The developmental selector *AS1* is an evolutionarily conserved regulator of the plant immune response

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The MYB-related gene *ASYMMETRIC LEAVES 1* (*AS1*) and its orthologs have an evolutionarily conserved role in specification of leaf cell identity. AS1 is expressed in leaf founder cells, where it functions as a heterodimer with the structurally unrelated AS2 proteins to repress activity of *KNOTTED 1-like homeobox* (*KNOX*) genes. AS1 therefore confines *KNOX* activity to the shoot apical meristem, where it promotes stem cell function through the regulation of phytohormone activities. Here, we show that loss-of-function mutations in *AS1* unexpectedly convey heightened protection against necrotrophic fungi. AS1 operates as a negative regulator of inducible resistance against these pathogens by selectively binding to the promoters of genes controlled by the immune activator, jasmonic acid (JA), damping the defense response. In contrast, AS1 is a positive regulator of salicylic acid (SA)-independent extracellular defenses against bacterial pathogens. Neither the absence of *AS2* nor *ERECTA* function, which enhances the morphological phenotype of *as1*, nor the conditional or constitutive expression of *KNOX* genes impacted disease resistance. Thus, the function of AS1 in responses to phytopathogens is independent of its AS2-associated role in development. Loss of function in the *AS1* orthologs *PHAN* in *Antirrhinum majus* and *NSPHAN* in *Nicotiana sylvestris* produced pathogen-response phenotypes similar to *as1* plants, and therefore the defense function of *AS1* is evolutionarily conserved in plant species with a divergence time of ≈125 million years.

Arabidopsis thaliana | asymmetric leaves 1 | disease resistance | plant defense response

An evolutionarily conserved genetic pathway controls specification of leaf cell fate at the shoot apical meristem and involves the *Arabidopsis ASYMMETRIC LEAVES1* (*AS1*), Antirrhinum *PHANTASTICA* (*PHAN*), *Nicotiana NSPHAN*, and maize *ROUGH SHEATH2* (*RS2*) genes. These genes encode orthologous MYB transcription factors that are expressed only in leaf founder cells and leaf primordia, where they are needed to repress *KNOTTED1-like homeobox* (*KNOX*) genes (1–4). *KNOX* genes are expressed within the shoot apical meristem, where they regulate the activity of gibberellin and cytokinin hormones to maintain indeterminacy (5–9). AS1 forms heterodimers with the structurally unrelated protein AS2 (10), and both proteins are required to prevent *KNOX* expression in developing leaves (9, 10). Ectopic *KNOX* expression produces lobed leaves, similar to those of *as1* and *as2* loss-of-function mutants (11), suggesting that it is responsible for this aspect of the *as1* or *as2* mutant phenotype.

A small number of genes that are needed for more general aspects of plant development have also been found to regulate disease resistance. For example, a potential transcriptional cofactor encoded by *AtTIP49a* promotes the plant defense response but is also needed for viability of female gametophytes and seedlings (12). Similarly, a potential component of a ubiquitin protein ligase complex encoded by *SGT1b* (*SUPPRESSOR OF THE G2 ALLELE OF skp1-4 1b*) is required for both plant disease resistance and auxin signaling (13).

The emerging evidence suggests that plant immune function discriminates between microbes that exhibit distinct pathogenic lifestyles (14). Thus, salicylic acid (SA)-dependent defenses largely underpin resistance against biotrophic pathogens, whereas jasmonic acid (JA)- and ethylene (ET)-based responses are required for protection against necrotrophic pathogens (15–18). Furthermore, these signaling networks are thought to be regulated in a mutually antagonistic fashion, suggesting that plants can prioritize specific defense mechanisms (19–20). Recently, a series of loss-of-function mutations have been uncovered that convey enhanced susceptibility to necrotrophic fungi (21–23). However, only *ocp3* (*overexpressor of cationic peroxidase 3*) and *jin1* (*jasmonate-insensitive 1*) have been reported to increase resistance against this class of pathogen (24, 25).

Here, we show that loss of *AS1* function conveys increased protection against necrotrophic fungal pathogens. Our findings suggest that *AS1* operates as a negative regulator of inducible defense responses against these pathogens by occupying the promoters of a subset of defense genes, damping their expression. In contrast, AS1 operates as a positive regulator of disease resistance against bacterial pathogens. These *AS1* functions are independent of its *AS2*- and *KNOX*-associated roles in development and are conserved in plant species with a divergence time of ≈125 million years.

Results

Forward Screen to Uncover Mutations that Convey Resistance Against *Botrytis cinerea*. To identify *Arabidopsis* mutants expressing increased resistance against the necrotrophic fungal pathogen *B. cinerea*, we screened the progeny of ≈10,000 plants carrying T-DNA insertions (26) by spraying with a suspension of *B. cinerea* spores. One mutant was found with increased resistance to *B. cinerea* and lobed leaves (Fig. 1 *A–D*). Increased pathogen resistance and altered leaf morphology behaved as a single recessive trait and cosegregated with insensitivity to glufosinate herbicide encoded by the T-DNA. Sequences flanking this insert were recovered by thermal asymmetric interlaced (TAIL)-PCR, revealing that the insertion was within the only intron of the *AS1* gene, and that the T-DNA prevented accumulation of *AS1* transcripts detectable by RT-PCR, consistent with a null mutation (data not shown). The mutation was confirmed as an allele of *AS1* in crosses to the classical mutation *as1-1* (27) and was therefore named *as1-4*. Furthermore, *as1-1* also conferred heightened resistance against *B. cinerea*, confirming the pleio-

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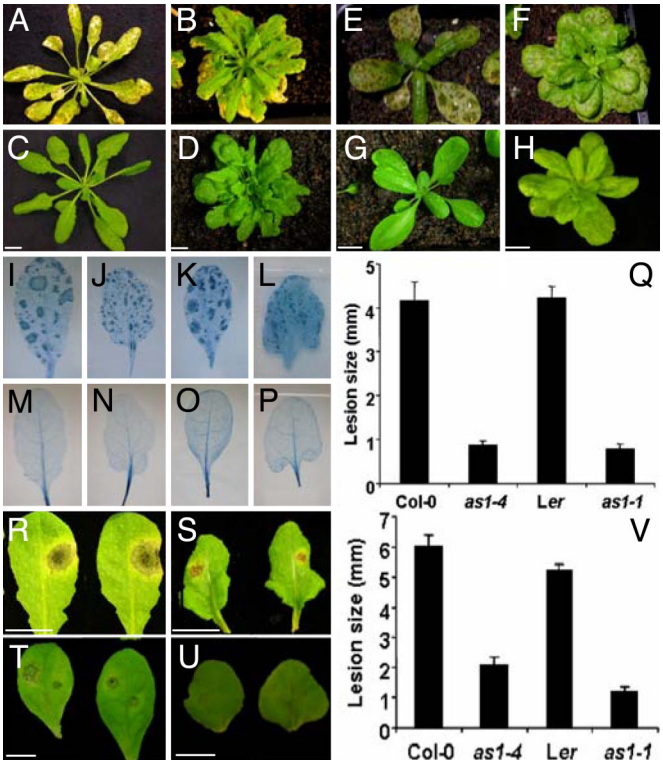


Fig. 1. Loss-of-function mutations in *AS1* increase disease resistance against necrotrophic fungi. (*A–H*) The *Arabidopsis* lines Col-0 (*A* and *C*), Col-0 *as1-4* (*B* and *D*), Ler (*E* and *G*), and Ler *as1-1* (*F* and *H*) were challenged with a spore suspension of *B. cinerea* (*A*, *B*, *E*, and *F*). For mock controls, the inoculation solution lacked spores (*C*, *D*, *G*, and *H*). All plants were scored for disease development at 96 hpi. (*I–P*) Col-0 (*I* and *M*), *as1-4* (*J* and *N*), Ler (*K* and *O*), and *as1-1* plants (*L* and *P*) were either challenged with a spore suspension of *B. cinerea* (*I–L*) or mock inoculated (*M–P*) as described above. Subsequently, these lines were stained with trypan blue, which marks dead plant cells, revealing lesion development. (*Q*) Quantification of the extent of *B. cinerea*-generated lesions in the indicated plant genotypes. (*R–U*) The *Arabidopsis* lines Col-0 (*R*), Col-0 *as1-4* (S), Ler (*T*), and Ler *as1-1* (*U*) were drop-inoculated with an *A. brassicicola* spore suspension and scored at 96 hpi. (*V*) Size of *A. brassicicola*-generated lesions in the indicated plant genotypes. Error bars represent 95% confidence limits. All experiments were repeated at least twice with similar results. (Scale bars, 1 cm.)

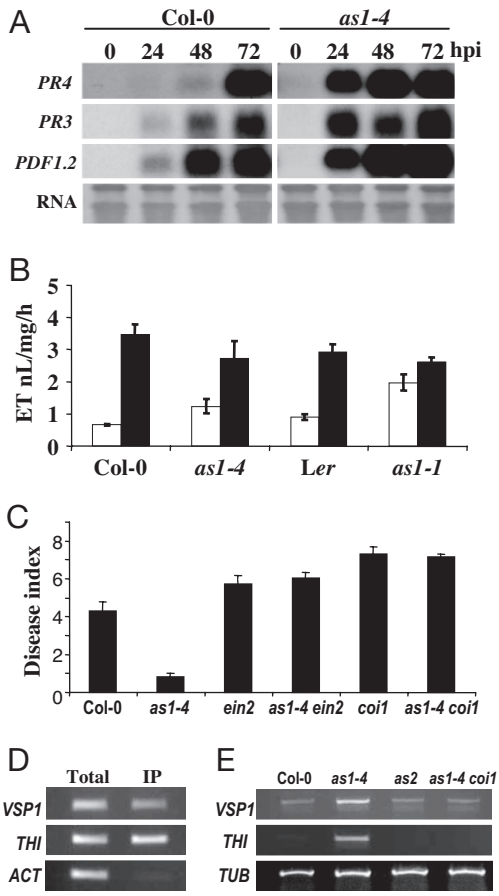


Fig. 2. Misexpression of JA/ET-dependent defense response genes in *as1* plants. (*A*) Northern blot analysis of *PDF1.2*, *PR3*, and *PR4* transcript accumulation in the indicated plant genotypes challenged with *B. cinerea* at the stated hpi. (*B*) Determination of the rate of ET production in the indicated plant lines. Noninoculated plants (empty bars) and *B. cinerea*-inoculated plants (filled bars). (*C*) The stated plant genotypes were challenged with *B. cinerea* and scored for disease development at 96 hpi. (*D*) Detection of the indicated gene-regulatory sequences by PCR in total and immunoprecipitated (IP) chromatin extracts by using an anti-GFP antiserum to enrich for chromatin containing PHAN-GFP. The actin (*ACT*) gene is used as a nonbinding control. (*E*) Accumulation of the listed gene transcripts in Col-0 wild-type and *as1* and *as2* mutant plants determined by RT-PCR. Tubulin (*TUB*) is a constitutively expressed control. Error bars represent 95% confidence limits. These experiments were repeated at least twice with similar results.

tropic roles of *AS1* in pathogen resistance and plant development (Fig. 1 *E–H*).

We monitored expression of the *B. cinerea ActA* gene, which correlates directly with the extent of *B. cinerea* growth (28). At 96 h postinoculation (hpi), *ActA* transcript accumulation in *as1-1* plants was reduced by 71% compared with wild type (data not shown). Furthermore, trypan blue staining of dead cells after *B. cinerea* inoculation revealed that *as1* mutants developed strikingly smaller lesions (reduced in size by ≈80% compared with wild type; Fig. 1 *I–Q*). Scoring overall symptom development with a disease index also showed *as1* mutations conveyed robust resistance against *B. cinerea* (data not shown). The level of protection against *B. cinerea* infection in *as1* plants was similar to that established by exogenous application of JA to wild-type plant lines [supporting information (SI) Fig. 5]. To investigate whether *as1* can restrict the growth of other necrotrophic fungi, *as1* and wild-type plants were challenged with *Alternaria brassicicola*. Both *as1-1* and *as1-4* mutants showed increased resistance, marked by a conspicuous decrease in lesion size (Fig. 1 *R–V*).

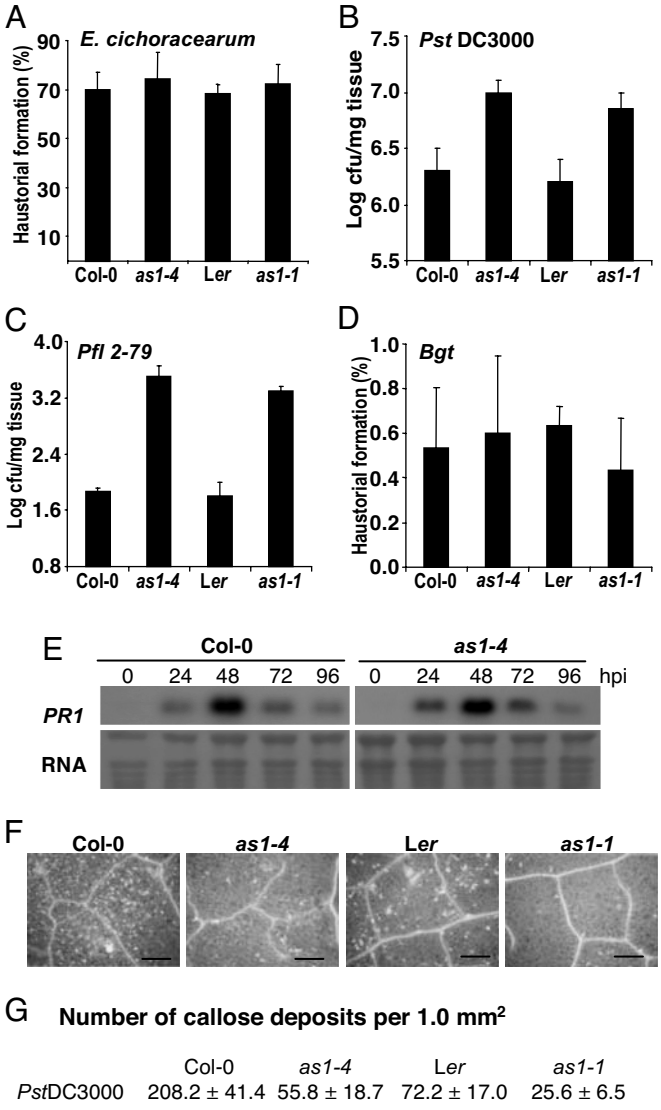
***as1*-Mediated Resistance Requires *COI1* and *EIN2*.** Resistance to necrotrophic pathogens is typically dependent on the plant hormones JA and ET (15–18). To examine whether increased resistance in *as1* mutants involved altered JA or ET responses, we monitored expression of defense marker genes that are up-regulated by these hormones. In *as1-4* mutants, induction of the JA/ET-dependent *PLANT DEFENSIN1.2* (*PDF1.2*), *PATHOGENESIS-RELATED3* (*PR3*), and *PR4* genes (15) was accelerated in response to *B. cinerea* infection (Fig. 2*A*). We therefore determined the concentrations of JA and ET during infection with *B. cinerea*. In noninoculated leaves, levels of ET were twice as high in *as1-1* mutants compared with wild type and 1.3 times higher in *as1-4* mutants but induced to the same levels in *as1* and wild type upon infection with *B. cinerea* (Fig. 2*B*). JA levels in uninfected and infected *as1* mutants were similar to wild type (data not shown). To examine if ET or JA signaling is required for *as1*-mediated disease resistance, we generated *as1* double mutants with either *ein2* (29) or *coi1* (30), which cause insensitivity toward ET or JA, respectively, and result in enhanced susceptibility to some necrotrophic pathogens (15, 16). In response to *B. cinerea*, *as1 ein2* and, particularly, *as1 coi1* plants

exhibited increased lesion development relative to the *as1* line (Fig. 2*C*) and were compromised in JA/ET-dependent gene expression (data not shown). Therefore, *as1*-mediated resistance against *B. cinerea* requires ET and, more significantly, JA signaling.

Identification of Gene Promoters Occupied by AS1. Because *as1* mutants showed increased induction of pathogen-induced marker genes, we tested whether AS1 might have a direct role in regulation of defense genes by using a chromatin immunoprecipitation (ChIP)-gene chip approach (31). A transgene expressing a PHAN-green fluorescent protein (GFP) fusion complemented both the developmental and defense-related phenotypes of *as1* mutants, revealing that PHAN is functionally equivalent to AS1 (SI Fig. 6). ChIP-chip utilizing antibodies against GFP suggested that AS1 occupied the promoters of at least 96 genes (SI Table 1). We analyzed the expression profiles of these genes, where possible, by interrogating array experiments documented in the TAIR database (www.arabidopsis.org/). The expression of 37% of interrogated genes was changed by at least 2-fold in response to *B. cinerea*/JA/ET (SI Table 2), consistent with AS1 having a direct role in regulation of genes that are involved in defense.

The well characterized JA-regulated genes *THIONIN* (*THI*) and *VEGETATIVE STORAGE PROTEIN* (*VSP1*) (17) were among the most significantly enriched target genes in ChIP-chip. Maximum *VSP1* and *THI* enrichment occurred with DNA sequences −350 to 400 and −150 to 250 bp, respectively, upstream of their respective start codons. In the case of *VSP1*, this sequence is 89 bp upstream of a known JA-responsive element (32). Primers designed to amplify these sequences were used to verify PHAN binding in three independent ChIP experiments, in which *THI* and *VSP1* were enriched 5.39- and 5.42-fold, respectively (Fig. 2*D*), and similar enrichment was obtained when a different antibody against GFP was used in immunoprecipitation (SI Fig. 7). *THI* and *VSP1* transcript accumulation was greater in *as1* compared with wild type, suggesting that AS1 binding is needed to repress these two target genes (Fig. 2*E*). *THI* and *VSP1* were not misexpressed in an *as1 coi1* double mutant, indicating that JA signaling is still required to induce *THI* and *VSP* expression in the absence of AS1 activity. In addition, *as2* mutants did not show increased *THI* and *VSP1* transcript levels, suggesting that the role of AS1 in disease resistance might be independent of AS2.

Response of *as1* Plants to Diverse Pathogens. We tested whether AS1 function affected responses to other pathogens. Resistance to a virulent biotrophic powdery mildew fungus, *Erysiphe cichoracearum* (33), was unaffected by the *as1* mutation (Fig. 3*A*). In contrast, *as1* mutants supported growth of the virulent hemibiotrophic bacterium, *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000 (34), to levels ≈7 times greater than wild type (Fig. 3*B*). Similarly, *as1* mutants were more susceptible to *Pseudomonas fluorescens* (*Pfl*) 2-79, for which wild-type *Arabidopsis* is a nonhost (35) (Fig. 3*C*). *Arabidopsis* is also a nonhost for the wheat powdery mildew fungus *Blumeria graminis* f.sp. *tritici* (*Bgt*) (36). In contrast to *Pfl*2-79, however, nonhost resistance against *Bgt* was not compromised in *as1* mutants (Fig. 3*D*). Basal and resistance (*R*) gene-mediated protection against two isolates of *Hyaloperonospora parasitica*, Noco2 (37) and Cala2 (38), also appeared not to be impacted by mutations in *AS1* (SI Fig. 8). Neither *PR1* expression (Fig. 3*E*) nor SA accumulation (data not shown) was affected in *as1* plants inoculated with *Pst*DC3000. However, callose deposition, a marker for SA-independent extracellular defense (39), was compromised in response to either *Pst*DC3000 (Fig. 3 *F* and *G*) or *Pfl*2-79 (data not shown).



G Number of callose deposits per 1.0 mm²

	Col-0	*as1-4*	Ler	*as1-1*
*Pst*DC3000	208.2 ± 41.4	55.8 ± 18.7	72.2 ± 17.0	25.6 ± 6.5

Fig. 3. Both nonhost and basal resistance is compromised in *as1* plants against attempted bacterial infection. (*A*) Extent of *E. cichoracearum* infection measured by determining the percentage of conidia that form a haustorium on the indicated plant lines. (*B*) Quantification of *Pst*DC3000 growth in the stated plant genotypes at 5 days postinoculation. (*C*) Growth of *Pfl*2-79 in the stated plant genotypes at 5 days postinoculation. (*D*) Extent of *Bgt* infection measured by determining the percentage of conidia that form a haustorium on the indicated plant lines. (*E*) Accumulation of *PR1* transcripts in wild-type Col-0 and Col-0 *as1-4* plants at the given times after *B. cinerea* inoculation. (*F*) Portions of leaves from the indicated plant genotypes stained with Aniline blue to detect callose deposition (white dots) after inoculation with *Pst*DC3000. (Scale bars, 250 μm.) (*G*) Quantification of callose deposition. Error bars represent 95% confidence limits. These experiments were repeated at least three times with similar results.

***AS1* Function in Responses to Phytopathogens Is Independent of Its *AS2*-Associated Role in Development.** AS2, a member of the LATERAL ORGAN BOUNDARIES (LOB) family of plant-specific proteins, forms heterodimers with AS1 and is likely to function in the same developmental process (9, 10). The *as2* and *as1* lines have similar morphological phenotypes (10), but whereas *as1* plants were more resistant to *B. cinerea*, *as2* mutants were as susceptible as wild type (Fig. 4*A*). Furthermore, analysis of the weak *as1-13* allele, which shows only mild morphological defects, revealed that this line still exhibited significant protection against *B. cinerea* (Fig. 4*A*). These findings suggest, firstly, that the

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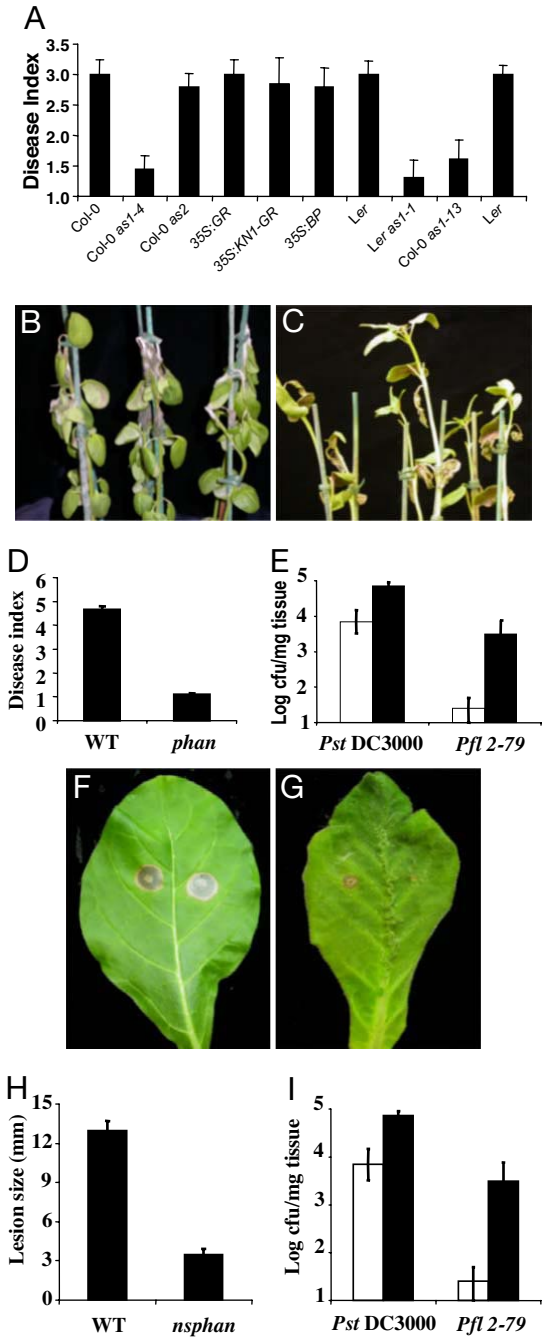

Fig. 4. *AS1* is an evolutionarily conserved regulator of plant immunity that functions independently of *AS2* and *KN1*. (*A*) The given *Arabidopsis* genotypes were spray-inoculated with a *B. cinerea* spore suspension. The *35S:GR* and *35S:KN1-GR* lines were exogenously treated with 1 μM dexamethasone 48 h before *B. cinerea* inoculation and disease symptoms scored at 96 hpi by using a disease index. (*B* and *C*) Wild-type *A. majus* (*B*) and *phan* (*C*) plants were challenged with *B. cinerea* and scored at 96 hpi. (*D*) Quantification of *B. cinerea* disease phenotypes in the stated plant genotypes by using a disease index. (*E*) Wild-type (empty bars) and *phan* (filled bars) *A. majus* plants were inoculated with either *Pst*DC3000 or *Pfl*2-79, and bacterial growth was determined at 5 days after inoculation. (*F* and *G*) Wild-type *N. sylvestris* plants (*F*) and an *N. sylvestris* antisense line (*G*) depleted in *NSPHAN* RNA were inoculated with *B. cinerea* and scored at 96 hpi. (*H*) Quantification of *B. cinerea* disease phenotypes in the stated plant genotypes. (*I*) Wild-type *N. sylvestris* (empty bars) and an *N. sylvestris* antisense line depleted in *NSPHAN* RNA (*nsphan*) (filled bars) were challenged with either *Pst*DC3000 or *Pfl*2-79 and bacterial growth was determined 5 days after inoculation. Error bars represent 95% confidence limits. These experiments were repeated three times with similar results.

resistance effects of *as1* mutations are not due to altered morphology and, secondly, that the role of AS1 in repressing JA-induced defense genes is separate from its AS2-dependent role in development.

The only recognized developmental function of *AS1* and *AS2* is to prevent expression of *KNOX* genes (1, 10), and misexpression of the *KNOX* gene *KN1* is sufficient to cause lobed leaves similar to those of *as1* or *as2* (11). Because the roles of AS1 in development and defense could be separated by their dependence on AS2, we predicted that *KN1* misexpression would not cause increased resistance to *B. cinerea*. To test this, we induced ectopic KNOX activity by using a fusion of KN1 to the rat glucocorticoid receptor under control of the constitutive *35S* promoter. This activity causes rapid dexamethasone-dependent changes in gibberellin and cytokinin levels and development of *as1*-like lobed leaves (11). Inducing KN1 activity before *B. cinerea* inoculation reduced the expression of a KN1 target gene, which encoded a gibberellin 20-oxidase (11) (data not shown) but did not confer increased resistance to *B. cinerea* (Fig. 4*A*). Constitutive expression of another *KNOX* gene, *BREVIPEDICELLUS* (*BP*) (40), that is an endogenous target of AS1 and AS2, gave a similar result. We also tested potential involvement of the ERECTA receptor-like kinase, which has multiple developmental roles mainly associated with cell growth. Although *er* mutations enhance the *as1* and *as2* mutant phenotype (10) and cause increased susceptibility to some bacterial pathogens (41), *er* mutants showed the same response to *B. cinerea* as wild type (Fig. 4*A*).

The Defense Function of *AS1* Is Evolutionarily Conserved. *AS1* is orthologous to *PHAN* in Antirrhinum (2) and *NSPHAN* in tobacco (4), which have conserved roles in repressing *KNOX* genes in leaves. We therefore determined whether the functions of AS1 in plant–pathogen interactions were also conserved. Wild-type *A. majus* showed strong disease development 10 days after spraying with a suspension of *B. cinerea* spores, whereas *phan* mutants had significantly reduced levels of disease (Fig. 4 *B–D*). Conversely, *phan* mutants supported an increase in *Pst*DC3000 and *Pfl*2-79 growth relative to wild type (Fig. 4*E*). Similarly, an antisense *N. sylvestris* line depleted in *NSPHAN* RNA (4) showed reduced lesion development in response to *B. cinerea* relative to wild type (Fig. 4 *F–H*) and increased *Pst*DC3000 and *Pfl*2-79 growth (Fig. 4*I*). Collectively, these data suggest that AS1 has an evolutionarily conserved role in plant–pathogen interactions.

Discussion

We have uncovered an unexpected role for the developmental regulator, *AS1*, in plant–pathogen interactions. Loss-of-function mutations in this gene convey strikingly increased resistance against both *B. cinerea* and *A. brassicicola*. Therefore, *AS1* may repress the inducible defense response against a relatively broad range of necrotrophic fungi. Our findings suggest that AS1 acts to suppress resistance to nectrotrophs by repressing the expression of JA-induced resistance proteins, including THI, which when overexpressed is sufficient to confer enhanced resistance to the necrotropic pathogen *Fusarium oxysporum* in *Arabidopsis* (42). Because AS1 associates physically with the promoters of JA-induced genes, this repression is likely to be direct. Target gene expression and increased resistance, however, remain dependent on functional JA signaling in the absence of AS1 activity. For at least one target gene, the promoter region occupied by AS1 is distinct from that required for response to JA, and *AS1* expression is not affected when JA signaling is disrupted. This observation suggests that induction by JA signaling and repression by AS1 act in parallel, converging on target gene promoters. One rationale for the repressive role of AS1 is that it damps the JA response, preventing premature deployment of the immunity response and its associated costs.

Epistasis analysis revealed that *EIN2* and, particularly, *COI1* were required for *as1*-mediated resistance against *B. cinerea*. This finding contrasts with resistances established by *opr3*, the only other reported loss-of-function mutation that conveys robust resistance against *B. cinerea* (24) or by overexpression of *ETHYLENE RESPONSE FACTOR1* (*ERF1*), which were both independent of *EIN2* (24, 43). AS1 is a known repressor of developmental target genes (1) and is implicated in epigenetic control because it interacts with a HIRA-like protein (44), homologues of which are needed to maintain silent chromatin in yeast and animals. JA/ET-dependent gene expression in *Arabidopsis* involves epigenetic control through histone modification (45), and therefore AS1 might act in this process. AS1, however, appears unlikely to function as a corepressor of many genes given the relatively low number of potential targets detected in ChIP-chip experiments and the limited pleiotropic effects of *as1* mutations.

Our findings suggest that *AS1* is a positive regulator of disease resistance against bacterial pathogens. Callose deposition in response to attempted bacterial infection was compromised in *as1* plants. However, neither *PR1* expression nor SA accumulation was altered. Thus, *AS1* appears to regulate bacterially triggered SA-independent extracellular defenses (which are known to be targets for suppression by effector proteins delivered via the type III secretory system) during bacterial pathogenesis (39). AS1 may therefore regulate defense responses against two distinct classes of phytopathogens.

AS2 functions as a heterodimer with *AS1* in leaf development (9, 10). Significantly, *as2* mutants did not show increased resistance against *B. cinerea*, implying that *AS1* acts independently of *AS2* in pathogen responses but not in development. The only known role of *AS1* and *AS2* in leaf morphogenesis is in repression of *KNOX* genes (1, 10). Ectopic KNOX activity encoded by *BP*, a target of AS1 repression (40), caused an *as1*-like morphology but did not increase resistance against *B. cinerea*, further supporting a function for *AS1* in disease resistance that is independent of its *AS2*-associated developmental role.

AS1 orthologs in Antirrhinum (2) and tobacco (4) have a conserved role in repressing *KNOX* genes in leaves. We have shown that their role in repressing resistance against *B. cinerea* is also conserved, and that they also have similar effects on resistance to hemibiotrophic bacterial pathogens. The role of *AS1* in immunity is therefore conserved in eudicot species with a divergence time of ≈125 million years. The ability to repress *KNOX* gene expression is likely to be more ancient because the morphological effects of *as1* mutations can be complemented by an *AS1*-like gene from Selaginella (46), which last shared a common ancestor with eudicots ≈400 million years ago. Although we have not tested when the role of *AS1*-like genes in disease resistance evolved, it is attractive to speculate that the ancestral role was developmental, and that *AS1*-like genes that were already expressed in lateral organs were later coopted to regulate defense responses.

A dual role in development and immunity is paralleled in animals in which the Spätzle-Toll-Cactus signaling pathway that controls dorsoventral patterning in Drosophila also regulates the innate immune response (47). However, it differs in involving a common transcriptional regulator in plants rather than redeployment of a signaling cassette as in Drosophila.

Methods

Plant Genotypes. The original *as1-1* mutant was identified in a Col-0 genetic background (1); this mutation was also introgressed into L*er*. The generation of activation T-DNA-tagged lines and *as1* double mutants was as described in ref. 26. The *A. majus* (2) and *N. sylvestris* (4) lines were reported previously.

Pathogen Maintenance and Inoculations. *B. cinerea* (PJH2) (26) and *A. brassicola* (MUCL20297) (15) were grown on oat meal and ½ potato dextrose agar media, respectively. Fungal spore density was adjusted to 5×10^5 spores per ml in ½ potato dextrose broth for *B. cinerea* and water for *A. brassicicola*. The resulting *B. cinerea* spore suspensions were sprayed onto 4-week-old *Arabidopsis* plants that were maintained at 100% humidity for 4 days. *A. brassicicola* was inoculated by applying single drops of spore suspension onto detached plant leaves. The disease index used to score *B. cinerea* infection was based on previously reported methodology (26). *Pst*DC3000 and *Pfl*2-79 were inoculated in 10 mM $MgCl_2$ at 5×10^5 cfu/ml (35). The fungal pathogens *E. cichoracearum* and *Bgt* were maintained and disease assays performed as described in refs. 26 and 36. *H. parasitica* inoculations were undertaken as reported in refs. 37 and 38.

RNA Analysis. Northern blot analysis was undertaken as reported in ref. 26. The *PR1*, *PR3*, *PR4*, and *PDF1.2* probes were amplified as described in refs. 15 and 26. The PCR primers for *AS1* were 5′-GCCTATTGACGAGAGTAAGTAC-3′ and 5′-CCACAAGCTCTGACAAGAACAC-3′. The PCR primers for *AtGA20ox1* were as reported in ref. 11.

Histochemical Analysis and Chemical Treatments. Lesion development was scored by staining leaves with lactophenol blue and clearing with saturated chloral hydrate as described in ref. 36. For callose deposition, *Arabidopsis* leaves were infiltrated with a bacterial suspension of 2.5×10^5 cfu/ml and detached after 20 h. Epifluorescence microscopy was used to detect callose after staining with aqueous aniline blue as described in ref. 39. To transiently express *KN1*, a conditional expression system was used in a Col-0 background. This system consisted of a C-terminal fusion of the *KN1* cDNA with the steroid-binding domain of the rat glucocorticoid receptor from pBI-ΔGR (11). A line expressing only ΔGR was used as a control.

Determination of SA, JA, and ET Levels. Free SA and SA-β-glucoside (SAG) levels were determined essentially as described in ref. 35. ET emission from 4-week-old plants was measured by gas chromatography with an HP5980 series II gas chromatograph with a flame ionization detector (Hewlett Packard) equipped with a Quadrex BTR-CW (50-m length, 0.32-mm diameter) and run at 80°C (48). JA concentrations were determined by gas chromatography-mass spectrometry as described previously (26).

ChIP and RT-PCR. Procedures are described in *SI Methods*.

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