Comparison of *Erysiphe cichoracearum* and *E. cruciferarum* and a Survey of 360 *Arabidopsis thaliana* Accessions for Resistance to These Two Powdery Mildew Pathogens

L. Adam,¹ S. Ellwood,² I. Wilson,¹ G. Saenz,³ S. Xiao,² R. P. Oliver,² J. G. Turner,² and S. Somerville¹

¹Department of Plant Biology, Carnegie Institution of Washington, Stanford, CA 94305, U.S.A.; ²Department of Biological Sciences, University of East Anglia, Norwich, NR4 7TJ, U.K.; ³Department of Plant and Microbial Biology, University of California, Berkeley 94720, U.S.A.

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In previous work, UEA1 and UCSC1, two geographically distinct, powdery mildew isolates, were recognized for their ability to infect *Arabidopsis thaliana*. We have clarified the identity of these isolates by determining their host ranges, reexamining their morphology, and comparing their DNA sequences for the 5.8S ribosomal RNA and two flanking internal transcribed spacer sequences. These experiments confirm that UEA1 is a member of *Erysiphe cruciferarum* and that UCSC1 belongs to *E. cichoracearum*. Interactions of the two *Erysiphe* isolates with 360 *A. thaliana* accessions were examined to provide a comprehensive profile of naturally occurring powdery mildew resistance in this weedy species. The majority of *A. thaliana* accessions (213) were susceptible to both isolates. Among the accessions exhibiting some degree of resistance, most (84) responded differentially to UEA1 and UCSC1 and the remainder were resistant to both isolates. Notably, resistance to UCSC1 cosegregated with *RPW7*, a locus previously demonstrated to confer resistance to UEA1 in Ms-0 × Landsberg (*erecta*) crosses. With this large collection of resistant accessions, questions about species specificity, genetic diversity and the evolution of resistance to powdery mildews can be addressed.

Additional keywords: cryogenic scanning electron microscopy.

Arabidopsis thaliana is widely recognized as a model host for pathology studies (Crute et al. 1994). In less than a decade, descriptions of *A. thaliana*-pathogen interactions have grown from simple reports of disease occurrence to detailed genetic and molecular characterizations of interactions with a number of biotrophic and necrotrophic pathogens (Kunkel 1996). Fungal diseases are considered the most economically significant diseases of plants and many fungi are known to infect *A. thaliana*, including members of the Plasmodiophoromycetes, Oomycetes, Ascomycetes, and Basidiomycetes. Genetic analyses of resistance to biotrophic fungal pathogens causing downy mildew (*Peronospora parasitica*), powdery mildew (*Erysiphe cruciferarum*, *E. cichoracearum*), and white blister rust (*Albugo candida*) have uncovered a large number of resistance loci ($n > 22$) throughout the *A. thaliana* genome (Holub 1997).

Both *E. cruciferarum* (Koch and Slusarenko 1990; Xiao et al. 1997) and *E. cichoracearum* (Adam and Somerville 1996) infect *A. thaliana*. Preliminary surveys revealed significant genetic diversity for powdery mildew resistance within *A. thaliana* (Adam and Somerville 1996; Xiao et al. 1997). Genetic analyses led to the identification of seven distinct resistance loci, referred to as *RPW* (recognition of powdery mildew) loci, on four of the five chromosomes (Adam and Somerville 1996; Xiao et al. 1997). Thus, like the better-characterized powdery mildew resistance in barley, powdery mildew resistance in *A. thaliana* is based on a number of distinct loci. These initial studies suggested that *A. thaliana* is a suitable model system for exploring powdery mildew resistance in plants, especially when coupled with the molecular genetic resources available for *A. thaliana*, such as map-based cloning and large-scale genome sequencing. Furthermore, the genomic colinearity apparent between *A. thaliana* and *Brassica* spp. (Kowalski et al. 1994; Lagercrantz et al. 1996) offers the possibility of identifying corresponding resistance genes in *Brassica* spp. utilizing genetic markers developed in *A. thaliana*. Additionally, the cloning of *A. thaliana* resistance genes may aid in the isolation of orthologues in unrelated species, as suggested by the finding that *A. thaliana* *RPW1* (= resistance to *Pseudomonas syringae* pv. *maculicola*, *avrRpm1*) ortho-

Corresponding author: Shauna Somerville
E-mail: Shauna@Andrew2.Stanford.Edu

Current address of L. Adam: Mendel Biotechnology, Inc., Hayward, CA 94545, U.S.A.

Current address of G. Saenz: Department of Biology, University of New Mexico, Albuquerque 87131, U.S.A.

Current address of R. P. Oliver: Carlsberg Laboratory, Department of Physiology, DK-2500 Copenhagen Valby, Denmark.

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L. Adam and S. Ellwood contributed equally to this project and are joint first authors.

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logues occur in soybean (Innes et al. 1993) and bean and pea (Dangl et al. 1992). Thus, information about the powdery mildew disease of *A. thaliana* will be of direct relevance to agronomic species.

As mentioned above, the two powdery mildew isolates that infect *A. thaliana* were tentatively identified as members of distinct species, *E. cichoracearum* and *E. cruciferarum*. Among the powdery mildews, perithecial characters such as number of asci, number of ascospores per ascus, and shape of cleistothecial appendages are normally used to assign an unknown isolate to a specific species (Junell 1967a; Parmelee 1977; Braun 1987). Morphological characteristics of the asexual state are also rich in diversity and can be used in the absence of the sexual state for classification purposes (Boesewinkel 1980). Only a few species can be classified based on a single asexual trait; however, combinations of traits can be used to classify isolates to the level of phylogenetic section and, in some instances, species (Ialongo 1992; Zeller 1995). More recently, DNA sequences of the genes encoding rRNAs (rDNA) have been used to classify members of the powdery mildews (Saenz et al. 1994; Zeller and Levy 1995; Saenz and Taylor 1999).

The powdery mildew pathogens that cause important diseases on cereals (*Blumeria graminis* [= *Erysiphe graminis*] f. sp.) exhibit highly specialized host ranges, with each form specialis causing disease on a distinct crop (Jørgensen 1988). For example, *B. graminis* f. sp. *hordei* infects only barley and *B. graminis* f. sp. *tritici* infects only wheat. Other species, such as *E. cichoracearum* and *E. polygoni*, are complex, polyphagous species (Salmon 1900; Junell 1967a; Yarwood 1978; Braun 1987). *E. cruciferarum* is the major pathogen to cause powdery mildew on crucifer species (Dixon 1981), while *E. cichoracearum* is the principal pathogen to cause powdery mildew on the Asteraceae (Salmon 1900; Junell 1967a; Braun 1987). Thus, host range, in spite of its limitations, can be a useful criterion for classifying several powdery mildews species in conjunction with morphological traits (Junell 1967a).

The primary goals of this study were to determine the species classification of powdery mildew isolates UCSC1 and UEA1 and to identify new sources of powdery mildew resistance among 360 *A. thaliana* accessions.

RESULTS

Classification of the UEA1 and UCSC1 powdery mildew isolates.

The taxonomy of the Erysiphales relies primarily on morphological characteristics of the sexual state. Unfortunately, cleistothecia, the fruiting bodies housing the asci and the ascospores, were not observed in either culture. However, because the taxonomic grouping of the isolates is critical to our interpretations of data collected in the general survey for resistance in *A. thaliana*, we extended our initial characterizations with more detailed comparisons of host range, morphological features of the asexual stage, and rDNA sequences.

Host range.

Host range is one of the most convenient characters to examine as a criterion for the classification of morphologically similar *Erysiphe* spp. Potential host species among the Brassicaceae, including *A. thaliana*, as well as members of the Apo-

cynaceae, Asteraceae, Balsaminaceae, Cucurbitaceae, and Solanaceae, were challenged with both UEA1 and UCSC1 (Table 1). The species were chosen in part because they had been reported to be hosts for either *E. cruciferarum* or *E. cichoracearum* (Amano 1986; Junell 1967a; Braun 1987). The disease reaction (DR) phenotype was scored by visual examination at 8 and 14 days post inoculation (d.p.i.) (Adam and Somerville 1996). In addition, plants resistant to UEA1 were examined by fluorescent microscopy with the lipophilic fluorochrome DiOC₆(3) to facilitate visualization of hyphae (Xiao et al. 1997).

The UEA1 isolate infected solely members of the Brassicaceae and most of the *Brassica* spp. cultivars tested were susceptible to UEA1. This observation is consistent with the classification of UEA1 as a member of *E. cruciferarum*. However, diversity for disease resistance was observed, with some cultivars of *B. rapa*, *B. oleracea*, and *Raphanus sativus* scored as highly resistant (DR = 0) to UEA1. Also, seven cultivars of *B. oleracea* supported only limited fungal growth (DR = 1). While susceptible *Brassica* spp. sustained profuse conidiation by UEA1, susceptible *A. thaliana* accessions (e.g., Col-0) supported relatively thin, sparse colonies. Koch and Slusarenko (1990) reported a similar observation for *A. thaliana* plants inoculated with a Swiss isolate of *E. cruciferarum*. When infected *A. thaliana* leaves were observed microscopically, however, thriving UEA1 mycelial growth and conidiation were apparent. Thus, despite the lack of obvious macroscopic symptoms, UEA1 successfully parasitized *A. thaliana* and was rated as a DR of 3 (Xiao et al. 1997).

The UCSC1 isolate displayed pronounced virulence on six members of the Cucurbitaceae. On tobacco, UCSC1 growth was weak and apparent only after a prolonged incubation period (i.e., 16 d.p.i.). Surprisingly, *A. thaliana* was the only Brassicaceae species tested that was fully susceptible to UCSC1, an important observation for the taxonomy of this isolate. In contrast to UEA1, UCSC1 formed conspicuous, well-developed, dense mycelial mats on the surface of inoculated *A. thaliana* leaves. On the susceptible accession, Col-0, UCSC1 growth was apparent as early as 5 d.p.i. and macroscopic evaluation was most reliable at 7 to 8 d.p.i., when colonies had coalesced and abundant conidiation had occurred.

Members of the Apocynaceae (*Vinca minor* cv. Pacifica Red), Balsaminaceae (*Impatiens balsamina* cv. Shady Lady), and Asteraceae (*Achillae milfoilum* cv. Milfoil-Cerise Queen, *Ageratum houstonianum* cvs. Adriatic and Blue Horizon, *Aster* sp. cv. Bouquet Powderpuffs, *Calendula officinalis* cvs. BonBon Apricot and Golden Prince, *Cosmos sulphureus* cv. Ladybird, *Dahlia pinnata* cv. Sunny Red, *Dendranthema chrysanthemum* cv. Spray Mix, *Gaillardia pulchella* cv. Red Flume, *Senecio cineraria* cv. Cindy, and *Tagetes patula* cv. Aurora-Yellow Fire) were also tested and found to be resistant to both UEA1 and UCSC1.

Morphological characteristics of the asexual state.

Microscopic examination by cryogenic scanning electron microscopy was used to identify distinctive morphological features of the asexual state that could be used to further differentiate UEA1 and UCSC1 (Fig. 1). Important asexual features traditionally used for classification purposes are as follows: (i) conidium size, shape, and surface features; (ii)

conidiophore type (euoidium, in which conidia are formed in chains, or pseudoidium, in which conidia are generated singly), the presence or absence of branching, and size and shape of the conidiophore; (iii) germination pattern; and (iv) appressorium shape (indistinct or nipple-shaped versus lobed) (Junell 1967a; Boesewinkel 1980; Braun 1987; Ialongo 1992; Shin and La 1993; Zeller 1995).

Conidia of UEA1 were ellipsoid to cylindrical in shape (38 to 46 × 13 to 16 µm) with a length/width ratio of approximately 3 (Fig. 1A, B). Small, wartlike projections and fine ridges covered the conidial surface. These extended along the appressoria and hyphae but were not present in regions of new growth. A characteristic, multilobed appressorium formed at one end of the conidium (Fig. 1A, 1A insert, and B) and was normally accompanied by the emergence of the first hypha from the same end of the conidium. The subsequent hypha developed from the opposite end (Fig. 1B). Along hyphae at regular intervals, multilobed appressoria were observed at the

points of penetration leading to haustoria formation (Fig. 1C). Initially, at the actively growing hyphal ends, the pattern of hyphal branching tended to be alternate but later opposite branches formed to give matching pairs. Hyphal branches of UEA1 typically emerged at less than 45 degrees (Fig. 1D). On *A. thaliana*, conidia were produced singly on short erect conidiophores (75 to 89 × 7.5 to 8.5 µm) composed of three cells rising perpendicularly from the upper hyphal surface (Fig. 1E). On *B. napus* cv. Cobra and on susceptible *Brassica* spp. generally, conidia were generated more rapidly and abundantly. Particularly when undisturbed, these mature conidia remained loosely attached to the conidiophore forming lengthy chains of conidia of similar shape and size (Fig. 1E insert). Such chains were rarely observed on *A. thaliana* (Fig. 1E). This fact together with the less prolific mycelial growth on *A. thaliana* may account for the difference in powdery mildew symptoms visible to the naked eye between susceptible *A. thaliana* and *Brassica* spp.

Table 1. Host range of *Erysiphe cruciferarum* UEA1 and *E. cichoracearum* UCSC1

Family/species	Commercial variety	DR score ^a	
		UEA1	UCSC1
Brassicaceae			
<i>Arabidopsis thaliana</i> Heynh.	Columbia (Col-0)	3 ^b	3
<i>Brassica juncea</i> (L.) Czerniak var. <i>rugosa</i>	Red Giant (mustard)	3	0
<i>Brassica napus</i> L. var. <i>pabularia</i>	Cobra (canola)	3	0
	Tower (canola)	3	— ^c
<i>B. napus</i> L. var. <i>napobrassica</i>	American Purple (rutabaga)	3	0
<i>B. oleracea</i> L. var. <i>acephala</i>	Dwarf Blue Scotch (kale)	1	0
	Winterbor (kale)	1	0
	Georgia (collard)	1	0
	Top Bunch (collard)	2	0
<i>B. oleracea</i> L. var. <i>botrytis</i>	Milkyway (hybrid cauliflower)	1	0
<i>B. oleracea</i> L. var. <i>capitata</i>	Copenhagen Market	2	—
	Elisa Hybrid (hybrid cabbage)	3	—
	Golden Cross (hybrid cabbage)	2	0
	Red Rookie (hybrid cabbage)	1	0
	Solarite (hybrid cabbage)	1	0
	Tasty Hybrid (hybrid cabbage)	2	0
	Tropic Giant (hybrid cabbage)	2	0
<i>Brassica oleracea</i> L. var. <i>gongylodes</i>	Express Forcer (kohlrabi)	0	—
	Purple Danube (kohlrabi)	1	0
<i>Brassica oleracea</i> L. var. <i>gemmifera</i>	Jade Cross (hybrid Brussels sprouts)	2	0
<i>Brassica rapa</i> L. var. <i>rapifera</i>	All Top (turnip)	3	0
	Purple Top White Globe (turnip)	3	0
	Seventop (turnip)	3	0
<i>Brassica rapa</i> L. var. <i>chinensis</i>	Jade Pagoda (hybrid Chinese cabbage)	0	0
	Pak Choi Joi Choi (hybrid pak-choi)	2	0
<i>Raphanus sativus</i> L.	Easter Egg (radish)	0	—
Cucurbitaceae			
<i>Cucumis melo</i> L. var. <i>inodorus</i>	Casaba Oasis (melon)	0	3
<i>Cucumis melo</i> L. var. <i>cantalupensis</i>	Ambrosia (cantaloupe)	0	3
<i>Cucumis sativus</i> L.	Salad Bush (cucumber)	0	3
<i>Citrullus lanatus</i> (Thunb.) Matsum & Nakai	Park's Whopper (watermelon)	0	3
<i>Cucurbita pepo</i> L.	Lumina (pumpkin)	0	3
<i>Cucurbita maxima</i> Duchesne ex Lam.	Kuta (squash)	0	3
Solanaceae			
<i>Nicotiana tabacum</i> L.	Madole (tobacco)	0	1

^a Disease reaction (DR) scores: 0, limited hyphal development with no conidiation (i.e., fully resistant phenotype); 1, low to moderate hyphal growth with sporadic conidiation; 2, abundant mycelial development with moderate or delayed conidiation; and 3, profuse mycelial development and conidiation (i.e., fully susceptible phenotype).

^b The most compatible interactions between UEA1 and *A. thaliana* were rated as a DR of 3 to reflect the fact that UEA1 is able to successfully parasitize *A. thaliana* and produce abundant conidiophores. Thus, on *A. thaliana*, a DR score of 3 reflects the fully compatible state for UEA1 and for UCSC1. As described in text, UEA1 infections on *A. thaliana* are difficult to evaluate by eye in part because UEA1 conidiophores bear a single conidium; therefore, UEA1 infections were scored microscopically rather than macroscopically by eye. On all other plant species, both UEA1 and UCSC1 infections were scored macroscopically.

^c Not determined.

As previously reported (Adam and Somerville 1996), UCSC1 had ovoid to ellipsoid conidia (26 to 33 × 16 to 19 μm) with a length/width ratio of less than 2 (Fig. 1F). UCSC1 conidia were smooth surfaced with germ tubes emerging at

one end bearing unlobed, club-shaped appressoria. The second germ tube usually developed from the opposite end as early as 24 h post inoculation (h.p.i.) (Fig. 1F, G). Hyphal appressoria were unlobed, indistinct, and often evident only as small pro-

Fig. 1. Cryogenic scanning electron micrographs illustrating morphological differences between *Erysiphe cruciferarum* UEA1 (left column) and *E. cichoracearum* UCSC1 (right column). **A**, At 12 h post inoculation (h.p.i.), conidium (c) is ellipsoid to cylindrical with a ridged surface. Appressorial germ tube (agt) is typically lobed but can be a complex (insert) multilobed structure. **B**, At 24 to 36 h.p.i., first hypha (hy) has emerged from the same end as the appressorial germ tube, which has continued to elongate. Third hypha followed from the distal end. **C**, At 2 days post inoculation (d.p.i.), lobed appressoria (ap) are present at regular intervals along the hyphae. **D**, At 3 d.p.i., secondary hyphae emerge at less than 45°, forming a network. **E**, At 7 d.p.i., conidia are produced singly from relatively short conidiophores (cp). On *Brassica napus* cv. Cobra in undisturbed conditions, conidia remain attached to conidiophore, forming chains (insert). **F** and **G**, At 12 to 36 h.p.i., conidia are ovoid to ellipsoid with a smooth surface. Appressorial germ tube is unlobed (club-shape) and often cannot be distinguished from the first hypha emerging at the opposite end. **H**, At 2 d.p.i., hyphal appressoria are unlobed, indistinct, and apparent as small protuberances along the hyphae. **I**, At 3 d.p.i., secondary hyphae emerge at a 45° to 90° angle from main hypha. **J**, At 7 d.p.i., abundant conidiation is apparent. Chains of 3 to 5 bulbous cells are produced, representing conidia at different differentiation stages.

tubercles along the hyphae (Fig. 1H). The third and fourth hyphae arose from conidia by 3 to 5 d.p.i. Early in development, hyphal branches generally alternated to either side at regular intervals (Fig. 1I) but were often found as opposing pairs later in development. These branches usually emerged at 45 to 90 degrees relative to the main hypha (Fig. 1I). The most striking difference between UCSC1 and UEA1 was in the type of conidiophore. Conidiophores of UCSC1 were significantly longer and conidia were formed in chains of 3 to 5 bulbous cells, representing conidia at different stages of differentiation (Fig. 1J). The UCSC1 conidiophore stalks (88 to 160 × 9.5 to 11 µm) consisted of three elongated cells including a curved foot cell.

Sequence comparison of DNA encoding the 5.8S rRNA, ITS1, and ITS2.

The rDNA gene cluster contains both highly conserved ribosomal subunits and variable internal transcribed spacer (ITS) regions that can be compared to evaluate the phylogenetic relationships among related fungal species and races within species (White et al. 1990; O'Donnell 1992). The nucleotide sequences for both isolates were obtained from polymerase chain reaction (PCR) fragments defined by the primers NS7 and ITS4 (White et al. 1990). Each sequence included a portion (UCSC1, 384 bp; UEA1, 363 bp) of the 3' end of the 18S rDNA, the complete 5.8S rDNA subunit (154 bp), and the two flanking internal transcribed spacers, ITS1 and ITS2, as well as a 57- to 60-bp portion of the nuclear large 28S rDNA (Fig. 2). ITS1 varied in size from 223 to 187 bp between UEA1 and UCSC1, respectively. The ITS2 region was also smaller in UCSC1 (165 bp) than in UEA1 (184 bp). As expected, the 3' end of 18S, the 5.8S, and 5' end of 28S rDNA sequences displayed a high degree of conservation between the two *Erysiphe* isolates, with 4, 1, and 2 bp changes in the respective segments. The occurrence of base pair changes in the conserved rDNA subunit genes in addition to a number of base pair changes in ITS1 and ITS2 confirmed, at the molecular level, that both isolates were from distinct phylogenetic groups (Saenz et al. 1994; Hirata and Takamatsu 1996; Saenz and Taylor 1999).

The sequences were aligned relative to each other, matching sequences of *B. graminis* f. sp. *hordei* and eight other powdery mildews for a phylogenetic reconstruction (Saenz and Taylor 1999) (Fig. 3). The tree is a strict consensus of the eight most parsimonious trees, which were found by a heuristic search employing the random stepwise addition option of PAUP (Swofford 1993). Branch support was determined by 1,000 bootstrapped data sets, which are displayed on the tree as numbers above the branches. The tree was 262 steps in length; the consistency index was 0.828, the retention index was 0.763, and the re-scaled consistency index was 0.632 (Farris 1989). This analysis showed that the UCSC1 and the UEA1 isolates are distinct *Erysiphe* spp. The UCSC1 isolate grouped with other *E. cichoracearum* isolates and *E. orontii*, while the UEA1 isolate grouped with *E. polygoni* and *E. convolvuli*.

Survey for powdery mildew-resistant *A. thaliana* accessions.

To expand our collection of powdery mildew resistant-accessions, we extended previous screens by incorporating an

extensive collection of *A. thaliana* accessions (360) from locations throughout the world. These accessions originate from a number of countries with a majority of accessions collected in Germany (156). Several groups of accessions, collected within only a few kilometers of one another, were also included in this survey to investigate the potential diversity within such groups. Accessions exhibiting a differential response to UEA1 and UCSC1 are listed in Table 2. Accessions that were susceptible (i.e., DR scores of 2 or 3) to both UEA1 and UCSC1 included the following: An-1, An-2, Ba-1, Bay-0, Bch-0, Bch-1, Bch-3, Bch-4, Bd-0, Be-0, Be-1, Bla-1, Bla-2,

Fig. 2. Alignment of rDNA sequences consisting of the 3' end of the 18S subunit, ITS1, 5.8S subunit, ITS2, and the 5' end of the 28S subunit. UEA1, *Erysiphe cruciferarum* UEA1; UCSC1, *E. cichoracearum* UCSC1. Hyphen denotes a gap; periods represent identical nucleotides; asterisks identify nucleotide differences between the two sequences.

Bla-3, Bla-5, Bla-6, Bla-11, Bla-12, Blh-2, Bs-1, Bs-2, Bscl-0, Bscl-1, Bscl-2, Bscl-4, Bu-4, Bu-6, Bu-7, Bu-9, Bu-13, Bu-17, Bu-20, Bu-25, Bur-0, Ca-0, Cal-0, Cat-1, Cha-0, Chi-0, Cnt-1, Co-2, Co-3, Col-0, Col-1, Col-3, Col-4, Col-5, Cvi-0, Da-0, Db-0, Db-1, Db-2, Db-3, Di-0, Di-1, Dijon-G, Dijon-M, Dr-0, Ei-6, En-2, Enkheim-D, Ep-0, Er-0, Erecta, Est-0, Est-1, Estland, Fi-1, Fi-4, Fr-2, Fr-3, Fr-4, Fr-5, Fr-6, Fr-7, Ga-2, Gc-0, Gd-1, Ge-1, Ge-2, Gie-0, Gr-1, Gr-2, Gr-4, Gr-6, Gre-0, Gü-0, Gü-1, H55, Ha-0, Hh-0, Hi-0, Hl-0, Hl-2, In-0, Is-0, Is-1, Ita-0, Je-1, Jl-1, Jl-4, Jm-1, Kb-0, Kin-0, Kl-0, Kl-1, Kl-2, Kl-3, Kl-4, Kl-5, Ko-2, Kro-0, La-0, Lan-0, Lc-0, Ler, Li-3, Li-5, Li-7, Li-8, Ll-1, Ll-2, Lm-2, Lö-1, Lö-2, Lu-0, Lu-1, Ma-0, Ma-2, Mc-0, Me-0, Mh-0, Mh-1, Mr-0, Mrk-0, Mv-0, Na-1, Nc-1, Nd-0, Nd-1, Nie-0, Nw-1, Nw-2, Nw-4, Ob-1, Ob-3, Old-1, Old-2, Or-0, Oy-0, Pa-0, Pa-1, Pa-2, Pa-3, Per-2, Per-3, Pf-0, Pla-0, Pr-0, Pt-0, Po-0, Ra-0, Ri-0, Rld-2, Rou-0, Rsch-0, Rsch-4, Ru-0, S96, Sav-0, Sei-0, Sf-1, Sf-2, Sg-2, Sh-0, Si-0, Ste-0, Sy-1, Ts-1, Ts-2, Ts-3, Ts-5, Ts-6, Tsh-0, Tsh-1, Tsu-0, Tu-0, Tu-1, Tul-0, Ty-0, Uk-2, Uk-3, Uk-4, Van-0, Vi-0, Wc-1, Wc-2, Wei-0, Wei-1, Wil-0, Wil-2, Wil-3, Ws, Ws-0, Ws-2, Wt-4, Wü-0, X-0, Xx-0, Xxx-0, Yo-0, Ze-0, and Zü-1.

In the analysis below, accessions were grouped into two broad categories: "susceptible" (i.e., accessions with DR scores of 2 or 3) or "resistant" (i.e., accessions with DR scores

of 0 or 1). Roughly 41% or 147 of 360 *A. thaliana* accessions were resistant to either UEA1 or UCSC1 (Table 3). Among this group, 84 accessions (57% of resistant accessions) exhibited species-specific resistance, with a greater proportion of accessions resistant to UEA1 (54) than to UCSC1 (30).

Among 144 accessions that were fully susceptible (DR = 3) to UEA1, 71% were also fully susceptible to UCSC1 (Table 3). A similar observation was made among the resistant class (DR = 0), in which 53% of the accessions rated at fully resistant to UEA1 were also fully resistant to UCSC1. A contingency test suggested that disease resistance to these two isolates tended to be positively associated ($P < 0.01$; Snedecor and Cochran 1967). Although a majority of accessions displayed similar DR scores when inoculated with either UEA1 or UCSC1, UCSC1 was more likely than UEA1 to be virulent on *A. thaliana* accessions.

Geographically, the ratio of resistant/susceptible accessions among the populations tested revealed no major bias when specific countries were considered. One exception was the U.S., where all 10 accessions were susceptible to both isolates. This bias may reflect the fact that *A. thaliana* is not native to the U.S. and the genetic base is narrow. Also, none of the 11 accessions collected in Italy were resistant to UCSC1.

Phenotypic variation for DR scores was observed among individual accessions collected at one site. For example, among 23 accessions collected at Burghaun/Rhon, Germany (i.e., the Bu-series), six were scored as resistant and eight were susceptible to both isolates. The remaining accessions exhibited a differential response to inoculations with UEA1 and UCSC1. At a few sites, all accessions gave similar DR phenotypes (e.g., Bscl-, Col-, and Db-), and may represent individuals from a population with limited genetic diversity.

Do *RPW4* and *RPW7* confer resistance to both UEA1 and UCSC1?

The general survey of *A. thaliana* accessions established that the accessions Su-0 and Ms-0 were resistant to both *Erysiphe* isolates. Previously, Su-0 was shown to carry resistance alleles effective against UCSC1 at *RPW4* on chromosome 4 (Adam and Somerville 1996). In an initial mapping population of 27 F_3 (Col-*gll* × Su-0) families, segregation analysis showed that resistance to UEA1 occurred as a single, nuclear locus (genetic model: 1:2:1, $\chi^2 = 2.48$, d.f.=2, $P > 0.25$) (Table 4). However, this locus was distinct from *RPW4* as resistance/susceptibility to the two *Erysiphe* isolates cosegregated in only 14 of 27 F_3 families (Contingency $\chi^2 = 4.15$, d.f. 4, $P > 0.5$) (Snedecor and Cochran 1967).

In the *A. thaliana* accession Ms-0, resistance to UEA1 depends on both *RPW6*, located on chromosome 5, and *RPW7* on chromosome 3 (Xiao et al. 1997). Neither resistance locus by itself conferred resistance to UEA1, as measured by an absence of asexual reproduction. On more careful examination, *RPW6*/*RPW6*, *rpw6*/*rpw6* and *rpw6*/*RPW6*, *RPW7*/*RPW7* genotypes exhibited an intermediate phenotype, in which asexual reproduction occurred but at a reduced level and necrosis of epidermal cells was observed. For mapping experiments, plants with an intermediate phenotype were classed as susceptible because conidiophores and asexual conidia were produced. Based on a population of 100 F_3 (Ms-0 × L-er) families, resistance to UCSC1 cosegregated absolutely with the *RPW7* locus (Table 5). This observation is made all the

Fig. 3. Phylogenetic analysis of UEA1, UCSC1, and nine other powdery mildew fungi based on the sequences shown in Figure 2 (Saenz and Taylor 1999). Strongly supported (95%) branches are shown in bold. Numbers shown are frequencies of branches in phylogenies estimated from bootstrap sampled data bases (Felsenstein 1985). Only values >60% are reported. *M. platani*, *Microsphaera platani*; *P. guttata*, *Phylactinia guttata*; *S. pannosa*, *Sphaerotheca pannosa*; *B. graminis hordei*, *Blumeria graminis* f. sp. *hordei*.

Table 2. *Arabidopsis thaliana* accessions showing full or intermediate resistance to *Erysiphe cruciferarum* UEA1 or *E. cichoracearum* UCSC1

Accession	Location - City (Country) ^a	DR score ^b		Accession	Location - City (Country) ^a	DR score ^b	
		UEA1	UCSC1			UEA1	UCSC1
Aa-0	Aua-Rhoen (GER)	2	1	Jm-2	Jamolice (CZR)	0	2
Ag-0	Argentat (FRA)	2	0	Kä-0	Karnten (AUT)	0	3
Ak-1	Achkarren/Freiburg (GER)	3	1	Kas-1	Kashmir (IND)	0	0
Al-0	Calver (UK)	1	3	Kil-0	Killeen (UK)	1	3
Ang-0	Angleur (BEL)	2	0	Kr-0	Krefeld (GER)	1	3
Ang-1	Angleur (BEL)	1	3	Ksk-1	Keswick (UK)	0	0
Bl-1	Bologna (ITA)	1	3	La-1	Landsberg-Warthe (POL)	0	0
Bla-10	Blanes/Gerona (ESP)	2	0	Le-0	Leiden (NTH)	1	3
Bla-14	Blanes (ESP)	1	1	Li-1	Limburg (GER)	0	2
Bla-4	Blanes/Gerona (ESP)	0	2	Li-10	Limburg (GER)	1	1
Blh-1	Bulhary (CZR)	1	2	Li-2	Limburg (GER)	1	2
Br-0	Brunn (Brno)/(CZR)	1	1	Li-6	Limburg (GER)	1	3
Bs-5	Basel (CHE)	1	1	Lip-0	Lipowicz/Chrzanow (POL)	1	1
Bu-0	Burghaun/Rhon (GER)	0	3	LI-0	Llagostera (ESP)	2	1
Bu-10	Burghaun/Rhon (GER)	1	3	Loh-0	Unknown	1	3
Bu-11	Burghaun/Rhon (GER)	0	3	Lz-0	Lezoux, Puy-de-Dome (FRA)	3	1
Bu-14	Burghaun/Rhon (GER)	1	3	Mau-6	Unknown	0	0
Bu-15	Burghaun/Rhon (GER)	1	2	Mir-0	Miramare/Trieste (ITA)	0	2
Bu-18	Burghaun/Rhon (GER)	0	0	Mnz-0	Mainz (GER)	3	1
Bu-19	Burghaun/Rhon (GER)	1	3	Ms-0	Moscow (RUS)	0	0
Bu-2	Burghaun/Rhon (GER)	1	1	Mt-0	Martuba/Cyrenaika (LBY)	2	1
Bu-21	Burghaun/Rhon (GER)	0	2	Mz-0	Merzhausen/Ts. (GER)	1	1
Bu-22	Burghaun/Rhon (GER)	1	1	No-0	Nossen (GER)	1	2
Bu-23	Burghaun/Rhon (GER)	3	0	Nok-0	Noordwijk (NTH)	0	0
Bu-24	Burghaun/Rhon (GER)	1	1	Nok-1	Noordwijk (NTH)	0	0
Bu-3	Burghaun/Rhon (GER)	2	0	Nok-2	Noordwijk (NTH)	1	3
Bu-5	Burghaun/Rhon (GER)	1	1	Nok-3	Noordwijk (NTH)	0	0
Bu-8	Burghaun/Rhon (GER)	0	1	Np-0	Nieps/Salzwedel (GER)	2	1
C24	Landsberg (GER)	0	0	Nw-0	Neuweilnau (GER)	0	0
Cha-1	Valais (CHE)	1	1	Nw-3	Neuweilnau (GER)	3	0
Chi-1	Chisdra (RUS)	1	1	Ob-0	Oberursel/Hasen (GER)	3	0
Chi-2	Chisdra (RUS)	1	3	Ob-2	Oberursel/Friedhof (GER)	1	3
Cit-0	Citou, Aude (FRA)	0	0	Ove-0	Ovelgoenne (GER)	0	2
Cl-0	Unknown	1	1	Oy-1	Oystese (NOR)	0	3
Co-1	Coimbra (PRT)	0	0	Per-0	Perm (RUS)	1	2
Co-4	Coimbra (PRT)	0	0	Per-1	Perm (RUS)	0	3
Condara	Tadjikistan (TKS)	1	0	Petergof	Petergof (RUS)	0	0
Ct-1	Catania (ITA)	0	3	Pi-0	Pitztal/Tirol (AUT)	2	1
Di-2	Dijon (FRA)	0	2	Pi-2	Pitztal/Tirol (AUT)	3	1
Do-0	Donsbach/Westerw. (GER)	0	3	Pla-1	Playa de Aro (ESP)	0	0
Dra-0	Drahonin (CZR)	3	0	Pla-2	Playa de Aro (ESP)	0	0
Dra-1	Drahonin (CZR)	0	0	Pla-3	Playa de Aro (ESP)	0	0
Dra-2	Drahonin (CZR)	0	0	Pla-4	Playa de Aro (ESP)	0	2
Edi-0	Edinburgh (UK)	1	1	Po-1	Poppelsdorf (GER)	1	3
Ei-2	Eifel (GER)	1	1	Pog-0	Point Grey, B.C. (CAN)	1	2
Ei-4	Eifel (GER)	0	0	Rak-2	Raksice (CZR)	0	2
Ei-5	Eifel (GER)	0	0	Rd-0	Rodenbach/Dill (GER)	2	1
El-0	Ellershausen (GER)	1	1	RLD1	Netherlands (NTH)	1	2
Ema-1	East Malling (UK)	1	3	Sah-0	Sierra Alhambra (ESP)	0	0
En-1	Enkheim/Frankfurt (GER)	1	3	Sap-0	Slapy (CZR)	1	2
Enkheim-T	Tadjikistan (TKS)	1	3	Se-0	San Eleno (ESP)	2	1
Es-0	Espoo (FIN)	3	0	Sg-1	St.Georgen (GER)	0	3
Et-0	Etraygues (FRA)	2	0	Shahdara	Palmiro-Alay, Tadjikistan (TKS)	0	0
Fe-1	Freiburg (GER)	1	2	SI-0	Unknown	1	0
Fi-0	Frickhofen (GER)	1	1	Sorbo	Tadjikistan (TKS)	0	0
Fl-1	Finland (FIN)	0	0	Sp-0	Berlin/Spandau (GER)	1	0
Fl-3	Finland (FIN)	1	1	St-0	Stockholm (SWE)	2	1
Ga-0	Gabelstein (GER)	0	0	Stw-0	Stobowa/Orel (RUS)	1	0
Ge-0	Geneva (CHE)	0	1	Su-0	Southport (UK)	0	1
Gö-0	Göttingen (GER)	2	1	Sue-0	Granada (ESP)	3	1
Gö-2	Göttingen (GER)	1	0	Sy-0	Isle of Skye (UK)	0	2
Gr-3	Graz (AUT)	0	2	Ta-0	Tabor (CZR)	0	2
Gr-5	Graz (AUT)	0	3	Te-0	Tenela (FIN)	2	1
Gy-0	La Miniere (FRA)	0	0	Ts-7	Tossa de Mar (ESP)	0	0
Hl-3	Holtensen (GER)	1	3	Uk-1	Umkirch (GER)	0	0
Hn-0	Hennetalsperre (GER)	1	1	Wa-1	Warschau (POL)	0	0
Hodja	Tadjikistan (TKS)	1	2	Wl-0	Wilbad (GER)	3	1
Hs-0	Hannover/Stroehen (GER)	3	1	Ws-3	Wassilewskija (BLR)	1	2
Je-0	Jena (GER)	0	1	Wt-1	Wietze (GER)	2	1
Je54	Unknown	0	2	Wt-2	Wietze (GER)	0	0
Jl-2	Vranov u Brno (CZR)	3	0	Wt-3	Wietze (GER)	0	3
Jl-3	Vranov u Brno (CZR)	1	1	Wt-5	Wietze (GER)	0	3
Jl-5	Vranov u Brno (CZR)	2	0	Zü-0	Unknown	1	1
Jm-0	Jamolice (CZR)	1	0				

^a AUT, Austria; BEL, Belgium; BLR, Belarus; CAN, Canada; CHE, Switzerland; CZR, Czech Republic; ESP, Spain; FIN, Finland; FRA, France; GER, Germany; IND, India; ITA, Italy; LBY, Libya; NOR, Norway; NTH, The Netherlands; POL, Poland; PRT, Portugal; RUS, Russia; SWE, Sweden; TKS, Tajikistan; UK, United Kingdom.

^b Disease reaction score: 0, fully, and 1, intermediate resistant; 2, intermediate, and 3, fully susceptible. See Table 1 footnotes 'a' and 'b' for complete description.

more noteworthy because 40 of the 100 F₃ families were selected as being recombinant between two markers, BGL1 and CDC2A, that define a 13-cM interval encompassing *RPW7*. Based on this observation, *RPW7* and the locus conferring resistance to UCSC1 are either the same or are separated by less than 0.5% recombination.

DISCUSSION

Classification of the UEA1 and UCSC1 powdery mildew isolates.

As a first step to resolving the classification of UEA1 and UCSC1 and establishing the phylogenetic relationship between these two isolates, we investigated their host ranges. Although the taxonomic value of host range is controversial, there are clear host limitations among *Erysiphe* spp. (Junell 1967a; Yarwood 1978; Braun 1987). *Brassica* spp. have been reported as hosts for a number of Erysiphales: Amano (1986) identified *E. arabidis*, *E. cichoracearum*, *E. communis*, *E. cruciferarum*, *E. rorippae*, *Leveillula cruciferarum*, *L. taurica*, *Microsphaera alni*, various *Oidium* spp., *Sphaerotheca fulginea*, *S. drabae*, and *S. macularis*. Several authors have independently reported *E. polygoni*, *E. arabidis*, *E. cruciferarum*, *E. orontii*, and *S. drabae* on crucifers (Spencer 1978; Plotnikova et al. 1998). In addition, one powdery mildew species may infect multiple host species. For example, *E. cichoracearum* has been narrowly defined by Braun (1987) as consisting of those powdery mildews infecting the Asteraceae, whereas others have suggested that all powdery mildews mor-

phologically similar to *E. cichoracearum* should be regarded as members of this species regardless of their host specialization (Salmon 1900; Schmitt 1955; Yarwood 1978). Amano (1986) alone has reported *E. cichoracearum* on more than 55 plant families.

The UEA1 isolate thrived exclusively on the Brassicaceae, with a relatively sparse growth pattern on susceptible *A. thaliana* accessions (Table 1). The pronounced susceptibility among *B. napus* cultivars to UEA1 may reflect the relatively recent evolution of this amphidiploid species from susceptible progenitors. *Brassica napus* likely arose from crosses between *B. rapa* and *B. oleracea*, which may have occurred only once or twice (Warwick and Black 1991). The *B. rapa* group, with the exception of its wild progenitor *B. campestris*, was susceptible to UEA1. The host range of UCSC1 was limited primarily to the Cucurbitaceae. The major exceptions were *A. thaliana* and *Arabidopsis lyrata* (D. J. Jacobson, personal communication), the only crucifers of the 22 tested that were found to be susceptible to UCSC1. This illustrates the capacity of some *Erysiphe* isolates, like UCSC1, to successfully propagate on particular host plant species, while being generally avirulent within the family. This example also illustrates the danger of relying solely on the host from which an isolate was recovered to define its host range (Schmitt 1955).

Morphological features at the asexual stage further distinguished UEA1 and UCSC1. Inclusion of UCSC1 into the genus *Erysiphe* is supported by the occurrence of sinuate conidial edges of immature conidia, a classification scheme developed by Shin and La (1993). *Erysiphe* spp. have been divided into three sections designated *Erysiphe*, *Golvinomyces*, and *Galeopsidis*. Section *Erysiphe* consists of species with lobed appressoria and conidiophores composed of one to three cylindrical cells followed by a single conidium released on maturation (Pseudoidium type) (Braun 1987; Zeller 1995).

Table 3. Summary of disease reaction (DR) scores^a

DR score with UCSC1	DR score with UEA1 ^b			
	0 ^c	1	2	3
0	32	6	6	6
1	4	21	11	7
2	13	11	41	29
3	11	19	41	102

^a Each cell indicates number of *Arabidopsis thaliana* accessions exhibiting the given DR score following inoculation with either *Erysiphe cruciferarum* UEA1 or *E. cichoracearum* UCSC1.

^b Contingency $\chi^2 = 161$ (d.f. = 9; $P < 0.01$) for the null hypothesis that the UEA1 DR score is independent of the UCSC1 DR score (Snedecor and Cochran 1967).

^c DR score: 0, fully resistant; 1, intermediate resistant; 2, intermediate susceptible; 3, fully susceptible. See Table 1 footnotes 'a' and 'b' for a more complete description of the scoring categories.

Table 4. Number of F₃ (Col-*gll* × Su-0) families with given genotype^a

F ₂ genotype with UEA1 ^b	F ₂ genotype with UCSC1 ^b			
	Σ	R	H	S
R	5	2	2	1
H	12	3	6	3
S	10	2	2	6
	27	Σ = 7	Σ = 10	Σ = 10

^a Each family was scored following inoculation with *Erysiphe cruciferarum* UEA1 and *E. cichoracearum* UCSC1. A minimum of 32 (two batches of 16–20) individuals from each family were scored to minimize effects of random sampling on detection of segregating families.

^b F₂ genotypes were inferred from the phenotypes of members of each F₃ family. Individual F₃ families were scored as "R" if all members of the family were resistant; "S" if all members were susceptible; and "H" if the family consisted of both resistant and susceptible members.

Table 5. Number of F₃ (Ms-0 × L-*er*) families with given genotype^a

F ₂ genotype with UEA1 ^b	Predicted genotype ^c		Σ	F ₂ genotype with UCSC1 ^b		
				R	H	S
R	<i>R6R6</i>	<i>R7R7</i>	6	6	0	0
H	<i>R6r6</i>	<i>R7R7</i>	12	12	0	0
	<i>R6R6</i>	<i>R7r7</i>	4	0	4	0
S	<i>R6r6</i>	<i>R7r7</i>	23	0	23	0
	<i>r6r6</i>	<i>R7R7</i>	8	8	0	0
	<i>r6r6</i>	<i>R7r7</i>	11	0	11	0
	<i>R6R6</i>	<i>r7r7</i>	4	0	0	4
	<i>R6r6</i>	<i>r7r7</i>	21	0	0	21
	<i>r6r6</i>	<i>r7r7</i>	11	0	0	11
			100	Σ = 26	Σ = 38	Σ = 26

^a Each F₃ family was scored following inoculation with *Erysiphe cruciferarum* UEA1 and with *E. cichoracearum* UCSC1. A minimum of 32 (two batches of 16–20) individuals from each family were scored.

^b F₂ genotypes were inferred from the phenotypes of members of each F₃ family. Individual F₃ families were scored as "R" if all members of the family were resistant; "S" if all members were susceptible; and "H" if the family consisted of both resistant and susceptible members.

^c The predicted genotypes at the *RPW6* and *RPW7* loci for the F₃ (Ms-0 × L-*er*) families were deduced from flanking molecular markers in previous work (Xiao et al. 1997). *R6* and *R7* are Ms-0-derived resistance alleles at *RPW6* and *RPW7*, respectively, and *r6* and *r7* are L-*er*-derived susceptibility alleles at *RPW6* and *RPW7*, respectively. *R7* alone confers resistance to *E. cichoracearum* UCSC1. *R6* and *R7* together, but not singly, confer resistance to *E. cruciferarum* UEA1 (Xiao et al. 1997).

The UEA1 isolate clearly belongs to the *Erysiphe* section. In section *Golvinomyces*, conidia are formed in chains (Euoidium type) typically consisting of three to five conidia, while the appressoria are nipple-shaped or indistinct. Isolate UCSC1 exhibits the features found in section *Golvinomyces*.

The rDNA analysis confirmed the morphological and host range-based identification of the isolates. UCSC1 clearly grouped into a phylogenetic branch with two different *E. cichoracearum* isolates and *E. orontii*, while UEA1 belonged to a separate group (Fig. 3). UEA1 was grouped loosely with *E. polygoni* and *E. convolvuli*, which is not so surprising given the morphological similarity of their asexual states.

Overall, host range and morphological characteristics support the classification of the UEA1 isolate as *E. cruciferarum*, the most prevalent species of *Erysiphe* to infect Brassicaceae (Dixon 1981). Taxonomically, the species *E. cruciferarum* is the result of a revision of *E. communis* by Junell (1967b), who recognized well-defined forms and host specialization among members of the complex compound species *E. polygoni* sensu Salmon. Currently, *E. polygoni* is restricted to *Erysiphe* spp. infecting plants of the genera *Polygonum* and *Rumex* (Braun 1987). The compound species *E. communis* as revised by Junell (1967b) remains unresolved. The rDNA sequences distinguished UEA1 from both *E. polygoni* Polygonum and *E. convolvuli* and support the present classification (Fig. 3; Saenz and Taylor 1999).

Using the same criteria as above, we confirmed our previous definition of the UCSC1 isolate as *E. cichoracearum*. It is important to realize that it is generally accepted that *E. cichoracearum*, as defined by Salmon (1900), is a complex composite species encompassing numerous races. Clear criteria enabling further subdivision into isolates or distinct species has proven to be problematic. Junell (1967a) followed by Braun (1987) subdivided *E. cichoracearum* into a number of distinct species and varieties based in part on host range as well as morphological differences. The intergrading of morphological differences, the variation of these traits when examined on distinct host species, and the overlapping host ranges make the circumscription of *E. cichoracearum* in the sense of Braun difficult to apply in the classification of UCSC1 at the species level. More recently, phylogenetic analyses based on rDNA sequences suggested that the restriction of *E. cichoracearum* to isolates infecting the Asteraceae is not valid (Saenz and Taylor 1999). We believe that until more precise criteria can be defined for the classification and taxonomy of this composite species, UCSC1 should be placed in *E. cichoracearum* in accordance with the broad definition of Salmon (1900). A morphologically similar isolate of powdery mildew that also attacks *A. thaliana* was recently identified as a member of the closely allied *E. orontii* (Plotnikova et al. 1998). This isolate can be distinguished from *E. cichoracearum* UCSC1 by its host range, which does not include cucurbit species, and by subtle morphological features such as shorter, straighter conidiophores and smaller conidia (Plotnikova et al. 1998).

Survey for powdery mildew-resistant *A. thaliana* accessions.

Our survey of 360 *A. thaliana* accessions for resistance to two *Erysiphe* isolates, *E. cichoracearum* UCSC1 and *E. cruciferarum* UEA1, provides a measure of the genetic resources

this model plant species has to offer. Previous studies of resistance to pathogens in naturally occurring plant populations suggest that diversity for disease resistance can occur both among individuals collected from one site and among individuals collected at diverse sites. In *Avena sterilis* and *A. barbata*, resistance to powdery mildew in plants collected from diverse areas of the Mediterranean occurred with greater frequency than among cultivated varieties (Zillinsky and Murphy 1967). Wild populations of *Hordeum spontaneum* showed a range of resistance types in which geographic distribution was determined in part by rainfall (Fischbeck and Jahoor 1991). Diversity in powdery mildew resistance phenotypes among *Senecio vulgaris* individuals was evident even in small 100 cm² plots (Bevan et al. 1993a). These surveys suggested a spatial mosaic of resistant genotypes with individuals carrying resistance alleles at a varying number of loci (Jana and Nevo 1991; Mastebroek and Balkema-Boomstra 1991; Bevan et al. 1993a; Jørgensen and Jensen 1997). We have found that the powdery mildew resistance trait was similarly variable and widely distributed among *A. thaliana* accessions.

A. thaliana accessions ranged from fully susceptible to fully resistant. However, a higher proportion were susceptible, with 59% of accessions susceptible to both isolates and 82% susceptible to at least one isolate. Others have observed that, among natural populations of wild species, susceptibility is often the predominant phenotype. Jana and Nevo (1991) found that, among 330 *H. spontaneum* lines collected at 14 sites in Iran, Turkey, and Syria, 55% were susceptible to an Israeli isolate of *B. graminis* f. sp. *hordei* and only 43% were susceptible to a mixture of a U.S. and a Japanese isolate of *B. graminis* f. sp. *hordei*. In the wild species, *Senecio vulgaris*, 90 to 99% of individuals were susceptible to at least one isolate of *E. fischeri* at various sites sampled near Glasgow or Wellesbourne (Bevan et al. 1993a). In spite of the prevalence of susceptible individuals, natural populations seldom succumb to widespread and devastating epidemics due to the spatial diversity for disease phenotypes occurring within a species (Bevan et al. 1993c). The persistence of disease susceptibility in populations has been ascribed variously to a pleiotropic fitness cost associated with resistance loci or to linkage to an undesirable trait (Bergelson and Purrington 1996). In support of the former possibility, Mitchell-Olds and Bradley (1996) demonstrated a 6% slower growth rate in *Peronospora parasitica*-resistant *Brassica rapa* genotypes in the absence of pathogen infection. We do not know whether there is a fitness cost associated with any *A. thaliana* powdery mildew resistance gene; however, detailed evaluations based on transgenic plants bearing cloned resistance genes may help resolve this issue (Bergelson and Purrington 1996).

Among the resistant accessions, 43% (63/147) were resistant to both *E. cichoracearum* UCSC1 and *E. cruciferarum* UEA1. A few studies have reported similar associations between resistances to different pathogens, although little is known of the genetic basis for this phenomenon. Mitchell-Olds et al. (1995) artificially selected for disease resistance to one pathogen and then assessed unselected resistance to unrelated pathogens. Lines selected for resistance to *Leptosphaeria maculans* also showed resistance to *Peronospora parasitica* and vice versa. These authors suggest that such multiple disease resistance could contribute to a durable defense not easily circumvented by rapidly evolving fungal

pathogens. For more closely related pathogens, like *E. cichoracearum* and *E. cruciferarum*, the multiple disease resistance observed in 43% of accessions may reflect the action of a broad, nonspecific form of resistance or this group of accessions may carry more than one resistance gene with each gene effective against one of the isolates (Bevan et al. 1993b; Jørgensen and Jensen 1997). Alternatively, the two powdery mildew isolates used in this study may harbor similar or identical avirulence factors, which are recognized by a common resistance gene. Addressing whether nonspecific resistance or the pyramiding of simple resistance loci contribute to powdery mildew resistance in this weedy species will be an interesting line of future inquiry.

As an initial step to dissecting the genetic bases of UCSC1 and UEA1 resistances in *A. thaliana*, we employed F₃ families that had been used previously to map *RPW4* and *RPW7*. Our studies suggest that Su-0 contains multiple resistance genes; *RPW4* is effective against UCSC1 and a distinct locus is effective against UEA1. The ability of Ms-0 alleles at *RPW7* to confer resistance to both isolates is more unusual. *RPW7* lies at the lower end of chromosome 3 in a 40-cM region that to date has yielded >11 other disease resistance and associated genes (Holub 1997). Systems such as the *MI-1* locus in barley (Jørgensen 1992), the *RPG1* locus in soybean (Ashfield et al. 1995), the *RPP* loci in *A. thaliana* (Reignault et al. 1996), the *Rp1* locus in maize (Hulbert et al. 1997), and the *Cf-9/Cf-4* complex locus in tomato (Parniske et al. 1997) provide evidence that resistance genes can be very closely linked. Thus, there are precedents for the idea that the *RPW7* locus may consist of a tight cluster of at least two distinct resistance genes, each with a different species specificity. Alternatively, the *RPW7* locus may consist of a single gene and the *RPW7* protein may recognize a component found in both isolates. Bisgrove et al. (1994) have demonstrated that *RPM1* (= *RPS3*) interacts with two distinct avirulence genes (*avrB* and *avrRpm1*) from *Pseudomonas syringae*. These alternatives will not be resolved until *RPW7* is cloned.

In addition to accessions that were resistant to both UEA1 and UCSC1, 57% (84/147) of resistant accessions were resistant to just one of these two powdery mildew isolates, with a greater number exhibiting resistance to UEA1 than UCSC1. The higher proportion of resistant accessions suggests that the UEA1 isolate may have coevolved with European *A. thaliana* accessions for a longer period of time. In one survey for powdery mildew resistance of *H. spontaneum* lines from the Middle East, a greater proportion of lines were resistant to an Israeli isolate of powdery mildew than to isolates from different parts of the world (Jana and Nevo 1991). However, in other studies this idea was not supported and hosts and pathogens from the same locality were more likely to be compatible than incompatible (Fischbeck and Jahoor 1991; Bevan et al. 1993a). An alternative explanation for the higher frequency of resistance to UEA1 is that *A. thaliana* is not a preferred host of UEA1, which was originally recovered from *B. napus*.

In conclusion, the UEA1 isolate was shown to be a member of *E. cruciferarum* Opiz ex Junell and the UCSC1 isolate a member of *E. cichoracearum* sensu Salmon. Inoculations of 360 *A. thaliana* accessions with these two powdery mildew isolates revealed a diversity of resistance phenotypes. In the long term, these resources can be used to determine the num-

ber of distinct resistance loci, the range of allelic variation at each locus, and the molecular mechanisms underlying genetic diversity for powdery mildew resistance.

MATERIALS AND METHODS

Plant cultivation.

Seeds for plants used to determine host range were purchased mainly from Park Seed (Greenwood, SC); *B. campestris* R 500 and cv. Tower were obtained from a collection maintained at the Carnegie Institution of Washington (Stanford, CA). The Nottingham *Arabidopsis* Stock Centre (Nottingham, U.K.) supplied the majority of the *A. thaliana* accessions. The following accessions were originally from the *Arabidopsis* Information Service seed bank (Frankfurt, Germany): Al-0, Be-0, Bl-1, Bla-10, Bur-0, C24, Co-1, Col-0, Ct-1, Cvi-0, Edi-0, Est-0, Ge-1, Gre-0, Kas-1, Kil-0, Kin-0, Li-6, Lip-0, Ll-0, Lm-2, Mh-0, Ms-0, Nd-0, No-0, Ob-2, Pa-1, Per-1, Pr-0, Po-1, Rsch-4, Se-0, Sei-0, Sf-1, Sg-2, Su-0, Sy-0, Ts-2, Tsu-0, Tu-1, Tul-0, Wa-1, Wil-2, Ws-0, Yo-0, Ze-0, and Zü-0.

Infection studies of *A. thaliana* accessions and host range determinations were performed at two geographic locations (i.e., Norwich, U.K., for UEA1 and Stanford, CA, for UCSC1) to prevent cross-contamination of the two isolates. Based on a limited number of UCSC1 infection studies performed at Norwich, no significant differences in disease scores were observed between experiments performed at the two locations. Although the disease scores are influenced to some extent by environmental conditions and inoculation densities, accessions scored as resistant (i.e., DR of 0 or 1) at Stanford were also scored as resistant at Norwich. Similarly, susceptible accessions (i.e., DR 2 or 3) were scored as susceptible at both locations.

A. thaliana seeds were sown onto autoclaved Levingtons Multipurpose compost (Ipswich, U.K.) or in pasteurized commercial compost (Sunshine mix no. 1; McCalif Grower Supplies, Ceres, CA). The pots were covered in plastic wrap to ensure high humidity and placed at 4°C for 24 to 48 h. Seeds for plants used to determine host range specificity were treated similarly but sown into a layer of fine sand to prevent damping off and to facilitate emergence. Plants at the U.K. location were grown in a controlled environment room with a 10-h day at 24°C under 110 $\mu\text{mol s}^{-1} \text{m}^{-2}$ (broad-spectrum 400 W fluorescent metal halide, Kolor Arc, lamps) and at 22°C at night. Similarly, plants at the U.S. location were grown in a controlled environment chamber with a 14-h photoperiod (100 to 150 $\mu\text{mol s}^{-1} \text{m}^{-2}$ in a 400 to 700 nm range) at 22°C (Somerville and Ogren 1982). To ensure uniform growth, *A. thaliana* seedlings (approximately a week after sowing) were transplanted in fresh soil at a density of 16 to 20 plants per 15.24-cm pot. The plants were allowed to grow further for 10 to 12 days before inoculation. Seedlings of other plant species were sown into 6- or 30-cm pots and thinned to the most suitable density.

Maintenance of fungal material.

The two *Erysiphe* isolates used in this study originated from different geographic locations and were identified independently based on their ability to infect and propagate on *A. thaliana*. The North American isolate, designated UCSC1

(University of California at Santa Cruz), was recovered from *A. thaliana* (Adam and Somerville 1996); while the European isolate, named UEA1 (University of East Anglia, Norwich, U.K.), was recovered from oil seed rape (*Brassica napus*) (Xiao et al. 1997). Both isolates were maintained by repeated subculturing on their respective susceptible hosts (e.g., Kuta squash or *A. thaliana* Col-0 for UCSC1 and Cobra oilseed rape or *A. thaliana* L-er for UEA1) and thus represent clonal derivatives of the original cultures. Infrequently, the cultures were taken through one to two rounds of single-colony isolation and tested on selected *A. thaliana* accessions to ensure the genetic homogeneity of the cultures.

Inoculation of plants.

Inoculation with *E. cruciferarum* isolate UEA1 was performed on *A. thaliana* plants at the six- to eight-leaf stage to minimize phenotypic variation in the expression of resistance during plant development. Previous observations suggested that very young leaves (i.e., cotyledons and the first two to four true leaves) and plants in flower display enhanced susceptibility to UEA1 and UCSC1. Inoculations were carried out by carefully brushing conidia from heavily infected sections of *B. napus* cv. Cobra leaves. This was carried out in a draft-free environment at a height of about 45 cm above the target leaves, in order to achieve an even distribution of conidia on settling. To ensure high humidity to stimulate spore germination, plants were watered prior to inoculation and subsequently placed in sealed propagator trays overnight. Inoculations with *E. cichoracearum* isolate UCSC1 were performed with modified settling towers as described by Adam and Somerville (1996) with the exception that heavily infected leaf segments of squash (*Cucurbita maxima* cv. Kuta) were used as a source of inoculum. Each isolate was occasionally subcultured on *A. thaliana*.

Plants (five to 10) of each *A. thaliana* accession were scored at 8 d.p.i. and again at 12 d.p.i. The DR phenotype of UEA1 was also evaluated at 8 d.p.i. by microscopic examination of leaf samples stained with the lipophilic fluorochrome DiOC₆(3) (Sigma, St. Louis, MO) (Duckett and Reed 1991). The DR phenotype with UCSC1 was evaluated macroscopically on leaves at 8 d.p.i. Resistant accessions were then evaluated microscopically as described for UEA1. This allowed direct comparison of host DR phenotypes in response to both *Erysiphe* isolates (Table 2). Each isolate was scored blind as a precaution against prejudicing the categorization of accessions. Susceptible accessions (DR = 2 to 3) were scored once and all other accessions were scored a minimum of two times. Plants of the F₃ (Col-*gll* × Su-0) and F₃ (Ms-0 × L-er) families were similarly evaluated. The scale for rating DR scores is described in detail in Table 1.

Fluorescence microscopy.

The fluorescent dye 3,3'-dihexyloxycarbocyanin iodide (DiOC₆(3)) was used to evaluate the growth and development of propagules together with host responses (Xiao et al. 1997). Whole or partial leaf samples were immersed in fresh aqueous solutions of 50 µg · ml⁻¹ DiOC₆(3) prepared from a stock solution in ethanol (0.5 mg of DiOC₆(3) · ml⁻¹, stored at 20°C; Duckett and Read 1991). Between 2 and 3 min of exposure normally gave an adequate level of stain absorption. The samples were then placed on slides and the excess stain solution

drawn off gently from the edges with tissue. The samples were viewed under an Optiphot-2 microscope (Nikon, Kingston upon Thames, U.K.) fitted with a epifluorescence filter B2A (450 to 490 nm excitation filter and a 520-nm barrier filter) and a Plan X 10 Phl/D1 objective lens.

Cryogenic scanning electron microscopy.

Leaves from *A. thaliana* Col-0 and *B. napus* cv. Cobra plants inoculated with the UEA1 isolate were collected for examination of distinguishing morphological features at 1, 2, 4, 7, and 14 d.p.i. Leaves of *A. thaliana* Col-0 were also inoculated with the UCSC1 isolate and collected daily from 1 to 7 d.p.i. The infected leaves were cut into pieces 2.5 to 5 mm in length and prepared for cryogenic scanning electron microscopy as described by Klecan et al. (1990).

Sequence comparison of rDNA.

PCR amplification was carried out on genomic DNA prepared from conidia collected from heavily infected susceptible host leaves at 12 d.p.i. A sterile, wooden applicator was used to collect the conidia from individual colonies, which were added to 300 µl of 0.9 M sorbitol, 0.1 M EDTA (pH 7.5), 50 mM DTT (dithiothreitol), and 0.5% Tween 20 (see step 4 of section 2.5.1; Matallana et al. 1992). DNA was prepared essentially as described in steps 4 to 13 of Matallana et al. (1992). From both isolates, single DNA fragments of approximately 950 bp (estimated on 1.2% agarose gel) were amplified with primers NS7 and ITS4 derived from the conserved 18S and 28S ribosomal genes (White et al. 1990). The PCR fragments from three independent DNA preparations were sequenced. Sequence alignments were performed with the software program DNASIS (Hitachi, San Bruno, CA).

Data analysis.

The UEA1 and UCSC1 rDNA sequences were incorporated into a subset of an existing alignment of powdery mildew fungi (Saenz and Taylor 1999). The data were analyzed by maximum parsimony methods in PAUP 3.1.1 (Swofford 1993). The trees were found by a heuristic search employing the random stepwise addition option of PAUP, given a random seed number and 100 replications. Branch support was determined by 1,000 bootstrapped data sets (Felsenstein 1985).

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