Interacting Signal Pathways Control Defense Gene Expression in *Arabidopsis* in Response to Cell Wall-Degrading Enzymes from *Erwinia carotovora*

Cecilia Norman-Setterblad,¹ Sabina Vidal,¹ and E. Tapio Palva^{2,3}

¹Department of Plant Biology, Swedish University of Agricultural Sciences, Box 7080, SE-750 07 Uppsala, Sweden; ²Department of Biosciences, Division of Genetics, and ³Institute of Biotechnology, University of Helsinki, Box 56, Helsinki, FIN-00014 Finland
Accepted 28 December 1999.

We have characterized the role of salicylic acid (SA)-independent defense signaling in *Arabidopsis thaliana* in response to the plant pathogen *Erwinia carotovora* subsp. *carotovora*. Use of pathway-specific target genes as well as signal mutants allowed us to elucidate the role and interactions of ethylene, jasmonic acid (JA), and SA signal pathways in this response. Gene expression studies suggest a central role for both ethylene and JA pathways in the regulation of defense gene expression triggered by the pathogen or by plant cell wall-degrading enzymes (CF) secreted by the pathogen. Our results suggest that ethylene and JA act in concert in this regulation. In addition, CF triggers another, strictly JA-mediated response inhibited by ethylene and SA. SA does not appear to have a major role in activating defense gene expression in response to CF. However, SA may have a dual role in controlling CF-induced gene expression, by enhancing the expression of genes synergistically induced by ethylene and JA and repressing genes induced by JA alone.

Additional keywords: elicitors, soft rot.

Plants have the ability to defend themselves against pathogen attack by activating series of both local and systemic defense responses. These responses may be triggered by a race/ cultivar specific recognition event, as in the gene-for-gene type of interaction, where resistance depends on matching of a dominant avirulence (*avr*) gene from the pathogen and a corresponding resistance gene (*R*) from the host (Flor 1971; Martin et al. 1993; Staskawicz et al. 1995). In addition to such specific resistance, plant defense responses can be activated by a range of unspecific biotic or abiotic elicitors (Nürnberg et al. 1994, 1995; Benhamou 1996). In both cases, the responses appear to involve transcriptional activation of defense genes and de novo synthesis of proteins, including enzymes involved in the biosynthesis of phytoalexins, defensins, and pathogenesis-related (PR) proteins (Bol et al. 1990; Kombrink and Somssich

1995; Penninckx et al. 1996). In many plant species, local infection by incompatible pathogens leads to the induction of a broad-spectrum disease resistance in uninfected parts of the plant, termed systemic acquired resistance (SAR) (Ryals et al. 1996). In tobacco and *A. thaliana*, the onset of SAR correlates with the local and systemic expression of a set of genes called SAR genes (Ward et al. 1991; Uknes et al. 1992). Several of these genes encode members of the family of PR proteins, in particular the secreted acidic isoforms, that can be used as molecular markers to evaluate the development of this response (Ward et al. 1991). Salicylic acid (SA) has been shown to play a key role in the signal transduction pathway leading to SAR and the expression of SAR genes (Malamy et al. 1990; Métraux et al. 1990; Ward et al. 1991). Direct evidence supporting this involvement comes from analysis of transgenic tobacco and *A. thaliana* plants expressing the *nahG* gene of *Pseudomonas putida*. The *nahG* gene product, salicylate hydroxylase, converts SA into its inactive form, catechol. The inability of these plants to accumulate SA leads to the incapability of developing SAR and SAR gene expression in response to pathogen invasion or SA treatment (Gaffney et al. 1993; Delaney et al. 1994; Lawton et al. 1995).

Recent studies by a number of laboratories have indicated the importance of SA- and SAR-independent defense responses. These include responses to fungi (Penninckx et al. 1996, 1998), *Erwinia carotovora* subsp. *carotovora* (Vidal et al. 1997, 1998), and growth-promoting rhizobacteria (Pieterse et al. 1996, 1998). A different form of induced systemic resistance (ISR) is triggered by nonpathogenic, root-colonizing rhizobacteria (*Pseudomonas fluorescens*) (Pieterse et al. 1996, 1998). In contrast to other systemic resistance responses, ISR does not result in PR gene activation.

Plant hormones like ethylene and jasmonates have been implicated in SA-independent defense responses. Ethylene levels have been shown to increase upon wounding or infection by pathogens, as well as from treatment with elicitors of defense responses (Boller 1991; Enyedi et al. 1992). Ethylene treatment induces several genes encoding PR proteins but, in contrast to SA, mainly the basic vacuolar isoforms (Boller 1988; Bol et al. 1990). Genetic evidence from ethylene-insensitive mutants of *A. thaliana* has shown that ethylene is not required for SAR (Lawton et al. 1995).

Corresponding author: E. Tapio Palva; Telephone: +356 9 191 59600; Fax: +358 9 191 59076; E-mail: tapio.palva@helsinki.fi

Cecilia Norman-Setterblad and Sabina Vidal contributed equally to this study.

Jasmonic acid (JA) and its methyl ester, methyl jasmonate (MeJA), accumulate both locally and systemically during the wound response of plants or upon elicitor treatment (Creelman et al. 1992; Gundlach et al. 1992). In addition, JA and MeJA induce the expression of genes involved in plant defense responses, including genes for phytoalexin biosynthesis (Gundlach et al. 1992), proteinase inhibitors (Farmer et al. 1992), thionins (Epple et al. 1995), and defensins (Penninckx et al. 1996). Recently, evidence has emerged regarding the involvement of ethylene and jasmonates in the defense response toward non-host-specific pathogens. Transgenic tobacco plants insensitive to ethylene and *A. thaliana* mutants unable to accumulate JA showed increased susceptibility to infection by *Pythium* spp., which normally are nonpathogenic fungi (Knoester et al. 1998; Vijayan et al. 1998). Both jasmonate and ethylene have been shown to play an important role in the ISR signaling pathway, although genes known to be activated by these phytohormones are not induced during ISR (Pieterse et al. 1998).

The Gram-negative bacterium *Erwinia carotovora* subsp. *carotovora* and other soft-rot erwinias constitute one of the most important groups of plant-pathogenic bacteria. This pathogen is the causal agent of potato soft rot and is destructive to a wide variety of plants under the right environmental conditions (Pérombelon and Kelman 1980; Kotoujansky 1987). Breeders have not yet identified any genetic traits of resistance that may be introduced into cultivars and the development of resistance to this pathogen seems to depend on generally induced defense mechanisms (Palva et al. 1993, 1994). The pathogenicity of *E. carotovora* subsp. *carotovora* is thought to be correlated with its ability to produce and secrete a large arsenal of plant cell wall-degrading enzymes, including pectinases and cellulases. These enzymes have been proposed to have a dual role: in addition to being the main virulence determinants of the pathogen (Collmer and Keen 1986; Kotoujansky 1987; Pirhonen et al. 1991), they also trigger plant defense (Davis and Ausubel 1989; Palva et al. 1993; Vidal et al. 1998). We have previously demonstrated that treatment of tobacco plants with acellular preparations of cell wall-degrading enzymes from *E. carotovora* subsp. *carotovora* induces both local and systemic expression of several genes encoding PR proteins (Palva et al. 1993; Vidal et al. 1997, 1998). Moreover, we have shown that these enzymes induce both local and systemic resistance responses to this pathogen in tobacco (Palva et al. 1993; Vidal et al. 1998). Although we demonstrated that exogenous SA induces resistance to *E. carotovora* subsp. *carotovora* (Palva et al. 1994), SA did not appear to be involved in the activation of defense mechanisms upon *E. carotovora* subsp. *carotovora* infection in tobacco. This suggested a role for other signal transduction pathways involved in plant resistance to *E. carotovora* subsp. *carotovora*.

In this study, we have further elucidated the role of both SA-dependent and SA-independent signal transduction cascades in the activation of defense gene expression upon treatment of *A. thaliana* plants with plant cell wall-degrading enzymes from *E. carotovora* subsp. *carotovora*. Our results show that ethylene and JA have a central role in regulating plant defense gene expression as well as resistance against *E. carotovora* subsp. *carotovora* in this interaction.

RESULTS

A. thaliana as a model system for studying the interaction between *E. carotovora* subsp. *carotovora* and plants.

Our earlier studies have shown that inoculation of tobacco plants with the phytopathogen *E. carotovora* subsp. *carotovora* or treatment of the plants with plant cell wall-degrading enzymes from this pathogen induces the expression of a number of basic PR proteins as well as increased systemic resistance to the pathogen in an SA-independent manner (Palva et al. 1993; Vidal et al. 1997, 1998). To facilitate further characterization of the signaling events that mediate the plant responses to *E. carotovora* subsp. *carotovora*, we employed *A. thaliana* as a model. Inoculation of *A. thaliana* leaves with *E. carotovora* subsp. *carotovora* results in the development of soft-rot symptoms that can be mimicked by treatment of plants with culture filtrates (CF) from *E. carotovora* subsp. *carotovora* containing the secreted plant cell wall-degrading enzymes from this pathogen (Fig. 1A).

To determine whether CF treatment could also enhance resistance against *E. carotovora* subsp. *carotovora* in *A. thaliana*, seedlings were pretreated with CF. Local application of CF to *A. thaliana* leaves was followed by inoculation of systemic leaves with *E. carotovora* subsp. *carotovora* suspensions. The effect of CF pretreatment on bacterial growth was clear (Fig. 1B). The number of viable bacteria was found to be 5 to 10 times higher in mock-treated plants than in plants pretreated with CF. This indicates, as previously shown in tobacco (Vidal et al. 1998), that CF can trigger enhanced systemic resistance (ESR) to further bacterial infection in *A. thaliana*. Taken together, these results indicate that *A. thaliana* provides a suitable model system to study plant defense responses to *E. carotovora* subsp. *carotovora*.

E. carotovora subsp. *carotovora* and CF induce local and systemic expression of defense genes in *A. thaliana*.

Pathway-specific defense genes have been described for ethylene-, JA-, and SA-dependent pathways, providing markers to study pathogen-induced signaling events. To establish the role of ethylene, JA and SA in the *A. thaliana* defense response to *E. carotovora* subsp. *carotovora*, five genes were employed as markers for the different pathways. These included the following: two ethylene-responsive genes, the gene for hevein-like protein (*HEL*) (Potter et al. 1993), and a basic chitinase (*CHIB*) (Samac et al. 1990); two JA-responsive genes, the gene for vegetative storage protein acid phosphatase (*AtVSP*) (Berger et al. 1995) and a defensin (*PDF1.2*) (Penninckx et al. 1996); and one SA-responsive gene, *PR-1* (Uknes et al. 1992). Expression of these genes was characterized by Northern (RNA) hybridization in local and systemic leaves following inoculation with bacteria or treatment with CF. Figure 2 illustrates the expression pattern of these genes in treated (local) and untreated (systemic) leaves 8 and 24 h after treatment. Expression of *HEL*, *AtVSP*, and *PDF1.2* was strongly induced by CF and bacteria in both local and systemic leaves already after 8 h. A somewhat weaker but prominent induction was seen for *CHIB* 24 h after treatment. In contrast, only a very weak induction of the SAR marker *PR-1* was observed in response to bacterial inoculation or CF treatment. *PR-1* was strongly induced in plants treated with SA whereas no transcript accumulation was evident for the other

genes tested. No significant increase of gene expression was detected in the mock-treated controls.

Taken together, these results demonstrate that *E. carotovora* subsp. *carotovora* induces both local and systemic expression of genes mainly responsive to ethylene or JA but not the SAR gene *PR-1*. Furthermore, similar induction of target genes was achieved both by treatment with CF and by inoculation with *E. carotovora* subsp. *carotovora* suspensions. Consequently, this enabled us to use CF as a tool to study responses of *A. thaliana* to this pathogen.

CF induction of defense related genes is dependent on ethylene and JA signaling pathways.

To clarify the role of ethylene and JA signal transduction pathways in the *A. thaliana* defense response to *E. carotovora* subsp. *carotovora*, we characterized the expression pattern of

HEL, *CHIB*, *PDF1.2* and *AtVSP* by Northern hybridization in several *A. thaliana* mutants after spray treatment with CF. Transcript accumulation for these genes was analyzed in wild-type plants (Col-0), the ethylene-insensitive mutants *etr1* and *ein2* (Bleecker et al. 1988; Guzmán and Ecker 1990), the JA-insensitive mutant *coi1* (Feys et al. 1994), and the LOX2 co-suppressed transgenic plant line S-12T (Bell et al. 1995). S-12T plants have reduced levels of lipoxygenase 2 and are affected in the production of JA in response to wounding (Bell et al. 1995). Accumulation of *HEL*, *CHIB*, *PDF1.2*, and *AtVSP* transcripts was strongly induced in wild-type plants 8 and 24 h after CF treatment, compared with control plants (Fig. 3). CF induction of *HEL* and *CHIB* genes was markedly reduced in the ethylene-insensitive mutants (*etr1*, *ein2*) and in the jasmonate insensitive mutant *coi1*, and somewhat reduced in the S-12T transgenic plants. CF-induced expression of *PDF1.2* was reduced to background levels in the *coi1* and S-12T plants and reduced in the ethylene mutants. In contrast, although accumulation of *AtVSP* transcript was blocked in the *coi1* plants and reduced in the S-12T plants, *AtVSP* exhibited a stronger CF induction in the ethylene-insensitive mutants than in the wild type.

Taken together, these results indicate that both ethylene and JA signal transduction pathways are involved in CF induction of *HEL*, *CHIB*, and *PDF1.2*. In contrast, induction of *AtVSP* in response to CF appears to be strictly dependent on the JA pathway.

SA is not required for CF-induced gene expression but may potentiate CF induction of ethylene- and JA-responsive defense genes.

To elucidate whether SA had any role in the regulation of the CF-inducible genes, we characterized the induction pattern of the marker genes in plants affected in SA accumulation and in SA signaling. CF-induced expression of *HEL*, *CHIB*, *PDF1.2*, *AtVSP*, and *PR-1* was characterized in wild-type plants (Col-0), transgenic NahG plants, and *npr1*-mutant

Fig. 1. Infection of *Arabidopsis thaliana* plants with *Erwinia carotovora* subsp. *carotovora*. **A**, Development of soft-rot symptoms 24 h after inoculation of *A. thaliana* leaves with 2 to 3×10^6 CFU of *E. carotovora* subsp. *carotovora* o.n.c. (overnight culture; left panel) or in a leaf treated with $10 \mu\text{l}$ of culture filtrate (CF; right panel). **B**, Effect on *E. carotovora* subsp. *carotovora* growth in planta after CF treatment of *A. thaliana* plants. *A. thaliana* plants were pretreated with $15 \mu\text{l}$ of CF in one leaf or with 20 mM MgCl_2 24 h before inoculation of a second leaf with 2 to 3×10^6 CFU of *E. carotovora* subsp. *carotovora* o.n.c. At indicated time points after inoculation, number of viable bacteria was determined from CF-treated (CF) or 20 mM MgCl_2 -treated (control) plants. Six independent experiments were carried out. SD indicated by error bars.

Fig. 2. *Erwinia carotovora* subsp. *carotovora* and culture filtrates (CF) induce local and systemic expression of defense genes in *Arabidopsis thaliana*. In vitro-grown *A. thaliana* plants (Col-0) were treated with CF from *E. carotovora* subsp. *carotovora* (CF), 2×10^6 CFU of *E. carotovora* subsp. *carotovora* suspensions (bacteria), or 20 mM MgCl_2 (CO). Treated (local, L) and untreated (systemic, S) leaf samples were collected 8 and 24 h after treatment. Salicylic acid (SA) treatments were done by spraying whole plants with 5 mM SA and samples were collected 8 h after treatment. Total RNA was extracted and analyzed by Northern (RNA) hybridization with specific probes for *HEL*, *CHIB*, *PDF1.2*, *AtVSP* and *PR-1*. To ensure equal loading of RNA the same filters were hybridized with a probe against ribosomal RNA (rDNA).

plants blocked in SA-induced expression of PR genes. The results in Figure 4 show that CF-induced accumulation of *HEL*, *CHIB*, and *PDF1.2* transcripts was slightly reduced in NahG plants, compared with wild-type plants. In contrast, this reduction was not evident in *npr1* mutants, where these transcripts accumulated at levels similar to or higher than those in wild-type plants. These data suggest that full CF induction of *HEL*, *CHIB*, and *PDF1.2* genes requires SA acting in an NPR1-independent manner. CF-induced accumulation of *AtVSP* transcripts was unaffected in *npr1* plants. In contrast, expression of this gene was enhanced in NahG plants, indicating that *AtVSP* could be negatively regulated by the presence of SA. *PR-1* was only weakly induced by CF treatment in wild-type plants and this induction was blocked in NahG plants or reduced in *npr1* plants.

CF-induced defense genes are synergistically regulated by ethylene and JA.

Our results (Fig. 3) suggested the involvement of both ethylene and JA signal pathways in CF-induced activation of plant defense genes. Previous studies have indicated that ethylene and JA act in concert to regulate the expression of the tobacco PR-gene osmotin and a defensin (*PDF1.2*) from *A. thaliana* (Xu et al. 1994; Penninckx et al. 1998). This prompted us to investigate whether CF induction of *HEL*, *CHIB*, *AtVSP*, and *PDF1.2* requires both ethylene and JA. To examine this, the expression of these genes was characterized after treatment of *A. thaliana* wild-type plants with CF, ethylene, MeJA, SA, or different combinations of ethylene, MeJA, and SA (Fig. 5). RNA was extracted from leaf tissue harvested 24 h after treatment and mRNA accumulation was analyzed by Northern hybridization. CF treatment of plants induced the expression of *HEL*, *CHIB*, and *PDF1.2* to a much higher extent than treatment with either ethylene or MeJA alone. However, when ethylene and MeJA were applied to plants in combination, the expression of *HEL*, *CHIB*, and *PDF1.2* genes increased synergistically, reaching levels similar to or higher than those obtained by CF treatment. In contrast, the expression of *AtVSP* was strongly induced by MeJA alone, reaching much higher levels of induction than after CF treatment.

When plants were treated with MeJA and ethylene in combination, the level of induction of *AtVSP* was markedly decreased. These results suggest that the induction of *HEL*, *CHIB*, and *PDF1.2* genes upon CF treatment could result from a synergistic effect between the ethylene and JA pathways. In contrast, the CF-induced expression of *AtVSP* appears to be strictly dependent on JA and repressed by the presence of ethylene. Treatment with SA did not induce any of the marker genes.

Treatment with SA in combination with ethylene induced the expression of *HEL*, *CHIB*, and *PDF1.2* to slightly higher levels than achieved by ethylene alone. No induction of *AtVSP* could be seen after this treatment. Similarly, treatment with SA, MeJA, and ethylene in combination enhanced the expression of *HEL*, *CHIB*, and *PDF1.2* whereas expression of *AtVSP* was reduced to background level. This suggests that SA could potentiate the synergistic effect seen on the induction of *HEL*, *CHIB*, and *PDF1.2* by MeJA and ethylene.

Fig. 4. Salicylic acid (SA) enhances culture filtrate (CF) induction of defense- but not wound-related genes. The transcript accumulation of *HEL*, *CHIB*, *PDF1.2*, *AtVSP*, and *PR-1* was characterized after CF treatment of wild-type plants (Col-0), transgenic NahG plants, or *npr1* mutants. Plants were spray treated with CF and leaf samples were collected at the time points indicated. Total RNA was extracted and analyzed by Northern (RNA) hybridization with gene-specific probes. To ensure equal loading of RNA, the same filters were hybridized with a probe against ribosomal RNA (rDNA).

Fig. 3. *Erwinia carotovora* culture filtrate (CF) induction of defense genes is dependent on ethylene and jasmonic acid (JA) signaling pathways. Transcript accumulation of *HEL*, *CHIB*, *PDF1.2*, and *AtVSP* after CF spray treatment was characterized in wild-type plants (Col-0), the ethylene-insensitive mutants *etr1* and *ein2*, the JA-insensitive mutant *coi1*, and LOX2 co-suppressed transgenic plants (S-12T). As a control (CO), wild-type plants (Col-0) were treated with 20 mM MgCl₂. Eight and 24 h after CF treatment, leaf samples were collected and total RNA was extracted. Transcript accumulation was analyzed in Northern (RNA) hybridization. To ensure equal loading of RNA the same filters were hybridized with a probe against ribosomal RNA (rDNA).

Ethylene and JA pathways are involved in the enhancement of local resistance against *E. carotovora* subsp. *carotovora*.

To establish the requirement for ethylene and JA in resistance to *E. carotovora* subsp. *carotovora*, we compared bacterial growth in wild-type (Col-0), *ein2* mutant, *coi1* mutant, and NahG transgenic plants. Plants were locally inoculated with 2 to 3×10^6 CFU and the number of viable bacteria was determined 24 h after inoculation. The results in Figure 6 show that *ein2* and *coi1* plants were more susceptible to *E. carotovora* subsp. *carotovora*, as the number of viable bacte-

Fig. 5. Culture filtrate (CF)-induced defense genes are synergistically regulated by ethylene and jasmonic acid (JA). *Arabidopsis thaliana* wild-type plants (Col-0) were treated with CF, 50 ppm of ethylene (Et), 4.6 μ mol of methyl jasmonate (MeJA), 0.25 mM SA (SA), a combination of 50 ppm ethylene and 4.6 μ mol of MeJA (Et+MeJA), a combination of 50 ppm ethylene and 0.25 mM SA (Et+SA), a combination of 4.6 μ mol of MeJA and 0.25 mM SA (MeJA+SA), or a combination of 50 ppm ethylene, 4.6 μ mol of MeJA and 0.25 mM SA (Et+MeJA+SA). Leaf samples were collected 24 h after treatment and analyzed by Northern (RNA) hybridization with specific probes for *HEL*, *CHIB*, *PDF1.2*, and *AtVSP*. Below, the amount of the corresponding RNA samples is indicated by hybridizing the same filters with a probe against ribosomal RNA (rDNA).

Fig. 6. Ethylene and jasmonic acid (JA) are involved in the enhancement of systemic resistance against *Erwinia carotovora* subsp. *carotovora*. *Arabidopsis thaliana* wild-type plants (Col-0), ethylene-insensitive mutant *ein2*, JA-insensitive mutant *coi1*, or transgenic NahG plants were inoculated with 2 to 3×10^6 CFU of *E. carotovora* subsp. *carotovora*. Twenty-four hours after treatment, the number of viable bacteria was determined. Each point is equal to eight independent experiments. SD are indicated with error bars.

ria reached 7 to 10 times higher levels than in the wild-type plants. In contrast, the lack of SA in the transgenic NahG plants did not result in significantly higher susceptibility to infection by this pathogen. In conclusion, these results suggest that ethylene and JA signaling are involved in resistance to *E. carotovora* subsp. *carotovora*. Although SA may contribute to potentiate this process, the lack of SA does not appear to influence the development of disease in plants infected with *E. carotovora* subsp. *carotovora*.

DISCUSSION

To elucidate the signal processes that activate plant defense in response to *E. carotovora* subsp. *carotovora*, we have employed *A. thaliana* as a model plant due to the availability of mutants and marker genes in pathogen-induced signal transduction pathways. In this study, we show that *A. thaliana* is susceptible to *E. carotovora* subsp. *carotovora* (Fig. 1A). Moreover, we show, in accordance with our previous results with tobacco (Vidal et al. 1998), that treatment of *A. thaliana* with acellular preparations of cell wall-degrading enzymes prior to bacterial inoculation leads to enhanced systemic re-

Fig. 7. Interacting signal transduction pathways control expression of *Arabidopsis thaliana* defense genes in response to cell wall-degrading enzymes (CF) secreted by *Erwinia carotovora* subsp. *carotovora*. *E. carotovora* subsp. *carotovora* triggers two distinct response pathways in *A. thaliana*. Synergistic action of ethylene and jasmonates is required for expression of *HEL*, *CHIB*, and *PDF1.2*. This induction is enhanced by the presence of salicylic acid (SA). Jasmonates alone mediate CF-induced expression of *AtVSP*. This pathway is inhibited by SA and ethylene.

sistance to *E. carotovora* subsp. *carotovora*. We therefore conclude that *A. thaliana* provides a suitable model system to study plant defense responses induced by this pathogen.

To elucidate the role of ethylene, JA, and SA signaling pathways in this interaction, we have characterized the expression of marker genes specific to these pathways in response to inoculation of plants with *E. carotovora* subsp. *carotovora* or treatment with CF from the pathogen. The results are summarized in the model presented in Figure 7, highlighting the importance of both ethylene and JA signaling in the plant response to *E. carotovora* subsp. *carotovora*. The experimental evidence for this conclusion is as follows. Bacterial inoculation or CF treatment of wild-type plants leads to both local and systemic expression of genes that are mainly responsive to ethylene or JA (Fig. 2) (*HEL*, *CHIB*, *PDF1.2*, and *AtVSP*). In contrast, *PR-1*, an SA-responsive gene, is only weakly induced upon CF treatment. The induction of *HEL*, *CHIB*, and *PDF1.2* genes by CF was reduced or blocked in ethylene-insensitive mutants as well as in the *coi1* mutant affected in the JA pathway, indicating that both signaling pathways are required for this response. In contrast, SA signaling did not appear to play a major role in this response since CF-induced expression of these genes was unaffected in *npr1* mutants and only marginally reduced in NahG plants.

Our results suggest that *E. carotovora* subsp. *carotovora* not only induces genes controlled by ethylene and JA but also JA-specific genes such as *AtVSP* exhibiting a JA-mediated but ethylene-independent induction by CF (Fig. 7). This gene has previously been shown to be strongly induced by MeJA, wounding (Berger et al. 1996), and plant cell wall-derived oligogalacturonide elicitors (OGAs) released by CF (Norman et al. 1999).

Our results suggest a synergistic action of ethylene and JA in CF-induced expression of defense genes (Fig. 7). Addition of ethylene or MeJA alone only results in a very modest induction of defense genes. In contrast, ethylene and MeJA added in combination synergistically induce the expression of *HEL*, *CHIB*, and *PDF1.2* genes, but not *AtVSP*, to levels achieved by CF treatment. This is in agreement with previous results showing that ethylene and MeJA synergistically induce the expression of osmotin, a tobacco PR-5 gene (Xu et al. 1994) as well as a defensin gene, *PDF1.2*, in *A. thaliana* (Penninckx et al. 1998). How MeJA or JA interact with ethylene and co-regulate gene expression is not known. However, it has been shown that ethylene and JA do not act in a sequential manner but rather via parallel and cooperative signaling events in the regulation of *PDF1.2* (Penninckx et al. 1998). EIN2 acts downstream of the ethylene receptor in the ethylene signal transduction pathway (Kieber 1997). Interestingly, ectopic expression of *EIN2* in *ein2* plants was sufficient to restore the ethylene signaling required for the JA induction of *PDF1.2* (Alonso et al. 1999).

Our results indicate that the SAR pathway does not play a major role in activating defense genes induced by *E. carotovora* subsp. *carotovora* or by CF treatment (Fig. 7). Only a weak accumulation of *PR-1* transcript, a very sensitive marker for the SAR pathway, was observed after CF treatment. Nevertheless, our results indicate that SA is involved in the regulation of genes that are strongly induced upon CF treatment (Fig. 7). SA appears to have a dual role in this regulation. First, it is required for the full CF induction of defense genes

(*HEL*, *CHIB*, and *PDF1.2*) as suggested by reduced expression of these genes in transgenic NahG plants. Second, CF treatment of NahG plants leads to an increased expression of a jasmonate-responsive gene (*AtVSP*), indicating that SA inhibits CF induction of this gene by blocking the JA pathway. Similar SA inhibition of the JA pathway was previously demonstrated by Doares et al. (1995) and Peña-Cortés et al. (1993).

The data presented here support the idea that SA, besides inducing SAR and SAR gene expression, may have an indirect role by potentiating the expression of other defense responses that are not directly responsive to SA but become induced upon pathogen attack (Mur et al. 1996; Shirasu et al. 1997). Interestingly, CF treatment of the *A. thaliana* mutant *npr1* induced *HEL*, *CHIB*, and *PDF1.2* expression to the same extent as, or even higher levels than, those obtained in wild-type plants. The results show that although the presence of SA may be necessary for full induction of ethylene- and JA-responsive genes, this induction does not require components downstream of SA in the signaling pathway, such as a functional NPR1. This is in contrast to ISR, which develops independently of SA but requires a functional NPR1 (Pieterse et al. 1998; Pieterse and van Loon 1999). Furthermore, ISR is not associated with PR gene expression (Pieterse et al. 1998; Pieterse and van Loon 1999), which distinguishes ISR from the defense response activated by *E. carotovora* subsp. *carotovora*.

In summary, our results indicate that *E. carotovora* subsp. *carotovora* or CF triggers two distinct defense pathways in Arabidopsis (Fig. 7). The first is mediated through the synergistic action of ethylene and jasmonates, potentiated by SA, and leads to expression of *PDF1.2*, *CHIB*, and *HEL*. The second pathway is strictly jasmonate mediated, inhibited by ethylene or SA, and controls expression of *AtVSP*. The latter could be triggered by recognition of OGAs and shares similarities with the wound response of plants.

Ethylene and JA are involved not only in triggering expression of plant defense genes but also in resistance against *E. carotovora* subsp. *carotovora* infection in *A. thaliana*. Evidence for this was provided by experiments showing that ethylene- (*ein2*) and jasmonate- (*coi1*) insensitive mutants were more susceptible to this pathogen than wild-type or transgenic NahG plants. Previous studies with *ein2* mutants have indicated that this mutation generates a lack of development of disease symptom upon infection, suggesting that ethylene is not involved in resistance responses, but instead promotes disease severity (Bent et al. 1992). In contrast to these observations, we show that the degree of *E. carotovora* subsp. *carotovora* growth in the *ein2* mutants was consistently enhanced, compared with bacterial growth in wild-type plants. A similar increase in susceptibility has also been observed in *ein2* mutants inoculated with the fungal pathogen *Alternaria brassicicola* (Penninckx et al. 1996).

In conclusion, ethylene and JA pathways appear to have a central role in regulating defense gene induction and resistance triggered by cell wall-degrading enzymes from *E. carotovora* subsp. *carotovora* (Fig. 7). As shown, induction of defense gene expression appears to be achieved by a combination of signals rather than the activation of single signal pathways. It is possible that when plants fail to accumulate high levels of SA necessary for induction of SAR and SAR gene expression upon pathogen attack, lower levels of SA might have a crucial role in modulating other types of defense

responses. Depending on the conditions, the activation of this type of defense mechanism by virulent pathogens such as *E. carotovora* subsp. *carotovora* may be enough to restrict pathogen growth in the infected plant.

MATERIALS AND METHODS

Plant material and growth conditions.

Seeds of *A. thaliana* ecotype Columbia (Col-0) were surface sterilized, sown on sterile MS-2 medium (Murashige and Skoog 1962), and replanted in 300-ml jars after germination. Plants were thereafter grown for 2 weeks in a Conviron growth chamber at 22°C with a 16-h light regime (120 to 170 $\mu\text{mol s}^{-1} \text{m}^{-2}$) before analysis. Treatments of plants were done under a constant light regime with the same conditions. Ethylene-responsive mutants *etr1-1* (Bleecker et al. 1988) and *ein2-1* (Guzmán and Ecker 1990) were obtained from the Arabidopsis Biological Resource Center (Columbus, OH; accession numbers CS237 and CS3071). The transgenic *A. thaliana* line expressing the *nahG* gene (Delaney et al. 1994) was provided by John Ryals (Ciba Geigy, Research Triangle Park, NC). The *npr1* mutant (Cao et al. 1994) was provided by Xinnian Dong (Duke University, Durham, NC). Transgenic LOX2-deficient *A. thaliana* line S-12T (Bell et al. 1995) was provided by Erin Bell (Texas A&M University, College Station). The JA-insensitive mutant *coi1* (Feys et al. 1994) was provided by John G. Turner (School of Biological Sciences, University of East Anglia, Norwich, UK).

Bacterial growth and inoculation of plants.

The *Erwinia carotovora* subsp. *carotovora* strain SCC3193, (Pirhonen et al. 1988) was cultured overnight at 28°C in L medium (Miller 1972). To evaluate bacterial growth in planta, plants were inoculated with 10 μl of a 10 $\times$ dilution of the bacterial overnight culture containing 2 to 3 $\times 10^6$ CFU. After inoculation, plants were incubated for the indicated time periods under high humidity conditions to facilitate bacterial growth. Bacterial growth in planta was evaluated by homogenizing the inoculated plants in 10 ml of 10 mM MgSO_4 and plating serial dilutions onto L agar plates. Each sample contained four plants. The number of viable bacteria was then counted in eight independent experiments.

Chemical applications and extracellular enzyme preparation and treatments.

CF from *E. carotovora* subsp. *carotovora* was prepared as previously described (Vidal et al. 1997). CF treatments were done by spraying plants with 2 ml of CF per jar. For analysis of systemic induction patterns, two leaves per plant were treated by applying 15 μl of CF or bacterial culture, pressing the tip of a pipette to cause a small wound (local). Two opposite leaves were analyzed for systemic defense gene expression. Leaf tissue corresponding to at least 12 plants was pooled and analyzed by Northern hybridization. Control plants were treated with 20 mM MgCl_2 in the same way. SA treatments were done by spraying plants with 2 ml of 5 mM SA per jar. Controls for this treatment were done by spraying 2 ml of sterile water. SA treatment in combination with other treatments was done by spraying plants with 2 ml of 0.25 mM SA. Ethylene treatments were performed by placing jars in an airtight chamber with 80% humidity containing air only

(control), or 50 ppm of ethylene. Ethylene concentration was measured by a photoionizer (PI 101 trace gas analyzer; HNU Systems, Newton, MA). MeJA treatments were done by placing a cotton stick containing 4.6 μmol of MeJA (Bedoukian Research, Danbury, CT) diluted in 96% ethanol, in a 300-ml jar, or only 96% ethanol as a control.

PCR amplification of target genes.

All DNA probes were generated by polymerase chain reaction (PCR) with *A. thaliana* genomic DNA as a template. The DNA fragment corresponding to the *HEL* gene was generated with primers corresponding to bases 131 to 155 and 571 to 595 of the cDNA sequence (Potter et al. 1993). A fragment of the coding region of the *A. thaliana* basic chitinase (*CHIB*) (Samac et al. 1990) was obtained with primers corresponding to bases 1625 to 1650 and 2137 to 2163 of the genomic sequence. The *PR-1* probe was obtained with primers corresponding to 191 to 220 and 466 to 495 of the cDNA sequence (Uknes et al. 1992). The probe for *AtVSP* (Potter et al. 1993) was generated with primers corresponding to bases 3 to 25 and 214 to 236 of the clone ATTS0751 (Höfte et al. 1992). The probe for *PDF1.2* (Penninckx et al. 1996) was generated with primers corresponding to bases 30 to 49 and 262 to 281 of the sequence of the expressed sequence tag T04323. All amplified fragments were cloned into the pCR 2.1 vector (Invitrogen, San Diego, CA).

RNA extractions and Northern hybridization analyses.

Total RNA from plant leaf tissue, corresponding to 12 plants, was prepared by phenol/chloroform extraction and LiCl precipitation essentially as described by Verwoerd et al. (1989) with an extra ethanol precipitation step added before the LiCl precipitation. Ten micrograms of total RNA was denatured in formamide and separated by electrophoresis through formaldehyde agarose gels (Sambrook et al. 1989). Filters were hybridized with [α - ^{32}P]dCTP-labeled probes generated by multipriming (Amersham, Buckinghamshire, UK) with an *EcoRI* fragment of the different clones as DNA template. Hybridizations were performed as previously described (Vidal et al. 1997). RNA gels were analyzed with ethidium bromide and blots were probed with a ribosomal probe to ensure equal loading of RNA in each sample.

ACKNOWLEDGMENTS

We wish to thank John Ryals for the generous gift of seeds of transgenic *A. thaliana* carrying the *nahG* gene, Xinnian Dong for the *npr1* mutant seeds, Erin Bell for the transgenic S-12T *A. thaliana* seeds, and John G. Turner for the *coi1* mutant seeds. We thank the Arabidopsis Biological Resource Center (Columbus, OH) for providing seeds from the ethylene response mutants. We are grateful to Mona Munther for preparing the plant material and to Marcos Montesano for help with ethylene treatments. This work was supported by SIDA, the Swedish Council for Forestry and Agricultural Research (SJFR), the Swedish Natural Sciences Research Council (NFR), and the Foundation in Memory of Oskar and Lili Lamm. The Nilsson-Ehle foundation is gratefully acknowledged. E. T. P. is also supported by the Academy of Finland, Biocentrum Helsinki, and the EU (contract no. ERBIC15-CT96-0908).

LITERATURE CITED

Alonso, J. M., Hirayama, T., Roman, G., Nourizadeh, S., and Ecker, J. R. 1999. EIN2, a bifunctional transducer of ethylene and stress responses in *Arabidopsis*. *Science* 284:2148-2152.

- Bell, E., Creelman, R. A., and Mullet, J. E. 1995. A chloroplast lipoxigenase is required for wound-induced jasmonic acid accumulation in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 92:8675-8679.
- Benhamou, N. 1996. Elicitor-induced plant defence pathways. *Trends Plant Sci.* 1:233-240.
- Bent, A. F., Innes, R. W., Ecker, J. R., and Staskawicz, B. J. 1992. Disease development in ethylene-insensitive *Arabidopsis thaliana* infected with virulent and avirulent *Pseudomonas* and *Xanthomonas* pathogens. *Mol. Plant-Microbe Interact.* 5:372-378.
- Berger, S., Bell, E., and Mullet, J. E. 1996. Two methyl jasmonate-insensitive mutants show altered expression of *AtVsp* in response to methyl jasmonate and wounding. *Plant Physiol.* 111:525-531.
- Berger, S., Bell, E., Sadka, A., and Mullet, J. E. 1995. *Arabidopsis thaliana Atvsp* is homologous to soybean *VspA* and *VspB*, genes encoding vegetative storage protein acid phosphatases, and is regulated similarly by methyl jasmonate, wounding, sugars, light and phosphate. *Plant Mol. Biol.* 27:933-942.
- Bleecker, A. B., Estelle, M. A., Somerville, C., and Kende, H. 1988. Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science* 241:1086-1089.
- Bol, J. F., Linthorst, H. J. M., and Cornelissen, B. J. C. 1990. Plant pathogenesis-related proteins induced by virus infection. *Annu. Rev. Phytopathol.* 28:113-138.
- Boller, T. 1988. Ethylene and the regulation of antifungal hydrolases in plants. Pages 145-174 in: Oxford Surveys of Plant Molecular and Cell Biology. B. J. Mifflin and H. F. Mifflin, eds. Oxford University Press, Oxford.
- Boller, T. 1991. Ethylene in pathogenesis and disease resistance. Pages 293-314 in: The Plant Hormone Ethylene. A. K. Mattoo and J. C. Suttle, eds. CRC Press, Boca Raton, FL.
- Cao, H., Bowling, S. A., Gordon, S., and Dong, X. 1994. Characterization of an *Arabidopsis* mutant that is nonresponsive to inducers of systemic acquired resistance. *Plant Cell* 6:1583-1592.
- Collmer, A., and Keen, N. T. 1986. The role of pectic enzymes in plant pathogenesis. *Annu. Rev. Phytopathol.* 24:383-409.
- Creelman, R. A., Tierney, M. L., and Mullet, J. E. 1992. Jasmonic acid/methyl jasmonate accumulate in wounded soybean hypocotyls and modulate wound gene expression. *Proc. Natl. Acad. Sci. USA* 89:4938-4941.
- Davis, K. R., and Ausubel, F. M. 1989. Characterization of elicitor-induced defense responses in suspension-cultured cells of *Arabidopsis*. *Mol. Plant-Microbe Interact.* 2:363-368.
- Delaney, T. P., Uknes, S., Vernooij, B., Friedrich, L., Weyman, K., Negrotto, D., Gaffney, T., Gut-Rella, M., Kessmann, H., Ward, E., and Ryals, J. 1994. A central role of salicylic acid in plant disease resistance. *Science* 266:1247-1250.
- Doares, S. H., Narváez-Vásquez, J., Conconi, A., and Ryan, C. A. 1995. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiol.* 108:1741-1746.
- Enyedi, A., Yalpani, N., Silverman, P., and Raskin, I. 1992. Signal molecules in systemic plant resistance to pathogens and pests. *Cell* 70:879-886.
- Epple, P., Apel, K., and Bohlmann, H. 1995. An *Arabidopsis thaliana* thionin gene is inducible via a signal transduction pathway different from that for pathogenesis-related proteins. *Plant Physiol.* 109:813-820.
- Farmer, E. E., Johnson, R. R., and Ryan, C. A. 1992. Regulation of expression of proteinase inhibitor genes by methyl jasmonate and jasmonic acid. *Plant Physiol.* 98:995-1002.
- Feys, B. J. F., Benedetti, C. E., Penfold, C. N., and Turner, J. G. 1994. *Arabidopsis* mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *Plant Cell* 6:751-759.
- Flor, H. H. 1971. Current status of the gene-for-gene concept. *Annu. Rev. Phytopathol.* 9:275-296.
- Gaffney, T., Friedrich, L., Vernooij, B., Negrotto, D., Nye, G., Uknes, S., Ward, E., Kessmann, H., and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science* 261:754-756.
- Gundlach, H., Müller, M. J., Kutchan, T. M., and Zenk, M. H. 1992. Jasmonic acid is a signal transducer in elicitor-induced plant cell cultures. *Proc. Natl. Acad. Sci. USA* 89:2389-2393.
- Guzmán, P., and Ecker, J. R. 1990. Exploiting the triple response of *Arabidopsis* to identify ethylene-related mutants. *Plant Cell* 2:513-523.
- Höfte, H., Desprez, T., Amselem, J., Chiapello, H., Caboche, M., Moisan, A., Jourjon, M.-F., Charpentier, L., Berthomieu, P., Guerrier, D., Giraudat, J., Quigley, F., Thomas, F., Yu, D.-Y., Mache, R., Raynal, M., Cooke, R., Grellet, F., Delseny, M., and Parmentier, Y. 1992. An inventory of 1152 expressed sequence tags obtained by partial sequencing of cDNAs from *Arabidopsis thaliana*. *Plant J.* 4:1051-1061.
- Kieber, J. J. 1997. The ethylene response pathway in *Arabidopsis*. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48:277-296.
- Knoester, M., van Loon, L. C., van den Heuvel, J., Hennig, J., Bol, J. F., and Linthorst, J. M. 1998. Ethylene-insensitive tobacco lacks nonhost resistance against soil-borne fungi. *Proc. Natl. Acad. Sci. USA* 95:1933-1937.
- Kombrink, E., and Somssich, I. E. 1995. Defense responses of plants to pathogens. *Adv. Bot. Res.* 21:1-34.
- Kotoujansky, A. 1987. Molecular genetics of pathogenesis by soft-rot *Erwinias*. *Annu. Rev. Phytopathol.* 25:405-430.
- Lawton, K., Weymann, K., Friedrich, L., Vernooij, B., Uknes, S., and Ryals, J. 1995. Systemic acquired resistance in *Arabidopsis* requires salicylic acid but not ethylene. *Mol. Plant-Microbe Interact.* 8:863-870.
- Malamy, J., Carr, J. P., Klessig, D. F., and Raskin, I. 1990. Salicylic acid: A likely endogenous signal in the resistance response of tobacco to viral infection. *Science* 250:1002-1004.
- Martin, G. B., Brommonschenkel, S. H., Chunwongse, J., Frary, A., Ganal, M. W., Spivey, R., Wu, T., Earle, E. D., and Tanksley, S. D. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. *Science* 262:1432-1436.
- Métraux, J. P., Signer, H., Ryals, J., Ward, E., Wyss, B. M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., and Inverardi, B. 1990. Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250:1004-1006.
- Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. p. 466.
- Mur, L. A. J., Naylor, G., Warner, S. A. J., Sugars, J. M., White, R. F., and Draper, J. 1996. Salicylic acid potentiates defence gene expression in tissue exhibiting acquired resistance to pathogen attack. *Plant J.* 9:559-571.
- Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15:473-497.
- Norman, C., Vidal, S., and Palva, E. T. 1999. Oligogalacturonide-mediated induction of a gene involved in jasmonic acid synthesis in response to the cell-wall-degrading enzymes of the plant pathogen *Erwinia carotovora*. *Mol. Plant-Microbe Interact.* 12:640-644.
- Nürnberger, T., Nennstiel, D., Hahlbrock, K., and Scheel, D. 1995. Covalent cross-linking of the *Phytophthora megasperma* oligopeptide elicitor to its receptor in parsley membranes. *Proc. Natl. Acad. Sci. USA* 92:2338-2342.
- Nürnberger, T., Nennstiel, D., Jabs, T., Sacks, W. R., Hahlbrock, K., and Scheel, D. 1994. High affinity binding of a fungal oligopeptide elicitor to parsley plasma membranes triggers multiple defense responses. *Cell* 78:449-460.
- Palva, T. K., Holmström, K.-O., Heino, P., and Palva, E. T. 1993. Induction of plant defense response by exoenzymes of *Erwinia carotovora* subsp. *carotovora*. *Mol. Plant-Microbe Interact.* 6:190-196.
- Palva, T. K., Hurtig, M., Saindrenan, P., and Palva, E. T. 1994. Salicylic acid induced resistance to *Erwinia carotovora* subsp. *carotovora* in tobacco. *Mol. Plant-Microbe Interact.* 7:356-363.
- Peña-Cortés, H., Albrecht, T., Prat, S., Weiler, E. W., and Willmitzer, L. 1993. Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta* 191:123-128.
- Penninckx, I. A. M. A., Eggermont, K., Terras, F. R. G., Thomma, B. P. H. J., De Samblanx, G. W., Buchala, A., Métraux, J.-P., Manners, J. M., and Broekaert, W. F. 1996. Pathogen-induced systematic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. *Plant Cell* 8:2309-2323.
- Penninckx, I. A. M. A., Thomma, B. P. H. J., Buchala, A., Métraux, J.-P., and Broekaert, W. F. 1998. Concomitant activation of jasmonate and ethylene response pathways is required for induction of a plant defensin gene in *Arabidopsis*. *Plant Cell* 10:2103-2113.
- Pérombelon, M. C. M., and Kelman, A. 1980. Ecology of the soft rot *Erwinias*. *Annu. Rev. Phytopathol.* 18:361-387.
- Pieterse, C. M. J., and van Loon, L. C. 1999. Salicylic acid-independent plant defense pathways. *Trends Plant Sci.* 4:52-58.

- Pieterse, C. M. J., van Wees, S. C. M., Hoffland, E., van Pelt, J. A., and van Loon, L. C. 1996. Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid accumulation and pathogenesis-related gene expression. *Plant Cell* 8:1225-1237.
- Pieterse, C. M. J., van Wees, S. C. M., van Pelt, J. A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P. J., and van Loon, L. C. 1998. A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. *Plant Cell* 10:1571-1580.
- Pirhonen, M., Heino, P., Helander, I., Harju, P., and Palva, E. T. 1988. Bacteriophage T4 resistant mutants of the plant pathogen *Erwinia carotovora*. *Microb. Pathog.* 4:359-367.
- Pirhonen, M., Saari-lahti, H., Karlsson, M.-B., and Palva, E. T. 1991. Identification of pathogenicity determinants of *Erwinia carotovora* subsp. *carotovora* by transposon mutagenesis. *Mol. Plant-Microbe Interact.* 4:276-283.
- Potter, S., Uknes, S., Lawton, K., Winter, A. M., Chandler, D., DiMaio, J., Novitzky, R., Ward, E., and Ryals, J. 1993. Regulation of a hevein-like gene in *Arabidopsis*. *Mol. Plant-Microbe Interact.* 6:680-685.
- Ryals, J. A., Neuenschwander, U. H., Willits, M. G., Molina, A., Steiner, H.-Y., and Hunt, M. D. 1996. Systemic acquired resistance. *Plant Cell* 8:1809-1819.
- Samac, D. A., Hironaka, C. M., Yallaly, P. E., and Shah, D. M. 1990. Isolation and characterization of the genes encoding basic and acidic chitinase in *Arabidopsis thaliana*. *Plant Physiol.* 93:907-914.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. A. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Shirasu, K., Nakajima, H., Rajasekhar, K., Dixon, R. A., and Lamb, C. 1997. Salicylic acid potentiates an agonist-dependent gain control that amplifies pathogen signals in the activation of defense mechanisms. *Plant Cell* 9:261-270.
- Staskawicz, B. J., Ausubel, F. M., Baker, B. J., Ellis, J. G., and Jones, J. D. G. 1995. Molecular genetics of plant disease resistance. *Science* 268:661-667.
- Uknes, S., Mauch-Mani, B., Moyer, M., Potter, S., Williams, S., Dincher, S., Chandler, D., Slusarenko, A., Ward, E., and Ryals, J. 1992. Acquired resistance in *Arabidopsis*. *Plant Cell* 4:645-656.
- Verwoerd, T. C., Dekker, M. M., and Hockema, A. 1989. A small-scale procedure for the rapid isolation of plant RNAs. *Nucleic Acids Res.* 17:2362.
- Vidal, S., Eriksson, A. R. B., Montesano, M., Denecke, J., and Palva, E. T. 1998. Cell wall-degrading enzymes from *Erwinia carotovora* cooperate in the salicylic acid-independent induction of a plant defense response. *Mol. Plant-Microbe Interact.* 1:23-32.
- Vidal, S., Ponce de León, I., Denecke, J., and Palva, E. T. 1997. Salicylic acid and the plant pathogen *Erwinia carotovora* induce defense genes via antagonistic pathways. *Plant J.* 11:115-123.
- Vijayan, P., Shockey, J., Levesque, C. A., Cook, R. J., and Browse, J. 1998. A role for jasmonate in pathogen defense of *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* 95:7209-7214.
- Ward, E. R., Uknes, S. J., Williams, S. C., Dincher, S. S., Wiederhold, D. L., Alexander, D. C., Ahl-Goy, P., Métraux, J.-P., and Ryals, J. A. 1991. Coordinate gene activity in response to agents that induce systemic acquired resistance. *Plant Cell* 3:1085-1094.
- Xu, Y., Chang, P.-F. L., Liu, D., Narasimhan, M. L., Raghothama, K. G., Hasegawa, P. M., and Bressan, R. A. 1994. Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell* 6:1077-1085.