Phenotypic and Genotypic Characterization of Interactions Between Isolates of *Peronospora parasitica* and Accessions of *Arabidopsis thaliana*

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The parasitic symbiosis between the wild crucifer *Arabidopsis thaliana* and the biotrophic oomycete *Peronospora parasitica* (downy mildew) provides an excellent opportunity to explore the evolution and molecular basis of plant defense. By describing the phenotypic and genotypic variation observed in this symbiosis, a foundation has been laid for investigating the process of genotype-specific recognition. Interaction phenotypes, incorporating aspects of both host response and parasite development, are described in this paper for combinations of 11 host accessions and seven parasite isolates. The timing and degree of asexual reproduction by the parasite varied among the combinations and provided an indirect assessment of colonization. Necrosis of host cells ranged from minute flecks visible macroscopically 7 days after inoculation (dai) to more extensive pits clearly visible 3 dai and often expanding until the entire cotyledon was necrotic 7 dai. Twelve host loci associated with different interaction phenotypes were postulated in part by studying segregation among F_2 progeny from crosses of a half-diallel among nine *A. thaliana* accessions; advanced generations including recombinant inbred lines were used to confirm the identity of loci. A single cross between two host accessions can be used to characterize and map several loci associated with isolate-specific recognition of *P. parasitica* and distinct interaction phenotypes. Partial dominance and genetic epistasis are postulated as being common features of this parasitic symbiosis.

Additional keywords: disease resistance, avirulence, gene-for-gene, Peronosporales, molecular genetics.

Genotype-specific interactions between *Arabidopsis thaliana* (L.) Heynh. and prokaryotic and eukaryotic parasites have provided the stimulus for the use of this wild crucifer to explore the molecular basis of cellular recognition by plants and ultimately to contribute to an understanding of the evolution of plant defense mechanisms (Crute *et al.* 1993; Dangl 1993; Debener *et al.* 1991; Koch and Slusarenko 1990a; Kunkel *et al.* 1993; Parker *et al.* 1993; Tsuji *et al.* 1991; Whalen *et al.* 1991; Yu *et al.* 1993). As a foundation for such investigation, this paper describes the phenotypic variation and genotypic specificity that has been observed in interactions between accessions of *A. thaliana* and variants of the biotrophic oomycete *Peronospora parasitica* (Pers. ex Fr.) Fr., which causes downy mildew.

Genotype specificity of interactions between a plant host and its parasites is a phenomenon commonly associated with pathosystems involving crop species (Crute 1985). Several well-documented studies such as those with flax rust and lettuce downy mildew (Flor 1971; Crute 1992) have demonstrated how simply inherited traits of the host and parasite largely determine the phenotypic outcome of an interaction. Genotype-specific interactions have also been found in nonagricultural pathosystems such as those involving wild relatives of flax and lettuce (Crute 1990; Burdon and Jarosz 1991). The specificity of interactions is often characterized by reciprocal variation where one isolate of the parasite is capable of prolific reproduction on a particular host accession (compatibility), but reproduction of a second isolate is restricted on the same accession (incompatibility). On a second host accession, restricted or copious reproduction is reversed for each of the two isolates. The specificity begins to appear increasingly complex as different patterns of interaction are identified among numerous combinations of parasite isolates and host accessions. This complexity suggests an enormous capability of plants to distinguish between self and non-self.

The matching traits of host and parasite associated with an incompatible interaction are thought to be controlled by alleles at single loci in each partner (Flor 1955; Ellingboe 1981). Incompatibility is considered to result from the product of a plant gene interacting directly with the specifically matching product of a parasite gene; further development of the parasite is somehow hindered because of the molecular recognition event itself or because recognition activates an antimicrobial defense response (Ellingboe 1982; Keen 1982; Callow 1984). The theory generally emphasizes the host's ability to recognize the parasite, but parasite development and the outcome of an interaction are most likely also actively directed by the parasite following recognition of specific molecular cues from the host.

A. thaliana is well suited for investigating genotype specificity and the predicted molecular recognition of parasitic

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symbiosis. This small crucifer is common in temperate climates, exhibits rapid and prolific reproduction when grown in an appropriate environment, and has a relatively small genome size comprising a low percentage of repetitive DNA (Redei 1975; Meyerowitz 1987). These attributes have made it an attractive species for fundamental research by an expanding international network of plant biologists. The possibility of rapid progress in plant pathology has been well demonstrated by several research groups currently investigating the genotype specific recognition of bacterial pathogens by *A. thaliana* (Davis *et al.* 1991; Debener *et al.* 1991; Dong *et al.* 1991; Tsuji *et al.* 1991; Whalen *et al.* 1991; Dangl *et al.* 1992b; Kunkel *et al.* 1993; Yu *et al.* 1993).

The association between *A. thaliana* and *P. parasitica* should also be useful for investigating the basis of genotype specificity and mechanisms of plant defense. Parasite germplasm is abundantly available because *P. parasitica* commonly occurs on *A. thaliana* throughout Europe (Rostrup 1913; Gustavsson 1959; Koch and Slusarenko 1990a; Holub *et al.* 1991). In southeast England, infected plants were observed in more than 25% of *A. thaliana* populations examined during two consecutive springs (Holub *et al.* 1993). We surveyed 31 populations of *A. thaliana* in northern England and southern Scotland during spring 1993, and 33% contained plants infected with *P. parasitica*. A similar occurrence of downy mildew was observed in populations of *A. thaliana* in northern Germany (T. Debener, personal communication). This suggests that, in some localities at least, parasitism is a significant feature of natural populations of *A. thaliana*. *P. parasitica* exhibits a high degree of host specialization. Most crucifer species are parasitized by *P. parasitica*; but variants of the symbiont are usually highly specialized to a given host

Table 1. Variation for interaction phenotype among 11 accessions of *Arabidopsis thaliana* following inoculation with seven isolates of *Peronospora parasitica*, with a summary of host and parasite loci postulated to explain the genotype specificity associated with each interaction phenotype

												P. parasitica isolate[c]						
												ATR[b]	*Emoy2*	*Cala2*	*Hiks1*	*Emwa1*	*Noks1*	*Wela3*	*Cand3*
												1	1	0	√	0	0	0	0
												2	0	1	0	0	0	0	0
												3	0	1	0	0	0	?	0
												4	1	0	0	√	0	0	?
												5	0	0	0	0	1	0	0
												6	0	0	0	0	0	1	?
												7	0	0	1	0	0	0	?
												8	1	0	0	?	0	0	?
												9	?	0	1	?	?	0	?
												10	√	1	√	0	√	0	?
												12	0	0	0	0	0	1	?
A. thaliana	*RPP*[b]																		
Genotype[a]	1	2	3	4	5	6	7	8	9	10	12[d]								
Nd-1	1	0	0	0	0	0	0	0	0	0	0		PN[e]	EH	PN	DH	EH	DM	EH
Col-5 (*gl1*)	0	1	0	1	0	1	1	0	0	0	0		FDL	FR	FR	FR	EH	FR	FN
Ler-1 (*er*)	0	0	0	√	1	0	√	1	0	0	0		FN	EH	FN	FN	CN	EH	FN
Oy-1	0	√	1	0	√	√	0	0	0	0	?		EH	FN	DH	DH	FN	FN	EH
Wei-1	0	0	0	0	0	0	0	0	1	?	0		DL[f]	DL	DL	DL	FDL	EH	DL
Ws-3	?	0	0	0	0	0	0	0	0	1	1		PN	CN	PN	DH	PN	FN	CN
Ksk-1	0	√	0	0	0	0	0	?	0	?	?		FN	FN	EH	FN	EH	FN	EH
Ema-1	√	0	0	0	?	0	0	0	0	0	0		PN	EH	PN	DH	FN	DH	EH
Cnt-1	√	0	0	?	?	?	?	?	?	0	?		PN	EH	PN	FN	FN	FN	EH
Rld-2	0	?	?	?	0	?	?	?	?	?	0		FN	FN	FN	FN	FDM	FN	FN
Tsu-1	0	0	0	0	0	?	0	0	0	?	?		EM	EM	EM	EM	EH	FN	EM

[a] Single plant genotypes selected randomly from the following accessions (top to bottom): Niederzenz (Nd-0), Columbia (Col-*gl1*) (Meyerowitz), Landsberg erecta (Ler-0), Oystese (Oy-0), Weiningen (Wei-0), Wassilewskija (Ws-0), Keswick (Ksk-0), East Malling (Ema-0), Canterbury (Cnt-0), Rschew (Rld-0), and Tsu (Tsu-0).

[b] *RPP* = recognition of *P. parasitica*; and *ATR* = *A. thaliana* recognized. *RPP* columns represent the loci in *A. thaliana* that have been identified genetically, whereas *ATR* loci (shown in rows) are inferred from a theoretical gene-for-gene relationship and thus correspond with numbers for *RPP* loci. Alleles within each *RPP* column or *ATR* row are indicated as follows: 1 = the matching alleles associated with an incompatible interaction and used to define the corresponding loci, √ = alleles determined by genetic analysis of the host to reside at a previously identified locus, ? = incompatibility exists which may be associated with that locus, but this has yet to be confirmed (i.e., additional gene pairs may need to be designated in the future), and 0 = alleles associated with full compatibility (i.e., do not contribute to recognition in the combinations shown). No gene pairs have been postulated to explain EM, DM, or DH phenotypes.

[c] Each isolate was derived from an oospore population. See Table 5 for further description.

[d] The corresponding loci *RPP11-ATR11* are identified by the interaction between Rld-2 and *Wela1* (Koch and Slusarenko 1990a). They are not shown here because crosses involving Rld-2 were not included in the half diallel analysis.

[e] Interaction phenotypes were characterized using an assessment of host and parasite characteristics: the emergence of sporangiophores of *P. parasitica* as early (E) 3 days after inoculation (dai) or delayed (D) >4 dai; the intensity of asexual sporulation as heavy (H; >20 sporangiophores per cotyledon), moderate (M; 10–20 sporangiophores), light (L; <10 sporangiophores), rare (R; <5 sporangiophores on <10% of inoculated seedlings), or none (N); and the type of response by *A. thaliana* as minute necrotic flecks (F) evident 7 dai, flecks clearly visible 3 dai which form necrotic cavities (C) 7 dai, or necrotic pits (P) observed as early as 3 dai and often expanding until much of cotyledon is necrotic 7 dai. See text for further detailed description of phenotypes.

[f] Unless otherwise specified as a FDL phenotype, the host response (chlorosis or necrosis) was too diffuse or variable to be characterized macroscopically. Examples of the DL phenotype will need to be characterized microscopically to reveal any differences between combinations of accession and isolate.

genus or species. This is the case for isolates collected from *A. thaliana*. In preliminary investigations, such isolates have been found to be capable of infecting and reproducing on *A. suecica*, but were not parasitic on *Brassicas* spp. or other wild crucifers commonly found growing alongside *A. thaliana* such as *Cardamine hirsuta* L. and *Capsella bursa-pastoris* (L.) Medic. Extensive reciprocal variation has been observed following inoculation of different genotypes of *A. thaliana* with several isolates of *P. parasitica* (Holub *et al.* 1993). It was also possible to characterize further the phenotypic variation among incompatible interactions with respect to the time of occurrence and degree of asexual sporulation by the parasite, and the symptoms of host response (Holub *et al.* 1993). Consequently, this variation for host response and parasite development could be combined for classifying a range of interaction phenotypes.

The primary objective of our research has been to unravel the complex nature and molecular basis of the genotype specificity and phenotypic variation in the parasitic symbiosis between *P. parasitica* and *A. thaliana*. We describe in this paper details of several different interaction phenotypes and present genetic evidence for the existence of individual host loci associated with the expression of these phenotypes. The loci were identified by studying segregation among F_2 progeny from crosses of a half-diallel among nine *A. thaliana* accessions, and by using advanced generations including recombinant inbred lines (Burr and Burr 1991; Reiter *et al.* 1992). Evidence is presented that demonstrates how a single cross between two host accessions can be used to examine the segregation of several loci associated with the expression of isolate specific and phenotypically distinct responses to infection by *P. parasitica*. A companion paper (Tor *et al.* 1994) presents molecular evidence that confirms the existence and position of these loci in the *A. thaliana* genome.

RESULTS

Characterization of interaction phenotypes.

A cotyledon assay conducted under controlled-environmental conditions was used to minimize nongenetic variation. This was essential for resolving clearly the inheritance of variation for interaction phenotype, particularly for detecting subtle differences between parents.

Interaction phenotypes indicative of any degree of hindrance to the development of *P. parasitica* were determined by comparisons with the fully susceptible phenotype. Full susceptibility of cotyledon tissue was characterized as the onset of asexual reproduction by the parasite 3 days after inoculation (dai), and sporulation becoming profuse (more than 20 sporangiophores per cotyledon) 7 dai when cotyledons were fully expanded and the first true leaf was beginning to appear. This phenotype is referred to as EH, an abbreviation for early and heavy sporulation (Table 1). From comparisons with noninoculated cotyledons, other characteristics of the EH phenotype were commonly a reduction in diameter of an infected cotyledon, a change in color to gray green, and some wilting of tissue 7 dai. Seedlings colonized extensively by *P. parasitica* were greatly reduced in vigor, but most of these seedlings were capable of subsequent sustained growth and reproduction when they were transferred to conditions of lower relative humidity (<95%) and long days (16-h photoperiod). Copious oospore production, evident 7 dai, was also a feature of the EH phenotype. Every isolate of *P. parasitica* tested thus far has been sexually competent and apparently homothallic. For routine assessment, only the timing and degree of asexual sporulation were recorded. For each isolate of *P. parasitica* studied, at least one accession of *A. thaliana* was identified which exhibited the EH phenotype following inoculation. Thus, a combination of isolate and accession that exhibited the EH phenotype was subsequently used as a guide for characterizing interaction phenotypes resulting from combinations of the same isolate with other accessions.

Among those interaction phenotypes where reproduction by *P. parasitica* was hindered, two major types of host response were observed (Fig. 1): Minute necrotic flecks that appeared to be associated strictly with the region of penetrated host cells; and conspicuous necrotic pits often expanding until the entire cotyledon was necrotic and thought to involve necrosis of host cells remote from ones that were penetrated.

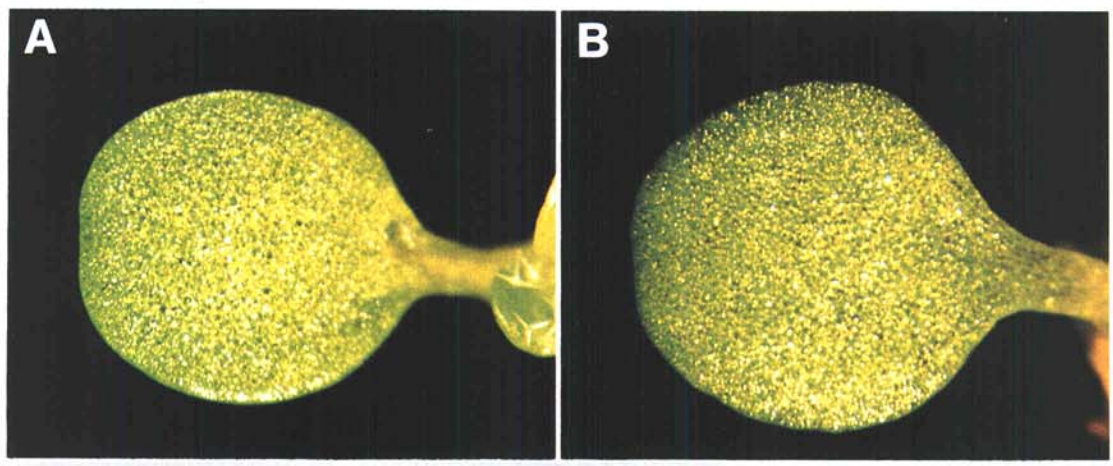

Fig. 1. Variation for interaction phenotype among combinations of *Arabidopsis thaliana* accessions and *Peronospora parasitica* isolates. Macroscopically visible characteristics were photographed 3 days after inoculation (dai)(A, C, E, G, I, K, M) and 7 dai (B, D, F, H, J, L, N): A, B, noninoculated (Col-3); C, D, early and heavy asexual sporulation (Nd-1 vs. *Cala2*); E, F, necrotic flecks with delayed and light asexual sporulation (Col-5 vs. *Emoy2*); G, H, necrotic flecks with no asexual sporulation (Col-5 vs. *Cala2*); I, J, necrotic cavities with no asexual sporulation (Ws-3 vs. *Cala2*); K, L, brown-centered, necrotic pits with no asexual sporulation (Ws-3 vs. *Emoy2*); M, N, and uniformly tan-colored necrotic pits with no asexual sporulation (Nd-1 vs. *Emoy2*).
(Continued on next page.)

A third intermediate type of host response described as necrotic cavities was also observed.

Necrotic flecks (F) on cotyledons was the host response most frequently observed. Over 50 accessions of *A. thaliana* were tested and each exhibited flecks in association with at least one of seven *P. parasitica* isolates (*Cala2, Emoy2, Emwa1, Hiks1, Cand3, Noks1,* and *Wela3*). No accession was found that exhibited an EH phenotype in combination with all of these isolates. Five accessions were found (Nd-1, Ws-3, Per-1, Ema-1, and Cnt-1) that exhibited pits when inoculated with either *Emoy2* or *Hiks1*; and two accessions were found that exhibited cavities (Ws-3 following inoculation with *Cala2*, and Ler-1 following inoculation with *Noks1*). Following inoculation with *Noco2*, the sexual parent of *Noks1* (not shown in Table 1), Ws-3 exhibited a CN phenotype and Ler-1 exhibited a FN phenotype. This compared with PN and CN for the same accessions, respectively, following inoculation with *Noks1*.

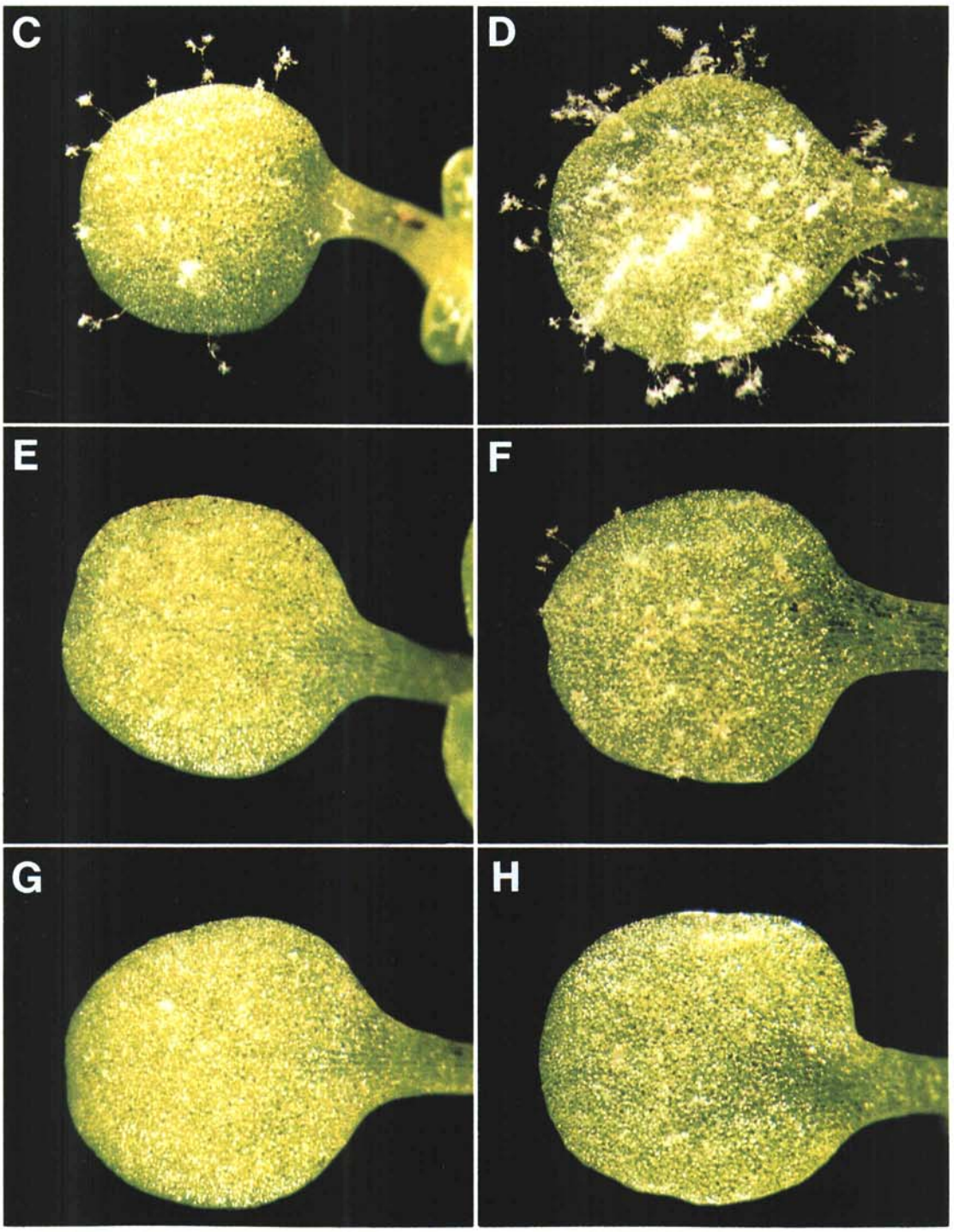

Fig. 1. *Continued from previous page.*

Interaction phenotypes involving flecks exhibited variation in the timing and intensity of asexual reproduction by *P. parasitica*. In some combinations of host accession and parasite isolate, sporulation was not evident 3 dai, but up to 10 sporangiophores were observed 7 dai. This type of sporulation was referred to as DL (abbreviation for delayed and light); and the interaction phenotype was referred to as FDL. When sporulation occurred only rarely in association with flecks, the phenotype was referred to as FR; and if sporulation was never observed but flecks were present, the phenotype was referred to as FN. The latter two phenotypes were only confirmed as such after assessing large numbers of inoculated seedlings for a given combination of accession and isolate. Hence, it was not possible to use these abbreviations for characterizing a heterogeneous population such as an F_3 family. The FR phenotype was defined by the observation of

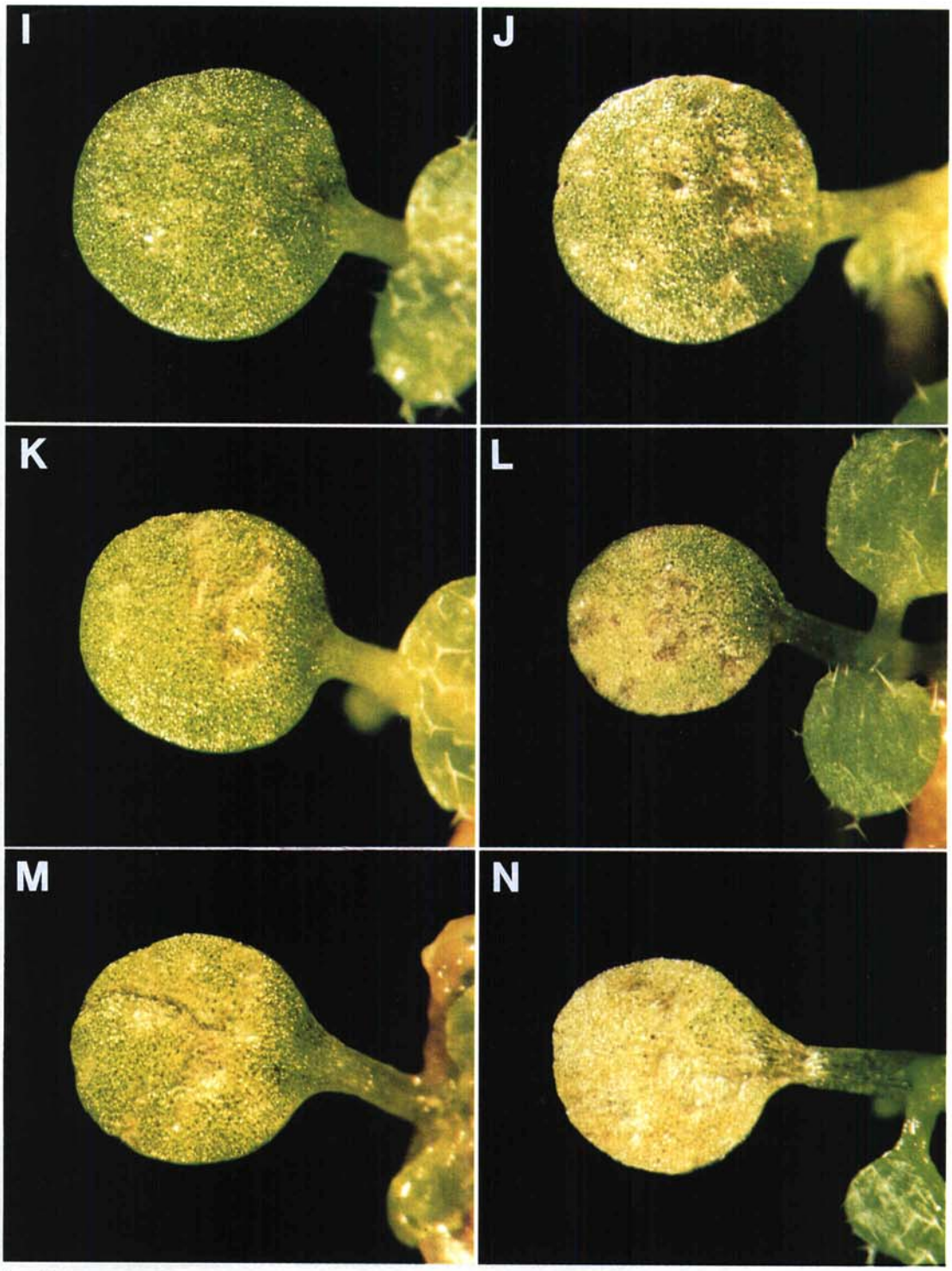

Fig. 1. *Continued from previous page.*

up to five sporangiophores 7 dai on fewer than 10% of inoculated plants. Combinations of accession and isolate which exhibited FDL, FR, or FN phenotypes are shown in Table 1.

The association between flecks and occasional, but hindered, sporulation suggested that some colonization of cotyledons by *P. parasitica* had occurred. From microscopic examination 3 dai, the flecks appeared to be associated with death of mesophyll cells. In the FDL phenotype exhibited by Col-5 inoculated with isolate *Emoy2*, necrosis appeared in a zone several host cells behind the advancing hyphal tip. Hyphae typically ramified throughout the cotyledon and colonization was sufficient for the production of up to 10 sporangiophores and several oospores per cotyledon. However, the hyphae appeared to be more sparse compared with a typical EH phenotype. Similarly in the FR phenotype exhibited by Col-5 inoculated with the isolate *Cala2*, hyphae were often observed to have advanced beyond necrotic hosts cells, but hyphal diameter was noticeably constricted and colony size was only occasionally sufficient to result in the production of one or two oospores. Production of a sporangiophore was rare. The least parasite growth associated with host cell necrosis was observed in the FN phenotype exhibited by Oy-1 following inoculation with *Cala2*. In this combination, fewer than 10 host cells became necrotic and growth of hyphae was usually arrested within the necrotic lesion that formed. No sexual or asexual reproduction was observed for this combination.

The occurrence of necrotic pits was the most extensive host response to inoculation with *P. parasitica* (Fig. 1). Sporulation was never observed in association with pits, so the interaction phenotype was referred to as PN. This phenotype, in common with the EH phenotype, could be scored unequivocally using inoculum concentrations as low as 50 sporangia per cotyledon. Necrosis was evident as early as 3 dai and involved a characteristic structural collapse of host tissue forming a depression or pit in the cotyledon. Colonization by *P. parasitica* was minimal, and host cell necrosis therefore occurred remote from the sight of penetration as the lesions had progressed well beyond the senescent parasite colony. Although the entire cotyledon often died and the seedling was stunted, necrosis always terminated before reaching the hypocotyl. Consequently, plants exhibiting the PN phenotype were capable of reproduction, quickly reviving with no apparent delay to seed production compared with noninoculated plants.

Subtle differences were observed in the types of necrotic pits produced by different combinations of accession and isolate. The combination of accession Nd-1 with isolate *Emoy2* typically resulted in tan-colored, necrotic lesions that were clearly visible 3 dai without obvious chlorosis. These lesions usually continued to expand and coalesce until the entire cotyledon was necrotic. The combination of Nd-1 and *Hiks1* was similar but appeared to progress more slowly, often becoming clearly visible 24 hr later. Combinations of accession Ws-3 with the same two isolates produced cotyledon lesions that typically had dark brown centers surrounded by a halo of chlorosis. These were clearly visible 3 dai, but lesion expansion often terminated before 7 dai, leaving some green or chlorotic tissue between necrotic lesions.

Necrotic cavities were considered to be another type of host response. This response was similar in appearance to flecks 3 dai, but the response typically developed into substantially larger lesions of collapsed tissue evident 7 dai. The lesions, often surrounded by a chlorotic halo, were invariably determinate in expansion and thus were not as extensive as pits. The name "cavity" was used to distinguish this host response as being qualitatively different from flecks or pits 7 dai. Apart from the distinction between flecks, cavities, and pits, it was not practical to further refine the classification of

Table 2. Segregation of interaction phenotypes among F_2 progeny from crosses of a half-diallel between nine accessions of *Arabidopsis thaliana* inoculated with *Peronospora parasitica* isolates *Emoy2* and *Cala2*

A. thaliana female parent[a]	*P. parasitica* isolate	*A. thaliana* male parent								
		Nd-1	**Oy-1**	**Col-5**	**Ler-1**	**Wei-1**	**Tsu-1**	**Ws-3**	**Ema-1**	**Ksk-1**
Nd-1	*Emoy2*	112:1:0:0[b]			55:42:5:3	24:8:1:1				83:19:1:1
	Cala2	0:0:16:197			0:0:0:28	0:13:13:14				0:35:22:8
Oy-1	*Emoy2*	47:5:9:13	0:1:11:113		0:15:16:4				30:6:0:2	0:63:16:2
	Cala2	0:58:4:6	0:258:0:0		0:440:70:66				0:29:2:9	0:139:1:0
Col-5	*Emoy2*	65:12:14:6	0:0:62:39	0:10:40:1	0:51:10:0	0:2:52:3		31:8:5:7	31:6:2:2	0:39:1:0
	Cala2	0:21:50:9	0:318:9:0	0:253:7:0	0:16:54:23	0:9:37:12		0:39:3:3	0:32:2:6	0:116:25:0
Ler-1	*Emoy2*				0:39:0:0					NA
	Cala2				0:0:8:70					NA
Wei-1	*Emoy2*		0:0:21:10		0:10:5:2	0:13:12:9				0:18:0:1
	Cala2		0:9:21:2		0:0:7:7	0:7:10:9				0:15:0:5
Tsu-1	*Emoy2*	19:1:1:0	0:2:1:16	0:20:17:18	0:14:6:0	0:1:4:15	0:1:9:18		23:5:1:5	NA
	Cala2	0:0:1:33	0:45:31:26	0:22:19:21	0:1:5:13	0:1:11:8	0:1:5:27		0:3:6:29	NA
Ws-3	*Emoy2*	69:0:0:0	72:11:7:8		26:11:1:1	36:13:5:0	NA	16:0:0:0	NA	NA
	Cala2	0:26:3:9	0:41:2:2		0:26:0:17	0:43:2:4	NA	0:17:0:0	NA	NA
Ema-1	*Emoy2*	140:0:0:0			29:5:0:1	26:4:3:1			18:0:0:0	NA
	Cala2	0:0:9:29			0:0:3:34	0:16:6:11			0:0:0:24	NA
Ksk-1	*Emoy2*									0:14:0:0
	Cala2									0:27:0:0

[a]Single plant genotypes selected randomly from the following accessions (top to bottom): Niederzenz (Nd-0), Columbia (Col-*gl1*) (Meyerowitz), Landsberg erecta (Ler-0), Oystese (Oy-0), Weiningen (Wei-0), Wassilewskija (Ws-0), Keswick (Ksk-0), East Malling (Ema-0), Canterbury (Cnt-0), Rschew (Rld-0), and Tsu (Tsu-0).

[b]Segregation of F_2 and S_2 progeny was tabulated in a ratio of four abbreviated classes of interaction phenotypes (P:N:D:E): P = necrotic pits without sporangiophores, N = no sporangiophores and any host response other than the production of pits (including N, FN, and CN phenotypes), D = delayed sporulation (including DL and DH phenotypes), and E = early sporulation (3 dai) becoming profuse 7 dai. Detailed description of phenotypes is provided in Table 1. NA = not available for testing.

host response using solely macroscopic characteristics. However, it should be possible in the future to reveal microscopic differences within each class of host response by means of histological and biochemical comparisons.

Genetic nomenclature.

Throughout the text, "locus" has been used to refer to the location of a postulated gene using evidence of recombination with previously defined loci. Corresponding loci for genotype specificities in the host and parasite were given names consistent with the hypothesis of recognition and intuitively descriptive of the organisms being investigated. Thus, specificity loci of *A. thaliana* have been named *RPP* loci (abbreviation of "recognition of *P. parasitica*," or else "recognized by *P. parasitica*"), and were numbered consecutively (i.e., *RPP1, RPP2*, etc.). Specificity loci of *P. parasitica* have been named *ATR* loci (abbreviation of "*A. thaliana* recognized," or else "*A. thaliana* recognition"), and were numbered the same as the corresponding *RPP* locus. This nomenclature is descriptive of an interaction regardless of which partner is actively recognizing the other.

Ideally, new loci should be named strictly on the basis of genetic recombination. However, some *RPP* loci have inevitably been named without such evidence in cases where different research groups are attempting to map loci that are associated with different specificities and from different accessions of *A. thaliana*. Consequently, there may eventually be examples where different specificities are in fact allelic (i.e. two previously designated loci are the same). At least two alleles, or variants of a gene, have already been designated at each locus: "1" represents the matching *RPP* and *ATR* alleles that were used to define the genetic basis of an interaction phenotype, and "0" represents the matching alleles associated with a compatible interaction or the lack of recognition at that locus. These have been named by hyphenation extension of the respective locus such as *RPP1-1* and *ATR1-1*, and *RPP1-0* and *ATR1-0*. When the molecular function of a particular allele has been demonstrated, a new nomenclature will need to be adopted that is more descriptive of function.

Half-diallel cross of *A. thaliana* accessions.

Cross of a half-diallel among nine parental genotypes of *A. thaliana* was produced for investigating the inheritance of different interaction phenotypes (Table 2). Accessions commonly used in laboratory research were included in this study: Columbia-*gl1* (Meyerowitz) (Col-5 *gl1*), Landsberg erecta (Ler-1), Niederzenz (Nd-0), Rschew (Rld-2), and Wassilewskija (Ws-0). Other accessions included: Oy-0 (Norway); Wei-0 (Switzerland); Tsu-0 (Japan) on which most isolates so far tested have been able to sporulate to some degree; and Ksk-1 and Ema-1 that were collected from wild UK populations. The parental genotypes used in the half-diallel were selected randomly from these accessions. In some cases, homogeneity of the original accession was uncertain so several genotypes were re-numbered (e.g., Oy-1 was selected from Oy-0) to ensure the future clarity in identifying the actual genotypes used in this study.

The F_2 populations of the half-diallel cross were tested for segregation of interaction phenotypes following inoculation with isolates *Emoy2* and *Cala2*. The phenotype of each inoculated seedling was characterized using host response and the number of sporangiophores produced per seedling 3 and 7 dai. Although a full characterization of phenotypes was possible for homozygous parental genotypes (Table 1), this was not feasible for a segregating F_2 population without testing F_3 progeny from every F_2 individual. For this reason, segregation data were tabulated into a ratio of four abbreviated phenotypic classes (P:N:D:E): P = necrotic pits without sporangiophores, N = no sporangiophores and any host response other than the production of pits (including N, FN, and CN phenotypes), D = delayed sporulation (including DL and DH phenotypes), and E = early sporulation (3 dai) becoming profuse 7 dai. Of these categories, the two extremes (P and E) were unequivocal. The N class, on the other hand, contained mostly individuals that exhibited flecks or cavities but probably also included occasional seedlings that escaped inoculation or infection. Similarly in the D class, most individuals exhibited light or at most moderate sporulation, but occasional individuals exhibited heavy sporulation (DH). Misclassified individuals in the N and D classes probably resulted from environmental variation or experimental error as distinct from genetic variation. The extent of nongenetic variation can

Table 3. Interaction phenotypes of recombinant inbred lines (F_8) from the cross Ws-1 × W100F (Ler) following inoculation with two isolates of *Peronospora parasitica*

Inbred class	Genotype combination[a]			Expected phenotype[b]		No. inbreds observed[c]
	RPP8	*RPP10*	*RPP10-C*	*Emoy2*	*Cala2*	
1	Ws	Ws	Ws	PN	CN	33
2	Ler	Ws	Ws	FN	CN	13
3	Ws	Ler	Ler	EH	EH	24
4	Ler	Ler	Ler	FN	EH	5
5	Ws	Ws	Ler	PN	EH	0
6	Ler	Ws	Ler	FN	EH	0
7	Ws	Ler	Ler	EH	CN	0
8	Ler	Ler	Ler	FN	CN	0

[a] All possible combinations of alleles at three host recognition loci. The allele from W100F (Ler) at locus *RPP8* on chromosome 5 and the allele from Ws-1 at *RPP10* on chromosome 3 are associated with recognition of isolate *Emoy2*. *RPP10-C* represents a third hypothetical allele from Ws-1 associated with recognition of isolate *Cala2*. Recombinants between *RPP10* and *RPP10-C* (classes 6 and 8) would prove the existence of a fourth locus.

[b] The Ws-1 allele at *RPP10* is associated with an PN phenotype (no asexual sporulation by *Emoy2* and necrotic pits on the host), whereas the W100F (Ler) allele at *RPP8* is associated with an FN phenotype (no sporulation by *Emoy2* and necrotic flecks on the host). It is assumed that an FN phenotype results when these alleles are combined in the same genotype (i.e., the Ler allele *RPP8.1* is epistatically dominant). The Ws-1 allele at *RPP10-C* is associated with a CN phenotype (no sporulation by *Cala2* and necrotic cavities on the host). W100F (Ler) exhibited an EH phenotype (early and heavy sporulation and no apparent host response) following inoculation with *Cala2*.

[c] Classes 2 and 8 share the same combination of phenotypes and differ only in the genotype at *RPP10*. Eight of the inbreds in class 2 exhibited a Ws-1 genotype at the loci *GL1* and m249 which flank *RPP10* and therefore probably carry the Ws-1 allele at *RPP10*. One inbred in class 2 exhibited a Ler genotype at the flanking loci. However, since this inbred carries the Ws-1 allele at *RPP10-C*, it probably carries the Ws-1 allele at *RPP10* also. The remaining four inbreds were recombinant between *GL1* and m249, and any one of these inbreds might actually belong in class 8. Similarly, classes 4 and 6 share the same combination of phenotypes differing only in the genotype at *RPP10*; however, all five inbreds in class 4 exhibited the Ler genotype at flanking markers and therefore probably carry the W100F (Ler) allele at *RPP10*.

be estimated using the data for interaction phenotypes among the self-pollinated populations.

Sufficient information for genetic interpretation is retained by classifying the segregating populations into four classes (P:N:D:E) (Table 2); however, assumptions must be made about the behavior of putative alleles. For example, the expression of a given allele associated with flecks may be partially dominant (dosage dependent) such that in the heterozygous condition the parasite grows sufficiently to allow some sporulation. This behavior would place a seedling in class D rather than class N, and interpretation is further complicated if on rare occasions a homozygous dominant individual exhibited a similar phenotype, as in the case of the FR phenotype. For simplicity, we have condensed segregation ratios further to two classes, thereby enabling calculation of chi-squared values based on expected ratios such as 3:1 or 15:1. Examples are provided that illustrate how the assumptions used to condense ratios can affect the genetic interpretation of the full phenotypic ratios. Nevertheless, by presenting the four class ratio in Table 2, it is possible for readers to examine the data according to their own assumptions, establish genetic hypotheses, and draw conclusions about the number and behavior of alleles predicted.

Genetic characterization of locus *RPP1* associated with necrotic pits and no asexual sporulation by *P. parasitica*.

Inheritance of the PN phenotype can be examined using F_2 populations of crosses between accession Nd-1 and any accession not exhibiting the PN phenotype following inoculation with isolate *Emoy2* (Table 1). Segregation of alleles associated with pits could be postulated by comparing the number of seedlings in the P class versus a total of the other classes (N, D, and E). When F_2 progeny from the cross between Oy-1 (EH phenotype) and Nd-1 were inoculated, the ratio observed was 47:27 (Table 2), suggesting that an incompletely dominant allele at a single locus may be associated with the PN phenotype. The locus for this putative allele was named *RPP1*. The allele from Nd-1 (*RPP1-1*) appears to be only partially dominant because the observed ratio was significantly different from the expected 3:1 ratio for a completely dominant allele ($\chi 2 = 5.21$, $P = 0.03$). A similar tendency for deviation from a 3:1 ratio was observed for two other crosses: Col-5 (FDL phenotype) × Nd-1 (65:32), and Nd-1 × Ler-1 (FN phenotype) (55:50). However, segregation among progeny from the cross Nd-1 × Wei-1 (DL phenotype) (28:10) indicated that the Nd-1 allele at *RPP1* is completely dominant ($\chi 2 = 0.04$, $P = 0.85$). Recombinant inbred lines (F_8) of the cross between Nd-0 and Oy-0 were used to support the hypothesis that Nd-1 carries a single allele associated with necrotic pits following inoculation with isolate *Emoy2*. Since a majority of such inbreds will be homozygous for any particular locus, it was possible to characterize fully the interaction phenotype (Table 1) for each inbred line. Fifty-five of 92 inbreds consistently exhibited the PN phenotype following inoculation with *Emoy2*; 46 inbreds would be expected if an allele at a single locus affects the PN phenotype and 69 inbreds if two independent alleles are effective. Thus, data from the inbreds support the more likely prediction that a single allele (RPP1-1) is associated with the PN phenotype of Nd-1 (for 1:1, $\chi 2 = 3.52$, $P = 0.06$; for 3:1, $\chi^2 = 11.36$, $P < 0.001$).

Two other accessions of *A. thaliana*, Ws-3 and Ema-1, were included in the half-diallel that also exhibited the PN phenotype when inoculated with isolate *Emoy2* (Table 2). In crosses between Ws-3 and other accessions not expressing the PN phenotype, a divergence from a 3:1 ratio (pits: no pits) was observed; whereas crosses between Ema-1 and the same non-PN accessions segregated with ratios more consistent with the expected 3:1. When Ws-3 and Ema-1 were crossed with Nd-1, all the F_2 progeny exhibited the PN phenotype. This suggests that the alleles associated with necrotic pits from each accession were probably at the same locus (*RPP1*). However, evidence presented below suggests that a single allele from Ws-3 is responsible for recognition of both *Emoy2* and *Cala2*. This implies that the Ws-3 allele differs in isolate specificity from the Nd-1 allele *RPP1-1*, and consequently, the former allele was named *RPP10-1*. The allele from Ema-1 associated with a PN phenotype appeared to exhibit the same isolate specificity as *RPP1-1* and was therefore named *RPP1-2*.

A genomic location of *RPP1* was fortuitously discovered using evidence that it is linked to the *GL1* locus for a recessive glabrous (without trichomes) allele on chromosome 3 (Koornneef *et al.* 1983). In the first crosses produced, Col-5(*gl1*) was used as a female parent so that the presence of trichomes at the F_1 generation would confirm that outcrossing had been successful. Among F_2 progeny of the cross between Col-5 and Nd-1, 73% exhibited parental phenotypes (glabrous and pits; or trichomes and no pits) following inoculation with isolate *Emoy2*. This suggested that *RPP1* and *GL1* were linked. A more precise map location for *RPP1* was subsequently determined in the interval delimited by GL1 and the RFLP locus identified by probe m249 (Tör *et al.* 1994).

The accession Nd-1 also exhibited the PN phenotype following inoculation with isolate *Hiks1*. This isolate has not yet been used to test F_2 progeny from the complete set of crosses in the half-diallel. However, F_3 families from the cross between Col-5 and Nd-1 were used to determine whether *RPP1-1* was associated with recognition of both *Emoy2* and *Hiks1*. Complete co-segregation was observed for 123 F_3 families, tested including examples of the *RPP1* locus for the homozygous Col-5 or Nd-1 alleles and in the heterozygous condition. A sample of families tested is provided (see Table 4 below). A similar test for recognition of both isolates by alleles from Ws-3 and Ema-1 has yet to be performed. For simplicity, we postulate that the same allele from Nd-1 (*RPP1-1*) is associated with recognition of *Emoy2* and *Hiks1*.

Genetic characterization of locus *RPP2* associated with necrotic flecks and the absence or rare occurrence of asexual sporulation by *P. parasitica*.

Three accessions from the half-diallel cross (Col-5, Oy-1, and Ksk-1) exhibited the FR or FN phenotype following inoculation with the isolate *Cala2*. Analysis using *Cala2* to test crosses between these accessions suggest that they share alleles at one locus associated with necrotic flecks. There were no seedlings exhibiting an EH phenotype among the F_2 progeny from each cross, although a low percentage of progeny exhibited a DL phenotype with fewer than five sporangiophores per seedling (none were observed exhibiting a DH phenotype): Col-5 × Oy-1 (0:318:9:0), Col-5 × Ksk-1 (0:116:25:0) and Oy-1 × Ksk-1 (0:139:1:0). A similar frequency of seedlings exhibiting the DL phenotype was ob-

served when self-pollinated progeny of Col-5 were inoculated with *Cala2*, but none were observed among self-pollinated progeny of Oy-1 or Ksk-1.

Inheritance of the FR phenotype in accession Col-5 following inoculation with *Cala2* can be examined using F_2 populations of crosses with accessions Nd-1 and Ler-1 (both exhibiting an EH phenotype). Most individuals in the D and N classes exhibited flecks, whereas individuals in class E did not. Using evidence of host response (ignoring evidence of sporulation), a chi-squared value can be calculated for the ratio of the total of seedlings in the N and D classes versus those in the E class. The observed ratio combined from both crosses (141:32) differed significantly from 3:1 ($\chi^2 = 3.90$, $P < 0.05$), but the existence of alleles at two independent loci was even less probable (for 15:1, $\chi^2 = 40.3$, $P < 0.001$). A logical conclusion would be that Col-5 carries a completely dominant allele at a single locus recognizing *Cala2*. If on the other hand, the observed ratio from both crosses is based on presence of sporangiophores (ignoring timing and degree of sporulation, and host response), a chi-squared value could be calculated by comparing the N class versus the total of D and E classes (37:136). In this case, the chi-squared values suggest that Col-5 carries a completely recessive allele at a single locus (for 1:3, $\chi^2 = 1.20$, $P < 0.05$). Using evidence of the full interaction phenotype (host response and parasite sporulation), we would predict that Col-5 carries a partially dominant allele (i.e., dosage dependent) at a single locus. This locus was named *RPP2* and has been mapped to an interval defined by molecular markers on chromosome 4 (Tör *et al.* 1994).

The number of loci carried by Oy-1 for recognition of *Cala2* is less clear because a greater majority of F_2 progeny from crosses with Nd-1 and Ler-1 (EH phenotype) exhibited a FN phenotype (0:498:74:72). If the segregation ratio is examined on a strict basis of presence or absence of sporangiophores (498:146), then the chi-squared value suggests that Oy-1 carries a completely dominant allele at a single locus (for 3:1, $\chi^2 = 1.86$, $P = 0.18$). However, if the total of progeny in N and D classes is compared to progeny in the E class (572:72), then the existence of a single locus is unlikely ($\chi^2 = 65.6$, $P < 0.001$). Recombinant inbreds (F_8) of the cross between Nd-0 and Oy-0 were used following inoculation with isolate *Cala2* to determine whether alleles at one or two loci were associated with the FN phenotype of Oy-1. The ratio of 79:3:16 was observed among the inbreds for classes N (FN and FR), DL, and EH, respectively. If Oy-1 carries an allele at a single locus for recognition of *Cala2*, then half of the inbreds would have exhibited the FN phenotype; a single allele is therefore unlikely (for 1:1, $\chi^2 = 36.7$, $P < 0.001$). If Oy-1 carries alleles at two independent loci, then 75% of the inbreds would have exhibited the FN phenotype; alleles at two loci are much more likely (for 3:1, $\chi^2 = 1.65$, $P < 0.20$). Using this evidence, two loci have been designated as *RPP2* (the locus in common with Col-5 and Ksk-1) and *RPP3*. However, the location of *RPP3* in the genome of *A. thaliana* has yet to be determined.

Table 4. Genotypes of F_2 progeny from a cross between *Arabidopsis thaliana* accessions Col-5 and Nd-1 and predicted from segregation within F_3 populations for loci associated with recognition of five isolates of *Peronospora parasitica*

A. thaliana accession[a]	*P. parasitica* isolate					*RPP* locus[c]					
	Emoy2	*Emwa1*	*Cala2*	*Wela3*	*Hiks1*	1	2	4	4*	6	7
Col-5	0:60:39:3[b]	0:136:0:0	0:46:5:0	0:49:0:0	0:93:6:0	1 1	1 1	1 1	1 1	1 1	1 1
Nd-1	167:4:0:0	0:18:25:86	0:1:16:40	0:6:36:27	78:0:0:0	2 2	2 2	2 2	2 2	2 2	2 2
1431	0:2:8:0	0:34:5:2	0:25:5:16	0:10:0:0	0:0:1:59	1 1	1 2	1 1	1 1	1 1	2 2
1433	8:0:2:0	0:16:5:1	0:13:6:3	0:9:0:0	24:3:3:3	1 2	1 2	?	1 2	1 1	1 2
1442	0:19:6:0	0:22:2:0	0:52:4:0	0:2:10:0	0:36:8:3	1 1	1 1	1 1	1 1	2 2	1 2
1444	0:5:5:0	0:23:0:0	0:29:9:11	0:7:4:0	0:0:12:20	1 1	1 2	1 1	1 1	1 2	2 2
1448	0:0:0:10	0:2:5:18	0:2:9:26	0:5:4:0	0:35:17:8	1 1	2 2	2 2	2 2	1 2	1 2
1459	0:0:0:6	0:2:7:10	0:0:1:30	0:6:5:0	0:0:8:22	1 1	2 2	2 2	2 2	1 2	2 2
1463	29:1:0:2	0:1:0:24	0:0:0:11	0:8:2:0	13:0:0:6	1 2	2 2	2 2	2 2	1 2	2 2
1466	21:0:0:0	0:3:11:11	0:0:0:11	0:1:5:4	25:0:0:0	2 2	2 2	?	2 2	2 2	?
1468	0:2:6:0	0:30:2:0	0:0:2:12	0:8:2:0	0:24:8:7	1 1	2 2	1 1	1 1	1 2	1 2
1472	0:0:0:20	0:2:2:21	0:0:2:25	0:4:6:0	0:0:6:22	1 1	2 2	2 2	2 2	1 2	2 2
1499	0:0:1:19	0:5:2:12	0:0:0:12	0:12:0:0	0:24:8:8	1 1	2 2	2 2	2 2	1 1	1 2
1521	14:0:0:0	0:1:5:20	0:0:0:10	0:7:4:1	23:1:0:0	2 2	2 2	?	2 2	1 2	?
1531	0:0:0:11	0:4:0:16	0:0:1:14	0:0:4:7	0:21:6:13	1 1	2 2	2 2	2 2	2 2	1 2
1534	0:10:2:3	0:17:4:1	0:15:2:1	0:7:2:2	0:15:4:3	1 1	1 2	1 2	1 2	1 2	1 2
1551	15:0:0:0	0:1:1:14	0:0:0:12	0:11:0:0	37:0:0:0	2 2	2 2	2 2	2 2	1 1	?
1554	16:0:0:0	0:3:3:16	0:0:0:26	0:0:4:7	48:2:0:0	2 2	2 2	?	2 2	2 2	?
1561	0:0:0:15	0:6:18:10	0:14:11:2	0:7:1:0	0:0:5:24	1 1	1 2	2 2	1 2	1 2	2 2
1563	0:13:1:0	0:22:0:0	0:14:1:0	0:1:11:0	0:0:0:29	1 1	1 1	1 1	1 1	2 2	2 2
1576	0:14:6:0	0:24:0:0	0:28:0:0	0:10:2:0	0:14:4:0	1 1	1 1	1 1	1 1	1 2	1 1

[a] Control accessions or else an F_3 population derived from a cross between Col-5 and Nd-1.

[b] Segregation of F_3 progeny (S_2 generation for controls) was tabulated in a ratio of four abbreviated classes of interaction phenotypes (P:N:D:E): P = necrotic pits without sporangiophores, N = no sporangiophores and any host response other than the production of pits (including N, FN and CN phenotypes), D = delayed sporulation (including DL and DH phenotypes), and E = early sporulation (3 dai) becoming profuse 7 dai.

[c] F_2 genotype predicted from interpretation of F_3 segregation ratio (P:N:D:E): 1 1 = homozygous condition of female parent (Col-5), 2 2 = homozygous condition of male parent (Nd-1), 1 2 = heterozygous condition. Allele *RPP1-1* from Nd-1 is associated with necrotic pits (P class) following inoculation with either isolate *Emoy2* or *Hiks1*; allele *RPP2-1* from Col-5 is usually associated with no asexual sporulation and necrotic flecks (N class) following inoculation with *Cala2*, and the same is true for allele *RPP7-1* from Col-5 following inoculation with *Hiks1*; and allele *RPP4-1* from Col-5 is usually associated with delayed, sparse production of sporangiophores (D class) following inoculation with either *Emoy2* or *Emwa1*. The column under 4 was predicted using data from inoculations with *Emoy2*, whereas the column under 4* was predicted using data from inoculations with *Emwa1*. A "?" is used where the genotype at a locus could not be scored due to the epistatic influence of the Nd-1 allele at *RPP1*.

The number of loci carried by Ksk-1 for recognition of *Cala2* is also uncertain. Among F_2 progeny of the cross between Nd-1 and Ksk-1, seedlings exhibiting the FN phenotype were most common following inoculation with *Cala2* (0:35:22:8) but not as prevalent as exhibited by Oy-1 (described above). If Ksk-1 carries only a single allele, then judging from the relatively small D class from this cross, the Ksk-1 allele is more dominant than the putative allele at *RPP2* in Col-5. It is also possible that Ksk-1 carries two alleles at closely linked loci that, acting in an additive manner, result in what appears to be a more dominant phenotype. Recombinant inbreds (F_6) derived from the cross between Wei-1 and Ksk-1 were also used to investigate the inheritance of the FR phenotype from Ksk-1. Following inoculation with *Cala2*, 41 of 87 inbreds exhibited the FN phenotype (for 1:1, $\chi^2 = 0.29$, $P = 0.61$). It was not possible to discriminate between an allele at a single locus and alleles at two closely linked loci.

Genetic characterization of *RPP* loci associated with delayed, light asexual sporulation by *P. parasitica*.

The combination of accession Col-5 and isolate *Emoy2* was used to determine the genetic basis for a phenotype involving delayed sporulation (Table 1). F_2 progeny from the cross between accessions Col-5 and Oy-1 segregated into classes D and E (0:0:62:39) following inoculation with *Emoy2*, which contrasted with progeny from self-pollinated Oy-1 (0:1:11:113) and Col-5 (0:10:40:1). These results suggest that an incompletely dominant allele is associated with the FDL phenotype of Col-5 (for 3:1, $\chi^2 = 10.75$, $P < 0.001$). This Col-5 allele is referred to as *RPP4-1*.

The accession Ler-1 appears to carry alleles at two independent loci of which one may be *RPP4-1*. Only class N and D progeny were observed among F_2 progeny from the cross between Col-5 and Ler-1 following inoculation with *Emoy2* (0:51:10:0). The 10 progeny in class D and none in class E suggested that these accessions share at least one allele associated with the DL phenotype. F_2 progeny of the cross between Oy-1 and Ler-1 segregated among classes N, D and E (0:15:16:4). The ratio for the total of progeny in the N and D classes compared to those in class E, suggests that Ler-1 carries an allele at a second locus other than *RPP4* (for 3:1, $\chi^2 = 3.75$, $P = 0.05$; for 15:1, $\chi^2 = 1.12$, $P = 0.29$). The existence of alleles at two loci in Ler-1, including one in common with Col-5, was supported by testing recombinant inbreds (F_8) of Ler-0 × Col-4. Thirty-one inbreds were inoculated with isolate *Emoy2*, and they segregated into two homozygous classes of FN and DL phenotypes (15:14). None exhibited the EH phenotype. The second locus in Ler-1, presumed to be associated with the FN phenotype, was named *RPP8* (information suggesting its location in the *A. thaliana* genome is provided below).

The accession Wei-1 also exhibited a phenotype involving delayed, light sporulation following inoculations with six out of seven *P. parasitica* isolates tested (Table 1). To determine whether this accession might carry isolate nonspecific resistance, recombinant inbreds (F_6) of Wei-1 × Ksk-1 were inoculated with isolates *Cala2* and *Hiks1*. Although half of the inbreds were not informative following inoculation with *Cala2* due to the apparent epistasis of *RPP2-2* from Ksk-1 (FN phenotype), 44 inbreds exhibited either DL or EH phenotypes in combination with this isolate. Of these 44 inbreds apparently lacking allele *RPP2-2*, 20 exhibited one or the other parental phenotype associated with the recognition of *Cala2* and *Hiks1* (DL and DL, or EH and EH, respectively) and 24 exhibited one or the other nonparental phenotype (DL and EH, or EH and DL, respectively). The locus from Wei-1 associated with recognition of *Cala2* has not been named because its location in the genome is yet unknown. However, the locus associated with recognition of *Hiks1* was named *RPP9* because it was observed to cosegregate with a previously defined locus on chromosome 1 associated with recognition of an isolate of *Albugo candida* (*RAC1*) (Crute *et al.* 1993). In any case, Wei-1 must carry at least two alleles at different *RPP* loci associated with qualitative inheritance of the DL phenotype and involved in isolate specific recognition of *P. parasitica*.

Table 5. Estimates of the frequency of genetic recombination between *RPP* loci and a locus *GL1* (glabrous) on chromosome 3 identified in a cross between accessions of *Arabidopsis thaliana* Col-5 (*gl1*) and Nd-1

Paired loci	Recombinant chromosomes[a]	Total number of chromosomes[b]	Recombination frequency (%)[c]
RPP1, *GL1*	19	164	12
RPP1, *RPP2*	135	294	49
RPP1, *RPP4*	107	236	41
RPP1, *RPP6*	84	190	44
RPP1, *RPP7*	108	232	47
RPP2, *GL1*	49	124	48
RPP4, *GL1*	40	108	50
RPP6, *GL1*	31	86	36
RPP7, *GL1*	44	206	49
RPP2, *RPP4*	39	244	11
RPP2, *RPP6*	78	190	41
RPP2, *RPP7*	51	182	34
RPP4, *RPP6*	73	188	39
RPP4, *RPP7*	46	152	49
RPP6, *RPP7*	50	150	33

[a]The genotype at each locus was recorded on the basis of 1 1 for the homozygous condition of the female parent (Col-5), 2 2 for the homozygous condition of the male parent (Nd-1), and 1 2 for the heterozygous condition (see Table 4 for examples). The number of recombinant chromosomes was then estimated by comparing two columns of data for genotypes at a pair of loci and totaling the number of changes from condition 1 to condition 2. For example, a family that was recorded as being 1 1 at *GL1* and 1 2 at *RPP1* would count as one recombination event between the loci; a 1 1 and 2 2 for each respective locus would count as two recombination events. In cases where both loci were in the heterozygous condition, it was not possible to distinguish between no recombination events or two. The estimates do not correct for this possible error.

[b]Twice the number of F_3 families scored for genotypes at the given pair of loci.

[c]Estimate of recombinant chromosomes divided by the total number of chromosomes, expressed as a percentage.

Genetic characterization of the locus *RPP10* associated with necrotic cavities and no asexual sporulation by *P. parasitica*.

Inheritance of the CN phenotype was determined using F_2 populations of crosses between accession Ws-3 and either of two accessions Nd-1 and Ler-1 (EH phenotype) following inoculation with isolate *Cala2*. For these crosses, seedlings exhibiting cavities were placed in class N, so segregation of alleles associated with this host response was postulated by comparing the number of seedlings in the N class with the total of classes D and E. Ratios of 26:12 and 26:17 were observed for crosses Ws-3 × Nd-1 and Ws-3 × Ler-1, respec-

tively (Table 2). The cross with Nd-1 suggests a completely dominant allele at a single locus ($\chi^2 = 0.88$, $P = 0.36$), but segregation among progeny from the cross with Ler-1 differed significantly from a 3:1 ratio ($\chi^2 = 4.84$, $P = 0.04$), suggesting that the allele is incompletely dominant.

Further evidence for an allele at a single locus from Ws-3 associated with recognition of *Cala2* was obtained using recombinant inbreds (F_8) of Ws-1 × W100F (Ler). Following inoculation with this isolate, 28 inbreds exhibited an EH phenotype, only eight exhibited a CN phenotype, and 38 exhibited a PN phenotype. The third class was unexpected because the host response was more extensive compared with 38 control seedlings of Ws-1, none of which exhibited a PN phenotype. No F_2 seedlings were observed exhibiting pits among the progeny of Ws-3 × Ler-1. Nevertheless, the CN and PN inbred classes of inbreds were both considered to carry the Ws-1 allele associated with recognition of *Cala2*. Although the ratio of CN and PN:EH appears to be skewed, it did not differ significantly from the expected 1:1 ratio for an allele at a single locus ($\chi^2 = 3.00$, $P = 0.09$). Most of the morphological markers that we observed segregating in this cross also exhibited skewed segregation in favor of the Ws-1 parent; the mean segregation of all nine morphological markers combined among the inbreds used for this investigation was 49:5:20 (Ws:Ws/Ler:Ler). Percentage recombination of this *RPP* locus with morphological markers from chromosomes 1, 2, 4, and 5 was as follows (chromosome-locus, percent recombination): 1-*AN*, 44; 1-*API*, 39; 2-*ER*, 38; 2-*PY*, 43; 4-*BP*, 47; 4-*CER2*, 53; and 5-*TT3*, 49. Linkage to loci on chromosome 3 (listed from top to bottom) including examples of those defined by RFLPs was as follows (locus, percent recombination): *HY2*, 41; m317, 28; m255, 21; *GL1*, 14; m249, 17; and m424, 41. The data for the molecular markers were produced by Reiter *et al.* (1992) and obtained from the database AATDB. Thus, as with *RPP1* described above, the interval delimited by loci *GL1* and m249 is thought to contain the locus for the allele from Ws-1 associated with recognition of *Cala2*.

Analysis of the cross between Ws-1 and W100F (Ler) was extended following inoculations of the same recombinant inbreds with other isolates. For example, the inbreds were used to determine whether the locus *RPP10* involved in recognition of *Cala2* is also involved in recognition of *Emoy2*. Table 3 lists the different phenotypic classes that were observed. Following inoculation with *Emoy2*, the PN phenotype was presumed to indicate presence of an *RPP10* allele from Ws-1 (i. e., classes 1 and 5), and the EH phenotype was presumed to indicate presence of an allele at this locus from W100F (Ler). Inbreds in classes 2, 4, and 8, however, exhibited a FN phenotype following inoculation with *Emoy2*. This is due presumably to recognition of *Emoy2* by the allele *RPP8-1* inherited from W100F (Ler). *RPP8* appears to be located on chromosome 5 because only two recombinants between *RPP8* and *TT3*, a locus on this chromosome associated with transparent testa, were observed among the inbreds included in Table 3. *RPP8* probably lies above *TT3* because the RFLP marker identified by probe m422 appeared to define the other side of the interval that carries *RPP8*.

Using evidence from this expected interval for *RPP8-1*, all the inbreds in class 2 appeared to have inherited this allele from W100F (Ler). On chromosome 3, however, eight of these inbreds exhibited a Ws-1 genotype at the loci *GL1* and m249 and therefore probably also carried the Ws-1 allele at *RPP10*. This suggests that the expression of allele *RPP10-1* from Ws-1 is influenced by the expression of *RPP8-1* because a FN phenotype was observed instead of a PN phenotype. One of the inbreds in class 2 exhibited a W100F (Ler) genotype at *GL1* and m249. This inbred also presumably carries the Ws-1 allele at *RPP10* because a CN phenotype was observed following inoculation with *Cala2* (i.e., *RPP10-1* was present). For this to be possible, there must have been two cross-over events, presumably in different inbred generations rather than a double cross-over in one generation. The remaining four inbreds in class 2 were recombinant between the flanking markers, so their genotype at *RPP10* remains uncertain. If any one of these four inbreds belongs in the recombinant class 8, it would prove that different *RPP* alleles were responsible for recognition of *Cala2* and *Emoy2*. All the in-

Table 6. Sources of isolates of *Peronospora parasitica* used in analyses of genotype specific recognition by *Arabidopsis thaliana*

Population	Location	Isolate[a]	Origin		Stock host[d]
			Inoculum[b]	Bait host[c]	
1	Canterbury, Kent, UK	*Cand1*	Sporangia from wild	wild (rosette leaf)	Nd-1
		Cala1	Sporangia of *Cand1*	Nd-0 (seedling)	Ler-1
		Cala2	Oospore of *Cala1*	Nd-0 (seedling)	Ler-1
		Cand3	Sporangia of *Cand1*	Nd-0 (seedling)	Nd-1
2	East Malling, Kent, UK	*Emoy1*	Oospore from wild	Oy-0 (seedling)	Oy-1
		Emoy2	Oospore from wild	Col-5 (seedling)	Oy-1
		Emwa1	Oospore from wild	Rld-0 (seedling)	Ws-3
3	Hillier Arboretum, Hampshire, UK	*Hiks1*	Sporangia from wild	wild (rosette leaf)	Ksk-1
4	Norwich, Norfolk, UK	*Noks1*	Oospore of *Noco2*[e]	Nd-0 (seedling)	Col-5
5	Weiningen, Switzerland	*Wela3*	Oospore of *Wela1*[f]	Ler-0 (seedling)	Ler-1

[a] Names indicate the location from which an isolate was collected (first two letters), the accession of *A. thaliana* used for maintenance of the isolate (last two letters), and a number distinguishing between different isolates with the same four-letter name.
[b] Sporangia were collected from fresh or frozen leaf tissue, oospores were from dried leaf tissue.
[c] Accession of *A. thaliana* or field host from which the isolate was obtained. For isolates generated originally from an oospore, the infection resulted in asexual sporulation on a single seedling and sporangia were then collected for maintenance on the designated stock host. For isolates generated from sporangia, the original inoculum was collected from a single rosette leaf (in the case of isolates obtained directly from a wild source) or from a single seedling.
[d] *A. thaliana* accession used for routine maintenance of the isolate.
[e] Oospore inoculum provided by J. Parker, Norwich, UK.
[f] Oospore inoculum provided by A. Slusarenko, Zürich, Switzerland.

breds in class 4 exhibited a W100F (Ler) genotype at loci *GL1* and m249 and therefore most likely carried the W100F (Ler) allele at *RPP10*. Consequently, no inbreds were placed in the recombinant class 6 that was expected to exhibit the same phenotypic pattern as class 2. Similar to the analysis using *Emoy2*, inoculations of the inbreds with isolates *Hiks1* and *Noks1* revealed an *RPP* locus in each case that mapped to the interval between *GL1* and m249.

There also appeared to be complications from epistasis associated with the presence of an allele inherited from W100F (Ler). Inoculations with *Wela3*, on the other hand, revealed a locus that mapped to chromosome 4. Using data from 93 inbreds, linkage to loci on chromosome 4 was as follows (locus, percent recombination): *BP1*, 32; m326, 12; m580, 10; m557, 17; *CER2*, 19; g3088, 42; and g3713, 39. Recombination with loci on the other four chromosomes was 40–53%.

Genetic relationships between *RPP* loci segregating among progeny from a single cross of *A. thaliana*.

F_3 families from the cross between Col-5 and Nd-1 were used to investigate the genetic relationship between alleles at *RPP1, RPP2, RPP4, RPP6,* and *RPP7*. These families were used for inoculations with several isolates, and thus enabled us to infer the genotype at each locus of individuals from the F_2 generation. Four parasite isolates and a set of up to 133 F_3 families were used to derive phenotypic ratios (P:N:D:E) (Table 4). Genotypes were predicted from interpretation of these phenotypic ratios using the following notation: 1 1 = homozygous condition of the female parent (Col-5), 2 2 = homozygous condition of the male parent (Nd-1), and 1 2 = heterozygous condition. For example, if all individuals from an F_3 family exhibited the EH phenotype following inoculation with both of the isolates *Emoy2* and *Cala2*, then the genotype would be 1 1 at *RPP1* and 2 2 at *RPP2*. The patterns of segregation associated with phenotypes at each of these loci were simple and qualitative in nature (e.g, pits versus no pits; and sporulation versus no sporulation).

Although the genotype at locus *RPP4* from Col-5 could be predicted for many F_3 families following inoculation with *Emoy2*, we found difficulty in predicting a genotype for some families because of the effect of alleles contributed to the cross by Nd-1. Expression of the PN phenotype associated with *RPP1* was thought to obscure the expression of the FDL phenotype associated with *RPP4*. Thus, families homozygous for the PN phenotype could not be scored following inoculation with this isolate for the genotype at *RPP4*. It is also possible that Nd-1 carries a second allele, associated with the DL phenotype but not located at *RPP4*. When recombinant inbreds (F_8) of Nd-0 × Oy-0 were inoculated with *Emoy2*, one of 92 inbreds was apparently homozygous at a locus associated with the DL phenotype. The low frequency of occurrence of this phenotype suggests that if an allele at a second locus exists, then this is closely linked to *RPP1*. As a consequence, errors are possible in predicting which of the two alleles (*RPP4-1* from Col-5 or the second allele from Nd-1) is associated with the DL phenotype of a particular F_3 family.

The same F_3 families of Col-5 × Nd-1 were also inoculated with isolate *Emwa1*. This isolate was obtained from the same oospore population as *Emoy2* but the interaction phenotype following inoculation of Nd-1 was DH rather than PN. We expected *Emwa1* would therefore prove useful for predicting the genotype at *RPP4* uncomplicated by epistasis of *RPP1-1*. This appeared to be the case because the genotype predicted for *RPP4-1* was the same for most F_3 families after inoculations with *Emoy2* and *Emwa1* (sample provided in Table 4). There were 10 exceptional F_3 families (e.g., 1561) among 79 tested against both isolates. These families probably indicate the expression of another allele that recognizes *Emoy2* but not Emwa1 (e.g., the Nd-1 allele suggested above which may be associated with a DL phenotype and linked to *RPP1-1*). Interestingly, there appeared to be greater hindrance of the parasite associated with recognition of *Emwa1* than in the case of *Emoy2* (i.e., FR phenotype rather than FDL). For simplicity, however, the same locus *RPP4* is thought to be associated with recognition of both isolates. This locus appears to be located near *RPP2* on chromosome 4 (Table 5). Hence, the segregation data obtained following inoculation with *Emwa1* was used for fine-scale mapping of an allele from Col-5 at *RPP4* (Tör *et al.* 1994).

Two additional isolates, *Hiks1* and *Wela3*, were used to determine the segregation of alleles from Col-5. When the F_3 families of Col-5 × Nd-1 were inoculated with isolate *Hiks1*, segregation of an allele associated with the FN phenotype could be observed at a locus named *RPP7*. In common with *RPP4-1*, expression of the allele *RPP7-1* was obscured by necrotic pits associated with *RPP1-1* from Nd-1. No isolate has been found yet which is diagnostic solely for the genotype at *RPP7* (i.e., comparable to the genotype at *RPP4* using isolate *Emwa1*). When the F_3 families were inoculated with isolate *Wela3*, segregation of an allele associated with a FN phenotype could be observed at a locus named *RPP6*. Although Nd-1 exhibited a DM phenotype when inoculated with this isolate, any recognition allele inherited from this parent could be ignored by scoring the effect of *RPP6-1* when the Nd-1 control seedlings exhibited sporulation (7 dai). The chromosome locations of *RPP6* and *RPP7* have yet to be determined (Tör *et al.* 1994). However, estimates of recombination demonstrate that neither is located at other *RPP* loci that have been identified using the cross between Col-5 and Nd-1 (Table 5).

Summary of *A. thaliana* alleles at *RPP* loci and corresponding *ATR* alleles of *P. parasitica*.

Alleles at *RPP* loci that have thus far been identified are summarized in Table 1. Additional evidence was required in some cases. Isolate *Hiks1*, *Noks1*, and *Wela3* were used to determine whether alleles at *RPP5* from Ler-1, and *RPP6* and *RPP7* from Col-5 were carried by other accessions included in crosses of the half diallel. Only the FN phenotype was observed among F_2 progeny of the cross Oy-1 × Ler-1 following inoculation with *Noks1* (0:105:0:0), suggesting that Oy-1 may carry an allele at *RPP5*. Similarly, only the FN phenotype was observed among F_2 progeny of the cross Col-5 × Ler-1 following inoculation with *Hiks1* (0:101:0:0), suggesting that Ler-1 may carry an allele at *RPP7*. Oy-1 probably carries *RPP6* because only DL sporulation was observed among a large number of progeny from the cross Col-5 × Oy-1 following inoculation with *Wela3* (0:607:31:0). Progeny exhibiting the EH phenotype were observed among F_2 progeny of the crosses Col-5 × Ws-3 (62:29:2:4) and Col-5 × Ema-1 (78:26:4:5) following inoculation with *Hiks1*, suggesting that neither Ws-3 nor Ema-1 carry an allele at *RPP7*.

Similarly, progeny exhibiting the EH phenotype were observed among F_2 progeny of the crosses Col-5 × Ws-3 (0:93:5:8) and Col-5 × Ksk-1 (0:89:6:6) following inoculation with *Wela3*, suggesting that neither Ws-3 nor Ksk-1 carry an allele at *RPP6*. The cross Ema-1 × Ler-1 was inconclusive following inoculation with *Noks1* (0:115:3:0). In this case, all progeny in the D class exhibited delayed sporulation with fewer than three sporangiophores. More seedlings need to be tested.

DISCUSSION

Genetic analyses of *A. thaliana* in this study have shown that single *RPP* loci associated with phenotypically distinct, isolate-specific recognition of *P. parasitica* can be defined. The 12 *RPP* loci revealed by interactions between a relatively small number of host accessions and parasite isolates suggests that a substantial capability exists in *A. thaliana* for recognition of this symbiont. These observations parallel similar reports from the numerous crop species that have been studied over the decades. The primary challenge now is to determine the molecular basis for recognition. As a prelude to providing this understanding, a positional cloning approach is now in progress to isolate alleles at seven of the *RPP* loci (Mauch-Mani *et al.* 1993; Parker *et al.* 1993; Tör *et al.* 1994).

Investigations aimed at providing a molecular understanding of parasite recognition by plants usually build on the premise that genotype combinations are either compatible (a virulent parasite isolate in combination with a susceptible host) or incompatible (an avirulent parasite isolate in combination with a resistant host). A binary classification is useful because it provides the means for defining the specificity of interactions. For this purpose, the phenotypic variation presented in Table 1 could be converted into two classes: compatible interactions could include EH, EM, DH, and DM phenotypes, and incompatible interactions could include all remaining phenotypes.

We have chosen to look beyond a binary classification because different phenotypes indicative of variation for incompatibility (FDL, FR, FN, CN, and PN) should eventually reveal important functional aspects of the parasitic symbiosis. At present, it is unknown whether the observed phenotypes illustrated in Figure 1 represent functionally different classes or a continuum of interactions resulting from a common mechanism. For example, the observed phenotypes may indicate the effect of nonspecific host responses (e.g., callose deposition, lignification, phytoalexin accumulation, or production of other secondary metabolites). It may be premature to assume that the parasite necessarily plays a passive role. The parasite may have evolved its own capability for recognizing host cues which serves as a prerequisite for the undetected colonization of the host. Failure of the parasite variant to recognize the host and develop optimally may contribute to an incompatible interaction. It is likely that investigations of numerous combinations of host and parasite will be required to resolve the full breadth of processes that have evolved in host-parasite interactions. As a starting point, it is perhaps fortuitous that we have been able to identify at least four *RPP* loci, associated with a range of interaction phenotypes, using the cross between Col-5 and Nd-1. Use of a single cross for such investigations will greatly improve the efficiency of isolating genes from *A. thaliana* that are associated with a range of different interaction phenotypes.

Plant defense against parasites has commonly been discriminated into one of two types: resistance that delays the onset of an epidemic (e.g., by restricting early parasite establishment), or resistance that retards the rate of epidemic development (e.g., by reducing parasite reproduction). The former has often been considered as synonymous with isolate specific resistance, often correlated with hypersensitive cell death; while the latter has often been considered to indicate durable, isolate nonspecific resistance. An exception to this idea of plant defense may be represented by the FDL phenotype observed in this study. In this case, "rate-reducing" resistance appears to be associated with hypersensitive death of host cells. A similar case has been observed in lettuce downy mildew with the host locus Dm7 (Crute and Norwood 1978).

The interaction phenotype that results when two or more *RPP* alleles at different loci are present and functional will be critical to our understanding of the mechanistic basis for genotype-specific recognition (Crute 1985). For example, epistatic relationships between *RPP* alleles might be simply explained by the sequence or timing of events (i.e., the influence of the epistatically dominant allele precedes and thereby obscures the effect of the hypostatic allele). At least four examples of epistasis were observed where the phenotype attributed to one allele appeared to obscure the phenotype attributed to a second allele at a different locus. It was postulated that *RPP1-1* (PN) and *RPP4-1* (FDL) both segregated among F_2 progeny from the cross between Col-5 and Nd-1 following inoculation with isolate *Emoy2*. However, the predominant PN phenotype suggested that the presence of *RPP1-1* could be detected regardless of the status of locus *RPP4*. We postulated a similar epistatic relationship between *RPP1-1* (PN) and *RPP7-1* (FR). On the other hand, two other examples suggested that the PN phenotype does not always obscure an FN phenotype. The interaction phenotypes observed for recombinant inbreds of Ws-1 × W100F (Ler) suggested that the allele *RPP8-1* (FN) could obscure phenotypic expression of *RPP10-1* (PN) following inoculation with *Emoy2* (Table 3). Following inoculation of the same inbreds with *Hiks1*, a similar epistatic relationship was suggested between the alleles *RPP7-2* (FR) from W100F (Ler) and *RPP10-1* (PN). Interestingly, this latter example contrasts with the reciprocal relationship that was observed between *RPP7-1* (FN) from Col-5 and *RPP1-1* (PN) from Nd-1. Regardless of such complexity, the epistatic relationships that have been postulated illustrate the importance of investigating phenotypic interactions between *RPP* loci, and the molecular interaction between several *RPP* alleles associated separately with different interaction phenotypes.

Gene dosage has been identified as an important feature of host alleles associated with isolate specific recognition (Crute 1985; Crute and Norwood 1986; Illot *et al.* 1989). The effect of dosage of an allele at a single locus, *RPP5* or *RPP6*, was suggested by observations of an intermediate F_1 phenotype associated with light sporulation following inoculations with *Noco2* and *Wela1*, respectively (Mauch-Mani *et al.* 1993; Parker *et al.* 1993). Segregation ratios reported in this paper indicate that partial dominance of alleles is common at several other *RPP* loci. Complete dominance may be exceptional, and when it does occur, it appears to be conditioned in

part by the genetic background. In future investigation, it will be instructive to determine whether increasing dosage of partially dominant alleles at two or more loci results in gradations of disease resistance (i.e., additive gene action). The interaction phenotype between *Cala2* and Oy-0, carrying *RPP2-3* and *RPP3-1*, could be used to investigate potential additivity. If dosage of *RPP* alleles at different loci can influence an interaction phenotype, then allele duplication might create what would appear in coarse-scale genetic analyses to be complete dominance of an allele at a single locus. Fine-scale dissection of that locus would be required to reveal that additivity of alleles at linked loci, rather than complete dominance at a single locus, was responsible for the observed phenotype.

The genetic model presented (Table 1) to explain the observed phenotypic and genotypic variation contains a conservative appraisal of host and parasite recognition loci that could be invoked from the available data. For example, matching gene pairs controlling the interaction phenotypes of EM, DM, or DH have not been postulated, and the proposed alleles at all the *ATR* loci are theoretical. While several accessions, such as Col-5, Ler-1, and Oy-1, are thought to carry functional alleles at several *RPP* loci, the accession Nd-1 is postulated to carry only a single allele. This most likely reflects the small sample of parasite isolates used thus far. As investigations continue, the full extent of functional alleles at *RPP* loci carried by a single accession should become increasingly evident. Additionally, it will be informative to determine the relative frequencies of alleles at *RPP* loci among a wider sample of *A. thaliana* accessions, particularly within natural populations known to harbor *P. parasitica*. The *RPP1* locus provides a notable example because the accession Ema-1 carries an allele identified as *RPP1-2* associated with recognition of isolate *Emoy2*. In this case, both the accession and the isolate were collected from the same location.

Future validation and evolution of the genetic model will benefit greatly from the characterization of additional parasite variants. Such variants will enable us to reveal more *RPP* loci or to discriminate between the action of alleles at two already postulated loci. For example, an isolate diagnostic for the recognition of *RPP3-1* in Oy-1 (i.e., lacking a functional allele at *ATR2*) would more readily facilitate validation and mapping of locus *RPP3*. The FR phenotype that is likely to be associated with both *RPP2-3* and *RPP3-1* from Oy-1 impedes experimental discrimination of these alleles. The usefulness of diagnostic isolates is illustrated by *Emwa1* that enabled the mapping of *RPP4* without epistatic complications from the allele *RPP1-1*. Additional isolates will also be necessary to determine whether the *RPP* loci identified thus far comprise two or more closely linked loci, each carrying a functional recognition allele. This has been clearly demonstrated by studies with downy mildew (*Bremia lactucae*) of lettuce in which the identification of parasite variants capable of discriminating a single host recognition locus revealed closely linked loci in the host (Illot *et al.* 1989).

New parasite variants should be sought among isolates collected from naturally occurring populations and sexual recombinants derived from oospore populations produced in the laboratory. In the latter case, it should be possible to derive progeny from controlled matings between previously characterized isolates and thereby provide essential genetic definition of putative *ATR* loci identified in the genetic model (Table 1). Furthermore, the origin of phenotypic variation among incompatible interactions described here may be attributable to genotypic variation in the parasite. For example, parasite variation is the most likely explanation for the apparent difference in phenotype associated with *RPP4-1* from Col-5 following inoculations with *Emoy2* (FDL) and *Emwa1* (FR). In another example, different phenotypes were associated with *RPP10* (PN, CN, or FN) depending on which isolate was used to inoculate Ws-3. The source of this variation may reside in the genotype of the parasite.

Detailed genetic investigation of the *RP1* locus of maize, involved in genotype specific recognition of *Puccinia sorghi* Schwien., demonstrates the potential complex nature of recognition loci in plants and the difficulty of proving allelism at a single locus (Hulbert *et al.* 1993). Although it is premature to estimate the number and organization of *RPP* loci in the genome of *A. thaliana*, it is already apparent that particular regions of several chromosomes play a key role in the capability of this wild crucifer to recognize variants of *P. parasitica*. The recognition specificities *RPP1, RPP2, RPP4, RPP8,* and *RPP9* define different loci on every chromosome except for chromosome 2. Due to the lack of evidence for recombination, *RPP5* and *RPP12* may be allelic with *RPP4*; similarly, *RPP10* may be allelic with *RPP1*. Nevertheless, these loci reinforce the importance of regions on chromosome 3 and 4 in the genotype specific recognition of *P. parasitica* by *A. thaliana.*

These observations of phenotypic variation and possible clustering of *RPP* loci will influence future research in at least two ways. If the complex nature of a locus such as *RPP1* or *RPP10* is to be investigated using mutation analysis, then attempts should be made to select classes of mutants that exhibit changes in isolate-specific recognition, and changes in interaction phenotypes. It may be possible, for example, to select mutants of the latter type that exhibit changes from a wild-type PN phenotype to a mutant FN or FDL phenotype; and conversely, changes from a wildtype FN phenotype to a mutant CN or PN phenotype. Secondly, further mapping of *RPP* loci can proceed by determining whether a new locus maps near or within the intervals for *RPP* loci already described.

Plant breeders have traditionally sought to use disease resistance genes in crop species that exert marked effects. Some such genes will have been derived from the ancient gene pool of cultivated varieties while others have been recently introgressed from wild sources during the era of plant breeding. One important effect of the deliberate efforts to improve crops is that the genes associated with the specific recognition of parasites probably represent a sub-set of those present in a naturally evolved pathosystem. If the range of interaction phenotypes conditioned by alleles at *RPP* loci are typical of a natural association between a host species and a parasitic symbiont, then the study of genotype specific recognition between *A. thaliana* and *P. parasitica*, without artefacts created by intentional domestication, is likely to prove illuminating.

MATERIALS AND METHODS

P. parasitica isolates.

A description of methods used to collect and store isolates of *P. parasitica* has been published elsewhere (Dangl *et al.*

1992a). Most isolates of *P. parasitica* were obtained from natural populations of *A. thaliana* found in the United Kingdom (Table 6). Some isolates were maintained as cultures of mass conidiosporangia having been obtained originally from inoculum that was washed from a single rosette leaf of a field collected plant. However, the principal isolates used for experimentation were obtained from oospore populations contained in dried and ground samples of infested leaf tissue. They are assumed in each case to be derived from infection of an individual seedling by a single oospore. Both natural and laboratory produced oospore populations were used as a source for the principal isolates *A. thaliana* germplasm.

The *A. thaliana* accessions used in this study were obtained from numerous sources: Ler-1, Col-5 (*gl1*), and Rld-2 were purchased from Lehle Seed (Arizona); Oy-0 (Oystese, Norway), Nd-0 (Niederzenz, Germany), Tsu-0 (Tsu, Japan), and Per-0 (Perm, Russia) were obtained from the Kranz collection via J. Dangl (Cologne, Germany); Ws-0 (Wassilewskija, Byelorussia) was obtained from the Nottingham *Arabidopsis* Stock Centre (UK); Wei-0 (Weiningen, Switzerland) was obtained from A. Slusarenko (Zürich, Switzerland); and Ema-0 (East Malling), Cnt-0 (Canterbury) and Ksk-0 (Keswick) were collected from natural populations in the United Kingdom.

A single plant was selected randomly from each accession and was subsequently used for pollinations in production of crosses for the half diallel analysis (Table 2). To ensure accurate reference in the future to the same genotypes, all of the plants selected for genetic analysis were given a new number designation. This was required in cases where the homogeneity of the original accessions was uncertain (e.g., Tsu-1 was selected from Tsu-0). A complete set of the genotypes used in our analyses has been deposited in the Nottingham and Ohio *Arabidopsis* stock centers for distribution. The third generation of self-pollination (S_3) was provided for each genotype. Nonstandardized names referred to previously in publications or prior distribution of seed are as follows: Ksk-1 = Kes37, Cnt-1 = Ca1176, and Ema-1 = EM197.

Recombinant inbreds were obtained from the following sources: F_8 Ws-1 × W100F (Ler) (produced by P. Scolnik *et al.*, Du Pont, USA) from the Nottingham *Arabidopsis* Resource Centre (UK), F_8 Nd-0 × Oy-0 from J. Dangl (Cologne, Germany), and F_8 Ler-0 × Col-4 from C. Dean (Norwich, UK). We produced our own recombinant inbreds (F_6) of Wei-1 × Ksk-1.

Growing plants.

Seed was sown on the surface of a soil mix consisting of six parts commercial peat-based seedling compost containing macronutrients (Levington F2 mix), one part vermiculite, and one part fine sand. The ingredients were mixed and sieved (5 mm mesh). After sowing, the soil was moistened from below by placing the pots in a pan of water. Pots were drained, placed in a tray, and covered with a clear polystyrene cloche. Pots remained covered for the duration of an experiment to maintain high humidity necessary for uniform seedling emergence and parasite infection and reproduction. The cloche also reduced the risk of cross-contamination between isolates of *P. parasitica*. Pots were stored for 5–14 days in the dark at 8° C before placing in the growth room. This served to vernalize sown seed, but also provided a convenient stock of plant material. Pots were transferred to walk-in growth rooms for germination at 18–20° C, under a 10-hr photoperiod at a photon flux density of 150–200 μE m^{-2} s^{-1}.

Plants used for inoculum production were grown in 7.5-cm pots at a density of 100–200 plants per pot. For experimentation, plants were grown in packs of 20 2- × 2- × 3-cm pots. A row of four pots was reserved for sowing control accessions of *A. thaliana* including one pot each of Oy-1, Nd-1, Col-5, Ler-1. Test material was sown in the remaining 16 pots, arranged in a row of four pots for each of four seed lines. Eight to 10 seeds were sown per pot. Plants were inoculated 7 days after the pots were transferred to the growth chamber.

Inoculation.

Once a culture was actively growing, it was maintained continuously on seedlings to provide reliable inoculum for experimentation. Inoculum was prepared as previously described (Dangl *et al.* 1992a) and adjusted to a concentration of 5×10^4 conidiosporangia per milliliter. Seven-day-old seedlings were inoculated by placing 1–2 μl of inoculum on each cotyledon, thereby delivering approximately 100–200 conidiosporangia per plant. The plants were placed in a clear plastic box to retain sufficient soil moisture and relative humidity for the duration of the experiment. There was no need to spray plants daily or add any additional water as recommended previously (Dangl *et al.* 1992a). Growth chamber conditions were the same as described above except the photon flux density was 75–100 μE m^{-2} s^{-1}. Relative humidity in the chamber fluctuated from 90–100%. *P. parasitica* grown on a fully susceptible accession typically began to sporulate three days after inoculation.

Characterization of interaction phenotypes.

Most characteristics of phenotypes could be seen with the naked eye, thereby making it practical to examine rapidly large numbers of inoculated seedlings. However, a dissecting microscope at low magnification (20×) was used for confirming phenotype identity and for counting sporangiophores. Microscopic observation at higher magnification was conducted to examine cotyledons for the extent of colonization by *P. parasitica* and the presence of oospores (sexual reproduction). Whole seedlings were placed in 70% ethanol for 24 hr to remove pigmentation and were subsequently transferred to lactophenol to soften and for storage. Cleared tissue was mounted in lactophenol and examined using phase- contrast and Normarski interference optics.

Pollination.

Cross pollination of *A. thaliana* accessions was performed on closed buds of flowers from the female parent plant. These were emasculated with the aid of forceps, and a dissecting microscope was used to confirm that pollen had not yet been released from the removed anthers. From the male parent plant, a dehiscing anther was removed with forceps and pollen was transferred to the stigma of the female parent. The same individual plant of each accession was used to make each cross included in the half-diallel.

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LITERATURE CITED

Burdon, J. J., and Jarosz, A. M. 1991. Host-pathogen interactions in natural populations of *Linum marginale* and *Melampsora lini* I. Patterns of resistance and racial variation in a large host population. Evolution 45:205-217.

Burr, B., and Burr, F. A. 1991. Recombinant inbreds for molecular mapping in maize: theoretical and practical considerations. Trends Genet. 7:55-60.

Callow, J. A. 1984. Cellular and molecular recognition between higher plants and fungi. Pages 212-237 in: Encyclopedia of Plant Physiology: Cellular Interactions. H. F. Linskens and J. Heslop-Harrison, eds. Springer-Verlag, Berlin, New York.

Crute, I. R. 1990. Resistance to *Bremia lactucae* (downy mildew) in British populations of *Lactuca serriola* (prickley lettuce). Pages 203-217 in: Pests Pathogens and Plant communities. J. J. Burdon and Leather, eds. Blackwell Scientific Publications, Oxford.

Crute, I. R. 1992. From breeding to cloning (and back again?): A case study with lettuce downy mildew. Annu. Rev. Phytopathol. 30:485-506.

Crute, I. R. 1985. The genetic basis of relationships between microbial parasites and their hosts. Pages 80-142 in: Mechanisms of Resistance to Plant Diseases. R. S. S. Fraser, ed. Martinus Nijhoff/Dr. W. Junk Publishers, Dordrecht.

Crute, I. R., Holub, E. B., Tör, M., Brose, E., and Beynon, J. L. 1993. The identification and mapping of loci in *Arabidopsis thaliana* for recognition of the fungal pathogens: *Peronospora parasitica* (downy mildew) and *Albugo candida* (white blister). Pages 437-444 in: Advances in Molecular Genetics of Plant-Microbe Interactions. E. M. Nester and D. P. S. Verma, eds. Kluwer Academic Publishers, Dordrecht.

Crute, I. R., and Norwood, J. M. 1978. Incomplete specific resistance to *Bremia lactucae* in lettuce. Ann. Appl. Biol. 89:467-474.

Crute, I. R., and Norwood, J. M. 1986. Gene-dosage effects on the relationship between *Bremia lactucae* (downy mildew) and *Lactuca sativa* (lettuce): the relevance to a mechanistic understanding of host-parasite specificity. Physiol. Mol. Plant Pathol. 29:133-145.

Dangl, J. L. 1993. The emergence of *Arabidopsis thaliana* as a model for plant-pathogen interactions. Pages 127-156 in: Advances in Plant Pathology. Vol. 10. J. H. Andrews ed. Academic Press,

Dangl, J. L., Holub, E. B., Debener, T., Lehnackers, H., Ritter, C., and Crute, I. R. 1992a. Genetic definition of loci involved in *Arabidopsis*-pathogen interactions. Pages 393-418 in: Methods in *Arabidopsis* Research. C. Koncz, N. Chua, and J. Schell, eds. World Scientific Publishing Co., Singapore.

Dangl, J. L., Ritter, C., Gibbon, M. J., Mur, L. A., Wood, J. R., Goss, S., Mansfield, J., Taylor, J. D., and Vivian, A. 1992b. Functional homologs of the *Arabidopsis Rpm1* disease resistance gene in bean and pea. Plant Cell 4: 1359-1369.

Davis, K., and Hammerschmidt, R., eds. 1993. *Arabidopsis thaliana* as a Model for Plant-Pathogenic Interactions. American Phytopathological Society Press, St. Paul, MN.

Davis, K. D., Schott, E., and Ausubel, F. M. 1991. Virulence of selected phytopathogenic pseudomonads in *Arabidopsis thaliana*. Mol. Plant-Microbe Interactions 4:477-488.

Debener, T., Lehnackers, H., Arnold, M., and Dangl, J. 1991. Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. Plant J. 1:289-302.

Dickman, M. B., and Mitra, A. 1992. *Arabidopsis thaliana* as a model for studying *Sclerotinia sclerotiorum* pathogenesis. Physiol. Mol. Plant Pathol. 41:255-263.

Dong, X., Mindrinos, M., Davis, K. R., and Ausubel, F. M. 1991. Induction of *Arabidopsis* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. Plant Cell 3:61-72.

Ellingboe, A. H. 1981. Changing concepts in host-pathogen genetics. Annu. Rev. Phytopathol. 19:125-143.

Ellingboe, A. H. 1982. Genetical aspects of active defense. Page 179-182 in: Active Defense Mechanisms in Plants. R. K. S. Wood, ed. Plenum Press, New York.

Flor, H. H. 1955. Host-parasite interaction in flax rust-its genetics and other implications. Phytopathology 45:680-685.

Flor, H. H. 1971. Current status of gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.

Gustavsson, A. 1959. Studies on Nordic Peronosporas, I. Taxonomic revision, Species on Cruciferae. Opera Botanica 3:79-116.

Holub, E., Crute, I., Brose, E., and Beynon, J. 1993. Identification and mapping of loci in *Arabidopsis* for resistance to downy mildew and white blister. Pages 21-35 in: *Arabidopsis* as a Model for Plant-Pathogenic Interactions. K. Davis and R. Hammerschmidt, eds. American Phytopathological Society, St. Paul, MN.

Holub, E. B., Williams, P. H., and Crute, I. R. 1991. Natural infection of *Arabidopsis thaliana* by *Albugo candida* and *Peronospora parasitica*. Phytopathology 81:1226.

Hulbert, S. H., Sudupak, M. A., and Hong, K. S. 1993. Genetic relationships between alleles of the *Rp1* rust resistance locus of maize. Mol. Plant Microbe Interact. 6:387-392.

Ilott, T. W., Hulbert, S. H., and Michelmore, R. W. 1989. Genetic analysis of the gene-for-gene interaction between lettuce (*Lactuca sativa*) and *Bremia lactucae*. Phytopathology 79:888-897.

Keen, N. T. 1982. Mechanisms conferring specific recognition in gene-for-gene plant parasite systems. Page 67-84 in: Active defence mechanisms in plants. R. K. S. Wood, ed. Plenum Press, New York.

Koch, E., Cox, R., and Williams, P. H. 1991. Infection of *Arabidopsis thaliana* by *Plasmodiophora brassicae*. J. Phytopathol. 132: 99-104.

Koch, E., and Slusarenko, A. 1990a. *Arabidopsis* is susceptible to infection by a downy mildew. Plant Cell 2:437-445.

Koch, E., and Slusarenko, A. J. 1990b. Fungal pathogens of *Arabidopsis thaliana* (L.) Heyhn. Bot. Helv. 100:257-267.

Koncz, C., Chua, N., and Schell, J., eds. 1992. Methods in *Arabidopsis* Research. World Scientific Publishing Co., Singapore.

Koornneef, M., van Eden, J., Hanhart, C. J., Stam, P., Braaksma, F. J., and Feenstra, W. J. 1983. Linkage map of *Arabidopsis thaliana*. J. Hered. 74: 265-272.

Kunkel, B. N., Bent, A. F., Dahlbeck, D., Innes, R. W., and Staskawicz, B. J. 1993. RPS2, an *Arabidopsis* disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. Plant Cell 5:865-875.

Meyerowitz, E. M. 1987. *Arabidopsis thaliana*. Annu. Rev. Genet. 21:93-111.

Norwood, J. M., Michelmore, R. W., Crute, I. R., and Ingram, D. S. 1983. The inheritance of specific virulence in *Bremia lactucae* (downy mildew) to match resistance factors 1, 2, 4, 6 and 11 in *Lactuca sativa* (lettuce). Plant Pathol. 32:177-186.

Parker, J. E., Szabo, V., Staskawicz, B. J., Lister, C., Dean, C., Daniels, M. J., and Jones, J. D. G. Phenotypic characterization and molecular mapping of the *Arabidopsis thaliana* locus, RPP5, determining disease resistance to *Peronospora parasitica*. Plant J. 4:821-831.

Redei, G. P. 1975. *Arabidopsis* as a genetic tool. Annu. Rev. Genet. 9:111-127.

Reiter, R. S., Williams, J. G. K., Feldman, K. A., Rafalski, J. A., Tingey, S. V., and Scolnik, P. A. 1992. Global and local genome mapping in *Arabidopsis thaliana* by using recombinant inbred lines and random amplified polymorphic DNAs. Proc. Natl. Acad. Sci. USA 89:1477-1481.

Rostrup, E. 1913. Danish Fungi. Copenhagen, Gyldendalske Boghandel.

Mauch-Mani, B., Croft, K. P. C. and Slusarenko, A. 1993. The genetic basis of resistance of *Arabidopsis thaliana* L. Heynh. to *Peronospora parasitica*. Pages 5-20 in: *Arabidopsis* as a Model for Plant-Pathogenic Interactions. K. Davis and R. Hammerschmidt, eds. American

Phytopathological Society, St. Paul, MN.
Tör, M., Holub, E. B., Brose, E., Musker, R., Gunn, N., Can, C., Crute, I. R., and Beynon, J. L. 1994. Map positions of four loci in *Arabidopsis thaliana* associated with isolate specific recognition of *Peronospora parasitica* (downy mildew). Mol. Plant-Microbe Interactions 7:214-222.
Tsuji, J., Somerville, S. C., and Hammerschmidt, R. 1991. Identification of a gene in *Arabidopsis thaliana* that controls resistance to *Xanthomonas campestris* pv. *campestris*. Physiol. Mol. Plant Pathol. 38:57-65.
Van den Ackerveken, G. F. J. M., Van Kan, J. A. L., and De Wit, P. J. G. M. 1992. Molecular analysis of the avirulence gene *avr9* of the fungal tomato pathogen *Cladosporium fulvum* fully supports the gene-for-gene hypothesis. Plant J. 2:359-366.
Whalen, M. C., Innes, R., F., B. A., and Staskawicz, B. J. 1991. Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. Plant Cell 3:49-59.
Yu, Guo-Liang, Katagiri, F., and Ausubel, F. M. 1993. *Arabidopsis* mutations at the RPS2 locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. Mol. Plant-Microbe Interact. 6:434-443.