Basic Compatibility of *Albugo candida* in *Arabidopsis thaliana* and *Brassica juncea* Causes Broad-Spectrum Suppression of Innate Immunity

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A biotrophic parasite often depends on an intrinsic ability to suppress host defenses in a manner that will enable it to infect and successfully colonize a susceptible host. If the suppressed defenses otherwise would have been effective against alternative pathogens, it follows that primary infection by the “suppressive” biotroph potentially could enhance susceptibility of the host to secondary infection by avirulent pathogens. This phenomenon previously has been attributed to true fungi such as rust (basidiomycete) and powdery mildew (ascomycete) pathogens. In our study, we observed broad-spectrum suppression of host defense by the oomycete *Albugo candida* (white blister rust) in the wild crucifer *Arabidopsis thaliana* and a domesticated relative, *Brassica juncea*. *A. candida* subsp. *arabidopsis* suppressed the “run-away cell death” phenotype of the lesion mimic mutant *lsd1* in *Arabidopsis thaliana* in a sustained manner even after subsequent inoculation with avirulent *Hyaloperonospora arabidopsis* (*Arabidopsis thaliana* downy mildew). In sequential inoculation experiments, we show that preinfection by virulent *Albugo candida* can suppress disease resistance in cotyledons to several downy mildew pathogens, including contrasting examples of genotype resistance to *H. arabidopsis* in *Arabidopsis thaliana* that differ in the R protein and modes of defense signaling used to confer the resistance; genotype specific resistance in *B. juncea* to *H. parasitica* (Brassica downy mildew; isolates derived from *B. juncea*); species level (nonhost) resistance in both crucifers to *Bremia lactucae* (lettuce downy mildew) and an isolate of the *H. parasitica* race derived from *Brassica oleracea*; and nonhost resistance in *B. juncea* to *H. arabidopsis*. Broad-spectrum powdery mildew resistance conferred by *RPW8* also was suppressed in *Arabidopsis thaliana* to two morphotypes of *Erysiphe* spp. following pre-infection with *A. candida* subsp. *arabidopsis*.

Additional keywords: oilseed mustard

Basic compatibility (Ellingboe 1976; Holub 2006) has been a term used to describe the intimate coevolved association that can exist between taxa (species or subspecies) of a plant and its adapted pathogens. To be adapted, the pathogen must intrinsically be able to evade or render ineffective the constitutive barriers and the inducible defenses of its compatible host. This pathogen also must possess the particular developmental and

metabolic characteristics that are necessary for it to be able to harness the host’s metabolism. Basic compatibility, however, can be observed only in the absence of natural intraspecific variation in disease resistance of the host, commonly mediated by pathogen receptor-like proteins, encoded by so-called resistance (R) genes, that are capable of detecting elicitor molecules produced by an otherwise virulent pathogen (deWit 2007; Holub 2001, 2007; Staskawicz et al. 1995).

Some plant pathogens have evolved the ability to enhance the susceptibility of their host toward normally avirulent variants of the same or different pathogen species. In some cases, suppression of “nonhost” resistance to biotrophic pathogens from other host species has been demonstrated. Yarwood (1951) first described this phenomenon more than 50 years ago and, along with subsequent reports, it appears to be a common attribute of true fungi such as basidiomycete rust and ascomycete powdery mildew pathogens (Gill 1965; Heath, 1980; Lyngkjaer and Carver 2000; Moseman and Greeley 1964; Moseman et al. 1965; Ouchi and Oku 1981; Yarwood 1977). Interestingly, these potent defense-suppressive fungi have contrasting life cycles: growth and reproduction of powdery mildews is confined primarily to epidermal cells, whereas rust fungi colonize the mesophyll, often following penetration via stomata, and later reemerge by rupturing the epidermis with the formation of spore-bearing pustules.

The filamentous oomycete *Albugo candida* is descended from an aquatic nonfungal ancestor and belongs to the kingdom Stramenopiles (along with brown algae and diatoms) (Sogin and Silberman 1998). Despite the separate phylogeny of oomycetes and true fungi (kingdom Eumycota), *A. candida* causes disease in a manner similar to rust fungi, including penetration by asexual inoculum via stomata, mesophyll colonization of a compatible host tissue, and reproduction via spore-bearing pustules. Hence, the disease caused by *A. candida* has been referred to as white blister rust (Holub et al. 1995). As with basidiomycete rusts, compatible host tissue colonized by *A. candida* often is associated with chlorophyll retention (green islands) and growth malformation that is indicative of hormonal disturbance (Crute et al. 1994).

In our study, we were interested in determining whether basic compatibility of *A. candida* with its natural hosts can, as a consequence, suppress defense against secondary infection by other pathogens, similar to the previous reports for basidiomycete rusts. *A. candida* has the capacity under field conditions to elevate the incidence and severity of infection by *Hyaloperonospora parasitica* (Brassica downy mildew; formerly *Peronospora parasitica*) (Constantinescu and Fatehi 2002) in cru-

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cifer hosts. Both organisms frequently grow intimately together in leaf or stem tissue of the same crucifer host (Bains and Jhoo 1985; Chaurasia et al. 1982; Sansome and Sansome 1974), and dual infections by both pathogens cause significant yield losses in oilseed mustard crops of *Brassica juncea* (Bains and Jhoo 1979). Sequential inoculations in laboratory experiments have suggested that preinfection with virulent *A. candida* can suppress downy mildew resistance in brassicas to avirulent isolates of either *H. parasitica* or *A. candida* (Rimmer et al. 2000; Singh et al. 2002). A similar observation was reported for suppression of resistance to *H. arabidopsis* (*Arabidopsis thaliana* downy mildew; renamed as a distinct species by Goker and associates [2004]; supported by Rehmany and associates [2000]) in *A. thaliana* following preinfection with *Albugo candida* (Holub et al. 1993) (Fig. 1A).

White rust in natural populations of *Arabidopsis thaliana* is caused by a distinct subspecies of *Albugo candida* subsp. *arabidopsis* (Borhan et al. 2008) and offers an attractive model for investigating the molecular basis of broad-spectrum defense suppression by a biotrophic oomycete. From previous molecular genetic analyses of downy mildew resistance, there are numerous examples of receptor-like genes in *Arabidopsis thaliana* that vary in different modes of defense regulation (Eulgem et al. 2004; Holub 2001; McDowell et al. 2000; Tör et al. 2002) and, thus, provide contrasting examples for our sequential inoculation experiments involving the same preinfection treatment with *Albugo candida* subsp. *arabidopsis*. In addition, mutation of a zinc-finger protein called lesion-simulating disease (LSD)1 provides a useful tool for determining the potency of cell death suppression by *A. candida* subsp. *arabidopsis*. This mutant exhibits a highly sensitive “runaway cell death phenotype” when stimulated by conditions such as shifting mutant plants from a short- to long-day environment, or inoculating leaves with virulent or avirulent pathogens (Dietrich et al. 1997). Once initiated, the cell death spreads rapidly and usually results in premature death of the plant. However, virulent *A. candida* can colonize and complete its life cycle unrestricted, without inducing cell death in cotyledons or leaves of *Arabidopsis thaliana lsd1* (Holub and Beynon 1997).

In this study, we used *lsd1* to determine how this mutant responds to secondary infection by an avirulent *H. arabidopsis* isolate following cell death suppression by *Albugo candida* subsp. *arabidopsis*. We quantified defense suppression by *A. candida* in sequential inoculation experiments using contrasting examples of both race and nonhost (species-level) resistance to downy mildew pathogens in *Arabidopsis thaliana*, and conducted similar experiments in a domestic relative, *B. juncea*, using a virulent isolate of *Albugo candida* race 2. In addition, we examined the impact of *A. candida* subsp. *arabidopsis* pre-

infection in *Arabidopsis thaliana* on broad-spectrum powdery mildew resistance (conferred by *RPW8*) (Xiao et al. 2001) and on temperature-sensitive resistance (accession Wassilewskaja [Ws-3]) to the hemibiotrophic fungus *Colletotrichum higginsianum* (O’Connell et al. 2004).

RESULTS

Albugo candida subsp. *arabidopsis* suppresses cell death in *lsd1* mutant *Arabidopsis thaliana* but not when elicited by a hemibiotroph.

Three avirulent isolates of *H. arabidopsis* (Cala2, Cand5, and Hiks1; all derived from U.K. *Arabidopsis thaliana*) and one *H. parasitica* isolate (P005; representing a race derived from *B. oleracea*) all induced rapid cell death following inoculation in cotyledons of *Arabidopsis thaliana* Ws-*lsd1*. Infected seedlings shriveled and collapsed within 48 h after inoculation (Fig. 2A, left cotyledon). In wild-type *Arabidopsis thaliana* Ws-3, *RPP1* confers resistance to all three of the *H. arabidopsis* isolates, whereas resistance to *H. parasitica* P-005 has not been genetically characterized. Similarly, *Arabidopsis thaliana* Col-*lsd1* seedlings shriveled and collapsed within 48 h of inoculation with avirulent *H. arabidopsis* isolates, including Cala2 (recognized in the Col-0 background by *RPP2*), Emwa1 (recognized by *RPP4*), Hiks1 (recognized by *RPP7*), and Cand5 (an example of polygenic resistance). An avirulent isolate of *Albugo candida* (A001; representing a race derived from *B. oleracea*) also elicited cell death in *Arabidopsis thaliana* Ws-*lsd1* that rapidly killed seedlings within 48 h of inoculation.

In contrast, the virulent isolate of *Albugo candida* subsp. *arabidopsis* (Acem1) colonized Ws-*lsd1* and Col-*lsd1* seedlings without eliciting cell death and, subsequently, sporulated in both genetic backgrounds (Fig. 2A, right cotyledon). Mutant *lsd1* plants in both genetic backgrounds readily became systemically infected with Acem1; examples were observed that survived several months without the initiation of cell death or flowering and eventually died after gradual senescence at the 8- to 10-leaf stage with rust pustules erupting on most tissues (Fig. 2B, red-bordered inset). A sequential inoculation experiment was performed in which Col-*lsd1* was inoculated with *A. candida* subsp. *arabidopsis* Acem1 and, 24 h later, with *H. arabidopsis* Hiks1. Low levels of Hiks1 sporulation were observed on cotyledons that also supported Acem1 blisters, without the induction of cell death (Fig. 2C). Interestingly, the Ws-3 virulent isolate of *H. arabidopsis* Emwa1 was effective in eliciting cell death in cotyledons of Ws-*lsd1*, but only 5 days after inoculation (dai), following extensive colonization and initiation of asexual sporulation (Fig. 2A, middle cotyledon).

Fig. 1. Preinfection (48 h) of wild-type *Arabidopsis thaliana* or *Brassica juncea* with a virulent isolate of *Albugo candida* (white blister rust) suppresses host defenses and, following secondary inoculation, enables avirulent mildew pathogens to colonize and reproduce. **A**, Sexual reproduction of avirulent *Hyaloperonospora arabidopsis* (*Arabidopsis* downy mildew; isolate Cala1) was observed in a juvenile leaf of *Arabidopsis thaliana* Columbia (Col-0), visible as hyaline, smooth, thick-walled oospores (left photograph, labeled Ho; revised from figure published in Holub et al. 1993) compared with the naturally golden-pigmented, rough-walled oospores of *Albugo candida* subsp. *arabidopsis* (labeled Ao). White rust pustules (labeled Ap) are also visible. Red dots indicate the relative location of *H. arabidopsis* oospores (leaf map on right). **B**, Cotyledons of *Arabidopsis thaliana* Col-5 inoculated solely with avirulent *H. arabidopsis* Cand5 and showing no symptoms (left) or sequentially inoculated with *Albugo candida* subsp. *arabidopsis* isolate Acem1 and Cand5 showing downy mildew sporangiothecia (center, upper surface) and white blisters (right, lower surface). **C**, Cotyledons of *Arabidopsis thaliana* Ms-0 inoculated with a powdery mildew fungus (morphotype similar to *Erysiphe cruciferarum*) showing no fungal sporulation (left) or sequentially inoculated with *Albugo candida* subsp. *arabidopsis* Acem1 and the same fungal isolate showing growth of powdery mildew (center, upper surface) white rust blisters (right, lower surface). **D**, Pitting and necrotic flecks on cotyledons of *Brassica juncea* line 560-1 inoculated with an avirulent isolate of *H. parasitica* (IP10, race derived from *Eruca sativa*) (left) and sporulation of IP10 on the upper surface (center) of a cotyledon that was pre-infected with a virulent *A. candida* race 2 isolate (blisters visible on lower surface, right). **E**, Juxtaposition of hyphae of *A. candida* (Ac) and *H. parasitica* (Hp) in the mesophyll of a sequentially inoculated cotyledon of *B. juncea* (line 560). Arrowheads indicate a characteristic large, lobed haustorium of Hp compared with the much smaller, stalked haustorium of *A. candida*. **F**, Scanning electron photomicrograph of an *Arabidopsis thaliana* Ws-*eds1* true leaf preinfected with *Albugo candida* subsp. *arabidopsis* Acem1 and, 48 h later, with *Bremia lactucae* B1096, showing a trichome characteristic of *Arabidopsis thaliana* and a single *B. lactucae* sporangiothecium with characteristic sterigmata (inset diagram, far right, from Webster 1980).

The fungus *C. higginsianum* IMI349061 was used to determine whether virulent *A. candida* Acem1 also could suppress cell death conferred by a hemibiotroph. We used Ws-3, which reportedly exhibits resistance to *C. higginsianum* at cool tem-

peratures (O'Connell et al. 2004). At 20°C, symptoms were clearly evident in Ws-3 cotyledons within 5 dai and moderate disease severity was observed 7 to 11 dai, indicating that the resistance was only partially effective under our experimental

conditions (Fig. 2D, upper right; Table 1). Preinfection (48 h) with *Acem1* had no significant effect on disease severity, neither enhancing nor reducing the partial resistance. A similar comparison was observed between both pathogen treatments in the *Ws-eds1*; however, this mutant appeared to be significantly more susceptible than *Ws-3* ($P < 0.001$) to the necrotrophic stage of *C. higginsianum* (Fig. 2D, lower photos; Table 1). Interestingly, this indicated that the temperature-sensitive resistance to *C. higginsianum* in *Ws-3* requires functional expression of the lipase-like protein *EDS1*.

Suppression of race-specific resistance in wild-type *Arabidopsis thaliana*.

Sequential inoculation experiments were undertaken to determine the capacity of *Albugo candida* subsp. *arabidopsis* isolate *Acem1* to suppress downy mildew resistance in *Arabidopsis thaliana* to different avirulent isolates of *H. arabidopsis*. *Arabidopsis thaliana* seedlings were preinoculated with *Acem1* 48 h before secondary inoculation with an avirulent *H. arabidopsis* isolate. In *Col-0* seedlings, *Acem1* significantly suppressed resistance to *H. arabidopsis* Hiks1, with an observed mean of 6.7 sporangioophores per cotyledon (Table 2). *Acem1* also significantly enhanced sporulation in *Col-0* by *Cand5* and *Cala2*, with each of these *H. arabidopsis* isolates producing more than eight sporangioophores per cotyledon (Table 2; Fig. 1B). In other genetic backgrounds, preinfection with *Acem1* enhanced sporulation to Hiks1 in *Ler-0* and to *Cala2* in *Ws-3* (Table 2).

Suppression of nonhost (species-level) resistance in *Arabidopsis thaliana*.

Isolates of *H. parasitica* derived from brassica hosts are broadly avirulent (species level) in wild-type *Arabidopsis thaliana*. An isolate of *H. parasitica* (P005, representing a race derived from *B. oleracea*) was used in a sequential inoculation experiment following inoculation with *Albugo candida* subsp. *arabidopsis* *Acem1* in both wild-type and mutant *Arabidopsis thaliana*. The highest level of sporulation was observed on sequentially inoculated cotyledons of the mutant *Ws-eds1* (Table 2). As expected from the previous report by Parker and associates (1996), this *Arabidopsis thaliana* mutant exhibited partial susceptibility to P005 in the absence of *Acem1*, producing a mean of 1.2 sporangioophores per cotyledon. However, preinfection (48 h) with *Acem1* enhanced sporulation by P005 in *Ws-eds1* approximately sevenfold. In wild-type *Arabidopsis thaliana*, *Acem1* also significantly ($P < 0.01$) suppressed resistance to *H. parasitica* P005. No sporulation of downy mildew was observed in the absence of *Acem1*, but a mean of 1.5 sporangioophores per cotyledon was observed in sequentially inoculated seedlings.

Albugo candida subsp. *arabidopsis* *Acem1* also suppressed resistance of *Arabidopsis thaliana* to the lettuce downy mildew pathogen *Bremia lactucae* (Table 2; Fig. 1F). In the absence of *Acem1*, *Bremia lactucae* did not sporulate in either wild-type *Arabidopsis thaliana* or mutant *Ws-eds1* seedlings, whereas *Acem1* enabled the sporulation of *Bremia lactucae* in wild-type (significant at $P < 1\%$) *Arabidopsis thaliana* and mutant *Ws-eds1* (significant at $P < 0.05\%$).

Suppression of downy mildew resistance in wild-type *Brassica juncea*.

Sequential inoculation experiments were performed on *Brassica juncea* line 560-1, using a virulent *Albugo candida* race 2 isolate (Bihar-SP, derived from *B. juncea*) for preinfection of cotyledons. Interestingly, sequential inoculations on *B. juncea* with virulent *H. parasitica* isolate IP05 revealed that the presence of *A. candida* Bihar-SP significantly ($P < 0.001$) increased the level of downy mildew sporulation in this com-

patible host-isolate interaction. Similarly, *A. candida* Bihar-SP suppressed genotype-specific resistance to an avirulent isolate of *H. parasitica* (IP00A, derived from *B. juncea*), enabling it to sporulate heavily on cotyledons (Table 3). In the absence of *A. candida* Bihar-SP, *H. parasitica* isolate IP00A produced extensive necrotic flecks and did not sporulate. A single dominant gene confers resistance to this downy mildew isolate in *B. juncea* line 560-1 (Nashaat et al. 1998).

In addition, experiments on *B. juncea* line 560-1 revealed that preinfection with *A. candida* isolate Bihar-SP suppressed resistance toward downy mildew pathogens collected from lettuce (*Lactuca sativa*) and other crucifer species (Table 3): *B. rapa*, *B. napus*, *B. oleracea*, *Eruca sativa*, *Arabidopsis thaliana*, and *Cherianthus cherii*, as well as from lettuce (*L. sativa*). Bihar-SP significantly enhanced ($P < 0.01$) the number of sporangioophores of *H. parasitica* IP00A (origin *B. juncea*), PP6 (origin *B. oleracea*), P003 (origin *B. napus*), IP09 (origin *B. rapa*), and IP10 (origin *E. sativa*) on sequentially inoculated *B. juncea*; heavy sporulation of the downy mildew pathogen was seen in each interaction. The effect of Bihar-SP on the number of sporangioophores produced by *H. parasitica* isolate WF1 (from *C. cherii*), was highly significant ($P < 0.004$). Again, heavy sporulation was seen on the upper surface of sequentially inoculated cotyledons. Examples of interaction phenotypes observed on seedlings of *B. juncea* are shown in Figure 1D. The *H. arabidopsis* isolate *Cala2* and the *Bremia lactucae* isolate BL/96 from lettuce were able to sporulate on preinfected *Brassica juncea* line 560-1 at very low frequency (Table 2). In the absence of *Albugo candida* Bihar-SP, all of the avirulent downy mildews tested produced necrotic "pitting" lesions on *B. juncea* line 560-1.

A. candida (including both *Acem1* and Bihar-SP) is distinguishable microscopically from *H. arabidopsis* and *H. parasitica* in cleared host tissue: *A. candida* produces minute, spherical haustoria which are attached to hyphae by thin necks, whereas the downy mildew parasites produce much thicker hyphae and club-shaped haustoria (Fig. 1E). In sequentially inoculated tissue of either *Arabidopsis thaliana* or *B. juncea*, the haustoria of downy mildew parasites typically were formed in host cells adjacent to ones that contained haustoria of either *Acem1* or Bihar-SP in their respective compatible host tissue (data not shown).

Suppression of powdery mildew resistance in *Arabidopsis thaliana*.

Albugo candida subsp. *arabidopsis* *Acem1* also suppressed resistance of *Arabidopsis thaliana* to powdery mildew, an ascomycete fungus. Resistant *Arabidopsis thaliana* Moscow (Ms-0) contains RPW8, which naturally confers broad-spectrum resistance to several powdery mildew species (Xiao et al. 2001). When this accession was inoculated solely with a powdery mildew isolate (morphotype similar to *Erysiphe cruciferarum*), no measurable fungal growth or sporulation was observed 7 dai; whereas 48 h of preinfection with *Acem1* resulted in 18% coverage of cotyledons with powdery mildew mycelium (Table 2; Fig. 1C). Similarly, transgenic *Arabidopsis thaliana* Col-0::RPW8 inoculated solely with the same powdery mildew isolate showed 11% cotyledon coverage, compared with up to 38% coverage in cotyledons that were preinfected for 48 h with *Albugo candida* subsp. *arabidopsis* *Acem1* (Table 2). The differences in powdery mildew between presence and absence of *Acem1* were statistically significant ($P < 0.01$) in both genetic backgrounds of *Arabidopsis thaliana*. With a second, highly virulent powdery mildew isolate (morphotype similar to *Erysiphe orontii*), RPW8 resistance appeared to be fully suppressed in transgenic Col-0::RPW8 (Fig. 3) following 48 h of preinfection by *Acem1*.

DISCUSSION

We have described examples demonstrating that basic compatibility of *Albugo candida* in two crucifer hosts involves broad-spectrum suppression of cell death and innate immunity, including the runaway cell death phenotype of the *Arabidopsis thaliana* *lsd1* mutant in a manner that is sustained even after secondary infection with an avirulent pathogen; several examples of genotype-specific resistance to downy mildew in *Arabidopsis thaliana* and *B. juncea* that, in the case of *Arabidopsis thaliana*, differ in the type of resistance (R) protein involved and the phenotypic manifestation of defense that is conferred; species-level (nonhost) resistance in *Arabidopsis thaliana* and *B. juncea* to an isolate of the *H. parasitica* race derived from *B. oleracea* and to *Bremia lactucae* (lettuce downy mildew);

Table 1. Mean disease severity caused by infection of *Arabidopsis thaliana* with the hemibiotrophic fungus *Colletotrichum higginsianum* (IMI349061) in seedlings that were noninoculated or preinfected (48 h) with *Albugo candida* subsp. *arabidopsis* (Acem1)^a

Accession ^b	Dai	Age ^c	No Acem1		Acem1 preinfection	
			Mean	SE	Mean	SE
Ws-3	7	7	4.0	±0.68	2.6	±0.50
	...	10	3.7	±0.69	4.0	±0.48
	11	7	4.0	±0.62	4.9	±0.49
	...	10	4.4	±0.43	4.3	±0.34
Ws-eds1	7	7	4.6	±0.53	4.0	±0.48
	...	10	4.1	±0.36	4.6	±0.34
	11	7	4.7	±0.56	4.6	±0.47
	...	10	4.7	±0.40	5.4	±0.35

^a Dai = days after inoculation when the disease severity was assessed using a six-class scale (where class 1 = no symptoms observed and class 6 = dead seedling) and SE = standard error. Summary of statistical analysis: Acem1 = not significant, accession and dai significant at $P < 0.001$.

^b *Arabidopsis thaliana* accession.

^c Seedling age in days when the seedlings were inoculated with *C. higginsianum*.

and broad-spectrum *RPW8*-mediated resistance (Xiao et al. 2001) to powdery mildew in *Arabidopsis thaliana*. The two *Albugo candida* isolates used in this study represent divergent groups within the species (Borhan et al. 2008) and, thus, demonstrate that suppression of downy mildew resistance is a species-level characteristic of *A. candida*. Interestingly, *A. candida* significantly enhanced sporulation of a virulent isolate of *H. arabidopsis* in *Brassica juncea*. However, preinfection with *A. candida* does not appear to protect a compatible host from fungal-mediated cell death, because this treatment had no measurable effect on the severity of disease caused by the hemibiotrophic fungus *C. higginsianum* in *Arabidopsis thaliana* Ws-3 (O'Connell et al. 2004).

We specifically chose isolates of *H. arabidopsis* for this study that are recognized by different RPP (resistance to *Hyaloperonospora parasitica*) proteins and are known to confer resistance through various defense pathways, as defined by molecular analyses of enhanced susceptibility mutants. For example, R genes in *Arabidopsis thaliana* Col-0 represent several interesting examples, including digenic RPP2 (that recognizes isolate Cala2), which confers resistance that is fully dependent on the expression of a lipase-like protein called EDS1 (Aarts et al. 1998); polygenic resistance (recognizing Cand5), involving at least three complementary R genes, which is fully dependent on a membrane-associated protein called NDR1 (Century et al. 1997; E. B. Holub, unpublished); and monogenic RPP7 (recognizing Hiks1), which confers cumulative resistance by altering regulation of EDS1, NDR1, and a further unknown defense component (McDowell et al. 2000). Defense signaling in each of these examples requires the expression of AtSGT1b, a protein that is conserved across kingdoms as a key component in ubiquitin-mediated proteolysis (Austin et al. 2002; Tör et al. 2002, 2003). For contrasting examples, resistance is conferred via an AtSGT1b-independent defense signaling pathway in the cases of Cala2, which is recognized by RPP1 in *Arabidopsis thaliana* Ws-3, and Hiks1, which is recognized by RPP27 in Ler-0 (Austin et al. 2002; Tör et al. 2002). *Albugo candida*

Table 2. Mean sporulation of avirulent downy mildew isolates (measured as the mean number of sporangioophores per cotyledons) and an avirulent powdery mildew isolate (percent leaf area covered with fungal hyphae and sporulation) in different *Arabidopsis thaliana* accessions that were either preinfected (48 h) or noninfected (control) by a virulent isolate of *Albugo candida* subsp. *arabidopsis* (Acem1)

Pathogen species	Isolate (original host)	Accession ^a	R gene (and defense regulation) ^b	No. of cotyledons	No Acem1	Acem1 preinfected	Significance
<i>Hyaloperonospora arabidopsis</i>	Cala2 (<i>Arabidopsis thaliana</i>)	Col-0	RPP2 (<i>AtSGT1b</i> , <i>EDS1</i>)	240	<0.1 (±0.00)	8.4 (±0.80)	<0.01
	Ws-3	Ws-3	RPP1-WsA (<i>EDS1</i>)	160	0.0 (±0.00)	6.0 (±0.60)	<0.01
	Hiks1 (<i>Arabidopsis thaliana</i>)	Col-0	RPP7 (<i>AtSGT1b</i> , <i>EDS1</i> , <i>NDR1</i> + other)	160	0.0 (±0.00)	6.7 (±0.60)	<0.01
	Ler-0	Ler-0	RPP7, RPP27 (<i>AtSGT1b</i> , <i>EDS1</i> , <i>NDR1</i> + other)	120	0.0 (±0.00)	2.4 (±0.60)	<0.01
	Cand5 (<i>Arabidopsis thaliana</i>)	Col-0	polygenic (<i>AtSGT1b</i> , <i>NDR1</i>)	260	0.1 (±0.08)	8.7 (±1.60)	<0.01
<i>H. parasitica</i>	P-005 (<i>Brassica oleracea</i>)	Col-0	nd	120	0.0 (±0.00)	1.5 (±0.30)	<0.01
	Ws-3	Ws-3	nd (<i>EDS1</i> + other)	40	0.0 (±0.00)	0.9 (±0.00)	<0.01
	Ws-eds1	...	...	80	1.2 (±0.30)	7.3 (±1.40)	<0.05
<i>Bremia lactucae</i>	BL/96 (<i>Lactuca sativa</i>)	Col-0	nd	40	0.0 (±0.00)	0.8 (±0.000)	<0.05
	Ws-3	Ws-3	nd	40	0.0 (±0.00)	0.8 (±0.00)	<0.01
	Ws-eds1	...	...	160	0.0 (±0.00)	1.8 (±0.60)	<0.01
	...	...	...	...	...	...	...
<i>Erysiphe</i> sp. ^c	Unnamed (<i>Arabidopsis thaliana</i>)	Col + <i>RPW8</i>	<i>RPW8</i> (<i>EDS1</i>)	...	11%	38%	<0.01
	...	Ms-0	<i>RPW8</i> (<i>EDS1</i>)	...	0%	18%	<0.01

^a *Arabidopsis thaliana* accession. Col + *RPW8* = Columbia transgenic line T-B6 (containing cosmid B6 and both functional copies of *RPW8*) described by Xiao and associates (2001).

^b R = resistance gene. RPP genes reviewed in Holub 2001, 2007; *AtSGT1b* = homologue of *SGT1* in yeast (Tör et al. 2002); *EDS1* = enhanced disease susceptibility (Aarts et al. 1998); *NDR1* = non-race-specific disease resistance (Century et al. 1997). Description of the resistance (R) genes is provided in the following references: Botella et al. 1998; Holub 2007; McDowell et al. 2000; Sinapidou et al. 2002; Tör et al. 2002; Xiao et al. 2001; nd = not determined.

^c Morphologically similar to *Erysiphe cruciferarum*; inoculum collected from resident population on *Brassica oleracea* and *Arabidopsis thaliana* plants in Warwick-HRI glasshouses.

Fig. 2. *Albugo candida* subsp. *arabis* (isolate Acem1) suppresses “runaway cell death” that is readily inducible in the *Arabidopsis thaliana lsd1* mutant but has no visible impact on seedling mortality caused by the necrotrophic stage of the hemibiotrophic fungus *Colletotrichum higginsianum* (IMI349061). **A**, Cotyledons of *Ws-lds1* mutant seedlings exhibit rapid cell death following inoculation with avirulent isolates of *Hyaloperonospora parasitica* (left, photographed 24 h after inoculation with isolate P005; entire seedling was dead 24 h later) or delayed cell death following heavy downy mildew sporulation when inoculated with a virulent isolate of *H. parasitica* (middle, 7 days after inoculation with isolate Emwa1; seedling was dead 48 h later); whereas no cell death or restriction of parasite reproduction occurs following inoculation with *Albugo candida* subsp. *arabis* Acem1 (right, 7 days after inoculation). **B**, Systemic infection of *Ws-lds1* by Acem1 protected the entire plant from the cell death phenotype (red box inset, the plant ceased at nine-leaf stage and survived for 6 months, eventually senescing similar to a systemically infected wild-type plant). **C**, Cotyledons of *Col-lds1* preinoculated with Acem1 followed 24 h later by inoculation with *Col*-avirulent *H. parasitica* (isolate Hiks1) did not exhibit cell death. **D**, Preinfection with Acem1 (48 h) provided no visible protection from seedling mortality inflicted by secondary infection by *C. higginsianum* in either wild-type Ws-3 or the *Ws-eds1* mutant.

subsp. *arabidopsis* (Acem1) suppressed resistance to downy mildew in all of the above examples to varying degrees. Similarly, a virulent isolate of *A. candida* race 2 (Bihar-SP) suppressed genotype-specific resistance of *B. juncea* to *H. parasitica* IP00A, which is conferred by a single dominant gene (Nashaat et al. 1998); interestingly, it also significantly enhanced susceptibility to a virulent *H. parasitica* isolate (IP05).

Although the ability of *A. candida* to suppress *R*-gene-mediated resistance is broad spectrum, it does not appear to be universal. Most notable is the rare *Arabidopsis thaliana* gene *RAC1* that confers white rust resistance to *Albugo candida* subsp. *arabidopsis*, including the Col-virulent isolate Acem1

used in our study. This gene encodes a Toll-interleukin receptor-like nucleotide-binding leucine-rich repeat (TIR-NB-LRR) receptor-like protein that is structurally similar to the molecularly characterized *RPP* genes mentioned above and also confers resistance via *EDS1*-signaling (Borhan et al. 2004). White rust resistance is an uncommon trait in *Arabidopsis thaliana*, occurring in <10% of *Arabidopsis thaliana* accessions across a worldwide collection, and this type of disease resistance is rarely conferred by *RAC1* (Borhan et al. 2001; Holub in press; Holub et al. 1995). Major advances have been achieved recently in the molecular characterization of oomycete “effector” proteins from *Phytophthora* spp. and *H. arabidopsis* that are

Table 3. Mean sporulation of a virulent and several avirulent downy mildew isolates in *Brassica juncea* cotyledons (accession 560-1) that were either preinfected (48 h) or noninfected (control) with a virulent isolate of *Albugo candida* race 2 (Bihar-SP)^a

Species, isolate ^d	Maintenance host ^e	Experiment 1 ^b			Experiment 2 ^c		
		None	Bihar-SP	Significance	None	Bihar-SP	Significance
<i>Hyaloperonospora parasitica</i>							
IP05 (<i>B. juncea</i>)	4.8 ± 0.7	2.9 ± 0.7	3.4 ± 0.4	ns	0.9 ± 0.1	1.6 ± 0.1	<0.001
IP00A (<i>B. juncea</i>)	3.4 ± 0.4	0	2.4 ± 0.5	0.03	0	1.3 ± 0.1	<0.001
IP09 (<i>B. rapa</i>)	3.6 ± 0.7	0	2.2 ± 0.3	0.03	0	1.6 ± 0.2	<0.001
PP6 (<i>B. oleracea</i>)	6.5 ± 0.5	0	2.7 ± 0.4	0.03	0	0.9 ± 0.1	<0.001
P003 (<i>B. napus</i>)	5.4 ± 0.6	0	3.5 ± 0.6	0.03	0	0.8 ± 0.1	<0.001
IP10 (<i>Eruca sativa</i>)	3.5 ± 0.5	0	1.6 ± 0.3	0.03	0	2.1 ± 0.2	<0.001
WF1 (<i>Cherianthus cherii</i>)	4.3 ± 0.8	0	0.1 ± 0.1	0.03	0	0.01 ± 0.003	<0.004
<i>H. arabidopsis</i>							
Cala2 (<i>Arabidopsis thaliana</i>)	4.3 ± 0.5	0	0.1 ± 0.1	0.03	0	0.01 ± 0.01	ns
<i>Bremia lactucae</i>							
BL/96 (<i>Lactuca sativa</i>)	21 ± 1.0	0	<0.01	ns	0	<0.01	ns

^a None = no Bihar-SP and Bihar-SP = preinfected.

^b Spore production was measured directly as the number of condiosporangia per square centimeter ($\times 10^4$) of cotyledon surface produced by the avirulent pathogen, 7 days after inoculation (dai); ns = not significant.

^c Sporulation was measured as the number of sporangiophores per square centimeter ($\times 10^3$) of cotyledon surface produced by the avirulent pathogen 7 dai.

^d Downy mildew species and isolate (original host). IP05 is virulent on accession line 560-1, and all other isolates are avirulent.

^e The respective susceptible host for each downy mildew isolates includes: *B. juncea*, PPBJ-1; *B. rapa*, RES-BR-263; *B. oleracea*, cv. Billabong; *B. napus*, cv. Capricorn; *Eruca sativa*, cv. Taramira; *Cherianthus cherii*, cv. Fairlady; *Arabidopsis thaliana*, Ws-eds1; and *L. sativa*, cv. Cobham Green.

Fig. 3. Preinfection (48 h) of cotyledons with *Albugo candida* subsp. *arabidopsis* (isolate Acem1) fully suppressed powdery mildew resistance conferred by *RPW8* in transgenic *Arabidopsis thaliana* Col-0. All seedlings shown in these photographs were inoculated with an isolate of powdery mildew fungus (a morphotype similar to *Erysiphe orontii*, producing long chains of conidia, top right; and highly virulent in Col-0, top left). A similar amount of hyphal growth and sporulation by the powdery mildew fungus was observed only in Col-*RPW8* that was preinfected with Acem1 (bottom center and right).

secreted from the parasite into the host and, if recognized by a matching *R* gene, can elicit innate immunity (Kamoun 2006). The current emphasis is focused on a large family of proteins with a shared “RLR” domain that generally is thought to indicate targeting of the so-called “effector” proteins for activity within the host cytoplasm (Whisson et al. 2007; Win et al. 2007). Numerous cytoplasmic effectors also are anticipated in oomycetes (Kamoun 2006; Whisson et al. 2007). Similarly, molecular characterization of *Albugo candida* effector proteins will be necessary to resolve whether *RAC1* enables *Arabidopsis thaliana* to recognize an *Albugo candida* subsp. *arabidopsis* effector that otherwise would suppress innate immunity in the absence of *RAC1*, or that defense suppression is an independent effector-mediated process which is preempted by *RAC1*-mediated recognition of an alternative avirulence protein. Identification of *RAC1* as a gene encoding a TIR-NB-LRR protein, which is predicted to be located in the cytoplasm, suggests that the defense suppression is initiated by an *A. candida* subsp. *arabidopsis* effector within the host cell. For instance, *RAC1* may guard the host protein targeted by the parasite suppressor.

The molecular basis of species-level (nonhost) resistance in *Arabidopsis thaliana* and *B. juncea* to downy mildew parasites from other host species is currently unknown. However, it is evident from *Arabidopsis thaliana* mutants such as *eds1* that parasite recognition (mediated by *R* genes and induced defense) most likely is involved. It also appears that oomycetes from brassicas and lettuce are at least partially pathogenically competent (i.e., equipped to complete their life cycle) in the “nonhost” *Arabidopsis thaliana* (Holub and Cooper 2004; Parker et al. 1996). In our current study, the *eds1* mutation in the *Arabidopsis thaliana* Ws-3 genetic background partially diminished nonhost resistance to the *H. parasitica* isolate P005 collected from *B. oleracea*, as previously reported (Parker et al. 1996). Preinfection of wild-type Ws-3 with *Albugo candida* subsp. *arabidopsis* also enhanced P005 susceptibility to a degree similar to that observed with P005 on its own in Ws-*eds1*. The combined effect in cotyledons of the *eds1* mutant following *A. candida* subsp. *arabidopsis* preinfection was as high a level of sporulation by the *H. parasitica* isolate as was observed for any normally compatible *H. arabidopsis* isolate in *Arabidopsis thaliana*. This indicates that isolates specifically adapted to alternative hosts (such as *B. oleracea*) are still fully competent pathogens in *Arabidopsis thaliana* once the defense process has been negated. Similar results were observed in *B. juncea* with *H. parasitica* isolates that were collected from other *Brassica* spp., including *B. oleracea*, *B. rapa*, and *B. napus*. Each of these isolates represented subtaxa that exhibit a high degree of specialization to their host species of origin (Sherriff and Lucas 1990).

In the most extreme example, the lettuce downy mildew isolate of *Bremia lactucae* BL/96 was capable of sporulating only in cotyledons that were preinfected with *Albugo candida*, and this was further enhanced in an *eds1* background. The low levels of sporulation in these examples and also in *Brassica juncea* suggest that either *Bremia lactucae* is not fully adapted as a parasite in crucifer hosts or there is residual resistance or inaccessibility that still constrains development of *Bremia lactucae*. Similar parasite or host factors may explain the very low level of sporulation by *H. arabidopsis* isolate Cala2 in *Brassica juncea* cotyledons that were preinfected with *A. candida* race 2.

Recently, research on nonhost resistance has begun to elucidate the host genes that govern inaccessibility rather than induced defense (Holub and Cooper 2004). For example, Yun and associates (2003) demonstrated that two lipase-like genes, *PAD4* and *EDS1*, are involved in the defense of *Arabidopsis*

thaliana against *Blumeria graminis* f. sp. *tritici* (barley powdery mildew). Lipka and associates (2005) subsequently demonstrated that *PEN2* (a glycosyl hydrolase) is a protein that restricts the ingress of pea (*E. pisi*) and barley powdery mildews, thus conferring inaccessibility or “preinvasion” defense. The *Arabidopsis thaliana eds1/pen2* double mutant was even more susceptible to both parasites, and supported sporulation by *E. pisi*. The authors concluded that pre- and post-invasion defense mechanisms both are involved in nonhost resistance to powdery mildew in *Arabidopsis thaliana*. Therefore, it will be interesting to determine whether *Albugo candida* subsp. *arabidopsis* is capable of suppressing the preinvasion mechanisms that restrict growth of *E. pisi* in *Arabidopsis thaliana*, or whether it can further enhance susceptibility of the *eds1 pen2* double mutant to *Blumeria graminis*. Additional sequential inoculation experiments with other types of pathogens such as hemibiotrophs or necrotrophs also should be attempted. For example, results with the hemibiotrophic fungus *C. higginsianum* from our study indicated that preinfection of *Arabidopsis thaliana* Ws-3 or Ws-*eds1* with *Albugo candida* subsp. *arabidopsis* Acem1 did not significantly affect the amount of seedling mortality caused by *C. higginsianum* isolate IMI 349061 at 20°C that was favorable for this hemibiotroph (O’Connell et al. 2004). This indicates that there are further limits (in addition to the *RAC1* example described above) to the suppressive effects induced in *Arabidopsis thaliana* by preinfection with *Albugo candida* subsp. *arabidopsis*, which will relate directly to the developmental stage of the host and environmental conditions.

The suppressive effect of *A. candida* appeared to be nonsystemic and sustained by the parasite at least until the onset of host senescence. The sustained effect of defense suppression is clearly evident in the survival of Col-*lsd1* and Ws-*lsd1* for several months following Acem1 infection, and despite secondary challenge with an avirulent downy mildew isolate. The effect was nonsystemic because enhanced downy mildew susceptibility was observed only when *A. candida* (readily visible by rust pustules) also was present in the same host tissue. Microscopic examination indicated that defense suppression may involve a local signal because haustoria produced by each of the oomycete parasites were never observed within the same host cell. Preliminary experiments with *Brassica juncea* (data not shown), in which one cotyledon was inoculated with *A. candida* race 2 and, 24 h later the opposite cotyledon was inoculated with an avirulent isolate of *H. parasitica*, showed no suppression of downy mildew resistance in the second cotyledon, suggesting that no long-distance signal for defense suppression is emitted by preinfection with *A. candida* infection.

The phenomenon of defense suppression is not limited to *A. candida* but extends into many other pathosystems. Over half a century ago, Yarwood (1951) found that bean rust (caused by a basidiomycete fungus) was able to suppress the resistance of bean toward viruses collected from other hosts. Similarly, Gill (1965) observed that sunflower plants preinfected with virulent rust, *Puccinia helianthi*, were more susceptible toward viruses, including *Tobacco necrosis virus*, *Cucumber mosaic virus*, *Artichoke latent virus*, and *Cabbage mosaic virus*. The breadth of this phenomenon also extends to monocotyledonous hosts in which powdery mildew acts as the suppressor; for example, wheat powdery mildew, *Blumeria graminis* f. sp. *tritici* (an ascomycete fungus), can suppress resistance in wheat to the barley powdery mildew pathogen *Blumeria graminis* f. sp. *hordei* (Moseman and Greeley 1964); and vice versa (Moseman et al. 1965). More recently, Olesen and associates (2003) found that *Blumeria graminis* f. sp. *avenae* also could grow on wheat and barley preinoculated with virulent formae speciales of *Blumeria graminis*. This pathosystem has provided an impres-

sive demonstration of how defense suppression is localized to host cells that are adjacent to ones preinfected by the virulent pathogen (Lyngkjær and Carver 2000). The suppression of disease resistance may involve highly conserved host targets among these diverse host–pathogen interactions. However, given the separate phylogeny of oomycetes and fungi, the underlying parasite determinants of defense suppression are likely to be distinct among the parasite lineages. As described above, molecular identification of defense suppressor proteins from oomycetes and fungi obviously is required to resolve this hypothesis.

The gene *RAC1* described above potentially could be used in experimental host lines to identify effector-like proteins that elicit cell death, assuming that this particular *R* gene may confer recognition of an *A. candida* subsp. *arabidopsis* suppressor (see discussion above). We are progressively identifying additional *R* genes that confer white rust resistance, which also could be used for similar experiments (Borhan et al. 2008). High-throughput assays using bombardment assays or bacterial delivery need to be devised which will enable reproducible screening of candidate oomycete effectors (Holub 2006; Rehmany et al. 2005; Rentel et al. 2008; Sohn et al. 2007).

Mutant screens of *Arabidopsis thaliana* for enhanced resistance to *Albugo candida* subsp. *arabidopsis* Acem1 may enable identification of candidate host proteins that are vulnerable to suppression by this parasite. Such screens have been used successfully for investigating compatibility of powdery and downy mildew parasites in *Arabidopsis thaliana* (Van Damme et al. 2005; Vogel et al. 2004). None of the published mutants, however, confer a measurable effect on *A. candida* subsp. *arabidopsis* compatibility (E. Holub, *unpublished*); therefore, direct screening for enhanced resistance mutants with *A. candida* subsp. *arabidopsis* will be important for future research.

Given the symptomology of *A. candida* infection, often including “green islands” and malformation of host tissue (Crute et al. 1994), reasonable criteria for identifying host regulators and candidate defense suppressors from *A. candida* would be evidence of interference with hormonal signaling networks, which are becoming more clearly defined, and particularly those implicated in responses mediated by levels of plant tissue auxin or cytokinin (Ferreira and Kieber 2005; Quint and Gray 2006). Thus, available hormonal signaling mutants should be used to assess the impact on compatibility with virulent and avirulent isolates of *A. candida*. This may provide useful information for verifying candidate defense suppressors by their expected correlative profile of hormonal imbalance.

Investigation of pathogen-mediated defense suppression potentially will be advanced in *Arabidopsis thaliana* with the premiere model oomycete *H. parasitica* (Holub 2006; Sohn et al. 2007). However, the fact that infection of the *Arabidopsis thaliana* *lsd1* mutant with a virulent isolate of *H. parasitica* can readily elicit runaway cell death in seedlings suggests that this parasite may not be able to sustain active suppression of host defenses. This also seems evident from previous description of “enhanced resistance” to virulent downy mildew in *Ws-lsd1*, from induction of cell death that trailed closely behind the growth of *H. arabidopsis* hypha in the mesophyll of true leaves (Dietrich et al. 1997). The striking contrast with virulent *Albugo candida* subsp. *arabidopsis*, which can sustain cell death suppression in *Arabidopsis thaliana* *lsd1*, is a compelling observation that, on its own, is enough to warrant the investment in oomycete genomics beyond *Phytophthora* spp. and *H. parasitica* which, according to Riethmüller and associates (2002), compose a narrow lineage within the Oomycetes. *Albugo candida* subsp. *arabidopsis* and its defense suppression capability represent an important and fascinating complement for downy mildew research in *Arabidopsis thaliana*.

MATERIALS AND METHODS

Pathogen isolates and handling.

A summary of the fungal and oomycete isolates used in this study is provided in Table 4. *Albugo candida* subsp. *arabidopsis* isolate Acem1 was maintained and prepared for experiments as described by Dangel and associates (1992). This isolate was used for all sequential inoculation experiments on *Arabidopsis thaliana*; inoculum was adjusted to 1.3×10^5 zoospores ml^{-1} . The *Albugo candida* race 2 isolate Bihar-SP was used for all experiments on *B. juncea*; inoculum was adjusted to 2×10^5 zoospores ml^{-1} . The *H. arabidopsis* isolates Cala2, Cand5, Noks1, and Hiks1 were maintained and prepared for experiments in *Arabidopsis thaliana* as described by Tör and associates (2002); inoculum was adjusted to 7×10^4 spores ml^{-1} .

H. parasitica isolates from their respective hosts (*B. juncea*, *B. oleracea*, *B. rapa*, *B. napus*, *Eruca sativa*, and *C. cherii*) were subcultured on 7-day-old susceptible seedlings. Sporulating cotyledons were removed and shaken in refrigerated distilled water; the spore solution then was sprayed onto 7-day-old host seedlings, which were placed in sealed propagators. Inoculated plants were placed in a growth room at 18°C with a 16-h photoperiod. Inoculum was adjusted to 1×10^5 spores ml^{-1} .

Table 4. Pathogen isolates and susceptible plant accessions used for maintaining cultures of each respective isolate

Pathogen species	Isolate name	Maintenance host (susceptible accession)	Source (reference)
<i>Albugo candida</i> “suppressors”			
<i>A. candida</i> subsp. <i>arabidopsis</i>	Acem1	<i>Arabidopsis thaliana</i> (Wassilewskija, Ws-3)	E. B. Holub (Holub et al. 1995)
<i>A. candida</i> race 2	Bihar SP	<i>Brassica juncea</i> (oilseed mustard, PPBJ-1)	A. O. Latunde-Dada (<i>unpublished</i>)
Avirulent “challengers”			
<i>Hyaloperonospora arabidopsis</i>	Cala2	<i>Arabidopsis thaliana</i> (Ws-eds1)	E. B. Holub (Holub et al. 1994; Parker et al. 1996)
	Cand5	<i>Arabidopsis thaliana</i> (Ws-eds1)	E. B. Holub (Holub et al. 1994; Parker et al. 1996)
	Hiks1	<i>Arabidopsis thaliana</i> (Ws-eds1)	E. B. Holub (Holub et al. 1994; McDowell et al. 2000)
	Noks1	<i>Arabidopsis thaliana</i> (Ws-eds1)	E. B. Holub (Parker et al. 1996)
<i>H. parasitica</i>	IP00A	<i>B. juncea</i> (oilseed mustard, PPBJ-1)	N. I. Nashaat, RRes (Nashaat et al. 1998)
	IP05	<i>B. juncea</i> (oilseed mustard, PPBJ-1)	N. I. Nashaat, RRes (Nashaat et al. 1998)
	P003	<i>B. napus</i> (oilseed rape cv. Capricorn)	J. A. Lucas (Sherriff and Lucas 1990)
	PP6	<i>B. oleracea</i> (cauliflower cv. Billabong)	J. A. Lucas (Silue et al. 1996)
	IP09	<i>B. rapa</i> (turnip, RES-BR-263)	N. I. Nashaat, RRes (Nashaat et al. 1998)
	WF	<i>Cheiranthus cherii</i> (wallflower cv. Fairlady)	J. A. Lucas (<i>unpublished</i>)
	IP10	<i>Eruca sativa</i> (rocket cv. Taramira)	N. I. Nashaat, RRes (<i>unpublished</i>)
<i>Bremia lactucae</i>	BL/96	<i>Lactuca sativa</i> (lettuce cv. Cobham Green)	E. B. Holub (obtained from I. R. Crute; <i>unpublished</i>)
<i>Erysiphe</i> spp.	Unnamed	<i>Arabidopsis thaliana</i> and <i>Brassica oleracea</i>	Glasshouse inhabitants at WHRI (<i>unpublished</i>)
<i>Colletotrichum higginsianum</i>	061	Plate culture	S. Sreenivasaprasad, Warwick HRI (O’Connell et al. 2004)

for experiments. For experiments in *Arabidopsis thaliana* using *H. parasitica* P005, the downy mildew inoculum was adjusted to 7×10^4 spores ml^{-1} .

Bremia lactucae BL/96 was subcultured on 7-day-old cotyledons of *L. sativa* L. (cv. Cobham green). Lettuce seed were sown on vermiculite soaked in a nutrient solution with 1% Rovral fungicide and placed in a 15°C growth room with a 12-h photoperiod.

Seven-day-old lettuce cotyledons were sprinkled with refrigerated distilled water; then, 14-day-old seedlings with heavy downy mildew from the previous culture were harvested and used to spread inoculum onto the wet surfaces of the new lettuce cotyledons. Inoculated lettuce cotyledons then were returned to the conditions used for seed germination. For experimentation, *Bremia lactucae* spores were suspended in chilled distilled water. The suspension was strained and centrifuged at $2,000 \times g$ for 5 min; the supernatant was removed and the pellet was resuspended in refrigerated distilled water. This procedure was repeated twice and the inoculum was adjusted to 5×10^4 sporangia ml^{-1} .

Powdery mildew inoculum from two different morphotypes of *Erysiphe* spp. was collected from leaves that occurred as natural epidemics on *Arabidopsis thaliana* and *Brassica oleracea* plants in glasshouses at Warwick-HRI. These included a morphotype similar to *Erysiphe cruciferarum* with short conidial chains, weakly virulent in *Arabidopsis thaliana* Col-0 and used for the experiment described in Table 2; and a morphotype similar to *E. orontii* with long conidial chains (Plotnikova et al. 1998), and highly virulent in Col-0 and used for the experiment shown in Figure 3. All inoculum was collected from *Arabidopsis thaliana* leaves. Further description of the inoculation method is described below.

The *C. higginsianum* isolate IMI 349061 (O'Connell et al. 2004) was subcultured on potato dextrose agar (PDA) at 20°C. Conidial suspensions were prepared from 10- to 12-day-old PDA cultures; inoculum was adjusted to 1×10^5 spores ml^{-1} in sterile distilled water.

Sequential infection experiments.

For experiments in *Arabidopsis thaliana*, 7-day-old cotyledons were inoculated first with an approximately 5- μl drop of *Albugo candida* subsp. *arabidopsis* Acem1 zoospore suspension using methods described by Holub and associates (1995). The inoculated seedlings were incubated for 2 days in a growth room at 15°C with a photoperiod of 12 h. The avirulent downy mildew isolate was introduced by placing an approximately 5- μl drop of inoculum on the same cotyledons and returning for incubation at the same growth conditions. Negative and positive controls were inoculated solely with the avirulent isolate, including resistant and susceptible (*Ws-eds1*) *Arabidopsis thaliana*.

Powdery mildew inoculations in *Arabidopsis thaliana* were made 48 h after inoculation with *Albugo candida* subsp. *arabidopsis*. Prior to powdery mildew inoculation, excess moisture was removed from cotyledons with tissue paper. Leaves covered with profuse powdery mildew sporulation were removed from infected plants, which were held over *Arabidopsis thaliana* seedlings while spores were brushed off the leaves with a fine paintbrush. The pots of sequentially inoculated seedlings were placed in a sealed propagator and incubated for 7 days in a 20°C growth room with a 10-h photoperiod. Viability of the two *Erysiphe* spp. was assessed by monitoring the germination of conidia on 5% agar plates (2 dai) that were incubated in the same conditions as for experimentation (data not shown).

For tests with *C. higginsianum*, *Arabidopsis thaliana* seedlings were inoculated with approximately 5- μl drops of this

culture (using methods described by O'Connell and associates [2004]) 2 dai with *Albugo candida* subsp. *arabidopsis* Acem1. Inoculated plants were incubated at 20°C in a growth room with a 10-h photoperiod for 7 days. Infection was scored 7 days after inoculation with *C. higginsianum*, and infected seedlings were categorized into classes with an assigned score. Categories included 1 = green cotyledons and adult leaves, 2 = green cotyledons no adult leaves, 3 = chlorotic or necrotic cotyledons with adult leaves, 4 = chlorotic or necrotic cotyledons and no leaves, 5 = shriveled cotyledons or leaves but turgid, and 6 = collapsed and macerated.

B. juncea line 560-1 was used for all sequential inoculation experiments on *B. juncea*. After a 24-h incubation period following the inoculation with *A. candida* race 2 (isolate Bihar-SP), cotyledons were inoculated with an avirulent downy mildew pathogen. Positive and negative controls were included; *B. juncea* line 560-1 was inoculated solely with Bihar-SP and solely with the avirulent oomycete. Inoculated seedlings were maintained in sealed plant propagators within growth cabinets as described above.

Assessing sporulation of sequentially inoculated cotyledons.

Sporulation of downy mildew isolates was assessed in *Arabidopsis thaliana* experiments 7 dai. An illuminated magnifier ($\times 2$) was used to count the number of sporangioophores per cotyledon up to 10, and estimating higher numbers as either medium (M, 11 to 19 sporangioophores) or heavy (H, ≥ 20 sporangioophores). A minimum of 20 seedlings was examined, as recommended by Mert (2001). Sporulation of the *H. parasitica* isolate P005 and the *Bremia lactuca* isolate BL/96 was confirmed by collecting sporangioophores from sequentially inoculated *Arabidopsis thaliana* and using these to inoculate the respective natural host of each isolate. Sporulation of these crop pathogens 7 days later confirmed their identity, confirming that they were not contaminants (data not shown).

Powdery mildew also was assessed using an illuminated magnifier. Sporulation was considered heavy if cotyledons had $\geq 90\%$ coverage, medium with 40 to 89% coverage, and sparse on cotyledons with $\leq 39\%$ coverage.

Two separate sequential inoculation experiments were performed on *Brassica juncea*. In the first experiment, sequentially inoculated cotyledons were excised 7 dai with the avirulent pathogen and shaken in vials with 1 ml of sterile distilled H_2O . Spores were counted using a hemacytometer and spore number then was estimated per square centimeter of cotyledon. In the second sequential inoculation experiment, cotyledons were excised 7 dai with the avirulent pathogen and were mounted for microscopy, which was used to assess the number of sporangioophores in several fields of view. This figure then was used to estimate the number of sporangioophores per square centimeter of cotyledon. The number of fields of view counted for sequential inoculations on *B. juncea* with downy mildews isolated from *Arabidopsis thaliana* and lettuce was increased to 90 to raise the probability of finding sporangioophores.

For microscopy, sequentially infected *B. juncea* cotyledons were cleared in a 3:1 (vol/vol) solution of ethanol and chloroform containing 0.15% trichloroacetic acid, stained in a 0.025% solution of cotton blue in lactophenol, and mounted on glass slides.

Statistical analyses.

Data analyses were performed using the computer package GenStat (Payne et al. 2007). For experiments on *Arabidopsis thaliana*, the heterogeneity of sporulation scores on sequentially inoculated cotyledons between repeated experiments was assessed. For experiments with *H. parasitica* P005 on *Arabidop-*

sis thaliana Ws-*eds1*, which sporulated in the absence *Albugo candida* subsp. *arabidopsis* Acem1, sporulation scores were assessed between repeated experiments with and without *A. candida* subsp. *arabidopsis* Acem1. This was achieved by analyzing the contingency table of numbers of cotyledons indexed by experiment and sporulation score, using a log-linear model. Where more than one experiment had been performed, data were pooled across experiments. For each different mildew isolate and each different accession of *Arabidopsis thaliana*, a contingency table indexed by presence or absence of *Albugo candida* subsp. *arabidopsis* Acem1 and by whether or not sporulation was observed was analyzed, again using a log-linear model. Finally, the data for *H. parasitica* P005 on Ws-*eds1* were reanalyzed as a contingency table indexed by the presence or absence of Acem1 and by sporulation score. In all cases, the effects of the margins of the contingency table were fitted and the significance of the interaction of interest was assessed by comparing the residual deviance to a χ^2 distribution on the appropriate number of degrees of freedom. For the *B. juncea* data, the significance of the differences between the levels of sporulation with and without preinfection with *A. candida* was assessed using Mann-Whitney U tests.

Mean severity scores from the *C. higginsianum* experiments were analyzed using restricted maximum likelihood (REML), weighted by the numbers of seedlings scored. REML is a generalization of analysis of variance, also suitable for unbalanced data. The significance of differences between treatments was assessed using Wald tests.

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