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# Expression profiling reveals *COI1* to be a key regulator of genes involved in wound- and methyl jasmonate-induced secondary metabolism, defence, and hormone interactions

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**Abstract**

The *Arabidopsis* gene *COI1* is required for jasmonic acid (JA)-induced growth inhibition, resistance to insect herbivory, and resistance to pathogens. In addition, *COI1* is also required for transcription of several genes induced by wounding or by JA. Here, we use microarray gene transcription profiling of wild type and *coi1* mutant plants to examine the extent of the requirement of *COI1* for JA-induced and wound-induced gene transcription. We show that *COI1* is required for expression of approximately 84% of 212 genes induced by JA, and for expression of approximately 44% of 153 genes induced by wounding. Surprisingly, *COI1* was also required for repression of 53% of 104 genes whose expression was suppressed by JA, and for repression of approximately 46% of 83 genes whose expression was suppressed by wounding. These results indicate that *COI1* plays a pivotal role in wound- and JA signalling.

## Introduction

Plants respond to environmental changes and biotic challenges by producing a number of signalling molecules that can in turn, trigger dramatic modifications in transcription and the physiological status of the cell. The jasmonate (JA) family of signalling molecules regulate responses to many biotic and abiotic stresses (Turner *et al.*, 2002, Devoto and Turner, 2003). These include defence against a variety of pathogens and insects (McConn *et al.*, 1997; Pieterse *et al.*, 1998; Staswick *et al.*, 1998; Thomma *et al.*, 1998; Ellis *et al.*, 2002). In addition, JAs can induce the production of secondary metabolites (Gundlach *et al.*, 1992; Feys *et al.*, 1994; Menke *et al.*, 1999; Brader *et al.*, 2001). In *Arabidopsis thaliana*, JA is required for stamen development, as evidenced by mutants that are unable to synthesise or perceive JA are also male sterile (Feys *et al.*, 1994; McConn and Browse, 1996).

Wounding causes a rapid and dramatic increase in JA (Creelman *et al.*, 1992). Because the application of JA to unwounded plants leads to the activation of wound-inducible genes, this suggests that JA plays a general role in the wound

response (Farmer and Ryan, 1990). However, JA-insensitive mutants of *A. thaliana* have revealed JA-independent wound signalling pathways (Titarenko *et al.*, 1997). These and other studies indicate that the wound response is a culmination of interactions between several pathways, including those for JA, ethylene, SA, and osmotic stress (Reymond *et al.*, 2000; Cheong *et al.*, 2002).

The known responses of *A. thaliana* to JA appear to require the gene *COI1*. This is based on studies on the *coi1* mutant, alleles of which were selected in screens for insensitivity to JA-induced growth inhibition; in general, the *coi1* mutants fail to express JA-induced genes (Feys *et al.*, 1994; Benedetti *et al.*, 1995; Ellis and Turner, 2002). *COI1* encodes an F-box protein (Xie *et al.*, 1998) and forms an integral part of an SCF-type E3 ubiquitin ligase that is predicted to target repressors of JA signalling to the proteasome for degradation (Devoto *et al.*, 2002; Xu *et al.*, 2002). COI1 binds a histone deacetylase, RPD3b, and may therefore regulate expression of genes involved in the JA response through moderation of the activity of RPD3b, and possibly other proteins (Devoto *et al.*, 2002). The *coi1-16* mutant displays a jasmonate-insensitive phenotype but has reduced fertility only at temperatures above 20 °C (Ellis and Turner, 2002). The *coi1-16* mutation is predicted to introduce a L245F mutation in a α-helix of the leucine rich repeats of COI1. Significantly, this mutant protein does not interact with RPD3b in yeast where the two proteins are expressed at high level (Devoto *et al.*, 2002), which provides supporting evidence for a functional link between COI1 and RPD3b.

*COI1* therefore appears to play a central role in JA signalling and in the wound response. Although the *COI1* gene regulates the known responses of *Arabidopsis* to JAs, the extent of this dependence has not been investigated systematically. Here, we investigate the requirement of *COI1* for wound- and JA-induced transcriptional regulation. We have used microarrays to compare the patterns of gene expression in response to wounding and JA in wild type and *coi1* backgrounds. We have identified gene families whose expression is co-ordinated by *COI1*-dependent JA signalling, and have identified the contribution of JA and *COI1* in the wound response.

## Materials and methods

### *Plant material and sample preparation*

*Arabidopsis* Col-*gl1* and *coi1*-16 seedlings were grown axenically on MS-agar medium for 10 days, and then wounded with blunt forceps or placed on MS-agar medium supplemented with 20 μM MeJA (Bedoukian Research Inc., Danbury, CT, USA). Samples were collected between 0.5, 6 h and 1, 6 and 48 h after wounding and MeJA treatment, respectively. As shown in Table SIII, the following samples were used for RNA isolation: Columbia untreated (5 replicates), Columbia wounded 0.5 h (1 replicate), Columbia wounded 6 h (2 replicates), Columbia MeJA 1 h (1 replicate), Columbia MeJA 6 h (3 replicates) Columbia MeJA 48 h (1 replicate) *coi1-16* untreated (3 replicates) *coi1-16* wounded 0.5 h (1 replicate) *coi1-16* wounded 6 h (2 replicates) *coi1-16* MeJA 1 h (1 replicate), *coi1-16* MeJA 6 h (2 replicates). Plants were grown with a 16-h light period at 22 °C until flowering.

Total RNA from seedlings and plants were isolated from frozen tissues using RNeasy Plant Mini kits (Qiagen, Crawley, UK) as described previously (Ellis and Turner, 2001). To control the biological variation, all tissue samples were a pool of at least 15 different plants. RNA from at least three independent samples was pooled.

### *Data acquisition and analysis*

For *Arabidopsis* GeneChip experiments (Affymetrix), RNA samples were extracted and subsequent cDNA synthesis, array hybridization, and overall intensity normalization for all of the arrays for the entire probe sets were performed as described by Zhu *et al.* (2001).

Gene expression data analysis was performed in the R language (Ihaka *et al.,* 1996) together with the Bioconductor package (Gentleman *et al.*, 2004). The cell intensity (CEL) files were obtained from the Affymetrix GeneChip© scanner and background correction, normalisation and data summaries were calculated using the GC-RMA model described in (Wu *et al.*, 2004).

A classical linear statistical modelling approach was taken to analyse the data (Searle *et al.*, 1971; Glonek and Solomon, 2004; Smyth, 2004). The methodology involved forming design

and contrast matrices in R defining three distinct experimental designs. The first was a $2 \times 2$ factorial design where the genotypes Col-*gl1* and *coi1*-16 were subjected to wounding and examined at 0.5 and 6 h. The second $2 \times 2$ factorial design used the same genotypes, but the treatment represented exposure to 20 $\mu$M MeJA. The time points for the second factorial design were 1 and 6 h. The $2 \times 2$ factorial designs allowed for a linear model to measure the effect of the interaction between treatments and the different genotypes over time. The third design was a replicated design comparing the two genotypes in the absence of treatments at the zero time point. The purpose of the third design was two-fold: to obtain estimates of the basal transcriptional differences between Col-*gl1* and *coi1*-16; and additionally estimate within-genotype variation. The former estimates allowed for gene-wise *post hoc* comparisons with corresponding expression levels subject to treatments and differing time points. The latter estimates contributed to the imputation of observations in the factorial designs in order to achieve balance (Wernisch *et al.*, 2003).

Prior to model fitting, non-specific filtering of the probe sets were performed. The premise of this technique was the removal of genes not deemed to show either enough differential expression or signal intensity to be useful in the analysis. As Northern blots were used as the confirmatory technique for differential expression; a natural floor value for the signal intensity of the probe sets was discovered to be approximately the intensity of certain Affymetrix control probes sets (AFFX-BioDn-5_at). Probes with fluorescence values below the flooring value were removed from further analysis.

As temporal differential expression was not of primary interest, but instead the effect of the genotypes coupled with the treatments, two distinct sets of linear models were fitted to each design, using least squares. By sets of linear models we referred to the fitting of a linear model separately for each gene. This approach, described in (Smyth *et al.*, 2004), accommodated differing gene variances.

The first set was a limited model, which had time as the only effect. The second set was a full model, which incorporates the effects of time, genotype and the time-genotype interaction. We selected our genes of interest by comparing the limited model to the full model on each gene using an ANOVA *F*-test. If the full model fit better than the limited model, we may conclude that the gene must be affected by the genotype.

Assessment of differential expression was achieved through a combination of empirical Bayes-derived moderated *F*- and *t*-statistics; and fold changes between genotypes. The statistics were ranked in terms of evidence against equal expression; and the *P*-values adjusted using the method by (Benjamini and Hochberg, 1995) with an expected false discovery rate at level 0.05.

Due to the large number of experiments as well as differentially expressed genes, mining of the results was performed with GeneSpring™ software (Silicon Genetics, Redwood City, CA, USA) and Cluster/Treeview (Eisen *et al.*, 1998). Affymetrix gene chip data files were imported into GeneSpring 5.0 software and were normalized according to the GeneSpring manual recommendations for Affymetrix chips. Data for each experimental treatment was compared to an untreated control that was harvested at the same time so as to eliminate circadian-regulated genes. Genes whose expression levels were less than 1 were floored to 1. Data for tables and figures were also restricted such that genes were considered only if they had a raw expression level of 100 and an Affymetrix flag calls of 'Present' in the sample with the greater value in the pairwise comparison. These cutoff levels were assigned because genes whose expression levels were less than 100 frequently had an Affymetrix flag call of 'Absent'. Genes whose expression was altered more than 2.5-fold over control values were considered to be differentially regulated. Gene lists defined as described above using GeneSpring 5.0 software were also grouped using Cluster/Treeview software (Eisen *et al.*, 1998). Raw data levels of genes whose expression was altered more than 2.5-fold over control values were log transformed, median centered, and normalized for both genes and arrays. The processed data then were subjected to the self-organizing map algorithm followed by complete linkage hierarchical clustering of both genes and arrays, using the Cluster/Treeview program as previously described (Eisen *et al.*, 1998, Zhu *et al.*, 2001 Glazebrook *et al.*, 2003).

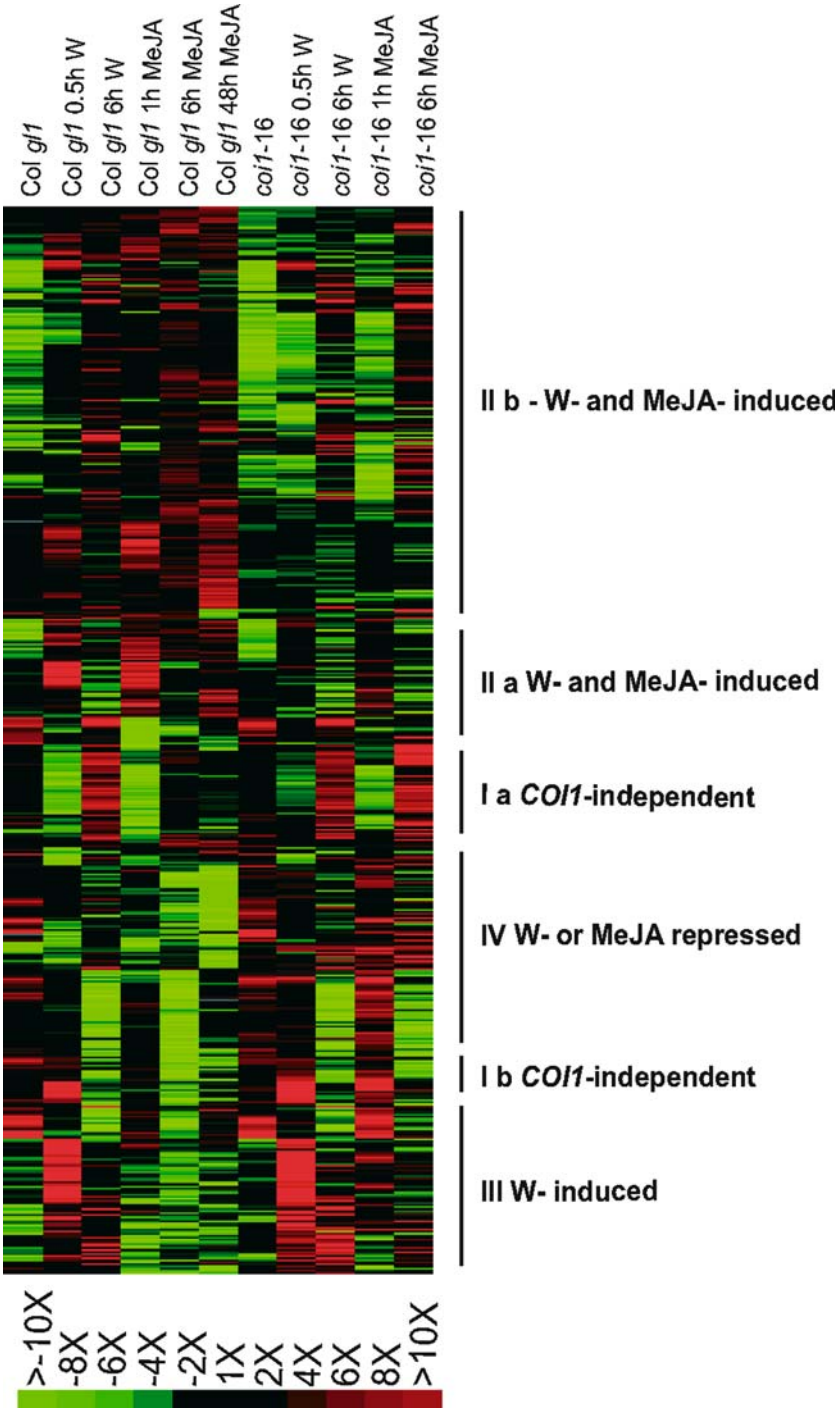


*Figure 1.* Clustering display of genes differentially regulated by wounding and MeJA in wild type seedlings. Five hundred and thirteen differentially expressed in response to wounding (0.5 h and 6 h) and MeJA (1 h, 6 h, and 48 h) were ordered. Groups Ia and b: *COI1* –independent; IIa and b: wound- and MeJa-induced; III: wound-induced; IV: wound- or MeJa-repressed.

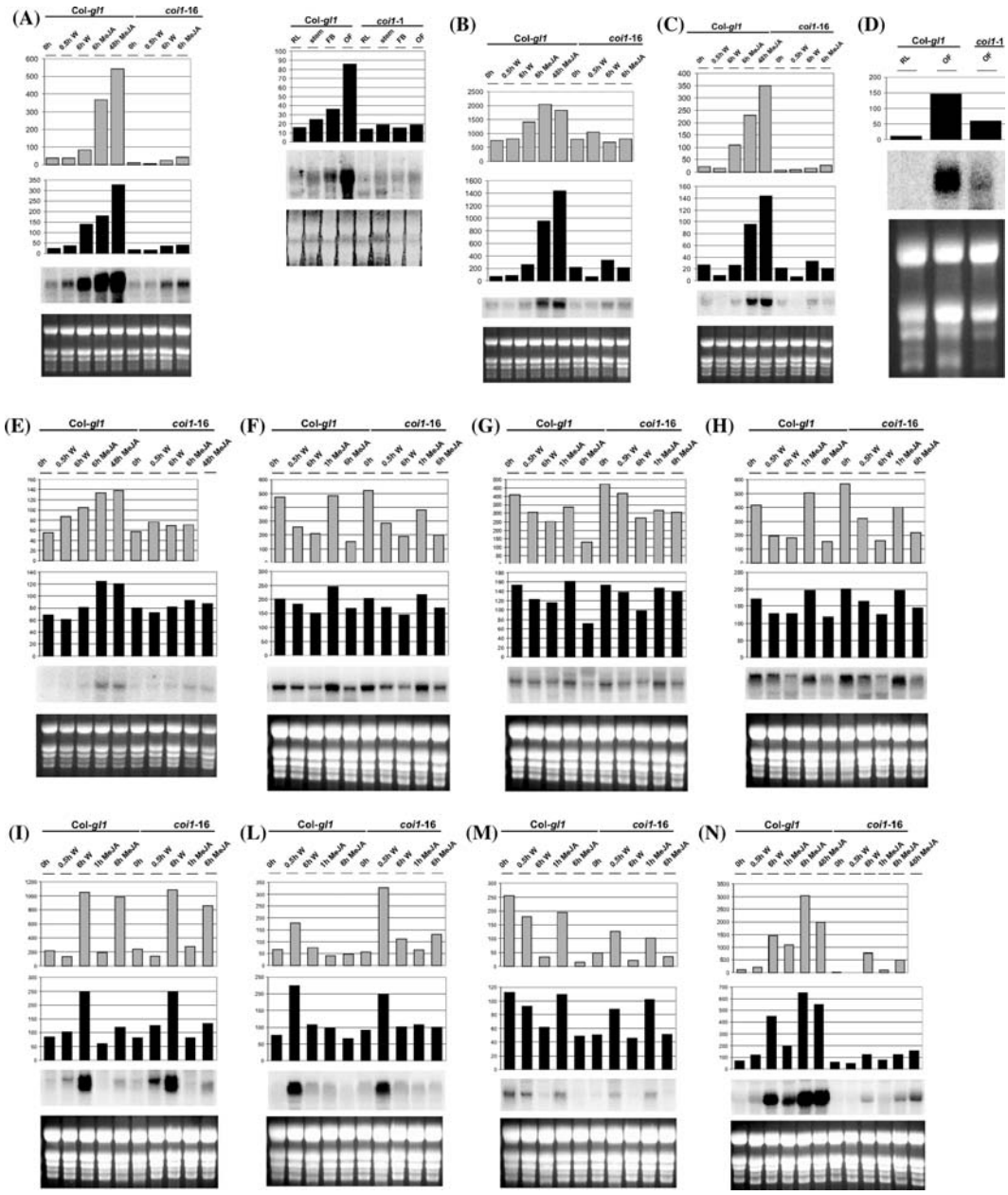


*Figure 2*. Comparison of raw expression levels and RNA gel blot analysis of genes involved in secondary metabolism and regulated by MeJA, wounding and *COI1* identified by transcriptome profiling. (A) At2g34810, putative berberine bridge enzyme; (B) At5g13930, chalcone synthase; (C) At4g22880, putative leucoanthocyanidin dioxygenase (LDOX); (D.) At2g24210, putative limonene cyclase; (E) At1g74020, putative strictosidine synthase; (F) At2g26690, putative nitrate transporter; (G) At3g23050, auxin-responsive protein AXR2/IAA7; (H) At4g37760, squalene epoxidase-like protein; (I) At2g28900, putative membrane channel protein; (L) At4g11280, ACC synthase 6 (ACS6); (M) At4g35770, senescence-associated protein; (N) At4g23600, tyrosine transaminase. A (left panel), B and C, E - N: differential expression in response to wounding (0.5 h and 6 h) and MeJA (1 h, 6 h, and 48 h) of wild type and *coi1*-16 plants; A (right panel) and D, differential expression in various organs of untreated wild type and *coi1*-1 plants. A 5-$\mu$g (A and B) or 10-$\mu$g (C – N) aliquots of total RNA isolated from each sample were utilized for Northern blots hybridized to cloned $^{32}$P labeled gene sequences. A (left panel) and B , F and G, H and I, L and M: the same membranes have been subjected to hybridization with two different DNA probes. Grey and black bars histograms represent raw expression levels of genes and mRNA levels obtained by quantification of the autoradiographic signals with PhosphorImager STORM840, respectively. W: wounding; RL: Rosette Leaves; FB: Flower Buds; OF: Opened Flowers.

*Table I.* Genes involved in secondary metabolism are regulated by MeJA, wounding and *COI1*. Genes induced by wounding or MeJA treatment and their fold induction over that of untreated plants are listed. Averaged values are given for genes that were present more than once on the microarray chip.

| Description | Pathway | Fold induction for Col-*gl1* seedlings | | | | | Ratio of basal expression levels of Col-*gl1* to *coi1-16* | Fold induction for *coi1-16* seedlings | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | W0.5h | W6h | JA1h | JA6 | JA48 | | W0.5h | W6h | JA1h | JA6h |
| Aminoacid synthesis and modification | | | | | | | | | | | |
| At4g39980 3-deoxy-D-arabino-heptulosonate y-phosphate synthase | chorismate biosynthesis | 1.4 | 1.9 | 2.9 | 8.7 | 3.9 | 1.3 | 0.7 | 2 | 1.2 | 3.7 |
| At5g05730 anthranilate synthase alpha subunit ASA1 | tryptophan biosynthesis | 2.5 | 1.6 | 4.6 | 6.9 | 5.4 | 1.6 | 1.2 | 1.8 | 2.2 | 3.4 |
| At5g17990 phosphoribosylanthranilate transferase | tryptophan biosynthesis | 1.6 | 2.2 | 2.5 | 6.8 | 4.5 | 1.4 | 1 | 1.9 | 1.2 | 2.6 |
| At1g07780 phosphoribosylanthranilate isomerase | tryptophan biosynthesis | 1 | 1.6 | 1.6 | 3.8 | 2.2 | 1.4 | 1.3 | 1.8 | 1.3 | 2.7 |
| At2g04400 putative indole-3-glycerol phosphate synthase | tryptophan biosynthesis | 1.9 | 2.1 | 4.1 | 6.2 | 3.3 | 1.3 | 1.1 | 2 | 1.2 | 2.8 |
| At3g54640 tryptophan synthase alpha chain | tryptophan biosynthesis | 1.5 | 2.5 | 2.1 | 6.9 | 2.7 | 1.4 | 1 | 2 | 1.1 | 3.3 |
| At4g27070 tryptophan synthase beta-subunit | tryptophan biosynthesis | 2.5 | 1.6 | 5.9 | 5.8 | 4.4 | 1.2 | 1.1 | 1.3 | 1.8 | 2.5 |
| At5g54810 tryptophan synthase beta subunit | tryptophan biosynthesis | 1.1 | 2.1 | 1.6 | 5.6 | 2.5 | 1.2 | 0.7 | 2.3 | 0.9 | 2.9 |
| At4g34200 Phosphoglycerate dehydrogenase - like protein | serine biosynthesis | 1.1 | 1.7 | 1.6 | 3.9 | 1.8 | 1 | 1 | 1.7 | 0.9 | 2.1 |
| At1g17740 phosphoglycerate dehydrogenase | serine biosynthesis | 1.1 | 2.6 | 2.3 | 6.8 | 3.4 | 1.2 | 0.9 | 2.8 | 1.1 | 2.8 |
| At4g35630 phosphoserine aminotransferase | serine biosynthesis; pyridoxal 5′-phosphate biosynthesis | 1.1 | 1.8 | 2 | 7.7 | 3.4 | 1.4 | 0.8 | 2.2 | 1.1 | 3.7 |
| At5g56760 serine acetyltransferase | cysteine biosynthesis; sulfur assimilation | 1.8 | 1.5 | 3.9 | 3.3 | 2.1 | 1.3 | 1.4 | 1.3 | 1.7 | 1.5 |
| At3g59760 cysteine synthase | cysteine biosynthesis; sulfur assimilation | 1 | 1.7 | 2.3 | 6.7 | 2.9 | 1 | 1 | 1.3 | 1 | 1.7 |
| At4g08870 similar to arginases | arginine degradation | 1.9 | 3.1 | 4.7 | 9.3 | 5.9 | 1.4 | 0.9 | 2.2 | 2.6 | 4.8 |
| At2g20340 tyrosine decarboxylase | amine metabolism | 1.5 | 0.8 | 8.2 | 3.4 | 3 | 1.1 | 0.8 | 0.7 | 1 | 1.2 |
| At2g24850 putative tyrosine aminotransferase | phenylalanine biosynthesis; tyrosine degradation | 11.4 | 14.6 | 18.8 | 25 | 44.8 | 2 | 6.4 | 13.4 | 3.3 | 14.6 |
| At4g23600 tyrosine transaminase like protein | phenylalanine biosynthesis; tyrosine degradation | 1.5 | 14.5 | 7.9 | 65.4 | 12.2 | 5.1 | 10 | 33.6 | 2.9 | 57.1 |
| At3g47340 glutamine-dependent asparagine synthetase | amine metabolism | 3.7 | 0.9 | 2 | 1 | 2.7 | 1.4 | 1.6 | 0.4 | 1.6 | 0.5 |
| Other nitrogenous compounds | | | | | | | | | | | |
| **At2g34810 putative berberine bridge enzyme** | isoquinoline alkaloids biosynthesis | 1 | 4.1 | 6.1 | 13 | 14.2 | 1.8 | 0.5 | 2.4 | 2.6 | 3.2 |
| **At1g74020 putative strictosidine synthase** | indole alkaloid biosynthesis | 1.5 | 1.9 | 2 | 2.5 | 1.4 | 1 | 1.1 | 1.1 | 1.4 | 1.2 |
| At4g39950 cytochrome P450-like protein | auxin biosynthesis | 1.8 | 2.7 | 2.8 | 9 | 5.1 | 3.2 | 0.8 | 4.5 | 1.7 | 8.2 |
| At4g23100 gamma-glutamylcysteine synthetase | glutathione biosynthesis | 1.3 | 1.5 | 1.8 | 3.3 | 1.9 | 1.1 | 0.8 | 1.4 | 0.8 | 1.7 |
| At5g27380 glutathione synthetase | glutathione biosynthesis | 1 | 1.8 | 1.1 | 4.4 | 2.2 | 1.2 | 0.8 | 2.2 | 0.7 | 2.3 |
| At2g22330 putative cytochrome P450 | auxin biosynthesis, glucosinolate biosynthesis | 1.9 | 2.2 | 4.8 | 7.7 | 2.9 | 2.8 | 1.6 | 2.2 | 1.8 | 5 |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Phenolics | | | | | | | | | | | | |
| At2g37040 phenylalanine ammonia lyase | phenylpropanoid biosynthesis | 1.2 | 0.8 | 2.2 | 1.4 | 3.2 | 1.1 | 0.9 | 0.7 | 0.9 | 0.9 |
| At4g37990 cinnamyl-alcohol dehydrogenase ELI3-2 | $NADP^+$ oxidoreductase | 0.9 | 3.8 | 0.8 | 2 | 1.2 | 0.8 | 0.9 | 3.1 | 0.8 | 0.9 |
| **At5g13930 chalcone synthase** | flavonoid biosynthesis | 1 | 1 | 1.6 | 2.8 | 3.1 | 1.2 | 1.1 | 0.6 | 1 | 1.4 |
| At5g42800 dihydroflavonol 4-reductase | anthocyanin biosynthesis | 1.1 | 2.8 | 2.7 | 16.7 | 6.1 | 2.3 | 1.1 | 3.9 | 2.5 | 15.5 |
| At2g38240 putative anthocyanidin synthase | anthocyanin biosynthesis | 6.4 | 208 | 10.7 | 86.9 | 30.2 | 7 | 0.8 | 3.3 | 9.3 | 2.4 |
| **At4g22880 putative leucoanthocyanidin dioxygenase (LDOX)** | anthocyanin biosynthesis | 0.7 | 3.6 | 3.3 | 11.5 | 8.7 | 2 | 1.2 | 3.1 | 1.8 | 8 |
| At4g27570 UDP rhamnose-anthocyanidin -3-glucoside rhamnosyltransferase-like | anthocyanin biosynthesis | 1.6 | 2.1 | 3.5 | 15.7 | 8.2 | 1.9 | 0.5 | 2.7 | 1.8 | 7.7 |
| Terpenoids | | | | | | | | | | | |
| **At2g24210 putative limonene cyclase** | monoterpene biosynthesis | 1.4 | 5.8 | 3.7 | 146 | 2.2 | 1.7 | 1 | 0.9 | 1.4 | 1.8 |
| At1g61120 similar to S-linalool synthase | monoterpene biosynthesis | 6.8 | 2.6 | 12.5 | 48.1 | 11.4 | 1.7 | 0.9 | 2 | 1.1 | 1.2 |
| At5g24140 squalene epoxidase homologue | brassinosteroid biosynthesis | 1.4 | 2.8 | 4.1 | 7.5 | 53.4 | 5.1 | 1 | 4.9 | 1 | 4.3 |

Genes from this table used in the experiment of Figure 2 are highlighted in boldface letters.

*RNA gel blot analysis and preparation of DNA probes*

Total RNA from seedlings and plants were isolated as described above. Three independent biological experimental replicates were performed for this analysis and the *Arabidopsis* GeneChip experiments. Extracted RNAs were subjected to electrophoresis on 1.5% formaldehyde/agarose gels and blotted to Hybond $^+$ XL membranes (Amersham). DNA probes for putative berberine bridge enzyme (At2g34810), chalcone synthase (At5g13930); putative leucoanthocyanidin dioxygenase (LDOX-At4g 22880), putative limonene cyclase (At2g24210), tyrosine transaminase (At4g23600), putative strictosidine synthase (At1g74020), senescence-associated protein (*SEN1*- At4g35770); inner mitochondrial membrane translocase (At2g28900), ACC synthase 6 (ACS6- At4g11280); putative nitrate transporter (At2g26690), squalene epoxidase-like protein (At4g 37760) and the auxin-responsive protein AXR2/ IAA7 (At3g23050), have been obtained from *Arabidopsis* (Col-0) genomic DNA by PCR amplification of full-length coding sequence (At2g34810, 1622 bp) or fragments corresponding to entire exons (At5g13930, 994 bp; At4g22880, 497 bp; At2g24210, 388 bp; At4g23600, 340 bp; At1g74020, 900 bp; At4g35770, 450 bp; At4g11280, 1000 bp; At2g26690, 510 bp; At4g37760, 560 bp) or 3′ UTR (At2g28900, 280 bp, At3g23050, 400 bp). Identity of the isolated fragments was confirmed by sequencing. Sequencing, Northern blots and hybridization procedures have been previously described (Ellis and Turner, 2002). Quantification of transcript levels was achieved by measurement of the scanning values of the autoradiographic signals with PhosphorImager STORM840.

## Results and discussion

*Arabidopsis* wild type and *coi1* plants were wounded or treated with MeJA, and changes in the expression of genes were examined by hybridizing RNA samples to an Affymetrix Gene chip representing over 8200 genes, as described by Zhu and Wang (2000). Gene expression data analysis was performed in the R language (Ihaka *et al.*, 1996) together with the Bioconductor package (Gentleman *et al.*, 2004) and GeneSpring™ software (Silicon Genetics, Redwood City, CA, USA).

Details are provided in the data acquisition and analysis section. When wild-type seedlings were wounded or treated with MeJA-treated, 513 unique genes were differentially expressed (Figure 1, Table SI). When *coi1-16* seedlings were wounded or treated with MeJA, a further 19 genes not present among genes affected in the wild type plants, were differentially expressed (Table SII). The *coi1-16* mutant (Ellis and Turner, 2002) was used to examine gene expression in young seedlings because it is conditionally male fertile and can be maintained as a homozygous line. By contrast, the *coi1-1* mutant is male sterile, and the homozygous mutant plants therefore have to be selected from a segregating population; this makes *coi1-1* unsuited for studies on seedlings.

The reproducibility of our microarray data was tested in independent biological replicates, each consisting of a different subset of treated and untreated wild type (Col-*gl1*) and *coi1-16* samples (Table SIII). RNAs prepared from each of the three replicate samples were pooled from at least three independent preparations of at least 15 individuals.

To further judge the reliability of the microarray data, the expression levels of genes known to be induced by MeJA were examined. The MeJA-inducible genes encoding vegetative storage protein (At5g24770 – Benedetti *et al*., 1995), thionin 2.1 (At1g72260 – Bohlmann *et al*., 1998), coronatine-induced protein (At1g19670 – Benedetti *et al*., 1998), myrosinase binding protein At1g52030 (Capella *et al*., 2001), and the jasmonate synthesis genes *LOX2* (At3g45140 – Bell and Mullet, 1993), allene oxide synthase (At5g42650 – Laudert and Weiler, 1998), and OPDA reductase (At2g06050 – Sanders *et al*., 2000; Stintzi and Browse, 2000) were induced by both MeJA and wounding (Table SI). Genes such as *PR5* (At1g75040) and *PDF1.2* (At5g44420), which require both MeJA and ethylene for high levels of expression (Xu *et al*., 1994; Penninckx *et al*., 1998), were not significantly upregulated by MeJA alone.

The consistency of the microarray data for key genes was confirmed by Northern blot analysis (Figure 2). Transcripts levels were analysed for: COI1-dependent induced genes such as putative berberine bridge enzyme (At2g34810), chalcone synthase (At5g13930), putative leucoanthocyanidin dioxygenase (*LDOX* – At4g22880), tyrosine transaminase (At4g23600) and putative strictosidine synthase (At1g74020); for COI1-dependent repressed genes such as senescence-associated protein (*SEN1*- At4g35770) and the auxin-inducible *AXR2*/*IAA7* (At3g23050); COI1-independent induced genes such as and inner mitochondrial membrane translocase (At2g28900) and ACC synthase 6 (*ACS6* – At4g11280); COI1-independent repressed genes such as putative nitrate transporter (*NTP2* – At2g26690), and squalene epoxidase-like protein (At4g37760). Transcripts levels were determined in response to wounding (0.5 h and 6 h) and treatment with MeJA (1 h, 6 h, and 48 h) in both wild type and *coi1*-16 plants. In all cases, in which the intensity of the chip signal was high, a good correlation between chip data and RNA gel blot data was observed (Figure 2). However, when the intensity of the hybridization on the chip was low (eg. At4g22880, At2g24210, At1g61120) hybridization could not be reproducibly detected by RNA gel blot techniques. Level of transcripts accumulation in various organs of untreated wild type and *coi1*-1 plants was also analysed for the COI1-dependent genes At2g34810 and At2g24210 (Figure 2).

### COI1 *regulates the expression of genes for secondary metabolism and defense*

JAs induce the production of secondary metabolites in several plant species (Gundlach *et al*., 1992). Previous macroarray experiments have identified that JA induces some genes for enzymes in secondary metabolism (Sasaki *et al*., 2001). The present study indicates that one of the principal effects of JA is to control secondary metabolism. Of the *COI1*-dependent genes that were strongly induced by both wounding and MeJA (Figure 1, groups IIa and b), approximately 20% encoded enzymes involved in the production of secondary metabolites or compounds such as amino acids that can serve as their precursors. These include genes for the production of a wide range of compounds including alkaloids, anthocyanin and other phenolic compounds, and terpenoid compounds (Table 1). The expression of over half of these genes was induced one hour after treatment.

Phenylalanine ammonia lyase regulates the first committed step in the synthesis of phenolics such as anthocyanins, flavonoids, and lignans. One *COI1*-dependent JA response in Arabidopsis is

an accumulation of anthocyanins (Feys *et al*., 1994). Significantly, several genes involved in the production of anthocyanins were upregulated by wounding and JA, including chalcone synthase (At5g13930), anthocyanidin synthase (At2g38240) and leucoanthocyanidin dioxygenase (At4g22880).

The terpenoids are derived from isopentenyl pyrophosphate and include such physiologically important compounds as plant hormones, phytosteroids, and essential oils. Wounding and MeJA both enhanced and suppressed the expression of several genes for terpenoid biosynthesis. The most highly upregulated genes from this class were the terpene synthase genes, limonene cyclase homolog (At2g24210) and *S*-linalool synthase (At1g61120). These genes may be important for the production of plant volatiles (Chen *et al*., 2003) that might play a role in plant–insect interactions or in plant defence. Interestingly, At2g24210 (*AtTPS10*) belongs to the terpenoid synthases gene family (Aubourg *et al*., 2002) and its expression is induced in *Arabidopsis* plants infested by *Pieris rapae* (van Poecke *et al*., 2001). We show here that higher accumulation of transcripts of limonene cyclase At2g24210 occurs in flowers (Figure 2). One of the closest homologues of *AtTPS10*, At4g16740 (*AtTPS03*), also shows highest expression in flowers and it has been shown to be upregulated in *Arabidopsis* leaves in response to wounding and jasmonate treatment (Faldt *et al*., 2003). Increased *AtTPS03* and *AtTPS10* transcripts correspond to increased levels of (E)-β-ocimene in the headspace of *Arabidopsis* plants (Faldt *et al*., 2003).

Another large class of secondary metabolites is the alkaloids. *Arabidopsis* is known to produce the simple alkaloid camalexin (Tsuji *et al*., 1992), but the synthesis of complex alkaloids has not yet been demonstrated in *A. thaliana* (Facchini *et al*., 2004). Nevertheless, several genes bearing homology to known alkaloid biosynthetic genes are present in the *Arabidopsis* genome (Fabbri *et al*., 2000). These genes may be involved in the production of as yet unidentified alkaloids. It is likewise possible that predictions of enzyme function based on sequence homology are insufficient and these genes may instead be involved in the synthesis of other, related compounds. MeJA induced the transcription of some of these genes, and those required for the synthesis of amino acids, which often serve as precursors of alkaloids (Table 1). These genes included 3-deoxy-D-arabino-heptulosonate phosphate synthase (At4g39980), the first enzyme in the shikimate pathway, a coronatine-inducible tyrosine aminotransferase (At4g23600), the first enzyme in the biosynthesis of tocopherols, which are known to be radical scavengers (Sandorf and Holländer-Czytko, 2002), five genes involved in tryptophan synthesis (Table 1), and the cytochrome p450 gene *CYP79B2* (At4g39950) involved in auxin and indole glucosinolate production (Mikkelsen *et al*., 2000; Zhao *et al*., 2002). Interestingly, a number of other genes involved in defence and alkaloid production, including the genes putative strictosidine synthase (At1g74020), putative berberine bridge enzyme (At2g34810), putative endochitinase (At2g43590), and basic endochitinase (At3g12500), were expressed at lower level in *coi1*-1 flowers than in wild type flowers (Figure 2 and Devoto and Turner unpublished), suggesting that JA may also function to protect vital reproductive tissues from pathogens. This is also in agreement with the observation that the phytotoxin coronatine, which is present in several pathovars of the plant pathogen *Pseudomonas syringae*, induced transcript accumulation of the elicitor-responsive gene encoding the berberine bridge enzyme of *Eschscholtzia californica* (Weiler *et al*., 1994). MeJA also induced transcription of genes involved in synthesis of serine, cysteine, and glutathione, and for the degradation of glucosinolates into toxic by-products including those encoding myrosinase binding protein (At2g39330) and acetone-cyanohydrin lyase (At2g23560 and At2g23620).

The transcription for all of these secondary metabolic genes was dependent on COI1 (Tables 1, SI, and Figure 2). The *coi1-16* allele used in this study is not a null allele (Ellis and Turner, 2002) and some increase in the transcription of most of these secondary metabolic genes was observed in response to wounding and MeJA. However, the transcript levels were typically induced 2–4-fold greater in the wild-type plants than in the *coi1-16* plants. Furthermore, basal expression levels in wild-type plants averaged 1.5-fold greater than in *coi1-16* plants.

Many of these secondary metabolites can inhibit growth of a broad spectrum of pathogens and herbivores (Schmeller *et al*., 1997; Rask *et al*., 2000; Wittstock *et al*., 2002). Some volatile compounds produced in response to JAs can

serve as insect attractants or repellents (Birkett *et al*., 2000). We therefore presume that these compounds contribute to the characterised JA-mediated defences against a broad range of pathogens and insect pests (McConn *et al*., 1997; Pieterse *et al*., 1998; Staswick *et al*., 1998; Thomma *et al*., 1998 Ellis *et al*., 2002). While secondary metabolites provide some defense to plants, many proteins also have anti-microbial or anti-insecticidal properties such as pathogenesis-related (PR) type genes, glucanases, chitinases, and defensins. Some of these are induced by JA and ethylene acting synergistically (Xu *et al*., 1994; Penninckx *et al*., 1998; Glazebrook *et al*., 2003; Lorenzo *et al*., 2003). Few of these genes were strongly upregulated by MeJA alone. Only thionin 2.1 (At1g72260) and beta-1,3-glucanase 2 (At3g57260) were induced by wounding and MeJA, whereas others genes including chitinases (At2g43590 and At3g54420), thaumatin-like protein PR5 (At1g75040), and *PDF1.2*, whose expression can be induced by a combination of ethylene and jasmonate, were not induced under these experimental conditions. Interestingly, although *PDF1.2* expression is abolished in the strong *coi1-1* mutant (Penninckx *et al*., 1998), expression was induced in the *coi1-16* seedlings by both wounding and MeJA. A similar phenomenon is observed in the *jin1* mutant that, like *coi1-16* plants, has reduced response to MeJA (Berger *et al*., 1996; Lorenzo *et al*., 2004). The induction of *PDF1.2* expression by MeJA is also greater in *jin1* plants than in wild type plants, suggesting that the level of jasmonate signal may be important in determining *PDF1.2* expression.

The expression of several peroxidases was enhanced, while that of others was suppressed, by wounding and by MeJA (Table SV). Peroxidases have diverse functions in plant defence response: they mediate the hypersensitive response (Feys and Parker, 2000), act as a secondary messenger for MeJA (Orozco-Cardenas *et al*., 2001), protect against ozone and superoxide radicals (Overmyer *et al*., 2000; Rao *et al*., 2000), and initiate the polymerisation of monolignans to lignin. The products of peroxidase genes that were induced by MeJA, including At1g49570, At4g11290, and At5g19890 are therefore candidates for enzymes that facilitate JA signalling and/or lignin synthesis.

*Signalling genes*

Of the 508 genes differentially expressed in response to MeJA and wounding in these microarray experiments, 59 were genes commonly involved in signal transduction (Table S IV).

Some members of the *MYB* family of transcription factors were upregulated by wounding and by MeJA, including *MYB34* (At5g60890), *MYB47* (At1g18710), *MYB44* (At5g67300), and *MYB75* (At1g56650). A dominant mutation in *MYB34* has previously been identified as *atr1*-D, which deregulates tryptophan biosynthesis (Bender and Fink, 1998). This gene, and the related *MYB47* (Kranz *et al*., 1998), were rapidly induced by both wounding and by MeJA. A T-DNA activation-tagged mutation in the gene *MYB75*, *pap1*-D, increases pigment production and upregulates genes for phenylpropanoid synthesis (Borevitz *et al*., 2000). Other *MYB* genes, such as CCA1 (At1g01060), were also repressed by wounding and/or MeJA. MYB proteins may work in conjunction with bHLH proteins to control transcription of pigmentation genes (Quattrocchio *et al*., 1998). Our results indicate that wounding and MeJA rapidly induce the expression of bHLH genes, such as At2g22770, while the bHLH gene At4g17880 was repressed by wounding.

Ethylene and JA can act antagonistically (Rojo *et al*., 1999; Ellis and Turner, 2002) and synergistically (Lorenzo *et al*., 2003, 2004) in regulating expression of different sets of genes. Wounding induced the ACC synthase 6 gene (ACS6 - At4g11280), involved in ethylene biosynthesis, in a COI1-independent manner (Table S1, Figure 2L). Furthermore, the ethylene response factors *ERF1* (At4g17500), *ERF2* (At5g47220) and *ERF4* (At3g15210), which mediate response to ethylene and synergistic interactions between MeJA and ethylene (Lorenzo *et al*., 2003), were induced by MeJA and wounding, but *ERF5* (At5g47230) expression was repressed. Possibly these genes are involved in secondary product formation, because the AP2/ERF-like protein *ORCA3* is required for the expression of terpenoid indole alkaloid production genes in response to JA in *Catharanthus roseus* (van der Fits and Memelink, 2000). Alternatively they may be directly involved in JA signalling and it may be significant therefore that *ERF1* mediates synergistic responses between JA and ethylene in

*Arabidopsis* (Lorenzo *et al*., 2003, 2004). Other AP2 domain containing genes that were induced by both treatments included *RAP2.6* (At1g43160), and *RAP2.9* (At4g06746).

Although Cheong *et al*. (2002) observed that many auxin-regulated genes were repressed by wounding, few were found to be downregulated by MeJA in this study. The auxin-inducible protein *AXR2/IAA7* gene (At3g23050) a negative regulator of auxin signalling (Nagpal *et al*., 2000), was repressed by wounding and MeJA in a COI1-independent manner while two auxin efflux carriers, At1g23080 and At5g57090, are induced by wounding or MeJA. In addition, several of the genes required for tryptophan and indole glucosinolate production also function in the synthesis of auxin (Zhao *et al*., 2002), suggesting that JAs have the potential to alter auxin levels.

Numerous heat shock genes and WRKY transcription factors have enhanced transcription in wounded mature rosette leaves (Cheong *et al*., 2002). The *WRKY70* gene has been recently demonstrated to be a common component in SA and JA-mediated signal pathways (Li *et al*., 2004). *WRKY70* is downstream of *NPR1* in a SA-dependent signal pathway and analysis of overexpressing lines revealed that it acts as an activator of SA-induced genes and as a repressor of JA-responsive genes (Li *et al*., 2004). In this study, expression of the transcription factor-like genes *WRKY15* (At2g23320) and *WRKY33* (At2g38470) were induced in wounded seedlings, but no *WRKY* genes were induced by MeJA. Likewise, the putative small heat shock protein At2g29500, heat shock protein 17.6A At5g12030, heat shock protein At3g46230, heat shock protein 17.6-II At5g12020, and 17.6 kDa heat shock protein At1g53540 were also induced in wounded seedlings in a *COI1*-independent manner, but not in MeJA-treated seedlings. It thus appears likely that these *WRKY* and *HSF* genes do not play a major role in the JA-mediated wound response.

*Genes repressed by MeJA*

A survey of the genes that were repressed by MeJA identified many genes that have been implicated in abscisic acid and drought stress response. These include the *ATHB-12* transcription factor (At3g61890, Lee and Chun 1998), the bZIP transcription factor *ABF3* (At4g34000, Kang *et al*., 2002) and *COR47* (At1g20440) and *LEA D113* (At5g06760). The nitrate transporter *NTP2* (At2g26690) and three members of the aquaporin family of transporters, At2g40900, At3g16240, and At4g17340, were also repressed by MeJA in a COI1-independent manner. These findings reinforce the role of jasmonic acid in osmotic homeostasis and are complementary to the study of Armengaud *et al*. (2004). However, other abiotic stress-responsive genes such as *RD21A* (At4g11320), *COR15b* (At2g42530), trehalose-6-phosphate phosphatase (At4g22590) and trehalose-6-phosphate synthase (At4g17770) were induced by MeJA and/or by wounding. These results are similar to those observed by Anderson *et al*. (2004), who found a complex relationship between abscisic acid and jasmonate signalling pathways. Responses to drought and pathogen challenge were generally antagonistic, whereas wounding resulted in generally positive interactions. The different expression patterns observed for abscisic acid-inducible genes in response to MeJA in this microarray study may therefore reflect the different functions of the individual genes. MeJA-repressed genes such as the aquaporins may therefore function in drought response whereas genes for trehalose synthesis may be involved in ABA-regulated wound response. Also repressed in a COI1-independent manner was a squalene epoxidase-like protein (At4g37760). A *Medicago truncatula* homologue of this gene has been functionally characterized and was demonstrated to be involved in the biosynthesis of the antimicrobial compounds triterpene glycoside saponins (Suzuki *et al*., 2002).

In addition, senescence-regulated genes were also repressed by wounding and MeJA. The expression of senescence associated gene *SEN1* (At4g35770) is repressed in Col-*gl1* (Figure 2M), but in the *coi1-16* background, this gene is induced by wounding and MeJA. This is similar to the expression pattern of *PDF1.2* and demonstrates the existence of COI1-independent JA signalling. Other genes included *SENESCENCE-ASSOCIATED GENE (SAG) 29*, (At5g13170) and a cysteine proteinase gene similar to *SAG12* (At2g27420). JA levels increase in senescing leaves and (He *et al*., 2002) and JA induces the expression of many SAGs (He *et al*., 2001) so it may seem counterintuitive that JA also represses SAG expression, However, previous studies have

demonstrated that expression profiles during natural senescence and induced senescence are not identical. Furthermore, most studies on senescence use mature leaf tissue and these genes may have a different function in young seedlings.

*The role of* COI1 *and MeJA in the wound response*

Of the 153 wound induced genes identified in this study, 80 were also induced by treatment with MeJA. Similarly, of the 84 wound repressed genes, 39 were also repressed by treatment with MeJA (Figure 3). Whilst the exact amount, timing, and localisation of JA synthesis in wounded seedlings is probably not exactly reproduced by the application of exogenous MeJA, this study indicates that approximately one half of the wound responsive genes may be responding to JA produced in the wounded plant. This reveals a potentially fundamental role for JA in the wound response. Of the 236 genes expressed differently in wounded plants, 106 (45%) apparently required *COI1* whereas other genes were *COI*1 independent (Figure 1, groups Ia and b; Table SVII). Of the 316 genes induced or repressed by MeJA, 233 (74%) required *COI1* (Figure 1, groups II and IV). However, transcription of 82 (26%) JA-responsive genes did not require *COI1*. Therefore, *COI1* regulates a considerable number of genes whose expression responds to MeJA. Many of the wound-induced genes were also repressed by treatment with MeJA, and/or were expressed at a higher level in the *coi1*-16 mutant (Figure 1, group III; Table SII).

We have depicted major categories of the wound signal transduction network mediated by JA and COI1 (Figure 4A). Wounding induced the production of secondary metabolism genes, vegetative storage genes, and some defense genes in a COI1-dependent manner and this was likely mediated by JA. Wounding also induced the production of ethylene biosynthetic genes and heat shock genes in a COI1-independent manner. Several drought response genes were repressed by wounding and MeJA independently of COI1, which may allow the plant to suppress the inhibitory effect of ABA-regulated genes on the JA signaling pathway (Anderson *et al*., 2004). In addition, genes were repressed by wounding and MeJA via COI1, including some genes whose expression is induced by senescence.

## Conclusions

A number of recent microarray studies have sought to define wound-, jasmonate-, and *COI1*-regulated genes. Differences in tissues analysed, treatment times, and genes probed make direct comparisons of individual genes difficult. However, by examining the cellular processes affected in these studies, common themes can be found. Many of these studies cite the upregulation of defence and stress response genes (Reymond *et al*., 2000; Schenk *et al*., 2000; Sasaki *et al*., 2001; Cheong *et al*., 2002; Glazebrook *et al*., 2003; Mandaokar *et al*., 2003; Puthoff *et al*., 2003; vanWees *et al*., 2003), amino acid synthesis and secondary metabolic genes (Schenk *et al*., 2000; Sasaki *et al*., 2001; Cheong *et al*., 2002; Feng

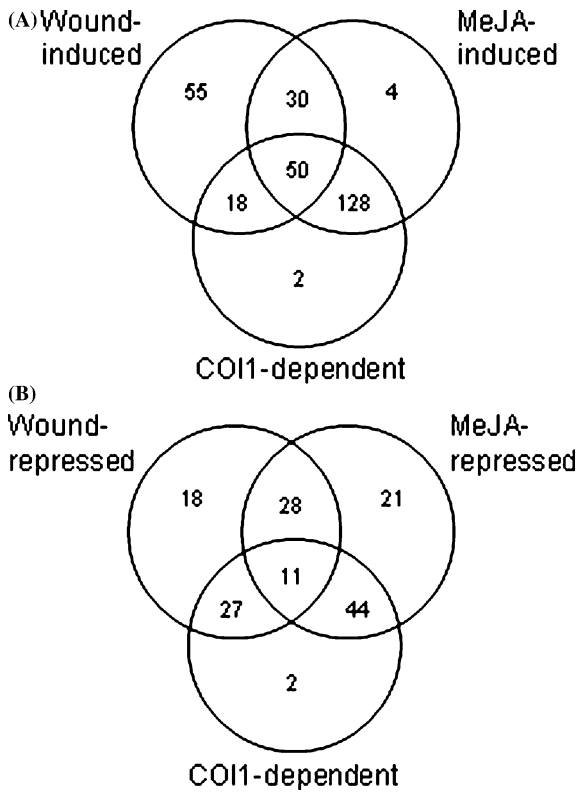


*Figure 3*. Venn diagrams of the number of genes differentially regulated at least 2.5 fold by wounding or MeJA treatment after 0.5h, 1h, or 6h, in wild type and *coi1*-16 backgrounds. (A) Genes upregulated by wounding or MeJA. (B) Genes downregulated by wounding or MeJA. *COI1*-independent genes were defined as those whose expression levels differed by less than 2.5 fold from that of similarly treated wild type plants.

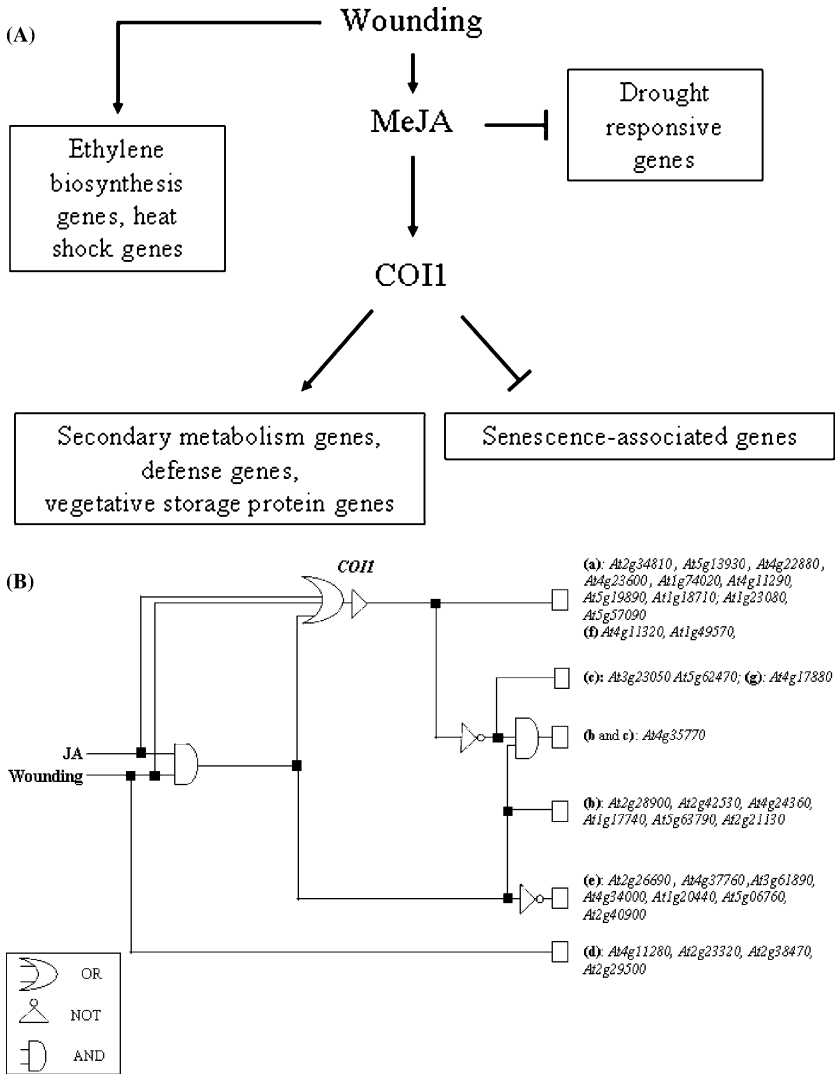


*Figure 4*. Signal transduction network mediated by jasmonate and COI1. (A) Representation of the major genes categories grouped by function. Details are described in the text. Arrows indicate induction of gene expression and blunt ends indicate repression. (B) Boolean representation (Devoto and Turner, 2005) of the signal transduction network mediated by jasmonate (JA), and COI1. A wound signal may induce the production of JA which will stimulate the expression of JA-responsive genes in a COI1-dependent or independent manner. Eight representative categories of genes are depicted. (a): MeJA, via COI1, has been found to induce the expression of genes such as At2g34810, At5g13930, At4g22880, At4g23600, At1g74020, At4g11290, At5g19890, At1g18710, At1g23080 and At5g57090. These genes are also induced by wounding and this might be mediated by JA. (b): MeJA and wounding also induce, independently of COI1, the expression of genes such as At2g28900, At2g42530, At4g24360, At1g17740, At5g63790, At2g21130 and At4g35770. (c): intriguingly, At4g35770 is also repressed by wounding and MeJA, in a COI1-dependent manner, similarly to the genes At5g62470 and At3g23050. (d): genes such as At4g11280, At2g23320, At2g38470, At2g29500 were induced by wounding but not by MeJA in a COI-independent manner. (e): MeJA and wound-mediated COI1-independent repression occurs for At2g26690, At4g37760, At3g61890, At4g34000, At1g20440, At5g06760, and At2g40900. (f): COI1-mediated, MeJA but not wound- induction, occurs for genes such as At4g11320 and, At1g49570. (g) genes such as At4g17880 were repressed by wounding only in a COI-dependent manner. AGI numbers and abbreviation of names of genes are in italics; genes whose expression has been analyzed by Northern blot are in bold italic. COI1, coronatine insensitive1. Boolean operator OR: alternative induction by wounding or JA or their combination.

*et al*., 2003; Mandaokar *et al*., 2003), and cell wall synthesis (Schenk *et al*., 2000; Cheong *et al*., 2002; Feng *et al*., 2003). In addition, numerous AP2/ERF (Schenk *et al*., 2000; Chen *et al*., 2002; Cheong *et al*., 2002; Feng *et al*., 2003; Lorenzo *et al*., 2003) and MYB transcription factors (Chen *et al*., 2002; Cheong *et al*., 2002) have been identified as being wound- and jasmonate-responsive. The *coi1* mutant has been used so far mainly to analyse transcriptional profile changes in response to pathogens such as *Pseudomonas syringae* (Chen *et al*., 2002; Cheong *et al*., 2002; Glazebrook *et al*., 2003), as *coi1* plants display enhanced resistance (Feys *et al*., 1994; Kloek *et al*., 2001), and *Alternaria brassicicola* (vanWees *et al*., 2003) or insect attack such as *Pieris rapae* (Reymond *et al*., 2000, 2004) to which *coi1* plants exhibit increased susceptibility (Thomma *et al*., 1998, 1999). These studies were consistent with the idea that JA is an important signalling molecule in response to these pathogens.

A number of interesting discoveries have arisen from this study. First, a substantial amount of JA-regulated transcription is *COI1*-dependent, supporting the idea that *COI1* acts as a principal control of JA signaling. Nonetheless, we have revealed that in *Arabidopsis*, expression of several JA induced genes might not require *COI1*. We have depicted, using Boolean symbolism (Genoud *et al.,* 2001; Devoto and Turner, 2005), representative categories of genes in Figure 4B which highlights the complexity of the signal transduction network mediated by JA and COI1. Second, JA-regulated and *COI1*-regulated genes account for approximately half of the wound-regulated transcription surveyed in this study. Genes that were induced both by wounding and MeJA generally required *COI1*, while ethylene-, SA-, and drought-responsive gene expression was occasionally suppressed by *COI1*. Third, it appears that one key function of JA and *COI1* is to initiate the expression of many secondary metabolic genes. For anthocyanin, glutathione, and indole glucosinolate synthesis, several pathway genes are coordinately regulated by MeJA and wounding. Jasmonic acid induces secondary product formation in many plants such as *Catharanthus roseus* (Gundlach *et al*., 1992) and it improves yield of the secondary product drugs (Roberts and Shuler, 1997). Few signaling genes have been previously linked to JA signal transduction. This study has identified several candidate genes that may be involved in JA perception and should assist the further definition of JA signaling mechanisms.

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## References

Anderson, J.P., Badruzsaufari, E., Schenk, P.M., Manners, J.M., Desmond, O.J., Ehlert, C., Maclean, D.J., Ebert, P.R. and Kazan, K. 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell 16: 3460–3479.

Armengaud, P., Breitling, R. and Amtmann, A. 2004. The potassium-dependent transcriptome of *Arabidopsis* reveals a prominent role of jasmonic acid in nutrient signaling. Plant Physiol. 136: 2556–2576.

Aubourg, S., Lecharny, A. and Bohlmann, J. 2002. Genomic analysis of the terpenoid synthase (AtTPS) gene family of *Arabidopsis thaliana*. Mol. Genet. Genomics 267: 730–745.

Bell, E. and Mullet, J.E. 1993. Characterization of an *Arabidopsis* lipoxygenase gene responsive to methyl jasmonate and wounding. Plant Physiol. 103: 1133–1137.

Bender, J. and Fink, G.R. 1998. A Myb homologue, ATR1, activates tryptophan gene expression in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 95: 5655–5660.

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.

Benedetti, C.E. and Arruda, P. 2002. Altering the expression of the chlorophyllase gene ATHCOR1 in transgenic *Arabidopsis* caused changes in the chlorophyll-to-chlorophyllide ratio. Plant Physiol. 128: 1255–1263.

Benjamini, Y. and Hochberg, Y. 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J. Roy. Stat. Soc. B 57: 289–300.

Birkett, M.A., Campbell, C.A., Chamberlain, K., Guerrieri, E., Hick, A.J., Martin, J.L., Matthes, M., Napier, J.A., Pettersson, J., Pickett, J.A., Poppy, G.M., Pow, E.M., Pye, B.J., Smart, L.E., Wadhams, G.H., Wadhams, L.J. and Woodcock, C.M. 2000. New roles for *cis*-jasmone as an insect semiochemical and in plant defense. Proc. Natl. Acad. Sci. USA 97: 9329–9334.

Bohlmann, H., Vignutelli, A., Hilpert, B., Miersch, O., Wasternack, C. and Apel, K. 1998. Wounding and chemicals

induce expression of the *Arabidopsis thaliana* gene Thi2.1, encoding a fungal defense thionin, via the octadecanoid pathway. FEBS Lett. 437: 281–286.

Borevitz, J.O., Xia, Y., Blount, J., Dixon, R.A. and Lamb, C. 2000. Activation tagging identifies a conserved MYB regulator of phenylpropanoid biosynthesis. Plant Cell 12: 2383–2394.

Brader, G., Tas, E. and Palva, E.T. 2001. Jasmonate-dependent induction of indole glucosinolates in *Arabidopsis* by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. Plant Physiol. 126: 849–860.

Capella, A.N., Menossi, M., Arruda, P. and Benedetti, C.E. 2001. COI1 affects myrosinase activity and controls the expression of two flower-specific myrosinase-binding protein homologues in *Arabidopsis*. Planta 213: 691–699.

Chen, W., Provart, N.J., Glazebrook, J., Katagiri, F., Chang, H.S., Eulgem, T., Mauch, F., Luan, S., Zou, G., Whitham, S.A., Budworth, P.R., Tao, Y., Xie, Z., Chen, X., Lam, S., Kreps, J.A., Harper, J.F., Si-Ammour, A., Mauch-Mani, B., Heinlein, M., Kobayashi, K., Hohn, T., Dangl, J.L., Wang, X. and Zhu, T. 2002. Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. Plant Cell 14: 559–574.

Chen, F., Tholl, D., D'Auria, J.C., Farooq, A., Pichersky, E. and Gershenzon, J. 2003. Biosynthesis and emission of terpenoid volatiles from *Arabidopsis* flowers. Plant Cell 15: 481–494.

Cheong, Y.H., Chang, H.S., Gupta, R., Wang, X., Zhu, T. and Luan, S. 2002. Transcriptional profiling reveals novel interactions between wounding, pathogen, abiotic stress, and hormonal responses in *Arabidopsis*. Plant Physiol. 129: 661–677.

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.

Devoto, A., Nieto-Rostro, M., Xie, D., Ellis, C., Harmston, R., Patrick, E., Davis, J., Sherratt, L., Coleman, M. and Turner, J.G. 2002. COI1 links jasmonate signalling and fertility to the SCF ubiquitin-ligase complex in *Arabidopsis*. Plant J. 32: 457–466.

Devoto, A. and Turner, J.G. 2003. Regulation of jasmonate-mediated plant responses in *Arabidopsis*. Ann. Bot. (Lond) 92: 329–337.

Devoto, A. and Turner, J.G. 2005. Jasmonate-regulated *Arabidopsis* stress signalling network. Phys. Plant 123: 161–172.

Eisen, M.B., Spellman, P.T., Brown, P.O. and Botstein, D. 1998. Cluster analysis and display of genome-wide expression patterns. Proc. Natl. Acad. Sci. USA 95: 14863–14868.

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 signalling pathways in *Arabidopsis thaliana* seeds and young seedlings. Planta 215: 549–556.

Ellis, C., Karafyllidis, I. and Turner, J.G. 2002. Constitutive activation of jasmonate signaling in an *Arabidopsis* mutant correlates with enhanced resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. Mol. Plant–Microbe. Interact. 15: 1025–1030.

Fabbri, M., Delp, G., Schmidt, O. and Theopold, U. 2000. Animal and plant members of a gene family with similarity to alkaloid-synthesizing enzymes. Biochem. Biophys. Res. Commun. 271: 191–196.

Facchini, P.J., Bird, D.A. and St-Pierre, B. 2004. Can *Arabidopsis* make complex alkaloids?. Trend. Plant Sci. 9: 116–122.

Faldt, J., Arimura, G., Gershenzon, J., Takabayashi, J. and Bohlmann, J. 2003. Functional identification of AtTPS03 as (E)-beta-ocimene synthase: a monoterpene synthase catalyzing jasmonate- and wound-induced volatile formation in *Arabidopsis thaliana*. Planta 216: 745–751.

Farmer, E.E. and Ryan, C.A. 1990. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. Proc. Natl. Acad. Sci. USA 87: 7713–7716.

Feng, S., Ma, L., Wang, X., Xie, D., Dinesh-Kumar, S.P., Wei, N. and Deng, X.W. 2003. The COP9 signalosome interacts physically with SCF COI1 and modulates jasmonate responses. Plant Cell 15: 1083–1094.

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

Genoud, T., Trevino Santa Cruz, M.B. and Metraux, J.P. 2001. Numeric simulation of plant signaling networks. Plant Physiol. 126: 1430–1437.

Glazebrook, J., Chen, W., Estes, B., Chang, H.S., Nawrath, C., Metraux, J.P., Zhu, T. and Katagiri, F. 2003. Topology of the network integrating salicylate and jasmonate signal transduction derived from global expression phenotyping. Plant J. 34: 217–228.

Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J., Hornik, K., Hothorn, T., Huber, W., Iacus, S., Irizarry, R., Leisch, F., Li, C., Maechler, M., Rossini, A.J., Sawitzki, G., Smith, C., Smyth, G., Tierney, L., Yang, J.Y.H. and Zhang, J. 2004. Bioconductor: Open software development for computational biology and bioinformatics. Genome. Biol. 5: R80.

Glonek, G.F.V. and Solomon, P.J. 2004. Factorial and time course designs for cDNA microarray experiments. Biostatistics 5: 89–111.

Gundlach, H., Muller, 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.

Harmer, S.L., Hogenesch, J.B., Straume, M., Chang, H.S., Han, B., Zhu, T., Wang, X., Kreps, J.A. and Kay, S.A. 2000. Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock. Science 290: 2110–2113.

Kang, J.Y., Choi, H.I., Im, M.Y. and Kim, S.Y. 2002. *Arabidopsis* basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. Plant Cell 14: 343–357.

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.

Kranz, H.D., Denekamp, M., Greco, R., Jin, H., Leyva, A., Meissner, R.C., Petroni, K., Urzainqui, A., Bevan, M., Martin, C., Smeekens, S., Tonelli, C., Paz-Ares, J. and Weisshaar, B. 1998. Towards functional characterisation of the members of the R2R3-MYB gene family from *Arabidopsis thaliana*. Plant J. 16: 263–276.

Ihaka, R. and Gentleman, R. 1996. R: A language for data analysis and graphics. J. Comput. Graph. Stat. 5: 299–314.

Laudert, D. and Weiler, E.W. 1998. Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling. Plant J. 15: 675–684.

Lee, Y.H. and Chun, J.Y. 1998. A new homeodomain-leucine zipper gene from *Arabidopsis thaliana* induced by water stress and abscisic acid treatment. Plant Mol. Biol. 37: 377–384.

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.

Liscum, E. and Reed, J.W. 2002. Genetics of Aux/IAA and ARF action in plant growth and development. Plant Mol. Biol. 49: 387–400.

Lorenzo, O., Piqueras, R., Sanchez-Serrano, J.J. and Solano, R. 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell 15: 165–178.

Lorenzo, O., Chico, J.M., Sanchez-Serrano, J.J. and Solano, R. 2004. Jasmonate-insensitive1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defence responses in *Arabidopsis*. Plant Cell 16: 1938–50.

Mandaokar, A., Dinesh Kumar, V., Amway, M. and Browse, J. 2003. Microarray and differential display identify genes involved in jasmonate-dependent anther development. Plant Mol. Biol. 52: 775–786.

McConn, M. and Browse, J. 1996. The critical requirement for linolenic acid is pollen development, not photosynthesis, in an *Arabidopsis* Mutant. Plant Cell 8: 403–416.

McConn, M., Creelman, R.A., Bell, E., Mullet, J.E. and Browse, J. 1997. Jasmonate is essential for insect defense in *Arabidopsis*. Proc. Natl. Acad. Sci. USA 94: 5473–5477.

Menke, F.L., Champion, A., Kijne, J.W. and Memelink, J. 1999. A novel jasmonate- and elicitor-responsive element in the periwinkle secondary metabolite biosynthetic gene Str interacts with a jasmonate- and elicitor-inducible AP2-domain transcription factor, ORCA2. Embo. J. 18: 4455–4463.

Mikkelsen, M.D., Hansen, C.H., Wittstock, U. and Halkier, B.A. 2000. Cytochrome P450 CYP79B2 from *Arabidopsis* catalyzes the conversion of tryptophan to indole-3-acetaldoxime, a precursor of indole glucosinolates and indole-3-acetic acid. J. Biol. Chem. 275: 33712–33717.

Nagpal, P., Walker, L.M., Young, J.C., Sonawala, A., Timpte, C., Estelle, M. and Reed, J.W. 2000. AXR2 encodes a member of the Aux/IAA protein family. Plant Physiol. 123: 563–574.

Orozco-Cardenas, M., Narvaez-Vasquez, J. and Ryan, C. 2001. Hydrogen peroxide acts as a second messenger for the induction of defense genes in tomato plants in response to wounding, systemin, and methyl jasmonate. Plant Cell 13: 179–191.

Overmyer, K., Tuominen, H., Kettunen, R., Betz, C., Langebartels, C., Sandermann, H. and Kangasjarvi, J. 2000. Ozone-sensitive *Arabidopsis* rcd1 mutant reveals opposite roles for ethylene and jasmonate signaling pathways in regulating superoxide-dependent cell death. Plant Cell 12: 1849–1862.

Penninckx, I.A., Thomma, B.P., 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.

Pieterse, C.M., vanWees, S.C., vanPelt, J.A., Knoester, M., Laan, R., Gerrits, H., Weisbeek, P.J. and vanLoon, L.C. 1998. A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10: 1571–1580.

Puthoff, D.P., Nettleton, D., Rodermel, S.R. and Baum, T.J. 2003. *Arabidopsis* gene expression changes during cyst nematode parasitism revealed by statistical analyses of microarray expression profiles. Plant J. 33: 911–921.

Quattrocchio, F., Wing, J.F., van derWoude, K., Mol, J.N. and Koes, R. 1998. Analysis of bHLH and MYB domain proteins: species-specific regulatory differences are caused by divergent evolution of target anthocyanin genes. Plant J. 13: 475–488.

Rao, M.V., Lee, H., Creelman, R.A., Mullet, J.E. and Davis, K.R. 2000. Jasmonic acid signaling modulates ozone-induced hypersensitive cell death. Plant Cell 12: 1633–1646.

Rask, L., Andreasson, E., Ekbom, B., Eriksson, S., Pontoppidan, B. and Meijer, J. 2000. Myrosinase: gene family evolution and herbivore defense in Brassicaceae. Plant Mol. Biol. 42: 93–113.

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: 707–720.

Reymond, P., Bodenhausen, N., VanPoecke, R.M., Krishnamurthy, V., Dicke, M. and Farmer, E.E. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. Plant Cell 16: 3132–3147.

Roberts, S.C. and Shuler, M.L. 1997. Large-scale plant cell culture. Curr. Opin. Biotechnol. 8: 154–159.

Rojo, E., Titarenko, E., Leon, J., Berger, S., Vancanneyt, G. and Sanchez-Serrano, J.J. 1998. Reversible protein phosphorylation regulates jasmonic acid-dependent and -independent wound signal transduction pathways in *Arabidopsis thaliana*. Plant J. 13: 153–165.

Rojo, E., Leon, J. and Sanchez-Serrano, J.J. 1999. Cross-talk between wound signalling pathways determines local versus systemic gene expression in *Arabidopsis thaliana*. Plant J. 20: 135–142.

Sanders, P.M., Lee, P.Y., Biesgen, C., Boone, J.D., Beals, T.P., Weiler, E.W. and Goldberg, R.B. 2000. The *Arabidopsis* DELAYED DEHISCENCE1 gene encodes an enzyme in the jasmonic acid synthesis pathway. Plant Cell 12: 1041–1061.

Sandorf, I. and Holländer-Czytko, H. 2002. Jasmonate is involved in the induction of tyrosine aminotransferase and tocopherol biosynthesis in *Arabidopsis thaliana*. Planta 216: 173–179.

Sasaki, Y., Asamizu, E., Shibata, D., Nakamura, Y., Kaneko, T., Awai, K., Amagai, M., Kuwata, C., Tsugane, T., Masuda, T., Shimada, H., Takamiya, K., Ohta, H. and Tabata, S. 2001. Monitoring of methyl jasmonate-responsive genes in *Arabidopsis* by cDNA macroarray: self-activation of jasmonic acid biosynthesis and crosstalk with other phytohormone signaling pathways. DNA Res. 8: 153–161.

Schenk, P.M., Kazan, K., Wilson, I., Anderson, J.P., Richmond, T., Somerville, S.C. and Manners, JM 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. Proc. Natl. Acad. Sci. USA 97: 11655–11660.

Schmeller, T.T., Latz-Bruning, B. and Wink, M. 1997. Biochemical activities of berberine, palmatine and sanguin-

arine mediating chemical defence against microorganisms and herbivores. Phytochemistry 44: 257–266.
Searle, S.R. 1971. Linear Models. Wiley, New York.
Smyth, G.K. 2004. Linear models and empirical Bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3: 1–25.
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.
Staswick, P.E., Tiryaki, I. and Rowe, M.L. 2002. Jasmonate response locus JAR1 and several related *Arabidopsis* genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. Plant Cell 14: 1405–1415.
Stintzi, A. and Browse, J. 2000. The *Arabidopsis* male-sterile mutant, opr3, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. Proc. Natl. Acad. Sci. USA 97: 10625–10630.
Suzuki, H., Achnine, L., Xu, R., Matsuda, S.P.T. and Dixon, R.A. 2002. A genomics approach to the early stages of triterpene saponin biosynthesis in *Medicago truncatula*. Plant J. 32: 1033–1048.
Thomma, B.P., Eggermont, K., Penninckx, I., 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. USA 95: 15107–15111.
Thomma, B.P., Nelissen, I., Eggermont, K. and Broekaert, W.F. 1999. Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicicola*. Plant J. 1999 19: 163–171.
Titarenko, E., Rojo, E., Leon, J. and Sanchez-Serrano, J.J. 1997. Jasmonic acid-dependent and -independent signaling pathways control wound-induced gene activation in *Arabidopsis thaliana*. Plant Physiol. 115: 817–826.
Tsuji, J., Jackson, E.P., Gage, D.A., Hammerschmidt, R. and Somerville, S.C. 1992. Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to *Pseudomonas syringae* pv syringae. Plant Physiol. 98: 1304–1309.
Turner, J.G., Ellis, C. and Devoto, A. 2002. The jasmonate signal pathway. Plant Cell 14(Suppl): S153–S164.
van derFits, L. and Memelink, J. 2000. ORCA3, a jasmonate-responsive transcriptional regulator of plant primary and secondary metabolism. Science 289: 295–297.
VanPoecke, R.M., Posthumus, M.A. and Dicke, M. 2001. Herbivore-induced volatile production by *Arabidopsis thaliana* leads to attraction of the parasitoid *Cotesia rubecula*: chemical, behavioral, and gene-expression analysis. J. Chem. Ecol. 27: 1911–28.
Weiler, E.W., Kutchan, T.M., Gorba, T., Brodschelm, W., Niesel, U. and Bublitz, F. 1994. The *Pseudomonas* phytotoxin coronatine mimics octadecanoid signalling molecules of higher plants. FEBS Lett. 345: 9–13.
Wernisch, L., Kendall, S.L., Soneji, S., Wietzorrek, A., Parish, T., Hinds, J., Butcher, P.D. and Stoker, N.G. 2003. Analysis of whole-genome microarray replicates using mixed models. Bioinformatics 19: 53–61.
Wittstock, U. and Halkier, B.A. 2002. Glucosinolate research in the *Arabidopsis* era. Trend. Plant Sci. 7: 263–270.
Wu, Z., Irizarry, R.A., Gentleman, R., Martinez-Murillo, F. and Spencer, F. 2004. A model-based background adjustment for oligonucleotide expression arrays. J. Am. Stat. Assoc. 99: 909–917.
Xie, D.X., Feys, B.F., James, S., Nieto-Rostro, M. and Turner, J.G. 1998. COI1: an *Arabidopsis* gene required for jasmonate-regulated defense and fertility. Science 280: 1091–1094.
Xu, Y., Chang, P., 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.
Xu, L., Liu, F., Lechner, E., Genschik, P., Crosby, W.L., Ma, H., Peng, W., Huang, D. and Xie, D. 2002. The SCF(COI1) ubiquitin-ligase complexes are required for jasmonate response in *Arabidopsis*. Plant Cell 14: 1919–1935.
Zhao, Y., Hull, A.K., Gupta, N.R., Goss, K.A., Alonso, J., Ecker, J.R., Normanly, J., Chory, J. and Celenza, J.L. 2002. Trp-dependent auxin biosynthesis in *Arabidopsis*: involvement of cytochrome P450s CYP79B2 and CYP79B3. Genes Dev. 16: 3100–3112.
Zhu, T. and Wang, X. 2000. Large-scale profiling of the *Arabidopsis* transcriptome. Plant Physiol. 124: 1472–6.
Zhu, T., Budworth, P., Han, B., Brown, D., Chang, H-S., Zou, G. and Wang, X. 2001. Toward elucidating the global gene expression patterns of developing *Arabidopsis*: Parallel analysis of 8300 genes by a high-density oligonucleotide probe array. Plant Physiol. Biochem. 39: 221–242.