that the peak of JA levels was detected in plants treated with 10 μM AA with no additional increase in these levels in plants treated with higher concentrations of AA (100 μM) (see Supplemental Figure 5B online). These data fully supports those obtained from transgenic lines where the JA levels were similarly increased in EP1 and EP2 plants that contain 0.45 and 0.25 mol % AA, respectively. These data collectively illustrate that increasing concentrations of exogenously applied or endogenously produced AA beyond the basal threshold levels is ineffective for further enhancement of JA levels. In addition, in agreement with the data obtained from EP lines, this increase in JA may be due to heightened expression levels of JA biosynthetic pathway genes in response to AA treatment. As expected, the expression levels of the JA biosynthetic gene *AOS* and the JA marker gene *VSP2* are increased in response to AA treatment (Figure 4B). Consistent with the data obtained from the EP transgenic lines, exogenous application of

(C) and **(D)** Hydroponically growing plants were inoculated with zoospores of *P. capsici.* Disease development was monitored at 48 h after inoculation by sporangia counts on roots in 10 microscope fields **(C)** and by colonization as measured by real-time quantitative PCR analysis of *P. capsici* DNA **(D)**. Asterisks denote a significant difference from the wild type (χ^2 = 5.45, P = 0.002 for sporangial counts; χ^2 = 14.4, P = 0.0002 for colonization) as determined by Wilcoxon rank sums test. Data are the means of 40 independent biological replicates ± SE.

(E) Bacterial growth in wild-type and EP1 plants inoculated with *Pst*. The bioluminescence was recorded 2 d after inoculation as photon counts per second (Cps). Asterisks denote a significant difference from the wild type (P = 0.001) as determined by *t* test. Data are means of four independent experiments ± SE.

[See online article for color version of this figure.]

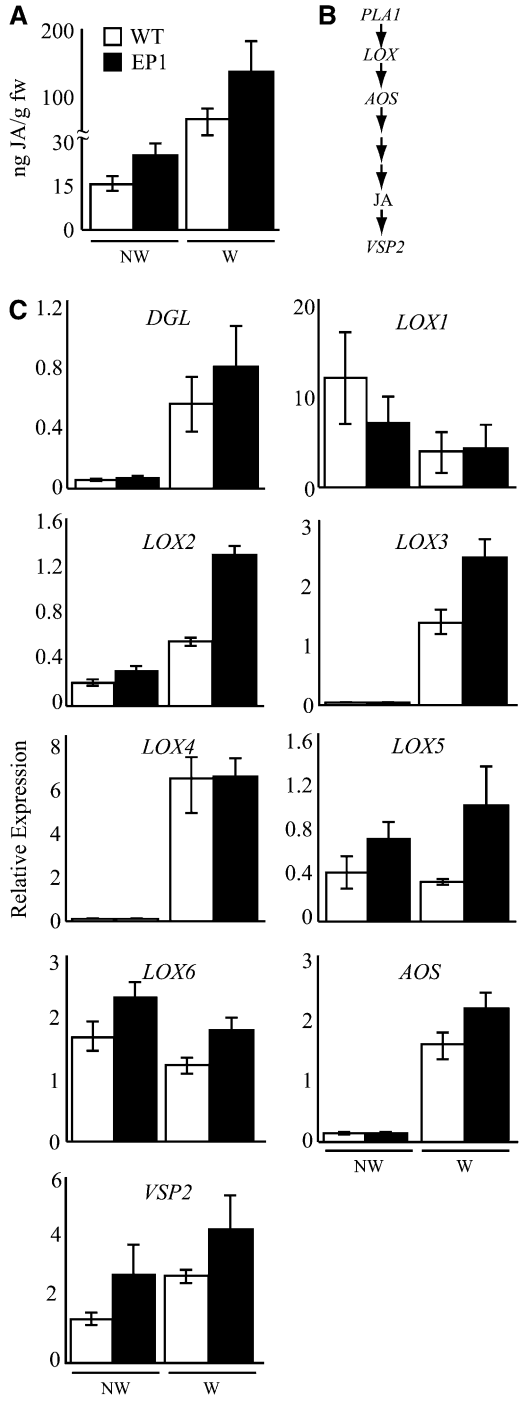

Figure 2. Levels of JA Pathway Transcripts and Metabolites Are Enhanced in EP Plants.

(A) Measurements of JA metabolite levels in the wild type (white bars) and EP1 (black bars) before (NW) and 90 min after wounding (W) show enhanced basal- and wound-induced levels of JA in EP plants. Data are means of six independent experiments ± SD.

(B) Simplified representation of JA-pathway genes.

(C) Total RNA was extracted from 4-week-old rosette leaves before (NW) and 90 min after wounding (W) and subjected to real-time quantitative PCR analysis. The transcript levels of each gene (*DGL*, *LOX1* through *6*, *AOS*, and *VSP2*) were normalized to At4g34270 (T1P41-like family protein) and At4g26410 (M3E9) measured in the same samples. Data are means of three biological replicates ± SE.

AA reduced SA levels and decreased the relative expression levels of *PAD4*, *ICS1*, *WRKY70*, and *PR1* (Figures 4C and 4D).

A substantial body of evidence has established that the SA and JA signaling pathways are mutually antagonistic and that this regulatory crosstalk may have evolved as a mechanism for fine-tuning the induction of defenses in response to different plant pathogens (Kunkel and Brooks, 2002; Bostock, 2005). To establish whether AA plays a direct role in modulating SA pathway transcripts and metabolite levels or an indirect role mediated through an antagonistic effect of JA on the SA pathway, we employed a JA-deficient line generated by a T-DNA insertion in *AOS* resulting in loss of *aos* function (Park et al., 2002). The *aos* loss of function mutant plants were treated with exogenous AA and subsequently analyzed for the SA metabolite pools and the expression levels of SA pathway genes. These analyses clearly established that application of AA in the absence of JA neither reduces SA metabolite levels nor significantly modifies the expression of SA pathway genes (Figures 5A and 5B).

AA-Mediated Enhanced Tolerance to *Botrytis* Is JA Dependent

We next examined whether exogenous application of AA alters tolerance of wild-type and *aos* mutant plants to *Botrytis* infection. These experiments were performed using different application strategies to rule out the possibility that direct contact with AA affected germination of conidia. In one approach, AA and *Botrytis* conidia were simultaneously spotted on single leaves per plant on the same and/or on two different spots. In the other approach, *Botrytis* conidia were spotted at a distance from the AA application site ~30 min after AA treatment, when the AA containing droplet was no longer visible. Visual symptoms together with lesion size measurements of mock- and AA-treated wild-type and *aos* mutant plants 3 d after inoculation with *B. cinerea* determined that, irrespective of the inoculation strategies, AA-treated wild-type leaves exhibit enhanced tolerance to infection, whereas mock-treated leaves do not (Figure 6A). Furthermore, in contrast with the wild-type plants, AA treatment did not alter the susceptibility of *aos* mutant leaves to infection, indicating that AA-mediated enhanced tolerance to *Botrytis* is JA dependent (Figure 6A). We next analyzed the JA and SA levels in these infected leaves and detected a twofold increase in JA levels in AA-treated wild-type leaves (Figure 6B). As expected, the JA levels in *aos* mutant leaves were below the detection levels under all the experimental conditions (Figure 6B). In contrast with heightened JA levels, the SA levels were reduced in AA-treated wild-type leaves infected with *Botrytis* (Figure 6C). These data are consistent with those obtained from AA-treated, uninfected leaves (Figures 4A). However, AA treatment did not alter the SA levels in *aos* leaves, suggesting that the AA-mediated signaling network underlying alteration of SA levels is JA dependent.

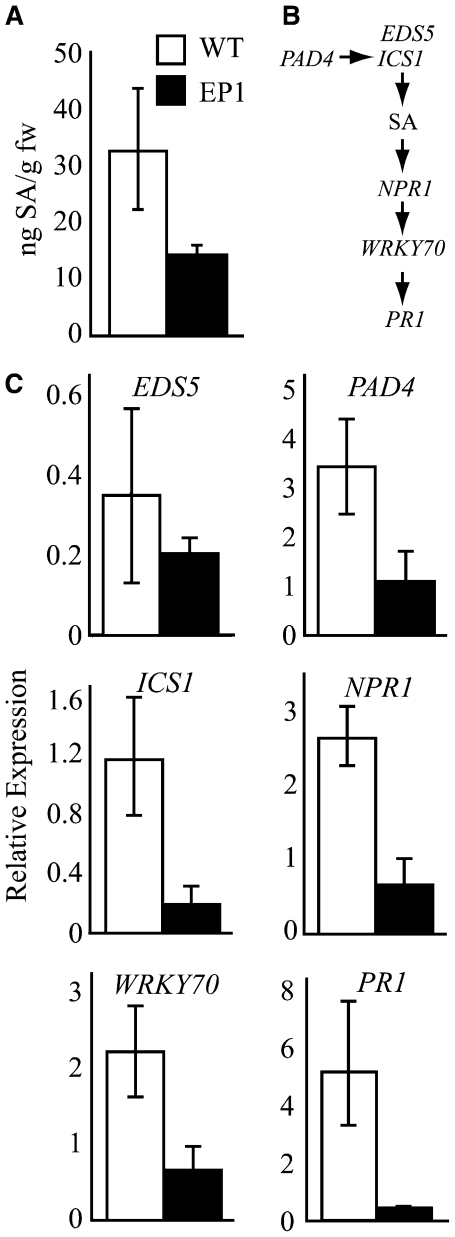

Figure 3. Expression of SA Pathway Genes and SA Metabolite Levels Are Reduced in EP Plants.

(A) Measurements of SA metabolite levels in unwounded wild type (white bars) and EP1 (black bars) show reduced SA levels in EP1 plants. Data are means of six independent experiments ± SD.

(B) Simplified representation of SA pathway genes.

(C) Total RNA was extracted from 4-week-old rosette leaves and subjected to real-time quantitative PCR analysis. The transcript levels of each gene, *EDS5*, *PAD4*, *ICS1*, *NPR1*, *WRKY70*, and *PR1*, were normalized to At4g34270 (T1P41-like family protein) and At4g26410 (M3E9) measured in the same samples. Data are means of three biological replicates ± SE.

AA Signaling Function(s) Is Not Limited to *Arabidopsis*

To determine whether exogenous application of the FAs employed in Supplemental Figure 5 online would modulate JA and SA metabolite levels in plants other than *Arabidopsis*, tomato (*Solanum lycopersicum*) leaves were treated with LA, ALA, EDA, ETrA, and AA. Subsequent measurements of these metabolites clearly showed that, similar to *Arabidopsis*, only AA treatment leads to enhanced levels of JA and reduced levels of SA in

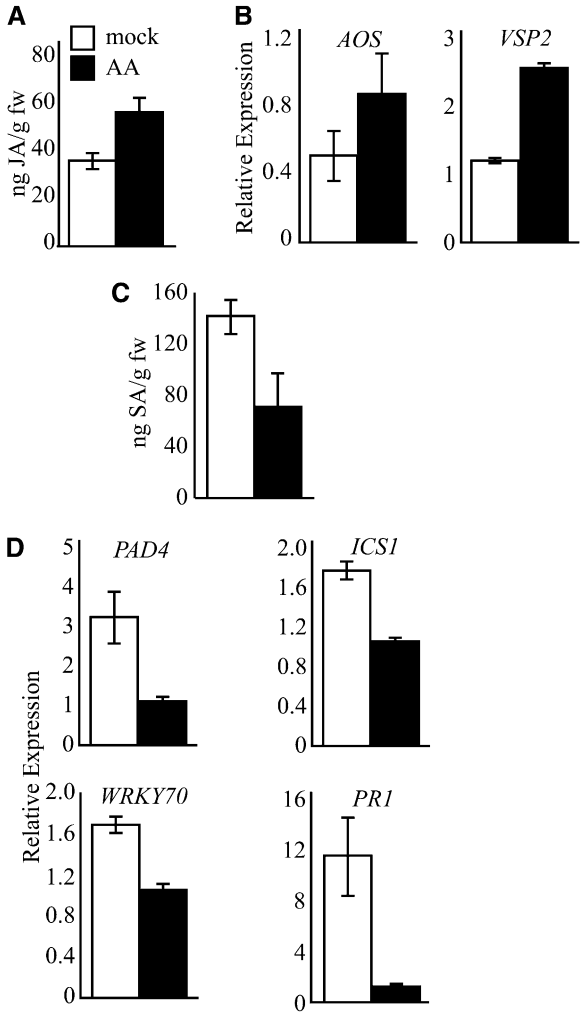

Figure 4. AA Coordinates Events Underlying Alteration in Expression of JA and SA Pathway Genes and Metabolites.

Measurements were performed on leaves exogenously treated with 10 μM AA (black bars) or mock treated with 0.02% ethanol (white bars).

(A) and **(C)** Measurements of JA and SA metabolite levels in AA- and mock-treated plants.

(B) and **(D)** Total RNA was extracted from AA- or mock-treated 4-week-old rosette leaves and subjected to real-time quantitative PCR analysis. The transcript levels of each gene, *AOS*, *VSP2*, *PAD4*, *ICS1*, *WRKY70*, and *PR1*, were normalized to At4g34270 (T1P41-like family protein) and At4g26410 (M3E9) measured in the same samples. Data are means of three biological replicates ± SE.

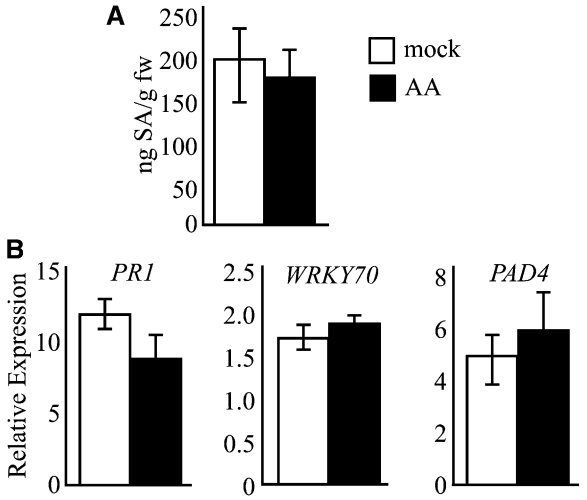

Figure 5. Alteration of SA Pathway Transcripts and Metabolites in Response to AA is JA Dependent.

(A) Measurements of SA metabolite levels in mock-treated (0.02% ethanol; white bars) and AA-treated (black bars) *aos* plants. Data are the means of six biological replicates ± SD.

(B) Total RNA was extracted from mock- or AA-treated 4-week-old rosette leaves of *aos* plants and subjected to real-time quantitative PCR analysis. The transcript levels of each gene, *PAD4*, *WRKY70*, and *PR1*, were normalized to At4g34270 (T1P41-like family protein) and At4g26410 (M3E9) measured in the same samples. Data are means of three biological replicates ± SE.

tomato leaves (Figures 7A and 7B). We next examined the tolerance of AA-treated tomato leaves to *Botrytis* infection. Visual symptoms and lesion size measurements of mock- and AA-treated tomato leaves 3 d after spot inoculation with *B. cinerea* clearly demonstrate that similar to the responses observed in *Arabidopsis*, AA-treated tomato leaves exhibit enhanced tolerance to infection (Figure 7C). These data provide additional compelling evidence for a signaling function of AA in plants other than *Arabidopsis* and potato, the latter species providing a model that led to the discovery of eicosapolyenoic acid elicitor action in plants (Bostock et al., 1981).

AA Induces General Stress-Responsive Genes

We previously identified a *cis*-element designated the rapid stress response element (RSRE) that responds to a wide range of stresses in a rapid and transient manner. Using transgenic plants in which multimerized RSREs drive the expression of the luciferase reporter gene (*4xRSRE:LUC*), we established the in vivo functionality of this element within general transcriptional stress responses induced by abiotic and biotic stresses (Walley et al., 2007). Therefore, we exploited the *4xRSRE:LUC* lines, together with the control empty vector lines (pATM-NOS) to determine whether exogenous application of any of the FAs employed in Supplemental Figure 5 online would induce expression of this general stress-responsive motif. Following application of each FA to a single leaf per plant, luciferase activity was monitored in 30 independent plants. The strongest luciferase activity was observed in leaves treated with AA (Figure 8A; see Supplemental Figure 6 online). The AA-induced expression of *4xRSRE:LUC* occurs only locally with a peak at ~90 min after the treatment (Figure 8A). We next employed real-time quantitative PCR to examine the expression levels of a subset of genes whose promoters contain the RSRE motif and whose expression is rapidly and transiently induced by wounding (Walley et al., 2007). Three of these genes, *ETHYLENE RESPONSE FACTOR #018* (*ERF#018*), *CCR4-ASSOCIATED FACTOR1* (*CAF1a*), and *BON ASSOCIATION PROTEIN1* (*BAP1*) were confirmed as AA-inducible genes (Figure 8B). These data indicate that application of AA sufficed to induce the expression of these otherwise stress-responsive genes and perhaps unknown genes that may play a role in multistress responses.

DISCUSSION

FAs and their derived metabolites are not only major structural and metabolic constituents of the cell, but they also function as modulators of a multitude of signal transduction pathways evoked by environmental and developmental changes. Remodeling the FA composition of membrane lipids can therefore alter

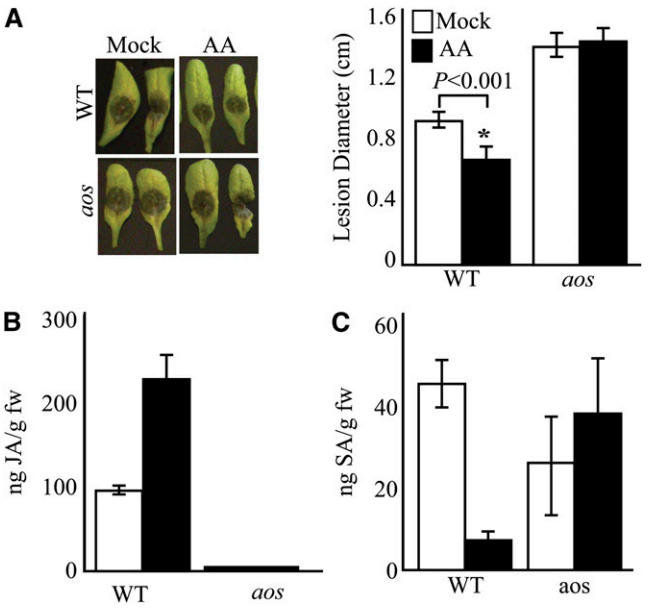

Figure 6. JA Is Required for AA-Mediated Enhanced Tolerance to *Botrytis*.

(A) Visual symptoms 3 d after spot inoculation with conidia of *B. cinerea*. Lesion size determined 3 d after inoculation. Data are means of 45 independent biological replicates ± SE. Asterisks denote a significant difference in AA- versus from mock-treated wild-type leaves ($P < 0.001$) as determined by *t* tests.

(B) and **(C)** Measurements of JA and SA levels in mock-treated (0.02% ethanol; white bars) and AA-treated (black bars) wild-type and *aos* mutant leaves 3 d after inoculation. Data are means of four independent biological replicates ± SE.

[See online article for color version of this figure.]

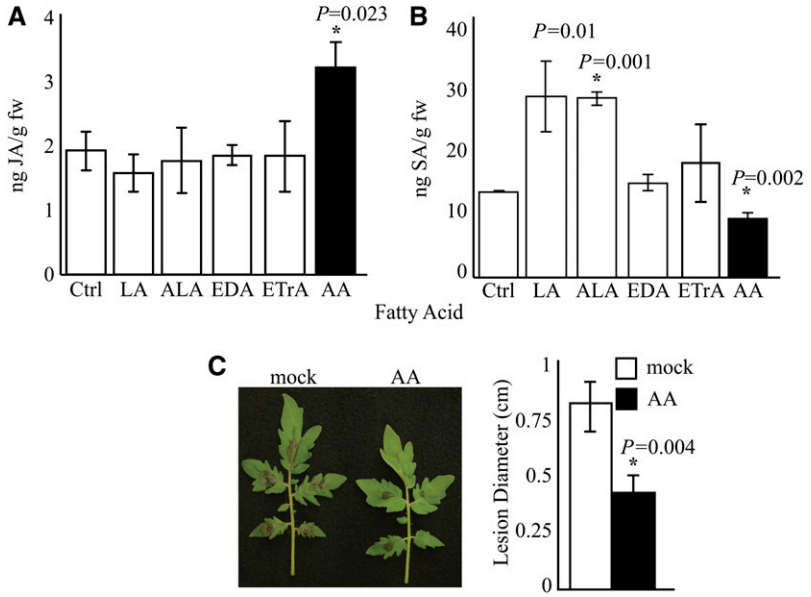

Figure 7. AA-Treated Tomato Leaves Have Altered JA and SA Metabolite Levels and Display Enhanced Tolerance to *Botrytis*.

(A) and **(B)** Measurements of JA and SA metabolite levels in tomato leaves treated with 0.02% ethanol (mock) or 100 μM various FAs [LA (18:2), ALA (18:3), EDA (20:2), ETrA (20:3), and AA (20:4)].

(C) Visual symptoms 3 d after spot inoculation with conidia of *B. cinerea*. Lesion size determined 3 d after inoculation. Data are means of 30 independent biological replicates ± SE. Asterisks denote a significant difference from mock treated ($P < 0.004$) as determined by *t* tests.

[See online article for color version of this figure.]

the metabolism as well as the complex interacting array of signaling cascades that affect a range of physiological responses.

This study was designed to explore the signaling function of FAs that are uncommon in plants but are present in animals and some pathogens, where release occurs during infection. Toward this goal, we examined plants engineered to produce minor levels of EDA, ETrA, and AA for alterations in components of defense response networks and tested them for resistance to a spectrum of biotic stresses. The results establish that even minor perturbations of cellular FA composition suffice to enhance the levels of JA, the phytohormone known to be central to activation of plant defense responses to a range of biotic challengers, including insect (aphid), fungal (*B. cinerea*), and oomycete (*P. capsici*) pathogens (Staswick et al., 1998; Glazebrook, 2005; Halim et al., 2006; Chehab et al., 2008). This increase in JA occurs despite the reduced levels of the FA precursor (18:3) in the EP2 compared with EP1 and wild-type plants. Instead, the enhanced level of JA appears to result from heightened expression of JA biosynthetic pathway genes, including *DGL*, *LOXs* (*2*, 3, 5, and 6), *AOS*, and the JA-responsive gene *VSP2*. These results, together with the enhanced expression of *HPL*, the main gene in the HPL branch of the oxylipin pathway, and the lack of any significant changes in the expression levels of *LOX1* and *4* indicate the broad but selective role of AA in potentiating regulation of the oxylipin transcriptional cascades. The physiological consequences of the AA-mediated alteration of the JA pathway are enhanced resistance to all biotic challenges examined except to *Pst*, a plant pathogen sensitive to SA-activated defense responses.

In contrast with JA-mediated responses, SA-mediated defense signaling is notably compromised in EP plants, as evidenced by their enhanced susceptibility to the SA pathway–inducing pathogen *Pst*. Consistent with this enhanced susceptibility, the expression of SA pathway genes and the levels of SA are considerably reduced in EP plants. These findings indicate that even seemingly insignificant changes in cellular FA composition directly or indirectly alters the coordinating events underlying JA and SA production and thereby leads to differential plant defense responses to various biotic stresses.

Of the pathogens used in this study, only *P. capsici* contains eicosapolyenoic acids. It could be argued that direct inhibition of *P. capsici* due to the presence of additional AA at the host-parasite interface, in the EP plants relative to wild-type plants, is sufficient to tip the balance to resist infection, as oomycetes can be inhibited by eicosanoids (Ricker and Bostock, 1994). However, this seems an unlikely explanation because *Pst*, which colonized EP plants better than wild-type plants, is also sensitive to eicosanoids and oxylipins (Prost et al., 2005). A more plausible explanation seems to be the differential impact on defense signaling networks as noted above.

Treatment of leaves with various free FAs established that only AA enhances the levels of JA apparently through heightened expression levels of JA pathway biosynthetic and thereby JA-responsive genes. This finding corroborated the earlier report that identified AA as a signaling molecule that coordinates events leading to enhanced expression of *VSP*, a JA-responsive gene (Staswick et al., 1998). These data together with those obtained from EP plants further advance the notion that AA can function as a signaling molecule that coordinates events underlying JA production to enhance plant resistance to a range of biotic stresses. Consistent with the data obtained from transgenic

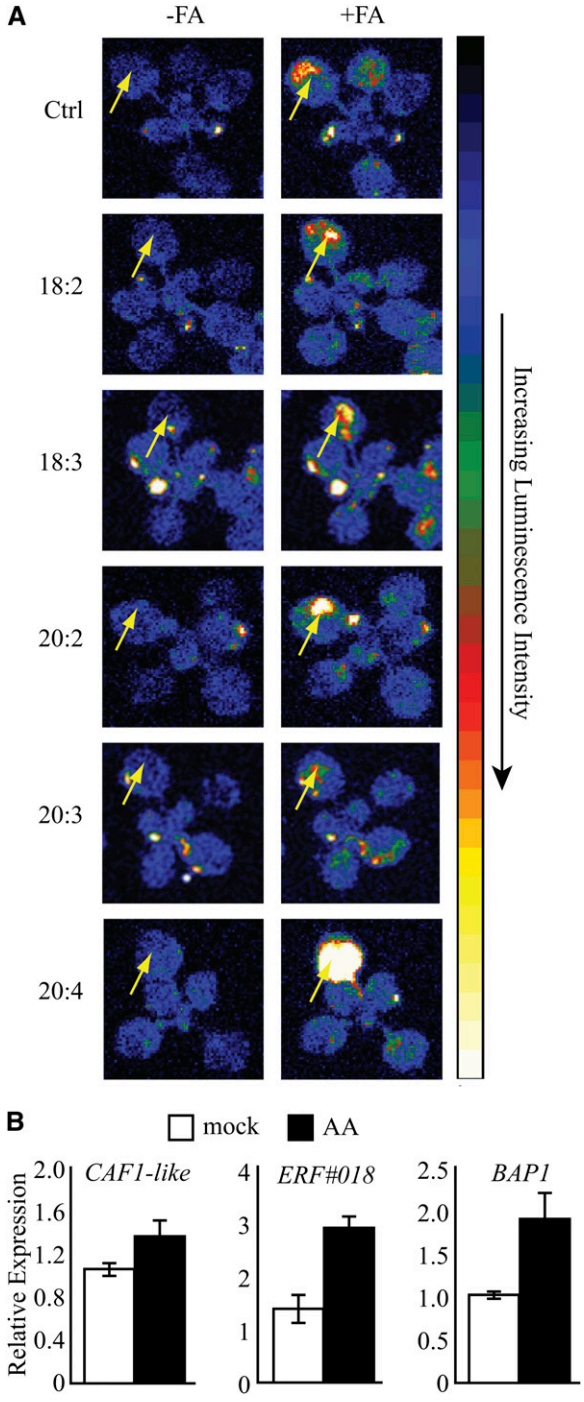

Figure 8. AA Elicits Expression of *4xRSRE:LUC* and Genes with the RSRE Motif.

(A) Image of individual *4xRSRE:LUC* transgenic plants before and 90 min after treatment with either 0.02% ethanol (mock) or different FAs (LA, ALA, EDA, EtrA, and AA). Images of empty vector lines, pATM-NOS (Ctrl) before and after AA treatment, are representative of the data obtained from mock experiments as well as exogenous application of other FAs.

(B) Total RNA was extracted from AA- (black bars) or mock-treated (white bars) 4-week-old rosette leaves and subjected to real-time quantitative PCR analysis. The transcript levels of *CAF1a*, *ERF#018*, and *BAP1* genes, which contain RSRE motifs in their promoter sequences, were normalized to At4g34270 and At4g26410 measured in the same samples. Data are means of three biological replicates ± SE.

lines, exogenous application of AA to both uninfected as well as *Botrytis*-infected wild-type plants reduced the expression of SA pathway genes and the levels of SA. The employment of a JA-deficient mutant line enabled us to uncouple the direct versus the indirect AA-mediated signaling function in reduction of SA and SA pathway genes. Collectively, these experiments unequivocally demonstrate that the reduced expression level of SA pathway genes and lowered SA metabolite levels are not mediated directly by AA, but indirectly through antagonist effects of the JA pathway, a well-established mechanism that allows plants to fine-tune the induction of their defense responses to different pathogens (Kunkel and Brooks, 2002; Bostock, 2005). Moreover, enhanced tolerance of AA-treated tomato leaves to *Botrytis* infection provides compelling evidence for signaling function of AA in plants other than *Arabidopsis* and potato.

To determine the regulatory role of FA in altering the general stress transcriptional network, we treated *4xRSRE:LUC* transgenic lines with a range of FAs. These experiments corroborated the earlier finding that AA is the signaling molecule that coordinates events underlying JA production and demonstrated that AA also potentiates the local and rapid activation of the RSRE. Furthermore, real-time quantitative PCR analysis of a selected group of genes that contain RSREs in their promoters showed that AA treatment also leads to enhanced expression levels of *ERF#018*, *CAF1a*, and *BAP1*. These findings clearly illustrate that AA not only elicits expression of the synthetic *4xRSRE:LUC* but also induces expression of genes with the native RSRE motif in their promoters. These data collectively advance the notion that as in animal systems, AA in plants can function as a signaling molecule that triggers both oxylipin-mediated defense responses and elicits general stress signaling networks.

This study indicates that although most plants do not produce AA, they nevertheless have evolved a capacity to perceive and respond to this ancient signaling molecule as part of their global defense-responsive network against biotic invaders. This signal acts reciprocally on the JA and SA pathways, concomitantly enhancing JA levels through enhanced expression of JA biosynthetic genes and suppressing SA levels indirectly through the well-known antagonism between these two pathways. In addition, more generally, this signal induces expression of genes bearing the RSRE regulatory element in their promoters. Our data thus expand the repertoire of signaling molecules known to trigger plant defenses and provide evidence that AA acts via a mechanism that regulates the general stress transcriptional network in addition to the JA-biosynthetic pathway.

METHODS

Generation of Eicosapolyenoic Acid Producing *Arabidopsis* Lines

The generation of the eicosapolyenoic acid–producing line referred to here as EP1, by sequential transformation with constitutively expressed C18-Δ^9-elongase, C20-Δ^8-desaturase, and C20-Δ^5-desaturase genes,

was described previously (Qi et al., 2004). Line EP2 was generated by a single step of transformation, using a plasmid made by adding the Δ^8- and Δ^5-desaturase genes to pCB302.3Δ9, a binary plasmid with the C18-Δ^9-elongase coding region in the cauliflower mosaic virus 35S promoter-nos terminator expression cassette of pCB302.3 (Fraser et al., 2004) as follows. pCB302Δ9 was first converted to destination vector pCB302Δ9G by insertion of a GATEWAY cassette (Invitrogen) into a unique *Hin*dIII site. Plasmid pENTR3FEC was made by cloning the PCR-amplified polylinker of pNEB193 (New England Biolabs; primers M13Fg, 5′-CACCGTAAAACGACGGCCAG-3′, and M13R, 5′-CAGGAAACAGC-TATGAC-3′) in pENTR/SD/D-TOPO (Invitrogen), then ligating a cauliflower mosaic virus 35S promoter-nos terminator expression cassette between the *Hin*dIII and *Eco*RI sites. The Δ^8-desaturase coding region was inserted into the expression cassette as a *Bam*HI-*Kpn*I fragment isolated from pBlueBacΔ8 (Qi et al., 2004) to create pENTRΔ8. The Δ^5-desaturase gene was PCR amplified from the pCAMBIA construct made by Qi et al. (2004) with primers (P35Sbeg, 5′-CACCAAGCTTGCATGC-CTGCA-3′; TnosEndH, 5′-AAGCTTCCCGATCTAGTAACAT-3′) that allowed the product to be cloned in pENTR/D-TOPO (Invitrogen), and then excised as a *Hin*dIII fragment for insertion into pENTRΔ8. The Δ8- and Δ5-desaturase genes from the resultant pENTRΔ8Δ5 were transferred to pCB302Δ9G by recombination in vitro using LR Clonase II (Invitrogen). The resultant plasmid, pCB302Δ9Δ8Δ5, was transferred to *Agrobacterium tumefaciens* strain GV3101 and used to transform *Arabidopsis thaliana* Columbia to BASTA resistance as described previously (Fraser et al., 2004; Qi et al., 2004).

Plant Growth and Wounding Treatments

Arabidopsis plants were grown in a 16-h-light/8-h-dark cycle at 22°C for most experiments, except plants used for bioassays. The latter were grown in a 12-h-light/12-h-dark photoperiod. Mechanical wounding of leaves was performed with a hemostat as previously described (Walley et al., 2007). Tissues harvested before and 90 min after wounding were immediately frozen in liquid nitrogen and stored at −80°C until use. Most experiments were performed on 4-week-old plants, unless stated otherwise.

To generate the tomato (*Solanum lycopersicum*) seedlings, tomato seeds were first surfaced sterilized with the following treatment sequence: 50% HCl (10 min) and rinsed with sterile deionized water, 10% trisodium phosphate (15 min), and rinsed three times in sterile deionized water and 70% ethanol (10 min) for 30 min and rinsed with sterile deionized water and 50% bleach (20 min) followed by sterile deionized water rinse (three times). Surface-sterilized seeds were started in germination paper in beakers containing sterile deionized water. Seedlings were then transferred to flats of potting soil and grown for an additional 2 weeks in a climate-controlled greenhouse with supplemental lighting until at least two true leaves had developed on each plant.

FA Analysis

Fatty acid methyl esters (FAMEs) were prepared from leaves using the previously described method (Browse et al., 1986). FAMEs were analyzed by gas chromatography–mass spectrometry using a Hewlett Packard 6890 series gas chromatograph coupled to an Agilent Technologies 5973 Network mass selective detector. Heptadecanoic acid (17:0) (Cayman Chemical) was added to the leaf extracts during preparation of FAMEs and provided an internal standard.

Plant Hormone Extraction and Quantification

Extraction of JAs (methyl jasmonate and JA) and SA was performed as previously described (Engelberth et al., 2004; Chehab et al., 2008). Dihydro-JA and deuterated SA (C/D/N Isotopes) were used as internal standards. The methyl ester derivatives were analyzed by gas chromatography–mass spectrometry operated in electronic ionization mode. Mass spectral analysis was done in selective ion monitoring mode. Fragment ions monitored were as follows: JA-ME 224, 151, 83; dihydro-JA-ME 96, 83; SA-ME 152; SA-D4-ME 156. Quantification calibration curves were generated by derivatization and analysis of known quantities of pure JA and SA (Sigma-Aldrich).

Expression Analysis

Total RNA from rosette leaves was isolated by TRIzol extraction (Life Technologies) and further purified using the Qiagen RNeasy kit with on column DNase treatment (Qiagen) to eliminate DNA contamination. RNA was reverse transcribed using Superscript III (Invitrogen). Real-time quantitative PCR was conducted in 50-μL reactions containing cDNA synthesized from 10 ng of total RNA, 1× iQ SYBR Green Supermix (Bio-Rad Laboratories), and 200 nM for each primer. Amplification and data analysis were performed as previously described (Walley et al., 2008). At4g34270 and At4g26410 were used as reference genes for the internal controls as previously described for transcript normalization (Walley et al., 2008). Primers are listed in Supplemental Table 1 online.

Fungal Pathogenicity Tests

Botrytis cinerea 'Grape' and 'B05.10' isolates were used in the assay. Preparation of inoculum and infection of *Arabidopsis* plants were performed as previously described (Rowe and Kliebenstein, 2007). Mature rosette leaves excised from 4-week-old *Arabidopsis* plants were placed in Petri dishes containing 1% agar. Leaves were inoculated with 5-μL droplets of 5×10^4 spores/mL in half-strength filtered organic grape juice and incubated at room temperature under ambient light conditions. Lesion diameter was measured from digital images of at least 30 infected leaves using Image J with scale objects included in images as previously described (Chehab et al., 2008; Walley et al., 2008).

Phytophthora capsici Culture and Inoculation

A pepper isolate of *Phytophthora capsici* (from Yolo County, CA; Bostock lab collection) was maintained on V-8 agar plates. We found this isolate to be capable of readily infecting *Arabidopsis* roots. Two-week-old plants maintained hydroponically in aerated half-strength Hoagland solution were inoculated by transferring the plants to a 24-well Cellstar multiwall plate (Greiner Bio-One) and placing the roots in 1 mL of half-strength Hoagland solution containing 10^5 zoospores of *P. capsici* per milliliter. Disease development was monitored at 48 h after inoculation and assessed as sporangia counts on roots in 10 microscope fields at ×200 magnification and by plant colonization as measured by quantitative PCR of *P. capsici* DNA.

P. capsici DNA Quantitation in Infected Host Tissue

Arabidopsis roots were flash frozen in liquid nitrogen and were used for DNA extraction using DNeasy Plant Mini kits (Qiagen). Pathogen DNA was quantified with real-time PCR using established primers for *P. capsici* (Cap-FW 5′-TTTAGTTGGGGGTCTTGTACC-3′ and Cap-RV1 5′-CCT-CCACAACCAGCAACA-3′ [Silvar et al., 2005]). Standard curves were verified for this system for pure *P. capsici* DNA ($R^2 = 0.99$) and *P. capsici* DNA amended with 100 ng *Arabidopsis* DNA ($R^2 = 0.99$).

Aphid Dual-Choice Assay

The choice assays were performed as previously described (Chehab et al., 2008). Briefly, green peach aphid (*Myzus persicae*) colonies were maintained on cabbage seedlings (*Brassica oleracea* var *capitata*) at

laboratory conditions (23 ± 3°C, 50% ± 20% relative humidity, and 16 h light). Choice bioassays were performed to identify the plants on which aphids prefer to deposit their nymphs. Female aphids were transferred using a fine hair brush and released into the center of a soil-containing pot, in which 11 plants were kept at laboratory conditions and examined every 24 h for two successive days. The location of the first deposited nymphs was recorded. The bioassays were performed with ~49 replicates and four trials.

Bacterial Pathogenicity Test

A bioluminescent strain of *Pseudomonas syringae* pv *tomato* DC3000 (*Pst*) was used to analyze susceptibility of wild-type and EP plants to a bacterial pathogen. *Arabidopsis* leaves were hand infiltrated with bacterial inocula of OD_{600} = 0.002 as previously described (Fan et al., 2008). Leaf discs (0.7 cm in diameter) were excised from inoculated leaves 2 days after inoculation. Bioluminescence was measured with a Micro-Lumat LB96P luminometer (EG and G Berthold) and recorded as photon counts per second. Thirty plants were analyzed in four independent experiments.

Application of Exogenous FAs

FAs were purchased from Cayman Chemical Co. FA stock solutions (300 mM) were prepared in 80% ethanol. Working solutions of indicated concentrations (see Supplemental Figures 3 and 4 online) were prepared in DI water as sonicated emulsion with ethanol content adjusted to 0.02%. For each measurement, 5 μL of emulsion with the required concentration was applied on leaves of growing plants.

Luciferase Imaging

Luciferase imaging of *4xRSRE:LUC* and control empty vector lines (pATM-NOS) were performed as previously described (Walley et al., 2007). Briefly, 10- to 14-d-old plants grown on soil were sprayed with 2.5 mM luciferin (Promega) in 0.001% Triton X-100 ~16 to 20 h prior to treatment. Subsequently, 5 μL of FA was applied to a single leaf per plant. For each treatment, at least 30 plants were imaged using an Andor DU434-BV CCD camera (Andor Technology). Images were acquired every 5 min over a 4-h period. Luciferase activity was quantified for a defined area as mean counts $pixel^{-1}$ exposure $time^{-1}$ using Andor Solis image analysis software (Andor Technology).

Statistical Analyses

To determine statistical significance of treatment effects or the effects of genotype when comparing wild-type versus EP plants, *t* tests were performed in most cases using Sigma Stat v3.5. To determine treatment effects on *P. capsici* colonization as measured by quantitative PCR and by sporangia counts on colonized roots, the Wilcoxon rank sums test was used to compare means because the data did not satisfy the analysis of variance criterion for normality. Statistical analyses were performed using JMP software version 8.0 (The SAS Institute). To investigate the effect of the trials and aphid types on probability of plant preference (wild type versus EP), Pearson χ^2 tests were performed. One-tailed binomial tests were performed to test the significance of the aphids' choices for nymph deposition (Zar, 1999).

Accession Numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: *PAD4* (At3g52430), *ICS1* (At1g74710), *NPR1* (At1g64280), *WRKY70* (At3g56400), *PR1* (At2g14610), *PLA1* (*DGL*) (At1g05800), *LOX1* (At1g55020), *LOX2* (At3g45140), *LOX3* (At1g17420), *LOX4* (At1g72520), *LOX5* (At3g22400), *LOX6* (At1g67560), *AOS* (At5g42650), *VSP2* (At5g24770), *HPL* (At4g15440), *CAF1a* (At3g44260), *ERF#018* (At1g74930), *BAP1* (At3g61190), and *EDS5* (At4g39030).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure 1. EP Plants Exhibit Altered Tolerance to Biotic Challengers.

Supplemental Figure 2. Levels of JA Pathway Transcripts and Metabolites Are Enhanced in EP Plants.

Supplemental Figure 3. Enhanced Expression Levels of *HPL* in EP Plants.

Supplemental Figure 4. Expression of SA Pathway Genes and SA Metabolite Levels Are Reduced in EP2 Plants.

Supplemental Figure 5. JA Production Is Induced by Exogenous Application of AA in a Concentration-Dependent Manner.

Supplemental Figure 6. Relative Luciferase Activity of *4xRSRE:LUC* Plants Treated with 10 μM of Various FAs.

Supplemental Table 1. List of Primers Used for Quantitative RT-PCR Analyses.

ACKNOWLEDGMENTS

We thank Peter Quail for his critical review of this manuscript. This work was supported by National Science Foundation Grant 0543904 to K.D.

Received January 5, 2010; revised September 16, 2010; accepted September 22, 2010; published October 8, 2010.

REFERENCES

Black, P.N., Faergeman, N.J., and DiRusso, C.C. (2000). Long-chain acyl-CoA-dependent regulation of gene expression in bacteria, yeast and mammals. J. Nutr. **130:** 305S–309S.

Blee, E. (2002). Impact of phyto-oxylipins in plant defense. Trends Plant Sci. **7:** 315–322.

Bostock, R.M. (2005). Signal crosstalk and induced resistance: Straddling the line between cost and benefit. Annu. Rev. Phytopathol. **43:** 545–580.

Bostock, R.M., Kuc, J.A., and Laine, R.A. (1981). Eicosapentaenoic and arachidonic acids from *Phytophthora infestans* elicit fungitoxic sesquiterpenes in the potato. Science **212:** 67–69.

Bostock, R.M., Schaeffer, D.A., and Hammerschmidt, R. (1986). Comparison of elicitor activities of arachidonic acid, fatty acids and glucans from *Phytophthora infestans* in hypersensitivity expression in potato tuber. Physiol. Mol. Plant Pathol. **29:** 349–360.

Bostock, R.M., Yamamoto, H., Choi, D., Ricker, K.E., and Ward, B.L. (1992). Rapid stimulation of 5-lipoxygenase activity in potato by the fungal elicitor arachidonic acid. Plant Physiol. **100:** 1448–1456.

Brodhagen, M., Tsitsigiannis, D.I., Hornung, E., Goebel, C., Feussner, I., and Keller, N.P. (2008). Reciprocal oxylipin-mediated cross-talk in the *Aspergillus*-seed pathosystem. Mol. Microbiol. **67:** 378–391.

Browse, J., McCourt, P.J., and Somerville, C.R. (1986). Fatty acid composition of leaf lipids determined after combined digestion and fatty acid methyl ester formation from fresh tissue. Anal. Biochem. **152:** 141–145.

Cao, H., Bowling, S.A., Gordon, A.S., and Dong, X. (1994). Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell **6:** 1583–1592.

Chandra-Shekara, A.C., Venugopal, S.C., Barman, S.R., Kachroo, A., and Kachroo, P. (2007). Plastidial fatty acid levels regulate resistance gene-dependent defense signaling in *Arabidopsis.* Proc. Natl. Acad. Sci. USA **104:** 7277–7282.

Chehab, E.W., Kaspi, R., Savchenko, T., Rowe, H., Negre-Zakharov, F., Kliebenstein, D., and Dehesh, K. (2008). Distinct roles of jasmonates and aldehydes in plant-defense responses. PloS One **3:** e1904.

Choi, D., Bostock, R.M., Avdiushko, S., and Hildebrand, D. (1994). Lipid-derived signals that discriminate wound- and pathogen-responsive isoprenoid pathways in plants: Methyl jasmonate and the fungal elicitor arachidonic acid induce different HMG-CoA reductase genes and antimicrobial isoprenoids in *Solanum tuberosum* L. Proc. Natl. Acad. Sci. USA **91:** 2329–2333.

Choi, D., Ward, B.L., and Bostock, R.M. (1992). Differential induction and suppression of potato 3-hydroxy-3-methylglutaryl coenzyme A reductase genes in response to *Phytophthora infestans* and to its elicitor arachidonic acid. Plant Cell **4:** 1333–1344.

Duan, H., Huang, M.Y., Palacio, K., and Schuler, M.A. (2005). Variations in CYP74B2 (hydroperoxide lyase) gene expression differentially affect hexenal signaling in the Columbia and Landsberg erecta ecotypes of *Arabidopsis.* Plant Physiol. **139:** 1529–1544.

Duplus, E., Glorian, M., and Forest, C. (2000). Fatty acid regulation of gene transcription. J. Biol. Chem. **275:** 30749–30752.

Engelberth, J., Alborn, H.T., Schmelz, E.A., and Tumlinson, J.H. (2004). Airborne signals prime plants against insect herbivore attack. Proc. Natl. Acad. Sci. USA **101:** 1781–1785.

Fan, J., Crooks, C., and Lamb, C. (2008). High-throughput quantitative luminescence assay of the growth in planta of *Pseudomonas syringae* chromosomally tagged with *Photorhabdus luminescens* luxCDABE. Plant J. **53:** 393–399.

Farmer, E.E., Weber, H., and Vollenweider, S. (1998). Fatty acid signaling in *Arabidopsis.* Planta **206:** 167–174.

Feussner, I., and Wasternack, C. (2002). The lipoxygenase pathway. Annu. Rev. Plant Biol. **53:** 275–297.

Fraser, T.C., Qi, B., Elhussein, S., Chatrattanakunchai, S., Stobart, A.K., and Lazarus, C.M. (2004). Expression of the *Isochrysis* C18-delta9 polyunsaturated fatty acid specific elongase component alters *Arabidopsis* glycerolipid profiles. Plant Physiol. **135:** 859–866.

Garcia-Pineda, E., Castro-Mercado, E., and Lozoya-Gloria, E. (2004). Gene expression and enzyme activity of pepper (*Capsicum annuum* L.) ascorbate oxidase during elicitor and wounding stress. Plant Sci. **166:** 237–243.

Gardner, R.W. (1995). Biological roles and biochemistry of the lipoxygenase pathway. HortScience **30:** 197–205.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. **43:** 205–227.

Halim, V.A., Vess, A., Scheel, D., and Rosahl, S. (2006). The role of salicylic acid and jasmonic acid in pathogen defence. Plant Biol. (Stuttg.) **8:** 307–313.

Huang, Z.H., Gu, D., and Mazzone, T. (2004). Oleic acid modulates the post-translational glycosylation of macrophage ApoE to increase its secretion. J. Biol. Chem. **279:** 29195–29201.

Hyun, Y., et al. (2008). Cooperation and functional diversification of two closely related galactolipase genes for jasmonate biosynthesis. Dev. Cell **14:** 183–192.

Jirage, D., Tootle, T.L., Reuber, T.L., Frost, L.N., Feys, B.J., Parker, J.E., Ausubel, F.M., and Glazebrook, J. (1999). *Arabidopsis thaliana* PAD4 encodes a lipase-like gene that is important for salicylic acid signaling. Proc. Natl. Acad. Sci. USA **96:** 13583–13588.

Kachroo, A., Lapchyk, L., Fukushige, H., Hildebrand, D., Klessig, D., and Kachroo, P. (2003). Plastidial fatty acid signaling modulates salicylic acid- and jasmonic acid-mediated defense pathways in the *Arabidopsis* ssi2 mutant. Plant Cell **15:** 2952–2965.

Kachroo, P., Kachroo, A., Lapchyk, L., Hildebrand, D., and Klessig, D.F. (2003). Restoration of defective cross talk in ssi2 mutants: role of salicylic acid, jasmonic acid, and fatty acids in SSI2-mediated signaling. Mol. Plant Microbe Interact. **16:** 1022–1029.

Kachroo, P., Shanklin, J., Shah, J., Whittle, E.J., and Klessig, D.F. (2001). A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc. Natl. Acad. Sci. USA **98:** 9448–9453.

Kachroo, P., Venugopal, S.C., Navarre, D.A., Lapchyk, L., and Kachroo, A. (2005). Role of salicylic acid and fatty acid desaturation pathways in ssi2-mediated signaling. Plant Physiol. **139:** 1717–1735.

Knight, V.I., Wang, H., Lincoln, J.-E., Lulai, E.C., Gilchrist, D.G., and Bostock, R.M. (2001). Hydroperoxides of fatty acids induce programmed cell death in tomato protoplasts. Physiol. Mol. Plant Pathol. **59:** 277–286.

Kunkel, B.N., and Brooks, D.M. (2002). Cross talk between signaling pathways in pathogen defense. Curr. Opin. Plant Biol. **5:** 325–331.

Li, J., Brader, G., and Palva, E.T. (2004). The WRKY70 transcription factor: A node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell **16:** 319–331.

Lorenzo, O., and Solano, R. (2005). Molecular players regulating the jasmonate signalling network. Curr. Opin. Plant Biol. **8:** 532–540.

Magnan, A., and Vervloet, D. (1999). Leukotriene inhibitors-(2): Place of leukotrienes in the pathophysiology of asthma and rhinitis. Rev. Fr. Allergol. **39:** 25–29.

McDowell, J.M., and Dangl, J.L. (2000). Signal transduction in the plant immune response. Trends Biochem. Sci. **25:** 79–82.

Nawrath, C., Heck, S., Parinthawong, N., and Métraux, J.-P. (2002). EDS5, an essential component of salicylic acid–dependent signaling for disease resistance in *Arabidopsis*, is a member of the MATE transporter family. Plant Cell **14:** 275–286.

Ozeretskovskaya, O.L., Varlamov, V.P., Vasyukova, N.I., Chalenko, G.I., Gerasimova, N.G., and Panina, Y.S. (2004). Influence of systemic signal molecules on the rate of spread of the immunizing effect of elicitors over potato tissues. Appl. Biochem. Microbiol. **40:** 213–216.

Park, J.H., Halitschke, R., Kim, H.B., Baldwin, I.T., Feldmann, K.A., and Feyereisen, R. (2002). A knock-out mutation in allene oxide synthase results in male sterility and defective wound signal transduction in *Arabidopsis* due to a block in jasmonic acid biosynthesis. Plant J. **31:** 1–12.

Pegorier, J.P., Le May, C., and Girard, J. (2004). Control of gene expression by fatty acids. J. Nutr. **134:** 2444S–2449S.

Preisig, C.L., and Kuć, J.A. (1985). Arachidonic acid-related elicitors of the hypersensitive response in potato and enhancement of their activities by glucans from *Phytophthora infestans* (Mont) Debary. Arch. Biochem. Biophys. **236:** 379–389.

Preisig, C.L., and Kuć, J.A. (1988). Metabolism by potato tuber of arachidonic acid, an elicitor of hypersensitive resistance. Physiol. Mol. Plant Pathol. **32:** 77–88.

Prost, I., et al. (2005). Evaluation of the antimicrobial activities of plant oxylipins supports their involvement in defense against pathogens. Plant Physiol. **139:** 1902–1913.

Qi, B., Fraser, T., Mugford, S., Dobson, G., Sayanova, O., Butler, J., Napier, J.A., Stobart, A.K., and Lazarus, C.M. (2004). Production of very long chain polyunsaturated omega-3 and omega-6 fatty acids in plants. Nat. Biotechnol. **22:** 739–745.

Ricker, K.E., and Bostock, R.M. (1992). Evidence for release of the

elicitor arachidonic acid and its metabolites from sporangia of *Phytophthora infestans* during infection of potato. Physiol. Mol. Plant Pathol. **41:** 61–72.

Ricker, K.E., and Bostock, R.M. (1994). Eicosanoids in the *Phytophthora infestans*-potato interaction: Lipoxygenase metabolism of arachidonic acid and biological activities of selected lipoxygenase products. Physiol. Mol. Plant Pathol. **44:** 65–80.

Rowe, H.C., and Kliebenstein, D.J. (2007). Elevated genetic variation within virulence-associated *Botrytis cinerea* polygalacturonase loci. Mol. Plant Microbe Interact. **20:** 1126–1137.

Rozhnova, N.A., Gerashchenkov, G.A., and Babosha, A.V. (2003). The effect of arachidonic acid and viral infection on the phytohemagglutinin activity during the development of tobacco acquired resistance. Russ. J. Plant Physiol. **50:** 661–665.

Schultz, J. (2002). Shard signals and the potential for phylogentic espionage between plants and animals. Integr. Comp. Biol. **42:** 454–462.

Shah, J. (2003). The salicylic acid loop in plant defense. Curr. Opin. Plant Biol. **6:** 365–371.

Silvar, C., Diaz, J., and Merino, F. (2005). Real-time polymerase chain reaction quantification of *Phytophthora capsici* in different pepper genotypes. Phytopathology **95:** 1423–1429.

Staswick, P.E., Yuen, G.Y., and Lehman, C.C. (1998). Jasmonate signaling mutants of *Arabidopsis* are susceptible to the soil fungus *Pythium irregulare.* Plant J. **15:** 747–754.

Straus, D.S., and Glass, C.K. (2001). Cyclopentenone prostaglandins: new insights on biological activities and cellular targets. Med. Res. Rev. **21:** 185–210.

Tjamos, E.C., and Kuć, J.A. (1982). Inhibition of steroid glycoalkaloid accumulation by arachidonic and eicosapentaenoic acids in potato. Science **217:** 543–544.

Upchurch, R.G. (2008). Fatty acid unsaturation, mobilization, and regulation in the response of plants to stress. Biotechnol. Lett. **30:** 967–977.

van Ryn, J., Trummlitz, G., and Pairet, M. (2000). COX-2 selectivity and inflammatory processes. Curr. Med. Chem. **7:** 1145–1161.

Venugopal, S.C., Jeong, R.D., Mandal, M.K., Zhu, S., Chandra-Shekara, A.C., Xia, Y., Hersh, M., Stromberg, A.-J., Navarre, D., Kachroo, A., and Kachroo, P. (2009). Enhanced disease susceptibility 1 and salicylic acid act redundantly to regulate resistance gene-mediated signaling. PLoS Genet. **5:** e1000545.

Walley, J.W., Coughlan, S., Hudson, M.E., Covington, M.F., Kaspi, R., Banu, G., Harmer, S.L., and Dehesh, K. (2007). Mechanical stress induces biotic and abiotic stress responses via a novel cis-element. PLoS Genet. **3:** 1800–1812.

Walley, J.W., Rowe, H.C., Xiao, Y., Chehab, E.W., Kliebenstein, D.J., Wagner, D., and Dehesh, K. (2008). The chromatin remodeler SPLAYED regulates specific stress signaling pathways. PLoS Pathog. **4:** e1000237.

Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defense. Nature **414:** 562–565.

Zar, J.H. (1999). Biostatistical Analysis. (Upper Saddle River, NJ: Prentice Hall).