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Constitutive Activation of Jasmonate Signaling in an *Arabidopsis* Mutant Correlates with Enhanced Resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*

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In *Arabidopsis* spp., the jasmonate (JA) response pathway generally is required for defenses against necrotrophic pathogens and chewing insects, while the salicylic acid (SA) response pathway is generally required for specific, resistance (*R*) gene-mediated defenses against both biotrophic and necrotrophic pathogens. For example, SA-dependent defenses are required for resistance to the biotrophic fungal pathogen *Erysiphe cichoracearum* UCSC1 and the bacterial pathogen *Pseudomonas syringae* pv. *maculicola*, and also are expressed during response to the green peach aphid *Myzus persicae*. However, recent evidence indicates that the expression of JA-dependent defenses also may confer resistance to *E. cichoracearum*. To confirm and to extend this observation, we have compared the disease and pest resistance of wild-type *Arabidopsis* plants with that of the mutants *coi1*, which is insensitive to JA, and *cev1*, which has constitutive JA signaling. Measurements of the colonization of these plants by *E. cichoracearum*, *P. syringae* pv. *maculicola*, and *M. persicae* indicated that activation of the JA signal pathway enhanced resistance, and was associated with the activation of JA-dependent defense genes and the suppression of SA-dependent defense genes. We conclude that JA and SA induce alternative defense pathways that can confer resistance to the same pathogens and pests.

Plants encounter a wide range of pathogens and pests and employ a small number of inducible resistance mechanisms that minimize losses through disease and infestation. These mechanisms include specific, resistance (*R*) gene-dependent defenses and broad-spectrum defenses. The defenses appear to be mediated by a number of small molecules, including salicylic acid (SA), jasmonic acid (JA), and ethylene (Feys and Parker 2000; Reymond and Farmer 1998). Several experiments, including those in tobacco (Felton et al. 1999), *Arabidopsis* spp. (Gupta et al. 2000), and tomato (Doares et al. 1995), suggest there is negative interaction between the SA and JA signal pathways.

SA is required for the execution of specific resistance to many different pathogens (Dempsey et al. 1999). Apparently, the host *R* gene product interacts with the corresponding avirulence (*Avr*) gene product from the pathogen, triggering defense responses, including the hypersensitive response (HR), production reactive oxygen intermediates (ROIs), and the production of SA which induces the expression of pathogenesis-related genes such as *PR1* (Ellis et al. 2000; Martin 1999). However, some *R* genes can confer resistance in the absence of SA accumulation (McDowell et al. 2000).

JA and ethylene regulate defense against a number of necrotrophic pathogens and insect pests, including the fungi *Alternaria brassicicola* (Thomma et al. 1998), *Botrytis cinerea* (Thomma et al. 1999), and *Pythium* spp. (Staswick et al. 1998; Vijayan et al. 1998); the bacterium *Erwinia carotovora* (Norman-Setterblad et al. 2000); and insect pests such as fungal gnats (McConn et al. 1997). JA-insensitive *coi1* plants have decreased susceptibility to *Pseudomonas syringae* pathogens (Feys et al. 1994; Kloek et al. 2001), while treatment with JA can reduce development of some diseases and infestations (Thomma et al. 2000; van Wees et al. 1999). Expression of JA- and ethylene-dependent defenses is associated with enhanced expression of several genes that encode antimicrobial proteins, such as Thionin2.1 (Epple et al. 1995), defensin PDF1.2 (Penninckx et al. 1998), and basic chitinase (Samac et al. 1990).

Mutants with altered JA signaling include *cev1*, which has constitutive expression of JA and ethylene responses (Ellis and Turner 2001), and *coi1*, which is insensitive to JA (Feys et al. 1994). Several mutants have been identified that express elevated levels of JA-regulated genes and constitutively express SA-regulated genes (Bowling et al. 1997; Clarke et al. 1998; Hilpert et al. 2001). However, *cev1* plants do not constitutively express SA-regulated genes. Therefore, *cev1* and *coi1* plants may prove useful tools for the further analysis of JA signaling.

The resistance of *Arabidopsis* spp. to powdery mildew pathogens can be conferred by the *R* gene *RPW8*, and SA is required for resistance (Xiao et al. 2001). Unexpectedly, therefore, *cev1* plants also have decreased conidiophore production following inoculation with three species of powdery mildew, in the absence of *RPW8* (Ellis and Turner 2001). This indicates that JA responses also can enhance resistance to powdery mildew. Other systems that involve SA signaling include the *Arabidopsis* spp.–*P. syringae* host–pathogen interaction (Kloek et al. 2001) and *Arabidopsis* infestation by the green peach aphid *M. persicae* (Moran and Thompson 2001).

Therefore, we have used the *Arabidopsis* mutants *cev1* and *coi1* to determine the possible contribution of JA responses to defense against a pathogen and a pest that normally induces SA-dependent defenses, and to clarify the role of JAs in defense against powdery mildews. For this we have quantified

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colonization of *cev1*, *coi1,* and wild-type plants inoculated with *P. syringae* pv. *maculicola* ES4326, infested by the phloem-feeding aphid *M. persicae*, and infected by *E. cichoracearum* UCSC1. Our results indicate that JA can contribute to defense against *E. cichoracearum* UCSC1, *P. syringae*, and aphids. Moreover, with the particular exception of *coi1* plants infected with *P. syringae*, resistance correlates with enhanced expression of JA defense-response genes and suppression of SA-dependent defenses.

RESULTS

Active JA signaling enhances resistance to *E. cichoracearum.*

cev1, coi1-16, and wild-type plants were inoculated with *E. cichoracearum* UCSC1, and colonization of leaf surfaces was quantified by counting the number of conidiophores produced in discrete colonies arising from single points of infection (Fig. 1A). *coi1*-16 is a novel allele that has responses to JA similar to *coi1*-1, except that it displays temperature-sensitive fertility and can be maintained as a pure breeding line (Ellis and Turner 2002). On all plants, spores had germinated by day 1, but conidiophores did not develop until 4 days post infection (dpi). After 6 dpi, many individual colonies had merged; therefore, further analysis was unfeasible. The numbers of conidiophores in colonies on *coi1*-16 plants increased faster than on wild-type plants. An unpaired students *t* test ($P < 0.05$) indicated that on days 5 and 6, the number of conidiophores per colony was statistically different for all three genotypes. Colonies on *cev1* plants were smaller than on the other plants and contained fewer conidiophores.

After 10 days, disease symptoms were more severe on *coi1*-16 plants than on the others (Fig. 1B). Infected *coi1*-16 leaves were covered with the characteristic white spore masses of powdery mildew infection and had become chlorotic. Wild-type plants showed white spore masses, but widespread chlorosis had not developed. By contrast, *cev1* plants contained only patches of powdery mildew. Although these patches contained mycelial growth, the density of conidiophores did not reach that on *coi1*-16 or wild-type plants (Fig. 1C). *cev1* plants permitted limited mycelial growth compared with wild-type plants, but did not produce ROI, as judged by incubation with diaminobenzidine (results not shown).

The *Arabidopsis* mutants *edr1* (Frye and Innes 2001) and *pmr1-pmr4* (Vogel and Somerville 2000) also provide enhanced resistance to powdery mildew and show increases in *PR1* expression following inoculation. To test the induction of defense-related genes, 5 days after leaves were inoculated with *E. cichoracearum,* samples of infected tissue were analyzed by RNA gel blot to compare the content of *PR1* transcripts, as a marker for SA defenses, and *PDF1.2* transcripts, as a marker for JA/ethylene defenses (Fig. 1D). Uninoculated *cev1* plants had low *PR1* expression and high *PDF1.2* expression. In inoculated *cev1* leaves, *PR1* expression remained low, but *PDF1.2* expression was reduced. By contrast, inoculated *coi1*-16 leaves had greatly increased *PR1* expression but *PDF1.2* expression could not be detected. Wild-type leaves had low expression of *PR1* and *PDF1.2* and this was not was visibly altered by infection.

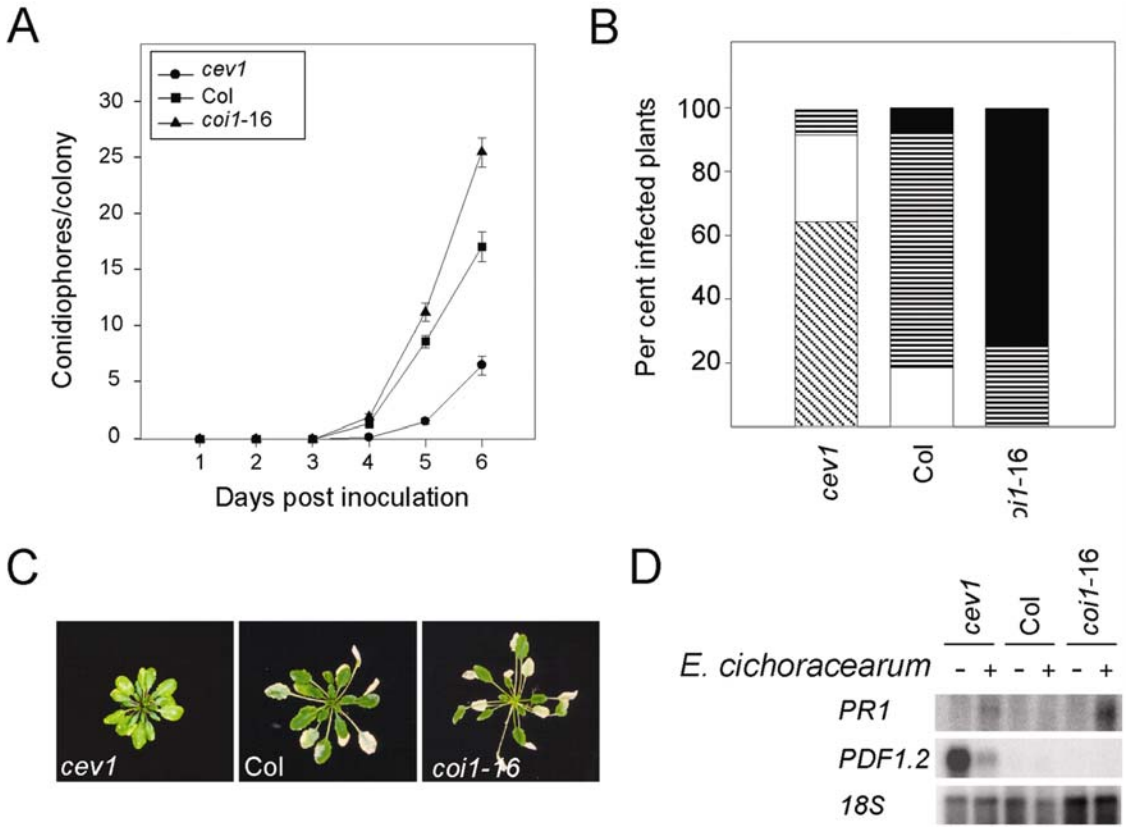

Fig. 1. *Erysiphe cichoracearum* UCSC1 infection on jasmonate (JA) response mutants. **A,** Six-week-old plants were inoculated with *E. cichoracearum* UCSC1. On the days indicated, leaves were fixed and cleared in a lactophenol solution, then stained with trypan blue. Mean conidiophores per colony were determined for approximately 50 colonies per data point. T-bars indicate standard error. **B,** Disease symptoms from at least 12 plants were scored 10 days post infection (dpi) using the following classifications: vertical lines = no visible fungal growth; diagonal lines = approximately 25% leaf surface covered with powdery mildew; white = approximately 50% leaf surface covered with powdery mildew; horizontal lines = approximately 100% leaf surface covered with powdery mildew; black = approximately 100% leaf surface covered with powdery mildew and leaves are completely chlorotic. **C,** Photographs of plants 10 dpi. **D,** Total RNA was extracted from plants before and at 5 dpi. Total RNA (1 μg) from each sample was electrophoresed, blotted onto nylon membrane, then probed with radiolabeled DNA probes as indicated; + = inoculated and – = uninoculated.

Active JA signaling enhances resistance to *P. syringae*.

Significantly smaller numbers of cells of *P. syringae* pv. *maculicola* ES4326 were recovered from inoculated *cev1* plants than from wild-type plants (Fig. 2A). By contrast, *coi1*-16 plants supported significantly less bacterial growth than either wild-type or *cev1* plants. These bacterial numbers were directly related to the symptoms at 5 dpi: small patches of chlorosis were observed on inoculated *cev1* leaves, water-soaked lesions surrounded by chlorosis were observed on wild-type leaves (Fig. 2B and C), whereas few or no disease symptoms could be detected on inoculated *coi1*-16 leaves.

At 2 dpi, samples of infected tissue were analyzed by RNA gel blot to compare the content of transcripts for *PR1* and *PDF1.2* (Fig. 2D). *PR1* mRNA accumulated after inoculation predominantly in wild-type and *coi1-16* plants. *PDF1.2* transcripts could be detected only in *cev1* plants, and the amount decreased after inoculation.

Active JA signaling enhances resistance to aphids.

To determine the effect of JA signaling on growth of aphid populations, two aphids were placed at the center of the rosette of individual 4-week-old wild-type, *cev1*, and *coi1*-16 plants. The plants were transferred from a short-day to a long-day environment, and the number of aphids on each plant was determined 7 days later. All plants initiated bolting 3 to 4 days after treatment, but none had produced opened flowers by the end of the 7-day period. Aphids were found mainly on the inflorescences and around the veins on the abaxial side of the leaves.

The number of aphids on *cev1* plants was significantly lower than on wild-type and *coi1*-16 plants (Fig. 3A). By contrast, *coi1*-16 plants had slightly more aphids than wild-type plants. Parallel experiments indicated that *coi1*-1 plants also supported more rapid growth of aphid populations than wild-type plants (data not shown). Leaves of wild-type and *cev1* plants sprayed with 500 μM methyl (Me)JA had smaller numbers of aphids than leaves sprayed with water. However, numbers of aphids on leaves of *coi1*-16 plants sprayed with MeJA were not statistically different, as judged by a student's *t* test, from numbers *on coi1*-16 leaves sprayed with water.

Aphids were removed from leaves after 7 days and leaf samples were analyzed by RNA gel blot to compare the content of transcripts for *PR1* and *PDF1.2*. *PR1* mRNA was not reliably observed in any of the samples (results not shown). Little difference was observed in the low level of *PDF1.2* transcripts found in control leaves and leaves colonized by aphids from wild-type and *coi1*-16 plants. However, the high constitutive expression of *PDF1.2* mRNA in *cev1* plants was slightly reduced in leaves colonized by aphids (Fig. 3B).

Regulation of expression of *PR1* and *PDF1.2*.

During the expression of resistance in *cev1* plants, *PR1* is not induced and constitutive expression of *PDF1.2* is slightly

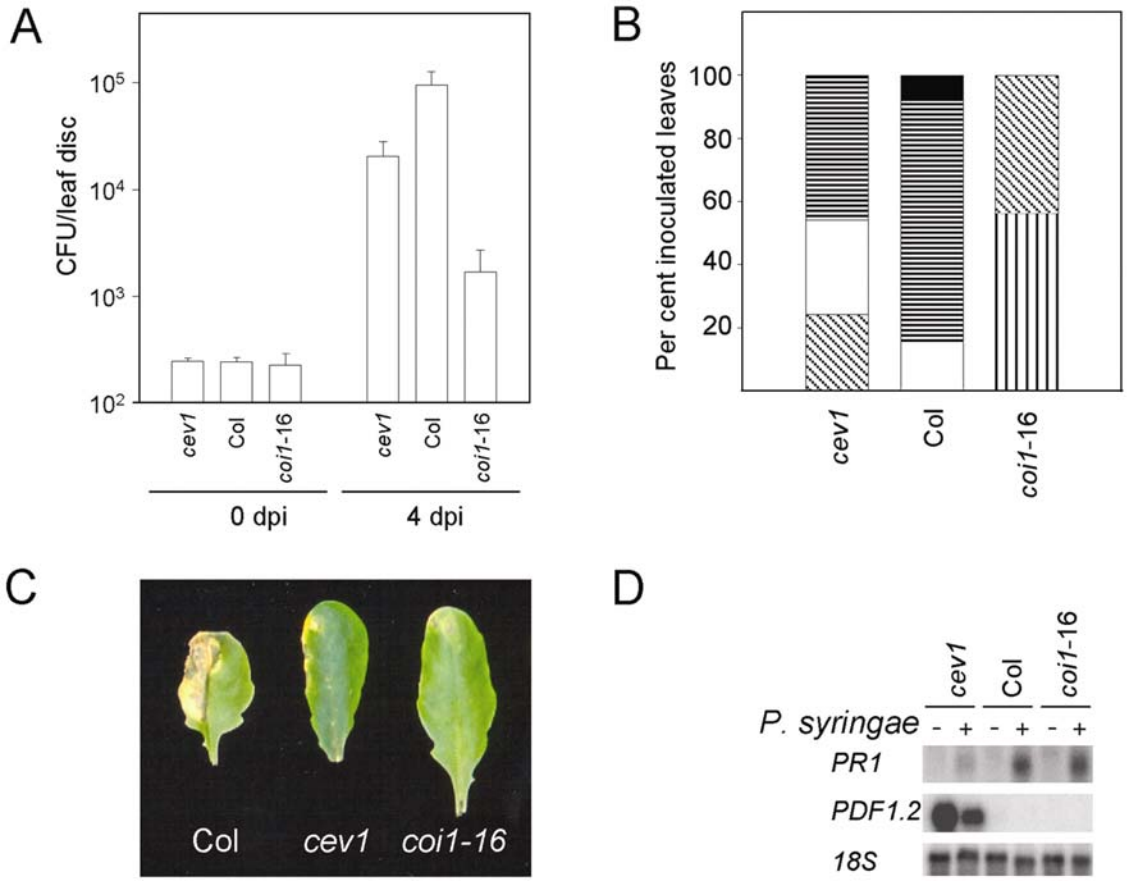

Fig. 2. Effect of jasmonate (JA) signaling on *Pseudomonas syringae* pv. *maculicola* ES4326 infection. Three leaves from 6-week-old plants were pressure infiltrated with approximately 10 μl of a bacterial suspension containing 10^5 CFU/ml. **B,** Mean CFU/leaf disc was determined from samples collected immediately after infiltration and 4 days post infection (dpi). Each data point was obtained using 12 leaves. T-bars indicate standard error. B, Disease symptoms were scored 5 dpi. as follows: vertical lines = no visible fungal symptoms; diagonal lines = approximately 25% of inoculated surface covered was chlorotic; white = approximately 50% of inoculated surface covered was chlorotic and contains small patches of water-soaked lesions; horizontal lines = approximately 75% of inoculated surface covered was chlorotic and contains large patches of water-soaked lesions; black = approximately 100% of inoculated surface covered was chlorotic and water-soaked. Data was obtained from 12 plants. **C,** Photographs of leaves taken 5 dpi. **D,** Total RNA was extracted from inoculated leaves 0 and 2 dpi. Total RNA (1 μg) from each sample was electrophoresed, blotted onto nylon membrane, then probed with radiolabeled DNA probes; + = inoculated and – = uninoculated.

suppressed (Figs. 1, 2, and 3). To determine whether the *cev1* mutation directly controls *PDF1.2* and *PR1* expression, or whether *PDF1.2* is induced and *PR1* is suppressed through activation of JA and ethylene signaling, we analyzed expression of these genes in the double mutants *cev1/cev1*;*coi1*-1/*coi1*-1 and *cev1/cev1*;*etr1/etr1*. We also examined the effect of SA on transcription of *PR1* and *PDF1.2* in *cev1* and *coi1*-16 plants to determine whether SA produced in response to infection could account for the reduced *PDF1.2* expression in infected *cev1* plants.

PDF1.2 was expressed constitutively in *cev1* plants but was suppressed in *cev1coi1*-1 and in *cev1etr1* plants (Fig. 4). Treatment of *cev1* plants with SA reduced *PDF1.2* expression. These results indicate that the expression of *PDF1.2* in *cev1* plants requires JA and ethylene signaling, and is suppressed by SA.

In *cev1coi1*-1 plants, *PR1* mRNA was expressed constitutively and expression was increased by treatment with SA. In other plants, *PR1* was expressed only in response to treatment with SA. SA-induced *PR1* expression was higher in *cev1coi1*-1 and in *coi1*-16 plants than in *cev1* and *cev1etr1*-3 plants. These results indicate that JA signaling, but not ethylene signaling, antagonize the induction of *PR1* by SA.

The *cev1etr1*-3 double mutants were extremely stunted and *cev1coi1*-1 plants developed extensive spontaneous necrosis, particularly in the older rosette leaves. Although *cev1coi1*-1 plants apparently had enhanced resistance to colonization by *E. cichoracearum*, *P. syringae,* and aphids, we do not present this data because before treatment the experimental tissue was already necrotic; therefore, we could not eliminate the possibility that the observed enhanced resistance of these plants was for trivial reasons.

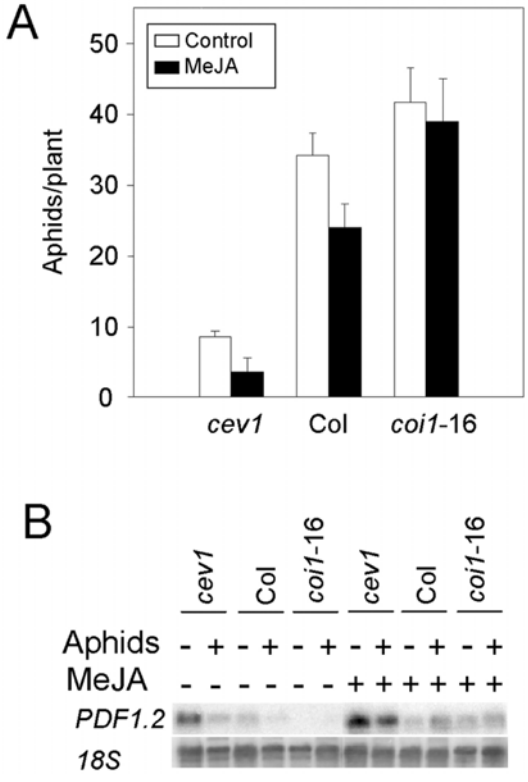

Fig. 3. Effect of jasmonate (JA) signaling on aphid growth. **A,** Two aphids were placed in the center of the rosette of 4-week-old plants. After 1 week, aphid numbers were determined. Plants were sprayed daily with either 0.001% methyl JA or water. Each data point represents results from 10 plants. T-bars indicate standard error. **B,** Total RNA was extracted from leaves on which aphids had fed for 1 week. Total RNA (1 µ) from each sample was electrophoresed, blotted onto nylon membrane, then probed with radiolabeled DNA probes.

DISCUSSION

We show here that JA signaling enhances resistance to the biotrophic fungus *E. cichoracearum*, to the bacterium *P. syringae* pv. *maucicola,* and to the aphid *M. persicae*. JA and SA signal pathways appear to be mutually antagonistic, and resistance to a particular pathogen previously has often been associated with either one or the other pathway. Resistance to *E. cichoracearum* and to *P. syringae* pv. *maculicola* previously has been causally linked to SA signaling, and *M. persicae* previously has been found to induce SA-dependent defenses (Kloek et al. 2001; Moran and Thompson 2001; Xiao et al 2001). Therefore, our results indicate that the JA and SA signal pathways regulate alternative disease resistance pathways that nevertheless can contribute to resistance to the same pathogen. Apparently the biological outputs of the JA and the SA pathway are broadly similar: that is, they both confer resistance to a broad and overlapping range of pathogens.

A crucial feature of our experimental evidence comes from analysis of *cev1*, which has constitutively active JA responses and is resistant to *E. cichoracearum*, *P. syringae* pv. m*aculicola*, and *M. persicae.* Some other mutants with constitutive expression of JA defenses also have constitutive activation of SA defenses (Bowling et al. 1997; Clarke et al. 1998; Hilpert et al. 2001). By contrast, *cev1* had activated JA-dependent defenses, but SA-dependent defenses were not expressed. The enhanced resistance of *cev1* plants to *E. cichoracearum* UCSC1, therefore, likely was due to the constitutive expression of JA-regulated genes.

Significantly, *coi1*-16 plants were more susceptible than wild-type plants to *E. cichoracearum* UCSC1. A role for JA in resistance to *E. orontii* previously had been ruled out on the basis that *jar1* plants are not more susceptible to *E. orontii* infection (Reuber et al. 1998). This discrepancy may be because JA perception is not completely blocked in *jar1* plants (Staswick et al. 1998) or perhaps *E. orontii* and *E. cichoracearum* activate slightly different defenses in the plant. Kloek and associates also concluded that *coi1*-20 plants were no more susceptible than wild-type plants to *E. cichoracearum* UCSC1. However, in that study, quantitative measurements were not reported. We find that the difference in conidiophore number on wild-type and *coi1* plants is significant but may have been overlooked in a visual assessment of susceptibility.

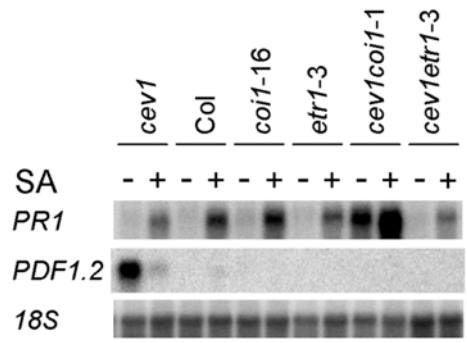

Fig. 4. RNA gel blot analysis of the effect of salicylic acid (SA) on defense gene expression in jasmonate (JA) and ethylene mutants. Total RNA was extracted from seedlings grown for 10 days on Murashige-Skoog (MS) medium, then transferred to MS medium supplemented with 50 µM SA for 2 days prior to harvest. Total RNA (2 µg) from each sample was electrophoresed, blotted onto nylon membrane, then probed with radiolabeled DNA probes as indicated; + = plus SA and – = minus SA.

It is intriguing that both insensitivity to JA and constitutive expression of JA responses can lead to decreased susceptibility to *P. syringae* pathogens (Feys et al. 1994; Kloek et al. 2001; this study). Furthermore, the exogenous application of MeJA also has been shown to decrease susceptibility to the avirulent pathogen *Pst*(avrRpt2) (van Wees et al. 1999). *coi1nahG* double mutant plants permit growth of *P. syringae* DC3000 and it has been suggested that the resistance of *coi1* plants to *P. syringae* is due in part to the hyperactivation of the SA signaling pathway in these plants (Kloek et al. 2001). These results indicate that the antimicrobial compounds produced by both JA and SA signaling pathways are capable of deterring *P. syringae* pathogens, although the latter appear more effective than the former.

The pathogenicity of *P. syringae* has been linked to its production of coronatine, a molecular mimic of JA and a virulence factor produced by strains of *P. syringae* (Bender et al. 1987; Feys et al. 1994, Hendrickson et al. 2000). Coronatine and JA induce many of the same genes, although coronatine is a more potent activator and causes pronounced chlorosis (Benedetti et al. 1995; Feys et al. 1994). Possibly a low level of induction of the JA pathway can induce sufficient defenses to inhibit bacterial growth, but the hyperinduction of the JA pathway and the concomitant suppression of SA-related defenses by coronatine decreases plant fitness to such an extent that it cannot mount a proper defense to the pathogen.

Like pathogen attack, insect herbivory causes widespread changes in plant gene expression (Hermsmeier et al. 2001; Reymond et al. 2000). Chewing insects have been demonstrated to induce many of the same genes induced by wounding or JA (Reymond et al. 2000). Aphids, however, do little damage to plant tissue and, on *Arabidopsis* spp., predominantly induce SA-regulated genes rather than JA-regulated genes, although the prior induction of SA-regulated genes was not found to cause a statistically significant decrease in aphid numbers (Moran and Thompson 2001).

We have demonstrated that the JA pathway also provides protection to *Arabidopsis* plants from aphid infestation: aphid numbers were lowest on *cev1* plants and highest on *coi1*-16 plants. In contrast with the results of Moran and Thompson (2001), aphid feeding in this study did not induce *PR1* gene expression, and may be accounted for by the lower numbers of aphids used in this study. Spraying the plants with MeJA significantly reduced the number of aphids on *cev1* and wild-type plants, again suggesting that augmenting JA defenses can enhance plant resistance to aphids. By contrast, the application of MeJA had little effect on the numbers of aphids on the JA-insensitive *coi1*-16 plants, indicating that MeJA has no direct effect on aphid viability.

E. cichoracearum UCSC1, *P. syringae*, and the aphid *M. persicae* all have been previously shown to activate SA responses (Kloek et al. 2001; Moran and Thompson 2001; Xiao et al 2001). For both *E. cichoracearum* UCSC1 and *P. syringae*, mutations that decrease SA signaling result in enhanced susceptibility. In this study, we have demonstrated that a reduction in JA signaling also results in enhanced susceptibility to *E. cichoracearum* UCSC1 and aphids. These results indicate that the JA pathway contributes to defense against those pathogens, in addition to previously characterized necrotrophs. This highlights the need to consider multiple defense pathways when studying host response to a particular pathogen.

Furthermore, it seems likely that the enhancement of the JA signaling pathway, such as in *cev1* plants, can provide defense against a broad spectrum of pests and pathogens. In the case of many pathogens, this defense is insufficient to completely repel the invaders, but it may serve to lessen disease severity.

MATERIALS AND METHODS

Plants and growth conditions.

etr1-3 seed was obtained from the Nottingham Arabidopsis Stock Centre. Double mutants were constructed and selected as described previously (Ellis and Turner 2001). Plants for RNA analysis were grown axenically in Murashige-Skoog (MS) (Murashige and Skoog 1962) medium for 10 days, then transferred to MS medium supplemented with 50 μM SA (Sigma, Poole, U.K.). Selection of *coi1*-1 plants was carried out on MS medium containing 50 μM MeJA (Bedoukian Research Inc., Danbury, CT, U.S.A.). Plants were grown in 16-h photoperiods at 22°C in a Versatile Environmental Test Chamber (Sanyo, Watford, U.K.). Plants for pathogen challenge were grown in soil for 5 to 6 weeks in 8-h photoperiods, then moved to 16-h photoperiods for inoculations.

Pathogen and pest challenge.

E. cichoracearum UCSC1 stocks were maintained on squash plants. *Arabidopsis* plants were inoculated by brushing spores from squash leaves with a small paintbrush. After 5 days, leaves were excised and cleared in lactophenol solution (Reuber et al. 1998). Conidiophores were stained with trypan blue and quantified visually using a light microscope.

P. syringae pv. *maculicola* ES 4326 was grown at 28°C in King's B medium overnight, then diluted to approximately 10^5 CFU/ml with 10 mM $MgSO_4$. Approximately 10 μl of bacterial suspension was pressure infiltrated into the abaxial side of 2 to 3 leaves per plant. A cork borer was used to collect samples for titration of bacteria. Samples were ground in 10 mM $MgSO_4$ and appropriate dilutions were plated onto King's B media.

Plants for aphid challenge were grown in soil for 6 to 7 weeks in 8-h photoperiods. Two adult *M. persicae* (green peach aphid) aphids were placed near the center of the rosette of each plant. Eight plants of each different mutant were kept on the same pot, allowing for aphid movement. Plants were kept in a ventilated Plexiglas chamber. All mutants were kept in the same covered trays so that aphids were forced to feed on the plants available to them, but were free to move within different plants. The plants were moved to a 16-h photoperiod for 7 days. Plants were sprayed daily with 500 μM MeJA or water until leaves were just wet. Aphid numbers then were recorded and samples taken for RNA. Aphids were removed from the leaf tissues before leaf excision by gentle brushing. In this experiment, each type of plant was grown in a separate tray, although experiments have been carried out with different types of plants randomly interspersed and similar results were obtained.

At least three independent trials were conducted for each pathogen and aphid experiment. Results from representative trials are shown.

RNA gel blot analysis.

Total RNA was prepared using an RNeasy kit (Qiagen, Hilden, Germany). RNA was electrophoresed and blotted as described previously (Benedetti et al. 1995). Blots were probed with random-primed ^{32}P-labeled DNA fragments (MegaPrime kit, Amersham, Little Chalfont, U.K.). *PR1*, *PDF1.2*, and *18S* probes were prepared as described earlier (Ellis and Turner 2001; Xiao et al. 2001). The RNA gel blots were repeated at least two times and similar trends were noted each time.

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LITERATURE CITED

Bender, C. L., Stone, H. E., Sims, J. J., and Cooksey, D. A. 1987. Reduced pathogen fitness of *Pseudomonas syringae* pv. *tomato* Tn5 mutants defective in coronatine production. Physiol. Mol. Plant Pathol. 30:273-283.

Benedetti, C. E., Xie, D., and Turner, J. G. 1995. COI1-dependent expression of an *Arabidopsis* vegetative storage protein in flowers and siliques and in response to coronatine or methyl jasmonate. Plant Physiol. 109:567-572.

Bowling, S. A., Clarke, J. D., Liu, Y., Klessig, D. F., and Dong, X. 1997. The cpr5 mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. Plant Cell 9:1573-1584.

Clarke, J. D., Liu, Y., Klessig, D. F., and Dong. X. 1998. Uncoupling PR gene expression from NPR1 and bacterial resistance, characterization of the dominant *Arabidopsis* cpr6-1 mutant. Plant Cell 10:557-569.

Dempsey, D. A., Shah, J., and Klessig, D. F. 1999. Salicylic acid and disease resistance in plants. Crit. Rev. Plant Sci. 18:547-575.

Doares, S. H., Narvaez-Vasquez, 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.

Ellis, C., and Turner, J. G. 2001. The *Arabidopsis* mutant cev1 has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell 13:1025-1033.

Ellis, C., and Turner, J. G. 2002. A conditionally fertile *coi1* allele indicates cross talk between plant hormone signaling pathways in *Arabidopsis thaliana* seeds and young seedlings. Planta 215:549-556.

Ellis, J., Dodds, P., and Pryor, T. 2000. Structure, function, and evolution of plant disease resistance genes. Curr. Opin. Pant Biol. 3:278-284.

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.

Felton, G. W., Korth, K. L., Bi, J. L., Wesley, S. V., Huhman, D. V., Mathews, M. C., Murphy, J. B., Lamb, C., and Dixon, R. A. 1999. Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. Curr. Biol. 9:317-320.

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.

Feys, B. J. F., and Parker, J. E. 2000. Interplay of signaling pathways in plant disease resistance. Trends Genet. 16:449-455.

Frye, C. A., and Innes, R. W. 1998. An *Arabidopsis* mutant with enhanced resistance to powdery mildew. Plant Cell 10:947-56.

Gupta, V., Willits, M. G., and Glazebrook, J. 2000. *Arabidopsis thaliana EDS4* contributes to salicylic acid (SA)-dependent expression of defense responses: Evidence for inhibition of jasmonic acid signaling by SA. Mol. Plant-Microbe Interact. 13**:**503-511.

Hendrickson, E. L., Guevera, P., Penalopez-Vasquez, A., Shao, J., Bender, C., and Ausubel, F. M. 2000. Virulence of the phytopathogenic *Pseudomonas syringae* pv. *maculicola* is rpoN dependent. J. Bacteriol. 182:3498-3507.

Hermsmeier, D., Schittko, U., and Baldwin, I. T. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (*Lepidoptera, Sphingidae*) and its natural host *Nicotiana attenuata.* I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs Plant Physiol. 125:683–700

Hilpert, B., Bohlmann, H., op den Camp, R., Przybyla, D., Miersch, O., Buchala, A., and Apel, K. 2001. Isolation and characterization of signal transduction mutants of *Arabidopsis thaliana* that constitutively activate the octadecanoid pathway and form necrotic lesions. Plant. J. 26:436-446.

Kloek, A. P., Verbsky, M. L., Sharma, S. B., Schoelz, J. E., Vogel, J., Klessig, D. F., and Kunkel, B. N. 2001. Resistance to *Pseudomonas syringae* conferred by an *Arabidopsis thaliana* coronatine-insensitive (coi1) mutation occurs through two distinct mechanisms. Plant J. 26:509-522.

Martin, G. B. 1999. Functional analysis of plant disease resistance genes and their downstream effectors. Curr. Opin. Plant Biol. 2:273-279.

McConn, M., Creelman, R. A., Bell, E., Mullet, J. E., and Browse, J. 1997. Jasmonate is essential for insect defense. Proc. Natl. Acad. Sci. U.S.A. 94:5473-5477.

McDowell, J. M., Cuzick A., Can C., Beynon J., Dangl J. L., and Holub E. B. 2000. Downy mildew (*Peronospora parasitica*) resistance genes in *Arabidopsis* vary in functional requirements for NDR1, EDS1, NPR1, and salicylic acid accumulation. Plant J. 2:523-529.

Moran, P. J., and Thompson, G. A. 2001. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. Plant Physiol. 125:1074-1085.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant. 15:473-497.

Norman-Setterblad, C., Vidal, S., and Palva, E. T. 2000. Interacting signal pathways control defense gene expression in *Arabidopsis* in response to cell wall-degrading enzymes from Erwinia carotovora. Mol. Plant-Microbe Interact. 13:430-438.

Penninckx, I. A. M. A., Thomma, B. P. H. J., Buchala, A., Metraux, 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.

Reuber, T. L., Plotnikova, J. M., Dewdney, J., Rogers, E. E., Wood, W., and Ausubel, F. M. 1998. Correlation of defense gene induction defects with powdery mildew susceptibility in *Arabidopsis* enhanced disease susceptibility mutants. Plant J. 16:473-485.

Reymond, P., and Farmer, E. E. 1998. Jasmonate and salicylate as global signals for defense gene expression. Curr. Opin. Plant Biol. 1:404-411.

Reymond, P., Weber, H., Damond, M., and Farmer, E. E. 2000. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. Plant Cell 12:707-719.

Samac, D. A., Hironaka, C. M., Yallaly, P. E., and Shah, D. M. 1990. Isolation and characterization of genes encoding basic and acidic chitinase in *Arabidopsis thaliana.* Plant Physiol. 93:907-914.

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.

Thomma, B. P. H. J., Eggermont, K., Broekaert, W. F., and Cammue, B. P. A. 2000. Disease development of several fungi on *Arabidopsis* can be reduced by treatment with methyl jasmonate. Plant Physiol. Biochem. 38:421-427.

Thomma, B. P. H. J., Eggermont, K., Penninckx, I. A. M. A., Mauch-Mani, B., Vogelsang, R., Cammue, B. P. A., and Broekaert, W. F. 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc. Natl. Acad. Sci. U.S.A. 95:15107-15111.

Thomma, B. P. H. J., Eggermont, K., Tierens, K. F. M.-J., and Broekaert, W. F. 1999. Requirement of functional ethylene-insensitive 2 gene for efficient resistance of *Arabidopsis* to infection by *Botrytis cinerea.* Plant Physiol. 121:1093-1101.

van Wees, S. C. M., Luijendijk, M., Smoorenburg, I., van Loon, L. C., and Pieterse, C. M. J. 1999. Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on expression of known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Plant Mol. Biol. 41:537-549.

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. U.S.A. 95:7209-7214.

Vogel, J., and Somerville, S. 2000. Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. Proc. Natl. Acad. Sci. U.S.A. 97:1897-1902

Xiao, S., Ellwood, S., Calis, O., Patrick, E., Li, T., Coleman, M., and Turner, J. G. 2001. Broad spectrum powdery mildew resistance in *Arabidopsis thaliana* mediated by RPW8. Science 291:118-120.