The Plant Journal (2004) **40**, 633–646 doi: 10.1111/j.1365-313X.2004.02236.x

Host and non-host pathogens elicit different jasmonate/ethylene responses in *Arabidopsis*

Laurent Zimmerli[1], Mónica Stein[1,2], Volker Lipka[3,†], Paul Schulze-Lefert[3] and Shauna Somerville[1,*]
[1]*Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305, USA,*
[2]*Department of Biological Sciences, Stanford University, Stanford, CA 94305, USA, and*
[3]*Department of Plant–Microbe Interactions, Max Planck Institute for Plant Breeding, Köln D-50829, Germany*

Received 15 June 2004; revised 22 August 2004; accepted 25 August 2004.
[*]For correspondence (fax +650 325 6857; e-mail ssomerville@stanford.edu).
[†]Present address: Center for Plant Molecular Biology, Plant Biochemistry, University of Tübingen, Auf der Morgenstelle 5, Tübingen D-72076, Germany.

Summary

***Arabidopsis* does not support the growth and asexual reproduction of the barley pathogen, *Blumeria graminis* f. sp. *hordei Bgh*). A majority of germlings fail to penetrate the epidermal cell wall and papillae. To gain additional insight into this interaction, we determined whether the salicylic acid (SA) or jasmonate (JA)/ethylene (ET) defence pathways played a role in blocking barley powdery mildew infections. Only the *eds1* mutant and *NahG* transgenics supported a modest increase in penetration success by the barley powdery mildew. We also compared the global gene expression patterns of *Arabidopsis* inoculated with the non-host barley powdery mildew to those inoculated with a virulent, host powdery mildew, *Erysiphe cichoracearum.* Genes repressed by inoculations with non-host and host powdery mildews relative to non-inoculated control plants accounted for two-thirds of the differentially expressed genes. A majority of these genes encoded components of photosynthesis and general metabolism. Consistent with this observation, *Arabidopsis* growth was inhibited following inoculation with *Bgh*, suggesting a shift in resource allocation from growth to defence. A number of defence-associated genes were induced during both interactions. These genes likely are components of basal defence responses, which do not effectively block host powdery mildew infections. In addition, genes encoding defensins, anti-microbial peptides whose expression is under the control of the JA/ET signalling pathway, were induced exclusively by non-host pathogens. Ectopic activation of JA/ET signalling protected *Arabidopsis* against two biotrophic host pathogens. Taken together, these data suggest that biotrophic host pathogens must either suppress or fail to elicit the JA/ET signal transduction pathway.**

Keywords: basal resistance, gene expression profiling, non-host resistance, powdery mildew.

Introduction

Although plants are constantly exposed to a wide variety of potentially pathogenic micro-organisms, disease occurrence is rare. Only a small proportion of well-adapted microbe species are able to infect target plant species and cause disease. A plant species susceptible to a pathogen species or subspecies grouping (e.g. pathovar or formae specialis) is termed a host to this parasite. The best-characterized form of resistance is gene-for-gene resistance, in which specific resistance genes (*R*-genes) in a host species confer resistance to specific genotypes or races of a pathogen based on the recognition of cognate pathogen avirulence gene products (Dangl and Jones, 2001). This form of resistance generally has a very narrow spectrum of action and often a short-term usefulness under agricultural conditions (Holub, 2001). Gene-for-gene resistance is associated with the activation of the salicylic acid (SA) signalling pathway and death of infected and adjacent cells (Cohn *et al.*, 2001). Less well characterized is non-host resistance in which an entire plant species is resistant to an entire pathogen species. This form of resistance is generally thought to be broad spectrum and durable under field conditions (Heath, 2000). Non-host resistance is presumed to be a complex, multi-component form of resistance, including both constitutive and inducible defences. Non-host resistance may also result from pathogen species being poorly adapted to the basic

physiology or growth habit of a plant species (Kamoun *et al.*, 1999; Thordal-Christensen, 2003).

The SA signal transduction pathway plays an important role in defence responses initiated by *R*-genes (Glazebrook, 2001); however, its contribution to non-host resistance is less clear. *NahG*-expressing *Arabidopsis*, which convert SA to catechol and thus do not accumulate SA (Lawton *et al.*, 1995), were compromised in non-host resistance to bacterial and fungal pathogens in some but not all plant species (Lu *et al.*, 2001; Mellersh and Heath, 2003; Van Wees and Glazebrook, 2003; Yun *et al.*, 2003). In separate experiments, sporulation by non-host white rust and downy mildew pathogens on Brassicaceae species showed a modest enhancement on *Arabidopsis eds1* mutants relative to wild type (Parker *et al.*, 1996).

The jasmonate (JA)/ethylene (ET) pathway has also been tested for its role in non-host resistance. Tobacco plants expressing the dominant mutant allele of the *Arabidopsis ETR1* gene were more susceptible to the non-host pathogen *Pythium sylvaticum*, while *Arabidopsis Etr1* mutants retained resistance to several non-host pathogens (Geraats *et al.*, 2003; Knoester *et al.*, 1998). In *Arabidopsis*, blocks in the JA (*coi1*, *jar1*) or ET (*ein2*, *Etr1*) pathways generally did not promote enhanced susceptibility to non-host pathogens, as measured by the establishment of functional feeding structures (Mellersh and Heath, 2003; Yun *et al.*, 2003). However, expression profiling of *Arabidopsis* responses to the non-host potato late blight pathogen (*Phytophthora infestans*) suggested that the JA signalling pathway was activated (Huitema *et al.*, 2003). This divergence may be explained by hypothesizing that the JA pathway alone is not sufficient to account for non-host resistance. Alternatively, the JA pathway may play a more important role in non-host resistance to potato late blight in *Arabidopsis* than it does in non-host resistance to wheat powdery mildew or various non-host rust pathogens.

A number of studies of the functions of a diverse group of genes have suggested roles in non-host resistance. Mutational studies have shown that *NHO1* and *PEN1* play a role in *Arabidopsis* non-host resistance to *Pseudomonas syringae* pv. *phaseolicola* and to barley powdery mildew respectively (Collins *et al.*, 2003; Kang *et al.*, 2003). In addition, gene silencing experiments in *Nicotiana benthamiana* suggest *SGT1* and the molecular chaperones HSP70 and HSP90 contribute to non-host resistance responses (Kanzaki *et al.*, 2003; Peart *et al.*, 2002). Collins *et al.* (2003) have speculated that the PEN1 (=SYP121) syntaxin prevents fungal ingress into plant epidermal cells. The remaining genes, like *EDS1*, *SGT1* and *NHO1*, are likely to participate in various non-host resistance mechanisms operating after penetration resistance has been breached. In addition, the host cytoskeleton is important, as actin polymerization inhibitors compromise non-host resistance to a variety of pathogens on barley, wheat, cucumber, tobacco and *Arabidopsis* (Kobayashi *et al.*, 1997; Yun *et al.*, 2003).

These studies suggest that plants mount active responses to non-host pathogens. Furthermore, some non-host pathogens terminate growth very early at the penetration stage, while others grow invasively to a limited extent on non-host plants suggesting that the early penetration-based resistance is not universally effective. Clearly, additional resistance mechanisms must operate to limit non-host pathogens on non-host plants. These results are consistent with the early ideas that non-host resistance is multi-component in nature (Heath, 2000). To extend our understanding of the various components that might contribute to non-host resistance in *Arabidopsis*, we compared the transcript profiles associated with non-host resistance to the barley powdery mildew and susceptibility to an *Arabidopsis* powdery mildew. The differential induction of the JA/ET pathway observed in the microarray experiments prompted us to re-examine the role of this signalling pathway in disease resistance to virulent biotrophic pathogens.

Results

Interaction between Arabidopsis *and the non-host pathogen, barley powdery mildew*

Arabidopsis is a host to the *Arabidopsis* powdery mildew, *Erysiphe cichoracearum*, and a non-host to the barley powdery mildew, *Blumeria graminis* f. sp. *hordei* (*Bgh*). Upon inoculation onto the Col-0 accession of *Arabidopsis*, *Bgh* sporelings germinated and produced appressoria. The majority (approximately 95%) of sporelings failed to penetrate leaf epidermal cells and fungal growth terminated during penetration attempts (Figure 1a). In contrast, most *E. cichoracearum* sporelings penetrated into epidermal cells and established haustoria (approximately 90%) (see also Adam and Somerville, 1996). Thus, a block at the penetration step, during the transition from surface to invasive growth, was the most prevalent form of resistance to the non-host barley powdery mildew in *Arabidopsis*. After 24 h, a few *Bgh* germlings (approximately 5%) were able to form haustoria in epidermal cells (Figure 1b). Occasionally, *Bgh* propagules with haustoria exhibited limited secondary hyphal growth on the leaf surface by 48 h post-inoculation (hpi). At this stage, growth arrested and successfully penetrated cells accumulated autofluorescent compounds (Figure 1c) and hydrogen peroxide (data not shown) in their walls and died (Figure 1d). Hydrogen peroxide accumulation at the tips of haustoria was observed in both host and non-host interactions (Figure 1e,f). Barley powdery mildew colonies never penetrated more than one epidermal cell and asexual reproduction was never observed on *Arabidopsis*.

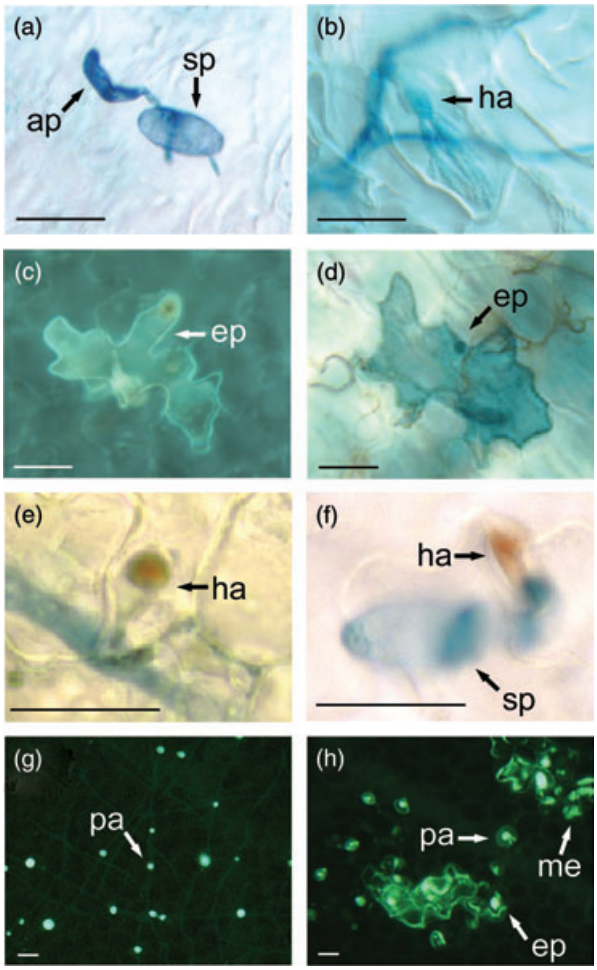

Figure 1. Development of the barley powdery mildew pathogen on *Arabidopsis* plants.
(a) *Bgh* spores (sp) germinate and produce appressoria (ap) 95% of the time on *Arabidopsis*.
(b) *Bgh* germlings are able to penetrate and establish haustoria (ha) at a low frequency.
(c, d) *Bgh*-penetrated epidermal cells (ep) produce autofluorescent compounds (c) and undergo cell death (d).
(e, f) Hydrogen peroxide accumulates at the tip of both *Erysiphe cichoracearum* (e) and *Bgh* (f) haustoria (ha).
(g, h) Callose deposition in response to *E. cichoracearum* occurs in discrete papillae (pa) (g), while callose deposition in response to *Bgh* is widespread and occurs in whole epidermal cells (ep) as well as in mesophyll (me) cells (h) (bar = 20 microns).

The most obvious cytological difference between the host and non-host interactions was in callose deposition. During the host interaction, callose deposition was limited to papillae, discrete, callose-rich, wall appositions, at penetration sites (Figure 1g). By contrast, in the non-host interaction, callose deposition also occurred in concentric rings around papillae, and around the entire attacked epidermal cells, as well as sometimes in epidermal and mesophyll cells adjacent to attacked cells (Figure 1h). This extensive callose response was observed at both successful and unsuccessful penetration attempts by *Bgh*. In addition, any *Bgh* haustoria that formed were rapidly encased in callose (data not shown).

Arabidopsis accessions that differed in their disease response to two compatible powdery mildew species, *E. cichoracearum* and *E. cruciferarum* (Adam *et al.*, 1999), were tested for variation in their response to two races of *Bgh*, K1 and A6. None of the 29 *Arabidopsis* accessions surveyed for growth of either K1 or A6 supported any more barley powdery mildew growth than Col-0 infected with race CR3 (Table S1). Of these *Arabidopsis* accessions, only Kas-1 showed an enhanced cell death response, with death of both attacked epidermal and the underlying mesophyll cells following inoculations by either K1 or A6. Kas-1 carries the resistance genes *RPW8.1* and *RPW8.2*, which confer resistance to a broad range of powdery mildews (Xiao *et al.*, 2001). Because Ms-0, which also carries *RPW8.1* and *RPW8.2*, did not exhibit this enhanced cell death phenotype, *RPW11* and *RPW12*, two powdery mildew resistance quantitative trait loci in Kas-1, either alone or in conjunction with *RPW8*, may enhance non-host defences against the barley powdery mildew pathogen (Wilson *et al.*, 2001). The failure to find a compatible *Bgh* race/*Arabidopsis* accession combination supports the contention that barley powdery mildew is a non-host pathogen of *Arabidopsis*.

To determine whether any of the known plant defence pathways affected the *Arabidopsis*/barley powdery mildew interaction, inoculations of various *Arabidopsis* mutants (Glazebrook, 2001) with the barley powdery mildew races K1 and A6 were monitored visually for the occurrence of enhanced hyphal growth. Among 29 mutants, including those defective in SA signalling, JA/ET signalling, and camalexin biosynthesis and those with activated defence responses (e.g. *cpr5-2*, *dnd1*, *edr1*), none supported visible increases in fungal growth relative to wild type (Table S2), suggesting that none of these mutants significantly compromised non-host resistance to *Bgh*. The *edr1* mutant, like the Kas-1 accession, exhibited an enhanced cell death response. Selected mutants with defects in the SA or JA/ET defence signalling pathways were evaluated in more detail. Two metrics of barley powdery mildew infection were quantified, penetration frequency (i.e. a measure of haustorium formation) and hyphal elongation frequency (i.e. a measure of the formation of functional haustoria able to support secondary hyphal growth) (Figure 2). None of the JA/ET pathway mutants tested *(coi1-1*, *ein2-1*, *jar1-1* and *Etr1-1)* had penetration or hyphal elongation frequencies significantly different from wild type. Of the SA pathway mutants or transgenics (*eds1-1*, *eds5-1*, *npr1-1*, *NahG*, *pad4-1*, *sid2-1*), only transgenic *NahG* plants and *eds1-1* mutants showed significant increases in penetration and hyphal elongation frequencies relative to Col-0 and Ws-0, their respective wild-type counterparts (Figure 2). As the SA biosynthetic mutant *sid2* did not exhibit a non-host infection

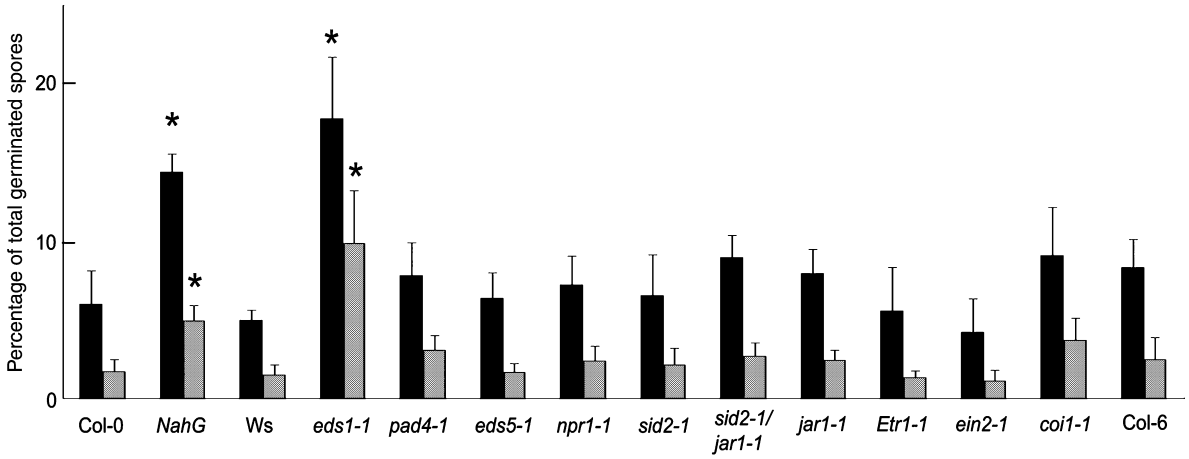

Figure 2. Growth of the non-host, barley powdery mildew pathogen on *Arabidopsis* mutants compromised in defences. Mean and standard deviation of the frequency of *Bgh* penetration (black bars) and hyphal elongation (grey bars) on *Arabidopsis*, expressed as a percentage of total germinated spores. *NahG*, *eds1*, *pad4*, *eds5*, *npr1 and sid2* are blocked in the salicylic acid pathway. *jar1*, *Etr1*, *ein2 and coi1* are blocked in the jasmonate/ethylene pathway. Wild-type controls were Ws-0 for *eds1*, Col-6 for *coi1* and Col-0 for all other mutants. Asterisks denote statistically significant differences between mutant and wild type by a Student's *t*-test ($P < 0.001$). Other comparisons to wild type were not significant (i.e. $P > 0.05$ by the Student's *t*-test).

phenotype, partial loss of penetration resistance in *NahG* transgenic plants could be due to 'catechol-mediated H_2O_2 production' as suggested by Van Wees and Glazebrook (2003) to explain the susceptibility of *NahG* transgenics to a non-host bacterial species or to other SA-independent changes in defence responses as reported by Heck *et al.* (2003).

The results in Figure 2 suggest that EDS1 has a distinct role in non-host resistance, unrelated to its role in *R*-gene triggered signal transduction via the SA pathway. In addition to these two roles, EDS1 also participates in basal defence responses that are activated in compatible host/pathogen interactions (Parker *et al.*, 1996). The double mutant with blocks in the JA (*jar1*) and SA (*sid2*) pathways did not support enhanced *Bgh* penetration or secondary hyphal growth. Collectively, these results suggest that neither the SA nor the JA/ET signal transduction pathways contribute significantly to penetration resistance to *Bgh*.

Barley powdery mildew elicited a stronger response from Arabidopsis *than* Arabidopsis *powdery mildew*

To reveal induced defences that might be elicited by a non-host pathogen, we used cDNA microarrays to monitor changes in gene expression in rosettes collected at 8, 18 and 24 hpi with spores of *Bgh* or *E. cichoracearum*. These time points corresponded to when mature appressoria formed and penetration is initiated in both pathogens (8 hpi), the first fully developed *E. cichoracearum* haustoria were visible (18 hpi), and callose deposition in response to *Bgh* attempted penetrations was pronounced (24 hpi) (Figure 1h). As attacked epidermal cells represent a small proportion of the cells of rosettes, the observed changes in gene expression may represent either very dramatic changes occurring only in attacked epidermal cells or changes in gene expression occurring in approximately all cells of the rosettes.

Although exceptions existed, two general trends in gene expression were noteworthy when the entire data set was considered. First, a number of genes responded similarly in host and non-host interactions. This observation was more pronounced among genes showing increased than among those with reduced expression following pathogen attack. Secondly, the changes in gene expression occurred more rapidly, were of greater magnitude, and were more likely to be statistically significant in the non-host interactions than the host interactions (Figure 3; Table S3).

Ninety-nine genes with statistically significant changes of expression were identified (Figure 3; Table S3). Of these, 89 genes were classified as differentially expressed between *Bgh*-inoculated and uninoculated plants. Fourteen genes were statistically significantly different between *E. cichoracearum*-infected and uninoculated plants, four of which overlapped with the genes differentially expressed in the *Bgh*-treated plants. These results suggest that the non-host powdery mildew elicits a broader and more pronounced response in *Arabidopsis* at these early time points. A discussion of specific genes showing statistically significant differential expression between inoculated and non-inoculated plants follows in the next sections.

General metabolism and photosynthesis genes were repressed by pathogen attack

Transcripts with reduced levels at both 18 and 24 hpi with the *Bgh* (Figure 3, cluster A) fell predominantly into the broad functional classes of photosynthesis, transcription or

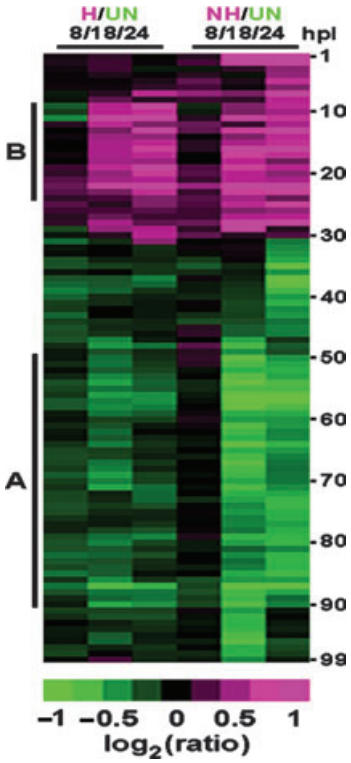

Figure 3. Hierarchical cluster analysis of differentially expressed genes. Hierarchical cluster analysis of 99 genes differentially expressed between control, uninoculated plants (UN) and either *E. cichoracearum*-(H) or *Bgh*-(NH) inoculated plants at 8, 18 or 24 hpi.
The average $\log_2$ (inoculated/uninoculated) value ($n = 4$ replicates) is given in each cell and colour-coded according to the scale at the bottom of the figure. The clusters labelled A and B are discussed in more detail in the text. The numerical values for the data in this figure are given in Table S3.

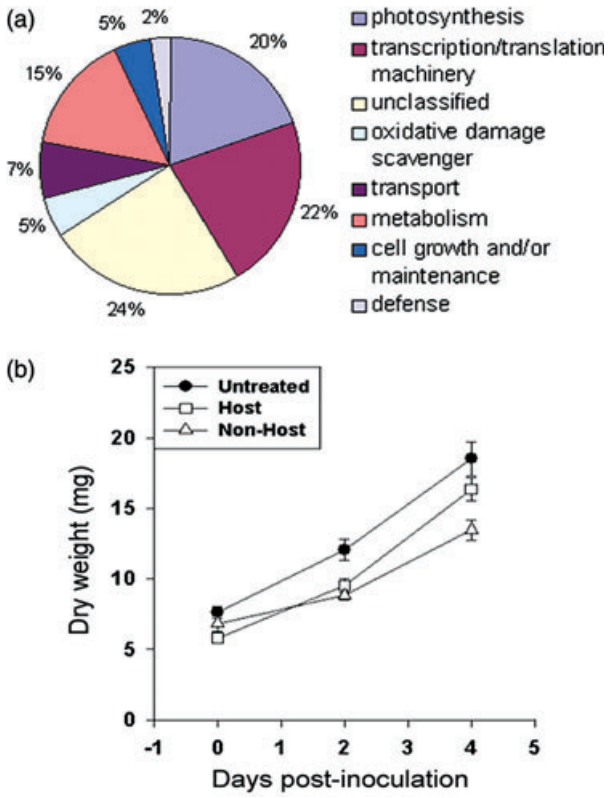

Figure 4. Transcripts with reduced levels following inoculation with the host (*Erysiphe cichoracearum*) or the non-host (*Bgh*) powdery mildew are enriched in photosynthesis and house-keeping functions.
(a) Distribution of cluster A genes (Figure 3) by functional category, expressed as percentage to the total number of genes in cluster A. The category, unclassified, refers to clones with no similarity to genes of known function.
(b) Effect of *E. cichoracearum* or *Bgh* inoculations on the growth of 3-week-old *Arabidopsis* plants. Data points in the graph are the mean and standard error of 24 plants. The experiment was repeated three times with similar results.

translation machinery, metabolism and transport (Figure 4a). Half of these genes encoded proteins predicted to reside in the chloroplast (data not shown). Consistent with the reduced transcript levels for components of photosynthesis and basic metabolism, *Bgh*-inoculated plants accumulated about 30% less dry weight at 4 dpi when compared with uninoculated, control plants (Figure 4b). The growth reduction following infection with *E. cichoracearum* was much more modest, which correlates with the limited number of cluster A transcripts with significantly reduced levels in plants infected by this pathogen (Table S3). Thus, the extent of repression of genes associated with general metabolism and photosynthesis was reflected in the reduction of plant growth following inoculation with the two pathogens.

Putative defence genes were induced by inoculation with barley powdery mildew and with Arabidopsis *powdery mildew*

Cluster B (Figure 3) was enriched in putative defence genes (e.g. genes encoding a glycosyl hydrolase similar to a β-1, 3-glucanase (Table S3; clone no. 9), putative disease resistance proteins (clone nos 13, 14), an AIG2-like protein (clone no. 22) and MAP kinase 3 (clone no. 21). To further evaluate the biological significance of these cluster B genes, we examined a 1000-base pair region upstream of the ATG translational start site of these genes for over-represented 6-mer elements. Among the elements enriched in occurrence were components of the *ocs* element (i.e. ATCTTA and ATTGAT) (Table 1). The *ocs* element serves as a binding site for *ocs* binding factors (OBF), and was originally described as a component of the promoter region of the *GST6* gene, encoding a glutathione S-transferase (Chen *et al.*, 1996; Lescot *et al.*, 2002). *ocs* elements mediate expression of *GST6* in diverse conditions such as treatments with auxin, SA or hydrogen peroxide (Chen and Singh, 1999). Moreover, CACTTT, an element of the OBP1-binding site that stimulates the binding of OBF proteins to the *GST6* promoter, was also over-represented in this cluster (Lescot *et al.*, 2002). The

Table 1 Over-represented oligomers in the1000-base pair regions upstream of the ATG sites of genes of cluster B

Oligomer	Absolute number of oligomer		Number of sequences containing oligomer		*P*-value from binomial distribution	Annotation[b]
	Query set	Genomic set	Query set	Genomic set[a]		
ATCTTA	24	26 455	16/16	16 512	2.03E-04	*Cis*-acting element involved in auxin, SA and oxidative stress response (*ocs*-element)
CCATTC[c]	17	10 757	11/16	8653	1.64E-03	*Cis*-acting element for a maximal elicitor-mediated activation
ATTGAT	25	36 921	16/16	20 010	4.4E-03	*Cis*-acting element involved in auxin, SA and oxidative stress response (*ocs*-element)
CACTTT	19	22 384	14/16	15 148	4.48E-03	*Cis*-acting element involved in *ocs*-elements regulation (OBP1 site)

[a]Of 28 088 sequences in the genomic set.
[b]From PlantCare at http://intra.psb.ugent.be:8080/PlantCARE/.
[c]From *Pisum sativum*. All other oligomers were described in *Arabidopsis*.

enriched 6-mer CCATTC is a component of an element required for maximal elicitor-mediated activation in pea (Lescot *et al.*, 2002; Seki *et al.*, 1996). We also looked for the consensus W-box sequence, TTGAC[C/T] (Eulgem *et al.*, 1999). The average number of W-boxes per gene in cluster B was 1.9 (TTGACC, $P = 4.64$ E-02; TTGACT, $P = 1.31$ E-01), while the average occurrence of this element in the upstream sequences of all *Arabidopsis* genes was 1.2. The over-representation of defence-associated putative *cis*-elements in the upstream regions of these genes supports the supposition that they have a role in plant defence.

CYTOCHROME P450 83B1 (*CYP83B1*) transcript (clone no. 7), a part of the glucosinolate-myrosinase metabolic pathway, accumulated to higher levels in *Bgh*-treated than in *E. cichoracearum*-treated plants (Bak *et al.*, 2001; Wittstock and Halkier, 2002). Other conditions known to induce *CYP83B1* gene expression in *Arabidopsis* include inoculation with the non-host potato late blight pathogen (Huitema *et al.*, 2003), wounding (Reymond *et al.*, 2000) and inoculation with the necrotroph *Alternaria brassicicola* (Schenk *et al.*, 2000). As this cytochrome is at the metabolic branch point between auxin and indole glucosinolate biosynthesis, auxin levels are likely to be modulated by flux to the glucosinolate pathway (Bak *et al.*, 2001). Consistent with this idea, genes for an auxin-regulated protein (clone no. 69) and an auxin-induced transcription factor (clone no. 59), and *AUX1* (clone no. 80) were significantly repressed only by inoculation with the non-host pathogen (Table S3).

The expression of defensins was correlated with non-host resistance

Among the transcripts that were uniquely induced following inoculation with the barley powdery mildew were two defensins (clone nos 1, 2) and a hevein-like protein precursor (*PR-4*) (clone no. 28), which are regulated by the JA/ET defence signalling pathway (Thomma *et al.*, 1998). Three other genes in this group, a hydroxyproline-rich glycoprotein (clone no. 3), the β subunit of tryptophan synthase (clone no. 26) and an NDR1/HIN1-like protein (clone no. 27), participate in cell wall strengthening (O'Connell *et al.*, 1990), phytoalexin biosynthesis (Zhao and Last, 1996) and gene-for-gene resistance (Varet *et al.*, 2003) respectively. In addition, four poorly characterized transcripts (clone nos 4, 5, 6, 8) were induced solely by *Bgh* inoculations (Table S3). The induction of genes belonging to various defence mechanisms suggests that *Arabidopsis* activates multiple defence pathways during the non-host interaction that were partially or totally silent during the compatible host interaction.

Defensins were of particular interest both because *PDF1.2* (clone no. 2) has been widely used as a marker for activation of the JA/ET signalling pathway and this signalling pathway is believed to have no role in compatible powdery mildew interactions (Reuber *et al.*, 1998; Schulze-Lefert and Vogel, 2000). To confirm the microarray results, we performed gene-specific RT-PCR analysis of individual members of the *PDF1* gene family (Thomma *et al.*, 2002). *PDF1.1*, *PDF1.2a/b/c* and *PDF1.3* transcripts accumulated to higher levels at 18 and 24 hpi in *Bgh*-treated tissues relative to uninoculated controls and to *E. cichoracearum*-infected tissues (Figure 5a). At 48 hpi, transcript levels for these genes returned to basal levels. Weak, transient increases of the *PDF1.2a/b/c and PDF1.3* transcript levels were observed at 18 hpi in *E. cichoracearum*-treated tissues. *PDF1.5* expression was unchanged and *PDF1.4* expression was modestly induced following inoculation with *Bgh* (Figure 5a). Neither *PDF1.4* nor *PDF1.5* was induced by inoculation with *E. cichoracearum*. Expression of *PDF1.2a* was also investigated in transgenic plants that carried a $P_{PDF1.2a}$:β-glucuronidase construct. *PDF1.2a* expression was observed only in plants inoculated with *Bgh* (Figure 5b). β-glucuronidase activity was detected in uninoculated sectors of inoculated leaves (data not shown) and at wound sites adjacent to the cut petiole (Figure 5b).

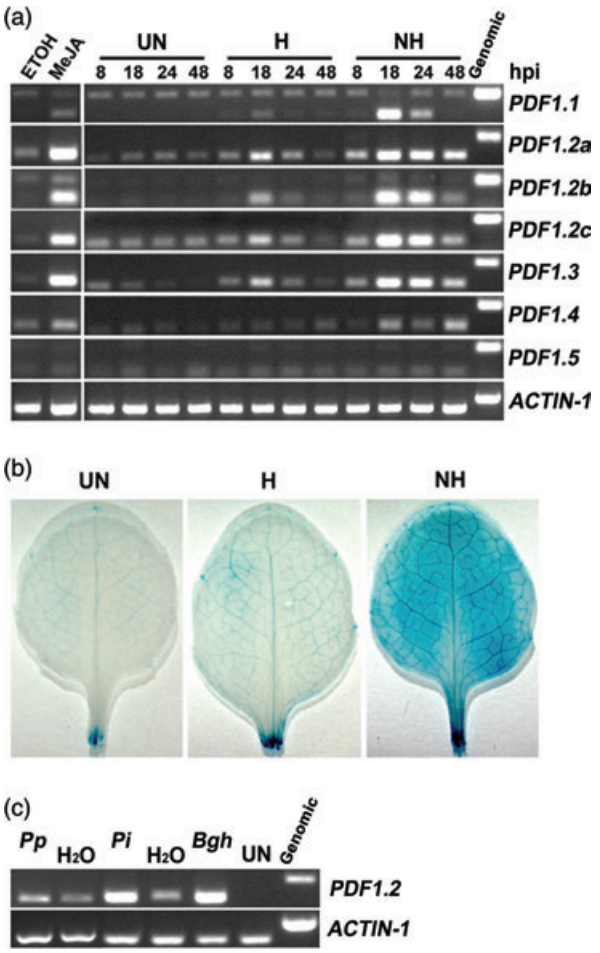

Figure 5. Comparison of the transcript levels of defensin gene family members in *Arabidopsis* in response to inoculations with non-host or host pathogens.
(a) Analysis of the expression of the *PDF1* gene family of defensins. Total RNA was extracted from untreated (UN), *Erysiphe cichoracearum*-(H) or *Bgh*-(NH) inoculated plants at the indicated times. The transcript levels of *PDF1* family of defensins along with *ACTIN-1* were assessed by RT-PCR. PCR was performed for 27 (*PDF1.2a/b/c*, *PDF1.3*) or 30 (*PDF1.1*, *PDF1.4*, *PDF1.5*, *ACTIN-1*) cycles. Note that the RT-PCR fragments are shorter than the corresponding genomic fragments, due to the removal of introns from the transcripts. Methyl-JA-treated *Arabidopsis* plants (21 days old) were used as a positive control for the induction of members of the *PDF1* gene family. This experiment was performed twice with similar results.
(b) Expression of *PDF1.2a* in response to powdery mildew inoculations. Representative leaves of 21-day-old transgenic *Arabidopsis* plants, containing the $P_{PDF1.2a}$:β-glucuronidase construct, either untreated (UN), or inoculated with *E. cichoracearum* (H) or with *Bgh* (NH). *Arabidopsis* leaves were stained for β-glucuronidase activity at 24 hpi (Jefferson *et al.*, 1987).
(c) RT-PCR analysis of *PDF1.2* expression in 14-day-old *Arabidopsis* plants at 24 hpi with the non-host, potato late blight (*Pi*) or the virulent, *Arabidopsis* downy mildew (*Pp*) pathogens. Water, mock-inoculated control plants (H_2O). The same RNA samples for *Bgh* (*Bgh*)-treated (24 hpi) and untreated (UN) from (a) were used as positive controls. PCR was performed for 25 cycles. Experiments were repeated twice with similar results.

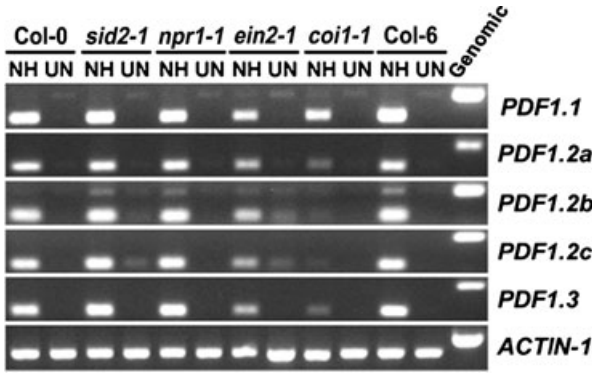

Figure 6. Expression of defensins in *Arabidopsis* mutants defective in defence response signalling.
Twenty-one-day-old plants of mutants defective in the salicylic acid (*sid2-1*, *npr1-1*), jasmonate (*coi1-1*) or ethylene (*ein2-1*) signalling pathways were inoculated with the *Bgh* (NH) and the transcript levels of members of the *PDF1* gene family were assessed at 18 hpi by RT-PCR. Uninoculated, control plants (UN). *ACTIN-1* was used as a control. Col-0 is the wild-type control for the *sid2-1*, *npr1-1* and *ein2-1* mutants, and Col-6 is the wild-type control for *coi1-1*. PCR was performed for 27 (*PDF1.2a/b/c*, *PDF1.3*) or 30 (*PDF1.1*, *ACTIN-1*) cycles. This experiment was performed twice with similar results.

To test whether the elevated accumulation of defensin transcripts was a general response to non-host pathogens, *Arabidopsis* plants were inoculated with the potato late blight pathogen, an oomycete rather than a fungal pathogen. This non-host pathogen also induced the accumulation of *PDF1.2* transcripts in *Arabidopsis* (Figure 5c). The levels of the *PDF1.2* transcripts were only marginally increased following infection with the virulent host oomycete, *Peronospora parasitica* (Figure 5c). Thus, the induction of defensins in *Arabidopsis* by non-host pathogens was not unique to *Bgh*.

All of the defensin genes induced following *Bgh*-treatment, except *PDF1.1*, were also induced by methyl-JA treatment (Figure 5a). To address whether the defensins were regulated via the JA/ET or another unknown signalling pathway in *Bgh*-treated plants, we inoculated the JA-insensitive mutant, *coi1-1*, and the ET-insensitive mutant, *ein2-1*, with *Bgh* and monitored transcript levels for the defensins, *PDF1.1*, *PDF1.2a/b/c* and *PDF1.3*. The induction of these transcripts by *Bgh* was reduced in the two mutants relative to wild type, most notably in the *coi1-1* mutant (Figure 6). Thus, a functional JA/ET pathway was necessary for the induction of most of the defensin genes by the non-host pathogen. Defensin gene expression in mutants defective in the SA defence signalling pathway such as *sid2-1* and *npr1-1* was unaffected (Figure 6).

Methyl-JA treatment protected Arabidopsis *against two obligate biotrophic host pathogens*

Virulent obligate biotrophic pathogens such as *E. cichoracearum* and *P. parasitica* did not elicit increased expression of defensin genes (Figure 5a,c, Nishimura *et al.*, 2003). In addition, the susceptibility of the *ein2-1* mutant to these

virulent pathogens resembled wild type (data not shown) and the *jar1* mutant is not compromised in basal resistance to the virulent *E. orontii* (Reuber *et al.*, 1998). These observations suggest that the JA/ET pathway does not play a role in compatible interactions between obligate biotrophic pathogens and *Arabidopsis*. However, it is still possible that, if activated ectopically, the JA/ET defence pathway would provide protection against host powdery mildews. Consistent with this hypothesis, treatment of *Arabidopsis* with methyl-JA significantly decreased the level of *E. cichoracearum* infection (Figure 7a). Importantly, *coi1-1* mutants were not protected by methyl-JA treatment, confirming that methyl-JA is not acting directly on *E. cichoracearum* to limit its growth (Schweizer *et al.*, 1993). To extend this test to another biotrophic pathogen, plants were inoculated with a virulent isolate of the *Arabidopsis* downy mildew, *P. parasitica*. Methyl-JA treatment of wild-type plants was also protective against downy mildew (Figure 7b). The *coi1-1* mutants treated with methyl-JA did not show enhanced resistance to downy mildew relative to untreated *coi1-1* mutants suggesting again that methyl-JA does not act directly on this oomycete pathogen. Thus, continuous treatment with methyl-JA can protect plants against obligate biotrophic pathogens. Similarly, the *cev1-1* cellulose synthase A3 mutant, in which the JA/ET signalling is constitutively activated, showed enhanced resistance to a wide range of pathogens, including *E. cichoracearum* (Ellis *et al.*, 2002a,b). A 2-day pre-treatment with methyl-JA was not sufficient to protect *Arabidopsis* plants against these two pathogens (data not shown; Thomma *et al.*, 1998), highlighting a requirement of sustained JA/ET-pathway activation for resistance.

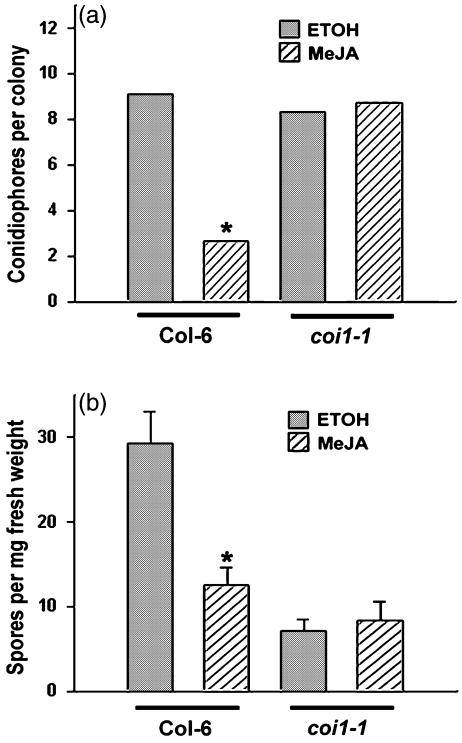

Figure 7. Methyl-jasmonate (JA) treatment protects *Arabidopsis* against obligate biotrophic pathogens.
Three-week-old Col-6 and *coi1-1* mutants were inoculated with either the host powdery mildew pathogen, *Erysiphe cichoracearum* (a), or the host downy mildew pathogen, *Peronospora parasitica* (b), and then incubated continuously in a closed chamber with either methyl-JA or ethanol as described in Experimental procedures.
(a) The number of *E. cichoracearum* conidiophores per colony was evaluated 6 dpi. Bars represent the mean with the standard error of 100 colonies from 12 plants per treatment. *Significantly different from control plants by an unpaired *t*-test ($P < 0.001$). This experiment was repeated three times with similar results.
(b) The number of *P. parasitica* spores per mg fresh weight was evaluated 7 dpi. Data represent the average and standard error of three independent experiments ($n = 12$ for each experiment). *Significantly different from control plants by an unpaired *t*-test ($P < 0.001$).

Unlike powdery mildew-infected *coi1-1* mutants, both ethanol-treated control *coi1-1* and methyl-JA-treated *coi1-1* were more resistant to downy mildew than wild-type plants (Figure 7b). Kloek *et al.* (2001) showed that *coi1-20* mutants were more resistant to the virulent bacterial pathogen, *Pseudomonas syringae* pv tomato DC3000, and that this resistance was associated with the hyperactivation of SA-dependent defences. The host downy mildew, like *P. syringae* pv tomato, may hyperactivate SA defences in the *coi1* mutant background. The differing growth habits of the downy mildew, an endoparasite, and powdery mildew, an ectoparasite, may explain the differential disease response of the *coi1-1* mutant to these two pathogens.

Discussion

Non-host resistance is the most common, durable and non-specific type of resistance observed in plant–pathogen interactions, making this type of resistance of great interest for agriculture (Heath, 2000). All *Arabidopsis* accessions surveyed were found to be highly resistant to barley powdery mildew infection indicating that barley powdery mildew is a non-host pathogen of *Arabidopsis*. A cytological comparison between *Arabidopsis* responses to the non-host powdery mildew, *Bgh*, and a host powdery mildew, *E. cichoracearum*, indicated that resistance occurred mainly at the penetration stage at the cell wall. However, at low frequency, functional haustoria able to support limited secondary hyphal growth were observed. Thus, the barley powdery mildew has the inherent capacity to successfully infect *Arabidopsis* (Figure 1b) (Collins *et al.*, 2003). This observation together with the exaggerated callose response and changes in plant gene expression suggest that cessation of *Bgh* growth on *Arabidopsis* is mediated by active non-host resistance responses and is probably not the consequence of missing compatibility or virulence factors. Of the *Arabidopsis* mutants deficient in different defence pathways, only transgenic *NahG* plants and *eds1* mutants

supported a moderate enhancement of penetration frequencies by the non-host pathogen relative to wild type (Figure 2). Yun *et al.* (2003) observed increased penetration by wheat powdery mildew on *pad4* and *npr1* mutants as well as on *NahG* and *eds1*, suggesting that the SA pathway appears to play a larger role in resistance to the wheat powdery mildew than to the barley powdery mildew. Similarly, different species of non-host rust fungi displayed different penetration frequencies on *Arabidopsis* mutants (Mellersh and Heath, 2003). Collectively these results suggest that resistance to biotrophic non-host powdery mildew and non-host rust fungi is an active process and that a major component of non-host resistance is penetration resistance. The SA and JA/ET pathways may contribute to resistance to some non-host pathogens, especially those pathogens for which penetration resistance is not very effective (Knoester *et al.*, 1998; Yun *et al.*, 2003). Notably, the non-host resistance in *Arabidopsis* to the wheat powdery mildew appears to consist primarily of EDS1- and actin microfilament-associated processes as this non-host pathogen can reproduce asexually on plants in which these two components are disrupted (Yun *et al.*, 2003).

The *Arabidopsis* transcriptional responses to host and non-host inoculations overlapped substantially. However, an earlier and stronger activation or repression of gene expression was observed after inoculation with the non-host powdery mildew. A majority of the genes were repressed by pathogen treatment, notably by *Bgh* treatment. Many of these genes are involved in the photosynthetic machinery and basic metabolism (Figure 4a). The repression of photosynthesis-related genes has also been observed in incompatible host–pathogen interactions (Matsumura *et al.*, 2003; Mysore *et al.*, 2003). Consistent with the reduced expression of photosynthesis genes, *Arabidopsis* growth was reduced in plants inoculated with *Bgh* relative to uninfected plants (Figure 4b), indicating that activation of non-host resistance poses a significant metabolic cost to the plant. This inverse relationship between growth and defence responses has been observed in other cases (Feys *et al.*, 1994; Wright *et al.*, 1995a,b), notably in plant mutants with constitutively activated defences (Bowling *et al.*, 1997; Ellis *et al.*, 2002b). Similarly, recognition of the flg22 peptide, a general elicitor from flagellum-containing bacteria, by the FLS2 receptor induces defence responses and leads to a growth penalty in *Arabidopsis* seedlings (Gomez-Gomez *et al.*, 1999). To explain this inverse relationship, Ehness *et al.* (1997) proposed that an extracellular invertase is induced by pathogen infection resulting in elevated monosaccharide levels, which in turn signal repression of genes encoding the photosynthetic machinery and activation of defence-related genes. Collectively, these observations indicate co-ordination of defence responses, including non-host resistance responses, and growth in plants, with metabolic resources shunted to defence responses and away from general metabolism during pathogen attack (Logemann *et al.*, 1995).

Transcripts that were elevated in both host and non-host interactions included defence-related genes (Table S3). Some of these genes shared upstream *cis*-elements that are associated with defence (Table 1). Among these genes, only *CYP83B1* was induced by inoculation with the potato late blight pathogen at 16 hpi (Huitema *et al.*, 2003). These induced genes are presumably activated by general elicitors and may be largely ineffective against the host pathogen, *E. cichoracearum*. Alternatively, these defences may not reach threshold levels needed to significantly retard *E. cichoracearum* growth. The shared responses are candidate components of basal resistance, which would explain why some genes conferring enhanced susceptibility phenotypes when mutated, like *EDS1*, also contribute to resistance to non-host pathogens (Parker *et al.*, 1996).

Among the genes that were preferentially activated in non-host interactions were plant defensin genes and this induction was dependent on the JA/ET signalling pathway. *PDF1.2* is also induced by inoculations with the non-host potato late blight pathogen (Figure 5c; Huitema *et al.*, 2003) and with necrotrophic pathogens (Thomma *et al.*, 1998). In contrast, *PDF1.2* is not induced in *Arabidopsis* by the wheat powdery mildew by 24 hpi (Yun *et al.*, 2003). The *coi1-1* and *ein2-1* mutants, in which induction of most of the defensins was impaired, did not permit enhanced *Bgh* penetration relative to wild-type plants (Figure 2). One interpretation of this observation is that the JA/ET-regulated defences act in conjunction with other defence mechanisms to restrict *Bgh* growth. Thus, only a concomitant disruption of multiple defence layers would reveal a function for JA/ET signalling in non-host resistance.

As originally proposed by Heath (2000) and more recently by Thordal-Christensen (2003), it seems likely that non-host resistance consists of preformed and inducible defences. Among the non-host defence mechanisms are penetration resistance, which appears to play a major role in the resistance of *Arabidopsis* to the barley powdery mildew, and basal resistance (i.e. those defences elicited by both virulent host pathogens and non-host pathogens). In this study, we have shown that the SA signalling does not appear to contribute significantly to non-host resistance to the barley powdery mildew. However, this pathway can play a more important role in other non-host interactions. Traditionally, the JA/ET pathway has been thought to play a significant role in plant defences against necrotrophic pathogens (Glazebrook, 2001). However, Huitema *et al.* (2003) and this work suggest that the JA/ET pathway is also activated during non-host interactions.

Heath (2000) has proposed that the evolution of a non-host pathogen to a host pathogen on a new plant species is a multi-step process, which might provide a rationale for the durability of non-host resistance. Thus,

E. cichoracearum must have acquired mechanisms to overcome penetration resistance as it became adapted to *Arabidopsis*. Other features of this evolutionary process appear to include insensitivity to induced basal defence responses (e.g. genes in Figure 3, cluster B). The JA/ET pathway may be part of the basal defence response that host powdery mildew pathogens fail to elicit or actively suppress. The failure to elicit or the ability to suppress non-specific defences is widely discussed as a necessary step in the evolution of pathogenicity on a new host species (Panstruga, 2003) and our results provide an illustration of this phenomenon (Figure 6).

Experimental procedures

Plant materials and growth conditions

Three-week-old *Arabidopsis thaliana* (L. Heyhn.) Columbia (Col-0) or Columbia *glabrous1* (Col-6) plants were grown in ProMix HP (Premier Horticulture, Red Hill, PA, USA). Plants to be infected with *Bgh* or *E. cichoracearum* were grown in growth chambers at 21°C with a 14-h photoperiod and a light intensity of about 100 μE m^{-2} sec^{-1}. For experiments presented in Tables S1 and S2, plants were grown as described by Collins *et al.* (2003). Plants used for *Arabidopsis* downy mildew and potato late blight infections were grown at 19°C with a 12-h light cycle and a light intensity of about 100 μE m^{-2} sec^{-1}.

The following mutants and transgenic plants were used in this study: *coi1-1* (Col-6 background) (Feys *et al.*, 1994), *npr1-1* (Cao *et al.*, 1994), *npr1-2* (NASC ID: N3801), *npr1-3* (NASC ID: N3802), *npr1-5* (NASC ID: 3724), *sid2-1* (Nawrath and Metraux, 1999), *pad1*, *pad2-1*, *pad3-1* (Glazebrook and Ausubel, 1994), *pad4-1*, *pad5* (Glazebrook *et al.*, 1997), *eds4-1*, *eds5-1*, *eds8*, *eds9-1* (Glazebrook *et al.*, 1996), *eds14*, *eds15*, *eds16* (Dewdney *et al.*, 2000), *eds1-1* (Ws-0 background) (Parker *et al.*, 1996), *eds1-2* (Aarts *et al.*, 1998), *ein2-1*, *ein3-1*, *ein4*, *ein5-1*, *ein6* (Roman *et al.*, 1995), *Etr1-1* (Bleecker *et al.*, 1988), *jar1-1* (Staswick *et al.*, 1992), *ndr1* (Century *et al.*, 1995), *cpr5-2* (Bowling *et al.*, 1997), *dnd1* (Yu *et al.*, 1998), *edr1* (Frye and Innes, 1998) and *NahG* (Lawton *et al.*, 1995) (all Col-0 background except as noted). The 29 *Arabidopsis* accessions listed in Table S1 are available from the Arabidopsis Biological Resource Centre or the Nottingham Arabidopsis Stock Centre.

Pathogen growth and inoculation

The *Arabidopsis* powdery mildew, *E. cichoracearum* UCSC1 (Adam *et al.*, 1999), was amplified on squash (cv. Kuta) and inoculations were performed as described in Wilson *et al.* (2001). The barley powdery mildew, *Bgh* CR3 (Moseman, 1968), was maintained on AlgerianS barley (Moseman, 1972) and isolates K1 (*AvrMla1*, *virMla6*, *virMla12*) and A6 (*virMla1*, *AvrMla6*, *AvrMla12*) were maintained as described in Shen *et al.* (2003). To inoculate with *Bgh*, 21-day-old *Arabidopsis* plants were placed in a settling tower of approximately 1 m in height and inoculated by dusting the conidia from heavily infected barley seedlings at 7 dpi into the top of the settling tower. After 10 min, the plants were placed in a dew chamber at 100% relative humidity and 18°C in the dark for 1 h and then returned to the growth chamber.

Peronospora parasitica Noco2 (Delaney *et al.*, 1995) was maintained by weekly culturing on Col-0. Seedlings were inoculated with a suspension of 1×10^5 conidia ml^{-1} (Vogel and Somerville, 2000). The *P. infestans* isolate 1306 (A1 mating type) (provided by H. Judelson, University of California, Riverside, CA, USA) was maintained on Rye B media (Caten and Jinks, 1968). *Phytophthora infestans* conidia were suspended in a solution of 0.2 g glucose per 20 ml water and added to 20 ml ice (−20°C) for 2 h at 4°C. This solution was then transferred to room temperature for 45 min to allow the ice to melt and was then sprayed onto rosettes of 14-day-old *Arabidopsis* plants (final concentration: 1×10^6 zoospores ml^{-1}) (Zimmerli and Collet, 1996).

Methyl-JA treatment

Plants (21 days old) were placed in sealed 5-l boxes containing 40 μl of either ethanol (control) or 100 mM methyl-JA (Sigma, Milwaukee, WI, USA) in ethanol. Plants were first inoculated and then treated with methyl-JA during the entire infection cycle. To evaluate the effect of a methyl-JA treatment prior to inoculation, plants were exposed to methyl-JA for 2 days, removed from the methyl-JA treatment boxes to a growth chamber and inoculated.

Cytology and quantification of fungal growth

Trypan blue staining of fungal conidia and aniline blue staining of callose were performed at 1 dpi (Adam and Somerville, 1996). For quantification of fungal growth, eight *Arabidopsis* leaves per genotype were stained with aniline blue with the addition of 250 μg of trypan blue per millilitre. Haustorial bodies, visualized as intracellular callose encasements, and secondary hyphal elongation were quantified in non-overlapping fields of view at 200× magnification, avoiding the edge and midvein regions of the leaf. Numbers were expressed as a percentage of total germinated conidia before being subjected to a Student's *t*-test.

For the experiments presented in Tables S1 and S2, fungal growth was monitored with a fluorescence stereomicroscope (MZFL III, Leica Mikrosysteme Vertrieb GmbH, Bensheim, Germany), while penetration success by non-host pathogens was detected by the occurrence of autofluorescence at infection sites as described in Collins *et al.* (2003).

To assess the effect of methyl-JA treatment, the number of *E. cichoracearum* conidiophores per colony was determined at 6 dpi on leaves from 21-day-old plants that were inoculated at a low density (average 1–2 conidia mm^{-2}) followed by trypan blue staining and visualization with a compound microscope at 100× magnification. In addition, 12-day-old plants were inoculated with *P. parasitica* conidia. Twelve pools of four plants each were collected and the number of conidia per mg of *Arabidopsis* fresh weight was determined for each pool at 7 dpi. Experiments were repeated three times. β-glucuronidase activity was determined in three independent experiments as described (Jefferson *et al.*, 1987).

RNA isolation and microarray preparation

Total RNA was isolated from liquid N_2-frozen *Arabidopsis* rosettes using the TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Poly(A)+ RNA purification, cDNA labelling and microarray hybridizations were as described by Scheible *et al.* (2003). The Y2000 AFGC DNA microarrays were designed from 11 300 *Arabidopsis* cDNA clones (Newman *et al.*, 1994) collected from public sources (first two biological replicates), and Y2001 AFGC DNA microarray contained 11 500 cDNA clones, (Newman *et al.*, 1994; White *et al.*, 2000) and 3000 gene-specific

amplicons (biological replicates 5 and 6). Additional information about the Y2000 and Y2001 AFGC microarrays can be found at the Arabidopsis Functional Genomics Consortium web site (http://arabidopsis.org/info/2010_projects/comp_proj/AFGC/index.html) (Wu *et al.*, 2001).

Microarray data analysis

Microarray slides were scanned and spot intensities were quantified as described (Ramonell *et al.*, 2002). The Cy3 (Channel 1) and Cy5 (Channel 2) intensities for each spot were normalized following the default normalization provided by the Stanford Microarray Database after spots flagged as bad were removed from the data sets (Gollub *et al.*, 2003). Data points with net (Cy3) or normalized net (Cy5) spot intensities of ≤350 were removed before further analysis. The data set was analysed with the Significance Analysis of Microarrays program (SAM) (Tusher *et al.*, 2001). A one-class analysis was performed for each treatment × time point combination separately. The values used were: $\Delta = 0.346$ with a false discovery rate of 1.85% (*Bgh*-treated at 18 hpi/uninoculated); $\Delta = 0.566$ with a false discovery rate of 6.21% (*E. cichoracearum*-treated at 24 hpi/uninoculated); and $\Delta = 0.498$ with a false discovery rate of 1.08% (*Bgh*-treated at 24 hpi/uninoculated). No other treatment × time point combinations produced false discovery rates of <10%. The three gene lists were combined to produce one gene list and the average $\log_2$(ratio) values for four replicates for each gene in the list for each of the six treatment × time point combinations are presented in Table S3. The selected genes were subjected to complete linkage hierarchical clustering using Cluster and Treeview software (Eisen *et al.*, 1998). The Munich Information Centre for Protein Sequences (MIPS) (http://mips.gsf.de/proj/thal/db/) resource was used to assign functions to genes. Known and putative functions and gene ontologies from selected genes were retrieved from The Arabidopsis Information Resource (TAIR) (http://www.Arabidopsis.org). Putative protein localizations were retrieved from the MIPS.

The 1000 bp sequences upstream of the start codons for selected genes were retrieved using the Sequence Bulk Download utility at TAIR (http://www.Arabidopsis.org/tools/bulk/sequences/index.htm). Motif analyses of groups of upstream sequences were conducted via Motif Finder at TAIR (http://www.Arabidopsis.org/tools/bulk/motiffinder/index.html).

RT-PCR analysis

First-strand cDNA was synthesized from RNase-free DNase I-digested (Qiagen, Valencia, CA, USA) total RNA by using M-MLV reverse transcriptase (Promega, Madison, WI, USA). PCR was performed on PTC-100 Programmable Thermal Controller (MJ Research, Inc., Waltham, MA, USA) with Ex Taq polymerase and manufacturer-supplied buffers (TaKaRa Bio via Fisher Scientific, Pittsburgh, PA, USA) using the following program: 25–30 cycles of 30 sec at 92°C, 30 sec at 61°C and 30 sec at 72°C. The software program Oligo 6.13 (Molecular Biology Insights, Cascade, CO, USA) was used to design gene-specific primers for the various defensins. Primers used were: *PDF1.1*, 5′-CAT GGC TAA GTC TGC TAC CAT CG-3′ (forward primer) and 5′-TGC AAG ATC CAT GTC GTG CTT TC-3′ (reverse primer); *PDF1.2a*, 5′-TAA GTT TGC TTC CAT CAT CAC CC-3′ (forward primer) and 5′-GTG CTG GGA AGA CAT AGT TGC AT-3′ (reverse primer); *PDF1.2b*, 5′-ACG CTG CTC TTG TTC TCT TTG CA-3′ (forward primer) and 5′-AAG TAC CAC TTG GCT TCT CGC AC-3′ (reverse primer); *PDF1.2c*, 5′-GTC TGC TAC CAT CAT CAC CTT CC-3′ (forward primer) and 5′-TTC CGC AAA CGC CTG ACC ATG TC-3′ (reverse primer); *PDF1.3*, 5′-TAT AAT CAT GGC TAA GTC TGC TG-3′ (forward primer) and 5′-AGT TGC AAG ATC CAT GTT TTG CC-3′ (reverse primer); *PDF1.4*, 5′-CAC TTA TGC TCT TCC TTT GCC TC-3′ (forward primer) and 5′-GAA GTA GCA GAA ACA TGC GAA AC-3′ (reverse primer); *PDF1.5*, 5′-GTT GCT CTT GTT CTC TTT GCT GA-3′ (forward primer) and 5′-CCA TGT CTC ACT TTC CCT TTT GC-3′ (reverse primer). Primers for *ACTIN-1* (OWB270/271) and *PDF1.2* (OBW240/241) were described by Penninckx *et al.* (1996). The *PDF1.2* (OBW240/241) primers amplify several members of the *PDF1.2* family. To ensure that the correct fragments had been amplified, all fragments were sequenced on an ABI Prism 310 Sequenator (Applied Biosystems, Foster City, CA, USA) following the manufacturer's instructions for labelling and sequencing.

Accession numbers

Microarray data are publicly available via the Stanford Microarray Database (http://genome-www5.stanford.edu//) under experiment ID nos 21091, 21092, 27687, 26648 (Cy5: *E. cichoracearum*-infected tissues; Cy3: uninoculated tissue; 8 hpi); 21355, 20767, 27689, 26880 (Cy5: *Bgh*-infected tissue; Cy3: uninoculated tissue, 8 hpi); 20777, 20783, 27384, 25723 (Cy5: *E. cichoracearum*-infected tissues; Cy3: uninoculated tissue; 18 hpi); 20772, 20795, 27385, 26611 (Cy5: *Bgh*-infected tissue; Cy3: uninoculated tissue; 18 hpi); 20776, 20788, 27638, 25686 (Cy5: *E. cichoracearum*-infected tissues; Cy3: uninoculated tissue; 24 hpi); 22034, 22202, 27272, 26885 (Cy5: *Bgh*-infected tissue; Cy3: uninoculated tissue; 24 hpi). These microarray data have also been deposited in the Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under accession number GPL1278.

Seeds of plants containing the $P_{PDF1.2a}$:β-glucuronidase construct have been deposited in the Arabidopsis Biological Resource Centre (http://www.arabidopsis.org). The *Bgh* isolates K1 and A6 can be obtained from Paul Schulze-Lefert.

Acknowledgements

We thank X. Dong (Duke University, USA) (*npr1-1)*, C. Nawrath (Fribourg University, Switzerland) *(sid2-1)*, J. Glazebrook (Torrey Mesa Research Institute, USA) *(pad4-1)*, J. Turner (University of East Anglia, UK) *(coi1-1)*, J. Parker (Sainsbury Laboratory, UK) (*eds1-1*), J. Ryals (Novartis, USA) (*NahG*), F. Ausubel and S. Ferrari (Massachusetts General Hospital, USA) (plants containing the $P_{PDF1.2a}$:β-glucuronidase construct) and the *Arabidopsis* Biological Resource Centre (Ohio State University, USA) *(eds5-1*, *eds1-1*, *ein2-1*, *Etr1-1*, *jar1-1)* for providing seed. We are also grateful to L. Friedrich (Syngenta, USA) and H.S. Judelson (Riverside University, USA) for providing *P. parasitica* and *P. infestans* isolates respectively. We thank T. Hamann and members of the Somerville lab. for critical comments. We also thank I. Xu and R. Ewing for upgrading the Motif Finder utility at TAIR, and B.H. Hou for excellent technical assistance. This work was supported in part by the Carnegie Institution of Washington, the International Research Management fund, the National Science Foundation and the US Department of Energy. Laurent Zimmerli was supported in part by a Swiss National Science Foundation fellowship and Monica Stein was supported in part by a Stanford Graduate Fellowship. This is manuscript number 1676 of the Carnegie Institution of Washington.

Supplementary Material

The following material is available from http://www.blackwellpublishing.com/products/journals/suppmat/TPJ/TPJ2236/TPJ2236sm.htm.

Table S1 Disease reaction scores of *Arabidopsis* accessions upon inoculation with host (*E. cichoracearum* or *E. cruciferarum*) or non-host (*B. graminis* f. sp. *hordei*) powdery mildews

Table S2 Disease reactions scores of *Arabidopsis* mutants upon inoculation with two different isolates of the non-host barley powdery mildew, *B. graminis* f. sp. *hordei*

Table S3 Log_2(ratio) values for clones selected by SAM analysis

References

Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J. and Parker, J.E. (1998) Different requirements for *EDS1* and *NDR1* by disease resistance genes define at least two *R* gene-mediated signalling pathways in *Arabidopsis*. *Proc. Natl Acad. Sci. USA*, **95**, 10306–10311.

Adam, L. and Somerville, S.C. (1996) Genetic characterization of five powdery mildew disease resistance loci in *Arabidopsis thaliana*. *Plant J.* **9**, 341–356.

Adam, L., Ellwood, S., Wilson, I., Saenz, G., Xiao, S., Oliver, R.P., Turner, J.G. and Somerville, S. (1999) Comparison of *Erysiphe cichoracearum* and *E. cruciferarum* and a survey of 360 *Arabidopsis thaliana* accessions for resistance to these two powdery mildew pathogens. *Mol. Plant Microbe Interact.* **12**, 1031–1043.

Bak, S., Tax, F.E., Feldmann, K.A., Galbraith, D.W. and Feyereisen, R. (2001) CYP83B1, a cytochrome P450 at the metabolic branch point in auxin and indole glucosinolate biosynthesis in *Arabidopsis*. *Plant Cell*, **13**, 101–111.

Bleecker, A.B., Estelle, M.A., Somerville, C. and Kende, H. (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. *Science*, **241**, 1086–1089.

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.

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

Caten, C.E. and Jinks, J.L. (1968) Spontaneous variability of single isolates of *Phytophthora infestans*. I. Cultural variation. *Can. J. Bot.* **46**, 329–348.

Century, K.S., Holub, E.B. and Staskawicz, B.J. (1995) *NDR1*, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. *Proc. Natl Acad. Sci. USA*, **92**, 6597–6601.

Chen, W. and Singh, K.B. (1999) The auxin, hydrogen peroxide and salicylic acid induced expression of the *Arabidopsis GST6* promoter is mediated in part by an *ocs* element. *Plant J.* **19**, 667–677.

Chen, W., Chao, G. and Singh, K.B. (1996) The promoter of a H_2O_2-inducible, *Arabidopsis* glutathione S-transferase gene contains closely linked OBF- and OBP1-binding sites. *Plant J.* **10**, 955–966.

Cohn, J., Sessa, G. and Martin, G.B. (2001) Innate immunity in plants. *Curr. Opin. Immunol.* **13**, 55–62.

Collins, N.C., Thordal-Christensen, H., Lipka, V. *et al.* (2003) SNARE-protein-mediated disease resistance at the plant cell wall. *Nature*, **425**, 973–977.

Dangl, J.L. and Jones, J.D. (2001) Plant pathogens and integrated defence responses to infection. *Nature*, **411**, 826–833.

Delaney, T.P., Friedrich, L. and Ryals, J.A. (1995) *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. *Proc. Natl Acad. Sci. USA*, **92**, 6602–6606.

Dewdney, J., Rueber, T.L., Wildermuth, M.C., Devoto, A., Cui, J., Stutius, L.M., Drummond, E.P. and Ausubel, F.M. (2000) Three unique mutants of *Arabidopsis* identify *eds* loci required for limiting growth of a biotrophic fungal pathogen. *Plant J.* **24**, 205–218.

Ehness, R., Ecker, M., Godt, D.E. and Roitsch, T. (1997) Glucose and stress independently regulate source and sink metabolism and defence mechanisms via signal transduction pathways involving protein phosphorylation. *Plant Cell*, **9**, 1825–1841.

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

Ellis, C., Karafyllidis, I., Wasternack, C. and Turner, J.G. (2002b) The *Arabidopsis* mutant *cev1* links cell wall signalling to jasmonate and ethylene responses. *Plant Cell*, **14**, 1557–1566.

Eulgem, T., Rushton, P.J., Schmelzer, E., Hahlbrock, K. and Somssich, I.E. (1999) Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors. *EMBO J.* **18**, 4689–4699.

Feys, B.J., 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.

Frye, C.A. and Innes, R.W. (1998) An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *Plant Cell*, **10**, 947–956.

Geraats, B.P.J., Bakker, P.A.H.M., Lawrence, C.B., Achuo, E.A., Höfte, M. and van Loon, L.C. (2003) Ethylene-insensitive tobacco shows differentially altered susceptibility to different pathogens. *Phytopathology*, **93**, 813–821.

Glazebrook, J. (2001) Genes controlling expression of defence responses in *Arabidopsis*: 2001 status. *Curr. Opin. Plant Biol.* **4**, 301–308.

Glazebrook, J. and Ausubel, F.M. (1994) Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens. *Proc. Natl Acad. Sci. USA*, **91**, 8955–8959.

Glazebrook, J., Rogers, E.E. and Ausubel, F.M. (1996) Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. *Genetics*, **143**, 973–982.

Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E.E., Crute, I.R., Holub, E.B., Hammerschmidt, R. and Ausubel, F.M. (1997) Phytoalexin-deficient mutants of *Arabidopsis* reveal that *PAD4* encodes a regulatory factor and that four *PAD* genes contribute to downy mildew resistance. *Genetics*, **146**, 381–392.

Gollub, J., Ball, C.A., Binkley, G. *et al.* (2003) The Stanford Microarray Database: data access and quality assessment tools. *Nucleic Acids Res.* **31**, 94–96.

Gomez-Gomez, L., Felix, G. and Boller, T. (1999) A single locus determines sensitivity to bacterial flagellin in *Arabidopsis thaliana*. *Plant J.* **18**, 277–284.

Heath, M.C. (2000) Nonhost resistance and nonspecific plant defences. *Curr. Opin. Plant Biol.* **3**, 315–319.

Heck, S., Buchala, A., Métraux, J.-P. and Nawrath, C. (2003) Genetic evidence that expression of NahG modifies defence pathways independent of salicylic acid biosynthesis in the *Arabidopsis–Pseudomonas syringae* pv. *tomato* interaction. *Plant J.* **36**, 342–352.

Holub, E. (2001) The arms race is ancient history in *Arabidopsis*, the wildflower. *Nat. Rev. Genet.* **2**, 516–527.

Huitema, E., Vleeshouwers, G.A.A., Francis, D.M. and Kamoun, S. (2003) Active defence responses associated with nonhost

resistance of *Arabidopsis thaliana* to the oomycete pathogen *Phytothphora infestans*. *Mol. Plant Pathol.* **4**, 487–500.

Jefferson, R.A., Kavanagh, T.A. and Bevan, M.W. (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. *EMBO J.* **6**, 3901–3907.

Kamoun, S., Huitema, E. and Vleeshouwers, V.G. (1999) Resistance to oomycetes: a general role for the hypersensitive response? *Trends Plant Sci.* **4**, 196–200.

Kang, L., Li, J., Zhao, T., Xiao, F., Thang, X., Thilmony, R., He, S.Y. and Zhou, J.-M. (2003) Interplay of the *Arabidopsis* nonhost resistance gene *NHO1* with bacterial virulence. *Proc. Natl Acad. Sci. USA*, **100**, 3519–3524.

Kanzaki, H., Saitoh, H., Ito, A., Fujisawa, S., Kamoun, S., Katou, S., Yoshioka, H. and Terauchi, R. (2003) Cytosolic HSP90 and HSP70 are essential components of INF1-mediated hypersensitive response and nonhost resistance to *Pseudomonas cichorii* in *Nicotiana benthamiana*. *Mol. Plant Pathol.* **4**, 383–391.

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.

Knoester, M., van Loon, L.C., Heuvel, J.V.D., Hennig, J., Bol, J.F. and Linthorst, H.J.M. (1998) Ethylene-insensitive tobacco lacks nonhost resistance against soil-borne fungi. *Proc. Natl Acad. Sci. USA*, **95**, 1933–1937.

Kobayashi, Y., Yamada, M., Kobayashi, I. and Kunoh, H. (1997) Actin microfilaments are required for the expression of nonhost resistance in higher plants. *Plant Cell Physiol.* **38**, 725–733.

Lawton, K., Weymann, K., Friedrich, L., Vernooij, B., Uknes, S. and Ryals, J. (1995) Systemic acquired resistance in *Arabidopsis* requires salicylic acid but not ethylene. *Mol. Plant Microbe Interact.* **8**, 863–870.

Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., Rouze, P. and Rombauts, S. (2002) PlantCARE, a database of plant *cis*-acting regulatory elements and a portal to tools for *in silico* analysis of promoter sequences. *Nucleic Acids Res.* **30**, 325–327.

Logemann, E., Wu, S.C., Schroder, J., Schmelzer, E., Somssich, I.E. and Hahlbrock, K. (1995) Gene activation by UV light, fungal elicitor or fungal infection in *Petroselinum crispum* is correlated with repression of cell cycle-related genes. *Plant J.* **8**, 865–876.

Lu, M., Tang, X. and Zhou, J.-M. (2001) Arabidopiss *NHO1* is required for general resistance against *Pseudomonas* bacteria. *Plant Cell*, **13**, 437–447.

Matsumura, H., Reich, S., Ito, A., Saitoh, H., Kamoun, S., Winter, P., Kahl, G., Reuter, M., Kruger, D.H. and Terauchi, R. (2003) Gene expression analysis of plant host-pathogen interactions by SuperSAGE. *Proc. Natl Acad. Sci. USA*, **100**, 15718–15723.

Mellersh, D.G. and Heath, M.C. (2003) An investigation into the involvement of defence signalling pathways in components of the nonhost resistance of *Arabidopsis thaliana* to rust fungi also reveals a model system for studying rust fungal compatibility. *Mol. Plant Microbe Interact.* **16**, 398–404.

Moseman, J.G. (1968) Reactions of barley to *Erysiphe graminis* f. sp. *hordei* from North American, England, Ireland, and Japan. *Plant Dis. Rep.* **52**, 463–467.

Moseman, J.G. (1972) Isogenic barley lines for reaction to *Erysiphe graminis* f. sp. *hordei*. *Crop Sci.* **12**, 681–682.

Mysore, K.S., D'Ascenzo, M.D., He, X. and Martin, G.B. (2003) Overexpression of the disease resistance gene *Pto* in tomato induces gene expression changes similar to immune responses in human and fruitfly. *Plant Physiol.* **132**, 1901–1912.

Nawrath, C. and Metraux, J.P. (1999) Salicylic acid induction-deficient mutants of *Arabidopsis* express *PR-2* and *PR-5* and accumulate high levels of camalexin after pathogen inoculation. *Plant Cell*, **11**, 1393–1404.

Newman, T., Debruijn, F.J., Green, P. *et al.* (1994) Genes galore: a summary of methods for accessing results from large-scale partial sequencing of anonymous *Arabidopsis* cDNA clones. *Plant Physiol.* **106**, 1241–1255.

Nishimura, M., Stein, M., Hou, B.-H., Vogel, J., Edwards, H. and Somerville, S. (2003) Loss of a callose synthase results in salicylic acid-dependent disease resistance. *Science*, **301**, 969–972.

O'Connell, R.J., Brown, I.R., Mansfield, J.W., Bailey, J.A., Mazau, D., Rumeau, D. and Esquerre-Tugaye, M.T. (1990) Immunocytochemical localization of hydroxyproline-rich glycoproteins accumulating in melon and bean at sites of resistance to bacteria and fungi. *Mol. Plant Microbe Interact.* **3**, 33–40.

Panstruga, R. (2003) Establishing compatibility between plants and obligate biotrophic pathogens. *Curr. Opin. Plant Biol.* **6**, 320–326.

Parker, J.E., Holub, E.B., Frost, L.N., Falk, A., Gunn, N.D. and Daniels, M.J. (1996) Characterization of *eds1*, a mutation in *Arabidopsis* suppressing resistance to *Peronospora parasitica* specified by several different *RPP* genes. *Plant Cell*, **8**, 2033–2046.

Peart, J.R., Lu, R., Sadanandom, A. *et al.* (2002) Ubiquitin ligase-associated protein SGT1 is required for host and nonhost disease resistance in plants. *Proc. Natl Acad. Sci. USA*, **99**, 10865–10869.

Penninckx, I.A., Eggermont, K., Terras, F.R., Thomma, B.P., De Samblanx, G.W., Buchala, A., Métraux, J.P., Manners, J.M. and Broekaert, W.F. (1996) Pathogen-induced systemic activation of a plant defensin gene in *Arabidopsis* follows a salicylic acid-independent pathway. *Plant Cell*, **8**, 2309–2323.

Ramonell, K.M., Zhang, B., Ewing, R.M., Chen, Y., Xu, D., Stacey, G. and Somerville, S. (2002) Microarray analysis of chitin elicitation in *Arabidopsis thaliana*. *Mol. Plant Pathol.* **3**, 301–311.

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

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.

Roman, G., Lubarsky, B., Kieber, J.J., Rothenberg, M. and Ecker, J.R. (1995) Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. *Genetics*, **139**, 1393–1409.

Scheible, W.R., Fry, B., Kochevenko, A., Schindelasch, D., Zimmerli, L., Somerville, S., Loria, R. and Somerville, C.R. (2003) An *Arabidopsis* mutant resistant to Thaxtomin A, a cellulose synthesis inhibitor from *Streptomyces* species. *Plant Cell*, **15**, 1781–1794.

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

Schulze-Lefert, P. and Vogel, J. (2000) Closing the ranks to attack by powdery mildew. *Trends Plant Sci.* **5**, 343–348.

Schweizer, P., Gees, R. and Mosinger, E. (1993) Effect of jasmonic acid on the interaction of barley (*Hordeum vulgare* L.) with the powdery mildew *Erysiphe graminis* f. sp. *hordei*. *Plant Physiol.* **102**, 503–511.

Seki, H., Ichinose, Y., Kato, H., Shiraishi, T. and Yamada, T. (1996) Analysis of *cis*-regulatory elements involved in the activation of a

member of chalcone synthase gene family (*PsChs1*) in pea. *Plant Mol. Biol.* **31**, 479–491.

Shen, Q.-H., Zhou, F., Bieri, S., Haizel, T., Shirazu, K. and Schulze-Lefert, P. (2003) Recognition specificity and RAR1/SGT1 dependence in barley *Mla* disease resistance genes to the powdery mildew fungus. *Plant Cell*, **15**, 732–744.

Staswick, P.E., Su, W. and Howell, S.H. (1992) Methyl jasmonate inhibition of root growth and induction of a leaf protein are decreased in an *Arabidopsis thaliana* mutant. *Proc. Natl Acad. Sci. USA*, **89**, 6837–6840.

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 defence-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proc. Natl Acad. Sci. USA*, **95**, 15107–15111.

Thomma, B.P., Cammue, B.P. and Thevissen, K. (2002) Plant defensins. *Planta*, **216**, 193–202.

Thordal-Christensen, H. (2003) Fresh insights into processes of nonhost resistance. *Curr. Opin. Plant Biol.* **6**, 351–357.

Tusher, V.G., Tibshirani, R. and Chu, G. (2001) Significance analysis of microarrays applied to the ionizing radiation response. *Proc. Natl Acad. Sci. USA*, **98**, 5116–5121.

Van Wees, S.C. and Glazebrook, J. (2003) Loss of non-host resistance of *Arabidopsis NahG* to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid. *Plant J.* **33**, 733–742.

Varet, A., Hause, B., Hause, G., Scheel, D. and Lee, J. (2003) The *Arabidopsis NHL3* gene encodes a plasma membrane protein and its overexpression correlates with increased resistance to *Pseudomonas syringae* pv. tomato DC3000. *Plant Physiol.* **132**, 2023–2033.

Vogel, J. and Somerville, S. (2000) Isolation and characterization of powdery mildew-resistant *Arabidopsis* mutants. *Proc. Natl Acad. Sci. USA*, **97**, 1897–1902.

White, J.A., Todd, J., Newman, T., Girke, T., Focks, N., Martinez de Ilárduya, O., Jaworski, J.G., Ohlrogge, J. and Benning, C. (2000) A new set of *Arabidopsis* ESTs from developing seeds: the metabolic pathway from carbohydrates to seed oil. *Plant Physiol.* **124**, 1582–1594.

Wilson, I.W., Schiff, C.L., Hughes, D.E. and Somerville, S. (2001) Quantitative trait loci analysis of powdery mildew disease resistance in the *Arabidopsis thaliana* accession Kashmir-1. *Genetics*, **158**, 1301–1309.

Wittstock, U. and Halkier, B.A. (2002) Glucosinolate research in the *Arabidopsis* era. *Trends Plant Sci.* **7**, 263–270.

Wright, D.P., Baldwin, B.C., Shephard, M.C. and Scholes, J.D. (1995a) Source–sink relationships in wheat leaves infected with powdery mildew. I. Alterations in carbohydrate metabolism. *Physiol. Mol. Plant Pathol.* **47**, 237–253.

Wright, D.P., Baldwin, B.C., Shephard, M.C. and Scholes, J.D. (1995b) Source–sink relationships in wheat leaves infected with powdery mildew. II. Changes in the regulation of the Calvin cycle. *Physiol. Mol. Plant Pathol.* **47**, 255–267.

Wu, S.-H., Ramonell, K., Gollub, J. and Somerville, S. (2001) Plant gene expression profiling with DNA microarrays. *Plant Physiol. Biochem.* **39**, 917–926.

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

Yu, I.-C., Parker, J. and Bent, A.F. (1998) Gene-for-gene resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *Proc. Natl Acad. Sci. USA*, **95**, 7819–7824.

Yun, B.-W., Atkinson, H.A., Gaborit, C., Greenland, A., Read, N.D., Pallas, J.A. and Loake, G.J. (2003) Loss of actin cytoskeleton function and EDS1 activity, in combination, severely compromises non-host resistance in *Arabidopsis* against wheat powdery mildew. *Plant J.* **34**, 768–777.

Zhao, J. and Last, R.L. (1996) Coordinate regulation of the tryptophan biosynthetic pathway and indolic phytoalexin accumulation in *Arabidopsis*. *Plant Cell*, **8**, 2235–2244.

Zimmerli, L. and Collet, G. (1996) Estimation de la résistance de la pomme de terre au mildiou par un test en chambre climatisée. *Rev. Suisse Agric.* **28**, 5–11.

Accession number for microarray data: Gene Expression Omnibus: GPL1278.