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Different requirements for *EDS1* and *NDR1* by disease resistance genes define at least two *R* gene-mediated signaling pathways in *Arabidopsis*

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ABSTRACT **The *Arabidopsis* genes *EDS1* and *NDR1* were shown previously by mutational analysis to encode essential components of race-specific disease resistance. Here, we examined the relative requirements for *EDS1* and *NDR1* by a broad spectrum of Resistance (*R*) genes present in three *Arabidopsis* accessions (Columbia, Landsberg-*erecta*, and Wassilewskija). We show that there is a strong requirement for *EDS1* by a subset of *R* loci (*RPP2*, *RPP4*, *RPP5*, *RPP21*, and *RPS4*), conferring resistance to the biotrophic oomycete *Peronospora parasitica*, and to *Pseudomonas* bacteria expressing the avirulence gene *avrRps4*. The requirement for *NDR1* by these *EDS1*-dependent *R* loci is either weak or not measurable. Conversely, three *NDR1*-dependent *R* loci, *RPS2*, *RPM1*, and *RPS5*, operate independently of *EDS1*. Another *RPP* locus, *RPP8*, exhibits no strong exclusive requirement for *EDS1* or *NDR1* in isolate-specific resistance to *P. parasitica*, although resistance is compromised weakly by *eds1*. Similarly, resistance conditioned by two *EDS1*-dependent *RPP* genes, *RPP4* and *RPP5*, is impaired partially by *ndr1*, implicating a degree of pathway cross-talk. Our results provide compelling evidence for the preferential utilization of either signaling component by particular *R* genes and thus define at least two disease resistance pathways. The data also suggest that strong dependence on *EDS1* or *NDR1* is governed by R protein structural type rather than pathogen class.**

Disease resistance in plants commonly is specified by genetically paired products that are encoded by plant resistance (*R*) genes and pathogen avirulence (*avr*) genes (1). Susceptibility to a particular pathogen race only can occur when either one of the genetic components is missing or inactivated. In many plant-pathogen combinations, this type of resistance is associated with localized cell necrosis at the site of attempted pathogen entry (the hypersensitive response). However, the precise mechanisms that lead to pathogen containment are not understood.

The cloning of *avr* genes from prokaryotic and eukaryotic plant pathogens and, more recently, corresponding *R* genes from several different plant species has provided some important insights to the process of pathogen recognition. For example, the tomato *Pto* gene specifying resistance to a bacterial pathogen encodes a functional serine/threonine protein kinase that specifically phosphorylates a second protein kinase, Pti1 (2). Pto also interacts with the corresponding bacterial Avr protein AvrPto in a yeast two-hybrid assay (3, 4), strongly supporting its role as the physiological receptor for the bacterial ligand. Other R proteins that are predicted to be localized either extracellularly or within the cytoplasm are structurally different to Pto and contain leucine-rich repeats (LRRs) (5), implicating protein–protein interactions as part of the recognition process. In the putatively cytoplasmic R protein members, the LRRs lie adjacent to sequences that constitute a nucleotide binding site (NBS) (5). The NBS/LRR class of R proteins can be subdivided into members that possess an amino-terminal leucine zipper (LZ) motif or those that have amino-terminal similarity to the cytoplasmic domains of the *Drosophila* Toll and mammalian interleukin 1 transmembrane receptors (referred to as the "TIR" domain) (1, 6).

The representation of a limited number of common structural motifs in the R proteins identified so far raises the possibility that they may function, at least in part, by similar signaling mechanisms. Indeed, the high level of sequence conservation between the tobacco *N* gene conferring resistance to a virus (7), the flax *L6* gene specifying rust fungus resistance (8), and the *Arabidopsis RPP5* gene for resistance to an oomycete pathogen (9) suggests conservation of resistance pathways between different plant species and differing pathogen types. A major goal now is to understand how early R protein-mediated recognition events lead to effective resistance.

The model crucifer *Arabidopsis* has been exploited as a host for bacterial, viral, fungal, and oomycete pathogens (10), and analysis of natural genetic variation between different plant accessions and pathogen isolates has led to the identification of distinct *R* loci. The cloned *RPS2* and *RPM1* genes, controlling race-specific resistance to the bacterial pathogen *Pseudomonas syringae*, encode proteins of the LZ-NBS/LRR class (11–13). In contrast, the *RPP5* gene mediating isolate-specific resistance to the biotrophic oomycete *Peronospora parasitica* encodes a protein of the TIR-NBS/LRR class (9).

Mutational analyses in *Arabidopsis* have identified other wild-type genes that are required for *R* gene-mediated resistance (14). A mutation in *NDR1* (*n*onrace-specific *d*isease *r*esistance) abolished resistance conferred by *RPS2* and *RPS5* to *P. syringae* expressing *avrRpt2* and *avrPph3*, respectively, as well as a dual specificity resistance encoded by *RPM1* to the bacterial genes *avrB* and *avrRpm1* (15). *ndr1* plants also were compromised in *RPP* gene-mediated resistance to several incompatible *P. parasitica* isolates, suggesting that the wild-type NDR1 protein may function at a common point downstream from the perception of these prokaryotic and eukaryotic pathogens (15). Another resistance signaling component is encoded by *EDS1* (*e*nhanced *d*isease *s*usceptibility) (16). A

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Abbreviations: LRR, leucine-rich repeats; NBS, nucleotide binding site; LZ, leucine zipper; Ler, Landsberg-*erecta*; Ws, Wassilewskija; Col, Columbia.
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mutation in *EDS1* abolished resistance conferred by several *RPP* loci but had no effect on *RPM1*-specified resistance, suggesting a function before the convergence of downstream pathways (16).

We thought it possible that the *ndr1* and *eds1* mutations may reflect the operation of distinct disease resistance signaling pathways and that particular R proteins exhibit a preference for one signaling mode. To address this possibility, we extended our analysis of the relative requirements for *EDS1* and *NDR1* to a wide spectrum of *Arabidopsis R* genes in three different genetic backgrounds (accessions). Our results show that the requirements for *EDS1* or *NDR1* appear to be mutually exclusive. Furthermore, the data suggest that preferential utilization of either EDS1 or NDR1 is determined more by R protein structure than pathogen type.

METHODS

Maintenance of Pathogen Isolates. *Pseudomonas syringae* pv. *tomato* DC3000 containing the avirulence genes *avrRps4* (17), *avrRpm1* (13), *avrRpt2* (11), or *avrPph3* (18) in the broad host range vector pVSP61 or DC3000 containing empty pVSP61 were cultured as described. The *Peronospora parasitica* isolates from *Arabidopsis* have been described before (16, 19–21). These were cultured on their corresponding compatible hosts as described (21).

Plant Material, Cultivation, and Pathogenicity Tests. Seeds of the *Arabidopsis* accession Wassilewskija (Ws-0) originally were obtained from K. Feldman (University of Arizona, Tucson, AZ). Columbia (Col-0 or Col-*gl*, containing the recessive mutation *gl1*) were obtained from J. Dangl (University of North Carolina, Chapel Hill, NC). Landsberg-*erecta* (Ler) seed were obtained from the Nottingham *Arabidopsis* Stock Centre (Nottingham, UK). The Ws *eds1-1* mutation has been described (16). The Ler *eds1-2*, *eds1-3*, and *eds1-4* mutant lines were isolated from fast neutron-bombarded M2 Ler seed obtained from Lehle Seeds (Tucson, AZ). The Col *ndr1-1* mutant line has been described (15). Mutant lines *eds1-3* and *eds1-4* were backcrossed once, and *eds1-1* and *eds1-2* were backcrossed twice, to their respective wild-type parent.

Cultivation of plants for bacterial inoculations, infiltration of leaves with bacterial suspensions, and bacterial growth assays were as described (16, 17). Plant cultivation for *P. parasitica* inoculations was as before (16). Seedlings were either sprayed with conidiospores (9) or individual cotyledons inoculated with droplets of conidiospore inoculum (20). Development of *P. parasitica* inside leaves or cotyledons was monitored by staining with lactophenol–trypan blue and examining under a light microscope (16).

Selection of Plant Genotypes. Plant genomic DNA was isolated as described (9). Ler *RPP5* and Col *RPP4* that are tightly linked on chromosome 4 were selected in F_2 progeny (see Table 1) by using codominant cleaved amplified polymorphic sequence primers for g4539 (http://genome-www.stanford.edu). Ler *RPS4* (17) and Ler *RPP8* (21) lie on chromosome 5 in a ≈15-cM interval between the cleaved amplified polymorphic sequence marker *DFR* and the microsatellite marker nga129. These two markers were used to select plants homozygous for Ler DNA in Col *ndr1-1* × Ler F_2 plants. Col *RPP2* was selected by using cleaved amplified polymorphic sequence primers for *AG* (≈1 cM centromeric to RPP2; Jim Beynon, University of London, Wye College, Kent, UK, personal communication). *EDS1* maps ≈12 cM telomeric to the RFLP marker m249 (16). Col-*gl* × Ws *eds1-1* F_2 plants were genotyped for *EDS1* or *eds1-1* by using m249-specific primers that detect a simple sequence length polymorphism (16). Genotypes subsequently were confirmed by using *EDS1*-specific cleaved amplified polymorphic sequence primers designed to detect a *Mse*I polymorphism between *EDS1* and *eds1-1* (A. Falk, B. Feys, and J.E.P., unpublished data). *EDS1* was distinguished from *eds1-2* in F_2 plants initially by using the m249-specific primers, as above. Genotypes were later confirmed by using *EDS1*-specific primers to detect a 900-bp deletion in *eds1-2* (A. Falk, B. Feys, and J.E.P., unpublished data). Homozygous *NDR1* or *ndr1-1* plants from Col *ndr1-1* × Ler F_3 families that had been phenotypically selected kindly were provided by K. Century (University of California, Berkeley). The *NDR1*/*ndr1-1* genotypes were confirmed by using *NDR1*-specific primers that detected a 1-kb deletion in *ndr1-1* (22).

RESULTS

Identification of Three New *eds1* Alleles. The *eds1* mutation in the *Arabidopsis* accession Wassilewskija (Ws-0) abolished resistance to several *P. parasitica* isolates mediated by *RPP1*, *RPP10*, and *RPP14* on chromosome 3 and *RPP12* on chromosomes 4 (16). The Ws *eds1* allele hereafter is denoted *eds1-1*. Three further defective *eds1* alleles were identified in two different screens of fast neutron-mutagenized seedlings of accession Landsberg-*erecta* (Ler). In the first screen, a loss of *RPP5*-mediated resistance to isolate Noco2 revealed two independent mutant alleles, *eds1-2* and *eds1-3*. In the second screen, a loss of *RPS4*-specified resistance to the compatible *Pseudomonas syringae* pv. *tomato* strain DC3000 expressing *avrRps4* (17) identified a fourth allele, *eds1-4*. It was significant that the requirement for *EDS1* in *RPS4*-specified resistance to a bacterial pathogen suggested a necessity for *EDS1* beyond *RPP* gene-mediated responses.

Resistance to Noco2 in wild-type Ler is conferred by a single gene, *RPP5* (9), and in Ws-0 by an unlinked gene, *RPP14* (19). Allelism between the new Ler *eds1* mutations and Ws *eds1-1* was tested by inoculating with Noco2 300 F_2 seedlings derived from crosses between each Ler mutant line and *eds1-1*. All F_2 progeny were Noco2-susceptible. Similarly, all F_2 seedlings from an *eds1-2* × *eds1-1* cross were susceptible to Cala2, a Ler-compatible *P. parasitica* isolate that is recognized by a single *R* locus, *RPP10*, in Ws-0 (20, 21). As expected, resistance and susceptibility to Noco2 in wild-type Ler × Ws-0 F_2 progeny segregated as a 15:1 ratio ($\chi^2 = 0.089$; $P < 0.01$). Resistance and susceptibility to Cala2 segregated as a 3:1 ratio ($\chi^2 = 0.314$; $P < 0.05$). These tests proved allelism and indicated that the four defective *eds1* alleles abolished the function of *RPP5*, *RPP10*, and *RPP14*.

***EDS1* Is Required by a Subset of *RPP* Loci.** To provide a quantitative assessment of the extent of colonization of Ws *eds1-1* and the three Ler *eds1* mutant lines by *P. parasitica* isolates Noco2 and Cala2, asexual conidiospore production was measured. This was compared with sporulation of each isolate on the naturally resistant and susceptible wild-type accessions, as indicated in Fig. 1. All *eds1* lines permitted high sporulation levels of Noco2 and Cala2 (Fig. 1 *A* and *B*).

We extended the analysis to two other *P. parasitica* isolates that are incompatible on Ler and for which corresponding *RPP* loci were identified previously (21). *P. parasitica* isolate Madi1 (corresponding to *RPP21*) and Emco5 (*RPP8*) are both compatible on Ws-0. As shown in Fig. 1*C*, resistance to Madi1 also was suppressed in the Ler *eds1* lines, indicating dependence of *RPP21* on *EDS1* function. Overall, Madi1 spore production was low, but levels were significantly higher in the mutant lines than in the naturally susceptible accession Ws-0. In all three *Arabidopsis*–pathogen combinations, *eds1-3* exhibited a less severe suppression phenotype than either *eds1-2* or *eds1-4* (Fig. 1 *A–C*), suggesting an incomplete loss of *EDS1* function in the *eds1-3* mutant background. In contrast to the clear suppression of resistance to Noco2, Cala2, and Madi1 by *eds1*, no detectable sporulation was observed in either *eds1-2*, *eds1-3*, or *eds1-4* after inoculation with Emco5 (Fig. 1*D*) even though this isolate was fully pathogenic on Ws-0.

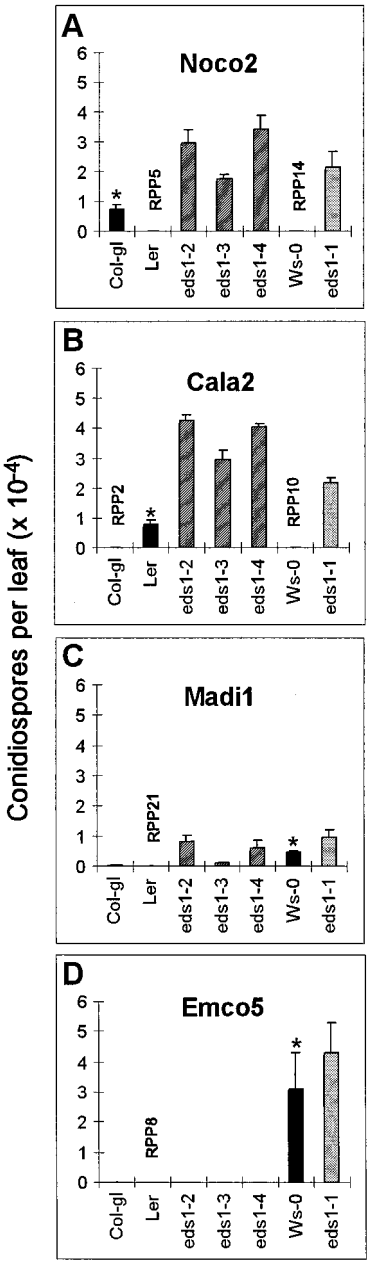

FIG. 1. Sporulation levels of different *P. parasitica* isolates on *eds1* mutant lines and wild-type *Arabidopsis* accessions. Conidiospore suspensions (4×10^4/ml) of each isolate were sprayed onto 2-week-old seedlings. Spores were harvested from leaves and counted after 7 days of incubation (see *Methods*). For each *P. parasitica* isolate tested, the genetically susceptible accession is marked with an asterisk and the resistant accession with the corresponding *RPP* gene. The experiment was repeated on 1-week-old seedlings with similar results.

The results in Fig. 1 showed that asexual reproduction of each *P. parasitica* isolate on *eds1* plants derived from a susceptible accession was significantly greater than on the corresponding compatible wild-type parent. This is consistent with an "enhanced disease susceptibility" phenotype previously observed on Ws *eds1-1* seedlings inoculated with the Ws-compatible isolate Emwa1 (16).

***RPP8*-Specified Resistance Is Weakly Suppressed by *eds1*.** The *RPP8*-specified response of Ler *eds1* seedlings to Emco5 appeared macroscopically to be as resistant as wild-type Ler seedlings (Fig. 1*D*). We therefore examined the inoculated plants for evidence of vegetative pathogen development. The extent of mycelium growth and plant cell necrosis was observed on a light microscope after staining seedlings with lactophenol–trypan blue (16). As shown in Fig. 2*A*, wild-type Ler leaves produced discrete necrotic lesions consisting of a few plant cells in response to attempted Emco5 penetration. In contrast, mycelial growth was more extensive in leaves of *eds1-2* seedlings, although the mycelium typically was surrounded by a trail of necrotic plant cells (Fig. 2*B*). Growth of Emco5 in *eds1-2* was very restricted compared with development in leaves of the natural Emco5-susceptible accession Ws-0 (Fig. 2*C*). A similar increase in pathogen growth was observed in *eds1-2* cotyledons and in leaves and cotyledons of *eds1-3* seedlings (data not shown). We concluded that *RPP8*-mediated recognition of Emco5 is impaired partially by the *eds1* mutation.

Requirements for *EDS1* or *NDR1* by Different *R* Genes Are Mutually Exclusive. The screen for mutations affecting *RPS4*-specified recognition of *avrRPS4* from *P. syringae* identified one defective *eds1* allele, *eds1-4*, demonstrating a requirement for *EDS1* beyond *RPP* gene-mediated disease resistance. Previous studies had shown that other *Arabidopsis R* genes, *RPM1*, *RPS2*, and *RPS5*, controlling recognition of bacterial *avr* determinants were suppressed by the *ndr1* mutation (15) and that *RPM1* functioned independently of *EDS1* (16), so we examined the relative requirements of these *R* loci for *EDS1* and *NDR1*.

In planta growth of *P. syringae* pv. *tomato*, strain DC3000 carrying different *avr* genes or an empty vector was used as an assay for function of the different *R* genes: in wild-type plants, a complementary *R* gene–*avr* gene combination leads to a significant reduction in bacterial growth (11, 17, 18). Growth of these bacterial strains in Ws-0 and Ws *eds1-1* plants, shown in Fig. 3*A*, was representative of results in all *eds1* mutant lines. Results showed that suppression of DC3000 carrying either *avrRpm1* or *avrRpt2* (corresponding to *RPM1* and *RPS2*, respectively) was the same in Ws-0 and *eds1-1* plants, whereas DC3000 carrying *avrRps4* (corresponding to *RPS4*) showed an increase in growth in *eds1-1* plants that was three orders of magnitude greater than in Ws-0 plants. The enhanced growth of DC3000 carrying *avrRps4* correlated with the formation of severe disease lesions in *eds1-1* leaves, whereas inoculated Ws-0 leaves remained symptomless (Fig. 2 *D* and *E*). Thus, although *RPM1* and *RPS2* do not require *EDS1*, *RPS4* has a strong dependence on *EDS1*. Growth of DC3000 carrying *avrPph3*, corresponding to *RPS5*, also was measured in Ws-0 and Ws *eds1-1* plants. In this experiment, the Ler *eds1* alleles were not tested because wild-type Ler does not contain the *RPS5* gene (18). Results (Fig. 3*B*) showed that *EDS1* is not required for *RPS5*-specified resistance.

The above experiments confirmed earlier observations (16) that *eds1* plants permit significantly more growth of the compatible strain DC3000 than wild-type *EDS1* plants. After 4 days, bacterial numbers in *eds1-1* leaves were reproducibly one to two orders of magnitude higher than in Ws-0 (Fig. 3). Disease symptom development after DC3000 inoculation was also more severe on *eds1-1* leaves (Fig. 2 *F* and *G*).

Previous experiments showed that the *ndr1-1* mutation abolished *RPM1*, *RPS2*, and *RPS5* functions in the accession Columbia (Col-0) (15). We therefore tested the effect of *ndr1-1* on Col-0 *RPS4*-specified resistance. Results from the growth assays (Fig. 4) showed that *RPS4* function was not significantly compromised by *ndr1-1*. We also tested the requirement of Ler-*RPS4* for *NDR1* in selected *ndr1* F_3 families derived from a Col *ndr1-1* × Ler cross. This analysis (results not shown) confirmed that both the Ler *RPS4* and Col *RPS4* wild-type alleles operate independently of *NDR1*.

Analysis of the requirements for *EDS1* or *NDR1* was extended to several mapped *RPP* loci that, if not present in the available mutant backgrounds, could be selected by using PCR-based polymorphic DNA markers (see *Methods* for details). Mutant lines or F_3 families, homozygous for the respective *RPP* locus and either the wild-type or mutant *eds1* or *ndr1*

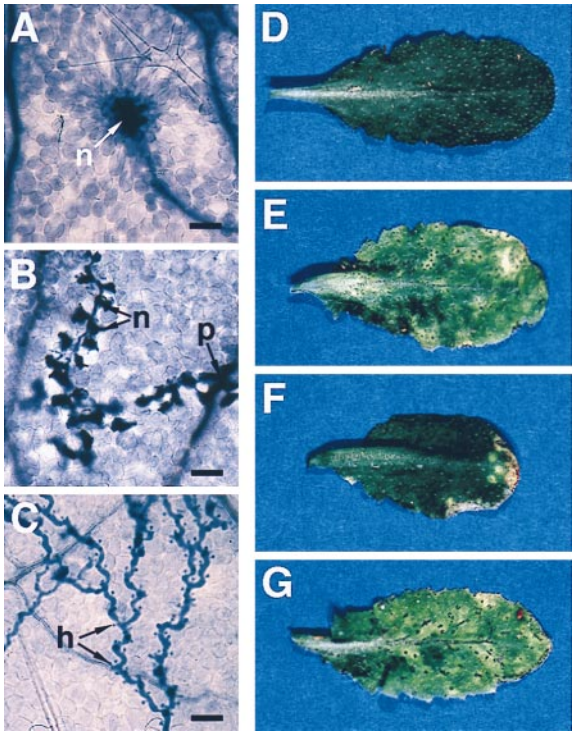

FIG. 2. Pathogen development and disease symptom expression in wild-type and *eds1* plants inoculated with *P. parasitica* or *P. syringae*. Development of *P. parasitica* isolate Emco5 is shown in *A–C*. Leaves were stained with lactophenol–trypan blue 5 days after inoculation and viewed under a light microscope to reveal pathogen mycelium and necrotic plant cells. (Bar = 50 μm.) Disease phenotypes of wild-type Ws-0 and Ws *eds1-1* leaves inoculated with *P. syringae* strain DC3000 or DC3000 expressing *avrRps4* are shown in *D–G*. Leaves were photographed 5 days after infiltration with suspensions of 1×10^5 bacteria/ml. (*A*) Ler wild-type, showing a discrete necrotic lesion (n) of plant cells surrounding an Emco5 penetration site. Resistance is conferred by *RPP8*. (*B*) Ler *eds1-2*, showing mycelium growing beyond the penetration site (p) but surrounded by a trail of necrotic plant cells (n). (*C*) Ws-0, lacking *RPP8*, is fully susceptible to Emco5. The mycelium forms haustoria (h) and grows systemically without associated plant cell death. (*D*) DC3000 expressing *avrRps4* causes no disease symptoms in Ws-0 due to resistance conferred by *RPS4*. (*E*) DC3000 expressing *avrRps4* causes severe disease symptoms in leaves of *eds1-1*. (*F*) DC3000 containing no functional *avr* gene elicits mild disease symptoms in Ws-0 leaves. (*G*) Disease symptom development in *eds1-1* leaves inoculated with DC3000 is more rapid than in Ws-0 leaves.

allele, were inoculated with the corresponding *P. parasitica* isolate. Leaves then were examined for evidence of pathogen asexual sporulation. As shown in Table 1, *RPP2*-specified resistance to *P. parasitica* isolate Cala2 and *RPP4*-specified resistance to Emwa1 in Col-0 (20, 21) were suppressed strongly by *eds1*. In contrast, Col *ndr1-1* seedlings inoculated with Cala2 (*RPP2*) or Emwa1 (*RPP4*) under the same conditions showed no increase in pathogen sporulation. Microscopic examination of lactophenol–trypan blue-stained, Cala2-inoculated leaves revealed no significant differences in pathogen vegetative growth between wild-type Col-0 and Col *ndr1-1* (not shown). *RPP5*-mediated resistance to Noco2 and *RPP8*-specified resistance to Emco5 in Ler were similarly not visibly affected by the *ndr1-1* mutation.

The results suggested that impairment of *RPP2*- and *RPP4*-mediated resistance that had been demonstrated previously in cotyledons of Col *ndr1-1* seedlings (15) may be an effect that is not measurable in leaves. We therefore inoculated individual *ndr1-1* cotyledons with Emwa1 conidiospore suspensions, as performed previously (15), and quantified sporangiophore and conidiospore numbers. The results in Table 2 show that there

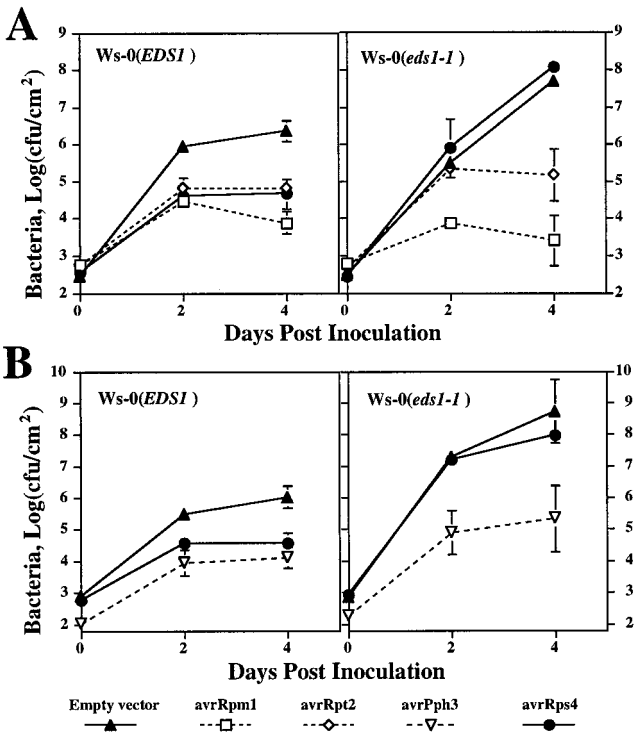

FIG. 3. Growth of different *P. syringae* strains in leaves of Ws-0 or Ws *eds1-1*. Leaves were infiltrated with suspensions of DC3000 containing an empty vector or expressing different *avr* genes, as indicated, and bacteria were recovered from leaves at various times after inoculation (see *Methods*). Results in *A* and *B* represent data from two separate experiments. These tests were repeated twice with similar results.

was a small but significant increase in sporangiophore production in *ndr1-1* cotyledons, consistent with the earlier observations (15). Spore numbers extracted from *ndr1-1* cotyledons increased ≈10-fold over Col-0 wild-type levels. However, Emwa1 spore numbers on *ndr1-1* cotyledons were <10% of those harvested from cotyledons of the naturally susceptible accession, Ws-0. A comparably low increase in Noco2 sporulation was observed in cotyledons of *RPP5/ndr1-1* F_3 seedlings compared with wild-type *RPP5/NDR1* F_3 seedlings selected from a Col *ndr1-1* × Ler cross (not shown). In contrast, no increased sporulation of Emco5 was observed in cotyledons of *ndr1-1*-selected seedlings (not shown). We concluded that *ndr1* has a minor but significant effect on resistance specified by strongly *EDS1*-dependent *RPP* genes.

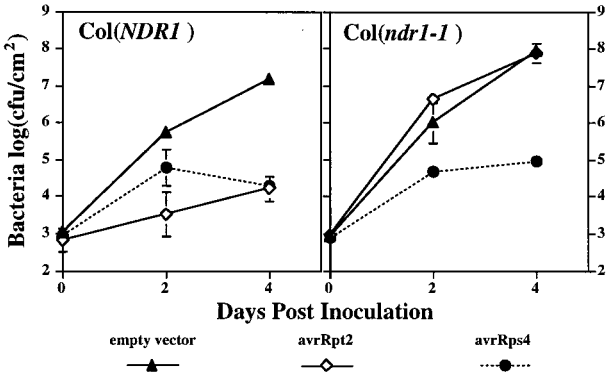

FIG. 4. Growth of different *P. syringae* strains in leaves of Col-0 or Col *ndr1-1*. Leaves were inoculated with DC3000 containing an empty vector, DC3000 expressing *avrRpt2*, or DC3000 expressing *avrRps4*, as indicated, and growth of bacteria was measured as in Fig. 3. Tests were repeated twice with similar results.

Table 1. Phenotypes of different *RPP* loci in combination with wild-type or mutant *eds1* or *ndr1* after inoculation of selected F_3 families with *P. parasitica*

RPP locus, *P. parasitica* isolate	Cross	F_3 families, *n*	Genotype*	Phenotype†
RPP2 (Cala2)	Col-*gl* × Ler-*eds1-2*	5	*RPP2, EDS1*	R
		5	*RPP2, eds1-2*	S
	Col-*ndr1-1*‡	–	*RPP2, ndr1-1*	R
RPP4 (Emwa1)	Col-*gl* × Ws-*eds1-1*	1	*RPP4, EDS1*	R
		2	*RPP4, eds1-1*	S
	Col-*ndr1-1*‡	–	*RPP4, ndr1-1*	R
RPP5 (Noco2)	Col-*ndr1-1* × Ler	5	*RPP5, NDR1*	R
		5	*RPP5, ndr1-1*	R
		2	*rpp5, NDR1*	S
RPP8 (Emco5)	Col-*ndr1-1* × Ler	5	*RPP8, NDR1*	R
		5	*RPP8, ndr1-1*	R
	Ws-0‡	–	*rpp8, NDR1*	S

*Each F_3 family was homozygous for the respective *RPP* locus and wild-type or mutant allele.
†Twenty to 30 9-day-old seedlings were spray-inoculated with *P. parasitica* conidiospores. Resistance (R, no sporulation) or susceptibility (S, medium to profuse sporulation) was scored with a hand lens on the first true leaves 7 days after inoculation.
‡Selfed Col *ndr1*, wild-type *Colgl*, or Ws-0 seedlings.

Altogether, the data are consistent with the preferential utilization of either *EDS1* or *NDR1* by particular *R* genes. *RPP8*-specified resistance to *P. parasitica* was not compromised strongly by either *eds1* or *ndr1*.

DISCUSSION

We present evidence for the operation of at least two *R* gene-specified signaling pathways that preferentially require either a functional EDS1 or NDR1 protein. *R* gene-mediated responses exhibiting a strong requirement for *EDS1* showed weak or no dependence on *NDR1*. Significantly, *RPS4* that recognizes a bacterial avirulence determinant, *avrRps4*, belongs to the *EDS1*-dependent class, revealing *EDS1* signaling function beyond *RPP* gene-mediated disease resistance. Although *P. parasitica* inoculations were performed on young (9-day- and 2-week-old) seedlings and bacterial assays were performed on older (4-week-old) plants, the lack of *NDR1* dependence by *RPS4* showed that the observed signaling trends are unlikely to be due to plant developmental effects.

NDR1 recently was cloned, and the *ndr1-1* mutation used in the present study was defined as a null allele due to an extensive deletion of the ORF (22). Similarly, cloning of *EDS1* has established that both *eds1-2* and *eds1-3* have extensive deletions of the *EDS1* ORF (A. Falk, B. Feys, and J.E.P., unpublished data). Because *eds1-3* lacks the 5′ promoter and amino-terminal amino acids, it was surprising that this allele appeared to have a slightly weaker suppression phenotype than *eds1-2* or *eds1-4* (Fig. 1). Successive *eds1-3* backcrosses to wild-type Ler should enable us to assess whether this is a feature of the mutant background. Overall, however, we conclude that the observed *eds1* and *ndr1* phenotypes are not due to leakiness of weak mutant alleles.

RPP5, a strongly *EDS1*-dependent gene, encodes a protein of the TIR-NBS-LRR class that has amino-terminal similarity to the cytoplasmic domains of the *Drosophila* Toll and mammalian interleukin 1 transmembrane receptors (6, 9). In contrast, *RPM1* and *RPS2*, both exhibiting *NDR1* dependence, have been assigned to the LZ-NBS-LRR class that possesses an amino-terminal domain containing a putative leucine zipper (1, 11-13). The functional significance of these domains is not understood, but a pattern emerges suggesting that pathogen recognition through an EDS1- or NDR1-dependent pathway may be directed by a particular R protein type. The structures of several recently cloned *Arabidopsis R* genes are consistent with this idea. *RPS4* (M. Hinsch, W. Gassmann & B.J.S., unpublished data) and a cluster of *RPP* genes comprising the *RPP1*, *RPP10*, and *RPP14* specificities (refs. 18 and 19; M. Botella, L. Frost, E.H., J. Beynon, J.E.P. & J. Jones, unpublished data) that are all strongly *EDS1*-dependent, encode proteins of the TIR-NBS-LRR class, whereas *RPS5*, an *NDR1*-requiring gene, encodes a LZ-NBS-LRR protein with strongest homology to RPS2 (23). The recently cloned *RPP8* gene that has no exclusive requirement for either *EDS1* or *NDR1* function encodes an NBS-LRR protein with a putative amino-terminal LZ motif (J. McDowell, M. Dhandaydham, and J. Dangl, personal communication). Thus, a strict separation of EDS1 or NDR1- pathway utilization based simply on the presence of the TIR or LZ domains is not valid. The partial attenuation of *RPP8*-specified resistance exhibited by *eds1* plants implicates minor EDS1 activity in this resistance response even though its loss ultimately can be compensated for to prevent successful pathogen colonization. Double *eds1*/*ndr1* mutant combinations should establish whether *RPP8* has a redundant requirement for *EDS1* and *NDR1* or mediates resistance through a different signaling pathway. It is notable that overall amino-terminal similarity between the "LZ" R protein members is less than that between the "TIR" R proteins, suggesting further mechanistic differences between the LZ class. In this respect, it is interesting that *ndr1* suppressed a hypersensitive response incited by high doses of DC3000 expressing *avrRpt2* but not *avrRpm1*, *avrB*, or *avrPph3* (15). Also, different early gene induction profiles were observed in the resistance responses specified by *RPS2* and *RPM1* (24, 25), implicating other levels of pathway discrimination beyond the separation of events described here.

Table 2. Asexual sporulation on cotyledons of different *Arabidopsis* lines inoculated with *P. parasitica* isolate Emwa1

Plant line	Sporangiophores/ cotyledon	Conidiospores/ cotyledon
Col-0	2.9 ± 0.2	70 ± 4
Col *ndr1-1*	6.5 ± 0.6	644 ± 18
Ws-0	>20	8236 ± 684
Ler	0	0

Individual cotyledons of 20–30 7-day-old seedlings were inoculated with 2-μl droplets containing ≈100 conidiospores. Spores were counted 7 days after inoculation.

A weak but significant impairment of resistance specified by the *EDS1*-dependent genes *RPP4* (Table 2) and *RPP5* was observed in cotyledons of *ndr1* plants. This points to a degree of cross-talk between the proposed *EDS1*- and *NDR1*-mediated pathways. We also noted that there was a slight (maximally 5- to 10-fold) but consistent trend toward increased growth of DC3000 expressing *avrRpt2* or *avrPph3* but not

avrRpm1 in leaves of *eds1* (Fig. 3). Likewise, *ndr1* plants supported 5- to 10-fold greater numbers of DC3000 expressing *avrRps4* than wild-type plants (Fig. 4). Although these differences were not statistically significant, it is possible that minor pathway cross-utilization also occurs between these strongly *EDS1*- and *NDR1*-mediated processes, as was clearly demonstrated in the *RPP4*-specified resistance response (Table 2). In the light of possible signaling variations associated with different "LZ" R proteins discussed above, it is notable that *EDS1* appears not to impinge at all on *RPM1*-controlled resistance.

In all compatible *P. parasitica–eds1* combinations tested, pathogen sporulation was significantly higher than in the corresponding wild-type susceptible plant (Fig. 1), revealing an "*e*nhanced *d*isease *s*usceptibility" (*eds*) phenotype. This was also apparent in the interaction between the compatible bacterial strain DC3000 and *eds1* plants (Fig. 3). Therefore, *EDS1* has a role in limiting pathogen growth both in certain *R* gene-mediated and compatible interactions. In our studies, *ndr1* plants were not significantly more susceptible to DC3000 than wild-type Col-0 (Fig. 4), suggesting a preferential requirement for *EDS1*, and not *NDR1*, in restriction of pathogen growth to this bacterial strain. Analyses with another compatible bacterial pathogen, *Xanthomonas campestris* pv. *campestris* strain 8004, showed that *eds1* plants were not significantly more susceptible than wild-type plants (data not shown). This is consistent with the notion that the "*eds*" phenotype exhibited by *eds1* may be limited to certain plant–pathogen combinations. It will be important to establish whether the biochemical role of EDS1 is the same in the maintenance of *R* gene-specified disease resistance and the growth limitation of a compatible pathogen.

Other "*eds*" loci have been identified by using various mutational screens, including assays for increased growth of a *P. syringae* isolate, PsmES4326 (26, 27). Phenotypic and genetic analyses of the different *eds* mutations point to the existence of complex, multiple pathways limiting growth of virulent pathogens. Significantly, a number of the *eds* mutations that have been characterized cause enhanced susceptibility to DC3000, like *eds1* (28). Also, they have no appreciable effect on *RPS2*-specified resistance and are not altered in their capacity to respond to inducers of systemic acquired resistance. This distinguishes them and *eds1* from mutations, such as *npr1* (29, 30), that compromise systemic resistance responses. One *eds* mutation, *pad4*, causes enhanced growth of PsmES4326 and also suppresses *RPP2*- and *RPP4*-mediated resistance (31), implicating a role for *PAD4* in these *EDS1*-dependent *R* gene responses. In that study, *RPP7* functioned independently of *PAD4*, suggesting a specificity in the requirement for PAD4 activity. Further analysis of these mutations and their corresponding wild-type gene products should help to unravel the complexity of particular *R* gene-specified pathways and how these interact with other processes limiting or promoting pathogen growth in plants.

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1. Baker, B., Zambryski, P., Staskawicz, B. & Dinesh-Kumar, S. P. (1997) *Science* **276,** 726–733.
2. Zhou, J., Loh, Y.-T., Bressan, R. A. & Martin, G. B. (1995) *Cell* **83,** 925–935.
3. Tang, X., Frederick, R. D., Zhou, J., Halterman, D. A., Jia, Y. & Martin, G. B. (1996) *Science* **274,** 2060–2063.
4. Scofield, S. R., Tobias, C. M., Rathjen, J. P., Chang, J. H., Lavelle, D. T., Michelmore, R. W. & Staskawicz, B. J. (1996) *Science* **274,** 2063–2065.
5. Jones, D. A. & Jones, J. D. G. (1996) *Adv. Bot. Res. Inc. Adv. Plant Pathol.* **24,** 89–167.
6. Rock, F. L., Hardman, G., Timans, J. C., Kastelein, R. A. & Bazan, F. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 588–593.
7. Whitham, S., Dinesh-Kumar, S. P., Choi, D., Hehl, R., Corr, C. & Baker, B. (1994) *Cell* **78,** 1011–1115.
8. Lawrence, G. J., Finnegan, E. J., Ayliffe, M. A. & Ellis, J. G. (1995) *Plant Cell* **7,** 1195–1206.
9. Parker, J. E., Coleman, M. J., Szabò, V., Frost, L. N., Schmidt, R., van der Biezen, E. A., Moores, T., Dean, C., Daniels, M. J. & Jones, J. D. G. (1997) *Plant Cell* **9,** 879–894.
10. Dangl, J. (1993) *Adv. Plant Pathol.* **10,** 127–156.
11. Bent, A. F., Kunkel, B. N., Dahlbeck, D., Brown, K. L., Schmidt, R., Giraudat, J., Leung, J. & Staskawicz, B. J. (1994) *Science* **265,** 1856–1860.
12. Mindrinos, M., Katagiri, F., Yu, G.-L. & Ausubel, F. M. (1994) *Cell* **78,** 1089–1099.
13. Grant, M. R., Godiard, L., Straube, E., Ashfield, T., Lewald, J., Sattler, A., Innes, R. W. & Dangl, J. L. (1995) *Science* **269,** 843–846.
14. Glazebrook, J., Rogers, E. E. & Ausubel, F. M. (1997) *Annu. Rev. Genet.* **31,** 547–569.
15. Century, K., Holub, E. B. & Staskawicz, B. J. (1995) *Proc. Natl. Acad. Sci. USA* **92,** 6597–6601.
16. Parker, J. E., Holub, E. B., Frost, L. N., Falk, A., Gunn, N. D. & Daniels, M. J. (1996) *Plant Cell* **8,** 2033–2046.
17. Hinsch, M. & Staskawicz, B. (1996) *Mol. Plant-Microbe Interact.* **9,** 55–61.
18. Simonich, M. T. & Innes, R. W. (1995) *Mol. Plant-Microbe Interact.* **8,** 637–640.
19. Reignault, P., Frost, L. N., Richardson, H., Daniels, M. J., Jones, J. D. G. & Parker, J. (1996) *Mol. Plant-Microbe Interact.* **9,** 464–473.
20. Holub, E. B., Beynon, J. L. & Crute, I. R. (1994) *Mol. Plant-Microbe Interact.* **7,** 223–239.
21. Holub, E. B. & Beynon, J. L. (1997) *Adv. Bot. Res.* **24,** 228–273.
22. Century, K. S., Shapiro, A. D., Repetti, P. P., Dahlbeck, D., Holub, E. & Staskawicz, B. J. (1997) *Science* **278,** 1963–1965.
23. Warren, R. F., Henk, A., Mowery, P., Holub, E. & Innes, R. W. (1998) *Plant Cell*, in press.
24. Reuber, T. L. & Ausubel, F. M. (1996) *Plant Cell* **8,** 241–249.
25. Ritter, C. & Dangl, J. L. (1996) *Plant Cell* **8,** 251–257.
26. Glazebrook, J. & Ausubel, F. M. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8955–8959.
27. Glazebrook, J., Rogers, E. E. & Ausubel, F. M. (1996) *Genetics* **143,** 973–982.
28. Rogers, E. E. & Ausubel, F. M. (1997) *Plant Cell* **9,** 305–316.
29. Cao, H., Bowling, S. A., Gordon, S. & Dong, X. (1994) *Plant Cell* **6,** 1583–1592.
30. Cao, H., Glazebrook, J., Clarke, J. D., Volko, S. & Dong, X. (1997) *Cell* **88,** 57–63.
31. Glazebrook, J., Zook, M., Mert, F., Kagan, I., Rogers, E. E., Crute, I. R., Holub, E. B., Hammerschmidt, R. & Ausubel, F. M. (1997) *Genetics* **146,** 381–392.