Genetics of Virulence in Californian Populations of *Bremia lactucae* (Lettuce Downy Mildew)

T. W. Ilott, M. E. Durgan, and R. W. Michelmore

Department of Vegetable Crops, University of California, Davis 95616. Present address of first author: The Plant Breeding Institute, Cambridge, England. Present address of second author: Bud of California Ag Research, Salinas 93902.

We are grateful to R. Brendler (Ventura/Oxnard) and W. Waycott (Salinas) for collecting isolates. We thank F. Zink, S. Hulbert, and B. Farrara for advice and technical assistance. We also acknowledge the financial support of USDA/CRGO Grant #84-CRCR-1-1470, the California Iceberg Lettuce Research Program, and the Meyers Foundation.

Accepted for publication 16 March 1987.

ABSTRACT

Ilott, T. W., Durgan, M. E., and Michelmore, R. W. 1987. Genetics of virulence in Californian populations of *Bremia lactucae* (lettuce downy mildew). Phytopathology 77:1381-1386.

Virulence phenotypes and sexual compatibility types were determined for 116 Californian isolates of *Bremia lactucae* collected between 1982 and 1986. All but one isolate could be grouped into one of three distinct pathotypes on the basis of their virulence phenotypes. Sexual compatibility type (SCT) was absolutely correlated with pathotype. All pathotype I isolates had the B_1 SCT, and all pathotype II and III isolates had the B_2 SCT. The genetic basis of avirulence was also studied for representative isolates of each pathotype. The avirulence genotypes and the lack of diversity in virulence phenotype suggested that the sexual cycle has not been important in the generation of variation in the Californian population of *B. lactucae*. This may have been because of the infertility of matings between the predominant pathotypes.

Additonal key words: *Lactuca sativa,* resistance.

Resistance to *Bremia lactucae* Regel, the diploid oomycete fungus causing the downy mildew disease of lettuce (*Lactuca sativa* L.), has long been a goal of lettuce breeding programs. Thirteen single dominant genes for resistance to *B. lactucae* have now been identified, and other incompletely characterized resistance factors also exist (11,14,15; B. Farrara and R. W. Michelmore, *unpublished*). This resistance is race-specific. Genetic analyses of both host and pathogen have shown that the interaction between lettuce and *B. lactucae* conforms to Flor's concept of a gene-for-gene relationship (4,7,22–24). In most cases, each resistance gene (*Dm*) is matched in the pathogen by a specific avirulence gene (*A*) (20,22–24). Avirulence is dominant to virulence.

Durable resistance to lettuce downy mildew, however, has not been achieved by the use of such resistance genes because of the variability of the pathogen population (3). Studies of European populations of *B. lactucae* have demonstrated the presence of numerous virulence phenotypes (1,3,6,8,9,16,19,25,29) and the potential of sexual reproduction and recombination to generate novel combinations of virulence genes (9). Other mechanisms of variation may also operate but have not been studied (2,9). Determinants of virulence to resistance genes not yet commercially deployed appear to be present at least at a low level in many pathogen populations (3,9); therefore, new combinations of virulence genes able to overcome the resistance in newly introduced cultivars rapidly evolve. The population structure and nature of variation of *B. lactucae* in California, however, may differ from that in Europe. Calmar, a cultivar containing the resistance genes *Dm*7, *Dm*5/8, and *Dm*13 (B. Farrara and R. W. Michelmore, *unpublished*), remained resistant for 13 yr in California (5), although downy mildew had been a severe problem before the introduction of Calmar. If variability in the Californian population of *B. lactucae* was as extensive as in Europe, the resistance in Calmar probably would have been overcome earlier.

The present study investigated the variability of the Californian population of *B. lactucae*. A survey of isolate virulence phenotype was conducted. In addition, the genetic basis of avirulence in representative isolates was analyzed to obtain information on the possible origins of isolates, the potential of the pathogen population to change, and mechanisms generating variation. This information was used to identify combinations of *Dm* genes that should provide at least transient resistance to downy mildew in California.

MATERIALS AND METHODS

Collection and maintenance of isolates. Isolates of *B. lactucae* were obtained over a 5-yr period from lettuce-producing regions in

©1987 The American Phytopathological Society

California and Arizona. Samples were collected by the authors from commercial fields and from breeding trials. In addition, samples were mailed to the authors by cooperators (R. Brendler and W. Waycott).

B. lactucae is a biotrophic fungus and cannot as yet be cultured axenically. It can be readily maintained, however, on lettuce seedlings and detached cotyledons. Procedures used in the subculture and maintenance of isolates were modified from those previously described (18). Conidia were washed from infected host tissue by shaking material in distilled water. Neomycin sulfate (Sigma Chemical Co.) at 1 ppm was included to inhibit bacterial contamination if the host material showed signs of decay. Conidia were pelleted using a low-speed centrifuge, and the supernatant, containing a water-soluble inhibitor of germination (17), was removed. The spores were resuspended in distilled water to a concentration of $6-10 \times 10^4$ conidia per milliliter. A chromatography spray unit (Sigma Chemical Co.) was then used to spray the suspension to runoff onto 7-day-old seedlings of Cobham Green, a cultivar in which no resistance genes have been detected. Seedlings had been grown on blotter paper moistened with Hewitt's solution (10), in sealed plastic GA-7 boxes (Magenta Corp., Chicago) kept in a growth room at 15 C, and illuminated for 14 hr daily with daylight fluorescent lamps (300 $\mu Em^{-2}s^{-1}$). Iprodione (20 mg L^{-1}) was included in the nutrient solution to prevent establishment of secondary pathogens. After inoculation, seedlings were incubated in the growth room described above.

After 7 days, asexual sporulation was visible on the seedlings. Single-conidial isolates were established from these using the method of Michelmore and Ingram (21). The virulence phenotypes of isolates were identical to that of their progenitor isolates taken from single plants. Consequently, late in the study, single-conidial lines were not generated as the procedure was time consuming. Isolates were subcultured as described above.

Isolates were stored at −80 or −20 C as asexual sporulations on seedlings or as pellets of conidia. Slow cooling (about 1 C min^{-1}) ensured good viability of conidia (often in excess of 90% germination several months after freezing). No changes in virulence phenotype have been observed as a result of freezing (*unpublished data*).

Determination of virulence phenotype. Each isolate was inoculated individually onto 7-day-old seedlings of a differential series of resistant cultivars (Table 1). The procedure employed was modified from that of Michelmore and Crute (18). Seeds of the differential cultivars were sown in clear plastic compartmented boxes (220 × 330 × 58 mm; Corth Plastics, Santa Clara, CA); seedlings were then grown and inoculated as described above. The seedlings were examined for the presence of asexual sporulation 7, 9, and 11 days after inoculation. Sporulation on the susceptible cultivar, Cobham Green, was profuse in all cases. Sporulation on other differential cultivars was usually either absent (an incompatible interaction) or profuse (compatible), depending on the virulence phenotype of the isolate. An incompatible interaction implied that the isolate had a gene for avirulence to match a particular resistance gene in the host cultivar. The few cases where sporulation was sparse or delayed are discussed later.

Determination of sexual compatibility type. *B. lactucae* is predominantly heterothallic, with two sexual compatibility types designated B_1 and B_2 (19). Homothallic isolates have also been described (21). The compatibility type of an isolate was determined by attempting crosses with isolates of known compatibility type. A hypodermic syringe and needle were used to inoculate a drop (approximately 0.05 ml) of a conidial suspension of the test isolate onto detached cotyledons of Cobham Green arranged in three clear plastic boxes. One box was then inoculated in the same way with a European isolate of compatibility type B_1, another with a B_2 isolate, and a third with no further isolate (to test for homothallism). After a 1-mo incubation in the growth room, the cotyledons are examined using a dissecting microscope (40×) for the presence of oospores. Isolates forming oospores in combination with the B_1 compatibility type isolate were designated B_2, and vice versa. No homothallic isolates were observed.

Genetic analysis of avirulence. Californian isolates were crossed to European isolates virulent on differential cultivars that were resistant to the Californian isolates. Pairings were also made between different Californian isolates. Cotyledons containing oospores were allowed to decay in the growth room for 4 wk, shaken in distilled water at 5 C for a further 4+ wk, and then macerated in a blender for 1 min to disrupt clumps of oospores. The resulting suspension of oospores was added to Hewitt's solution, which was then used in the cultivation of seedlings of Cobham Green. Approximately 10^4 oospores were added to each box of seedlings. After 7–10 days, asexual sporulations, resulting from infection of seedlings by germinating oospores, were visible on individual cotyledons. In most cases between one and three seedlings were infected per box (about 50 seedlings); each infection was assumed to have resulted from a single oospore. If more than three seedlings were infected per box, new boxes of seedlings were set up to which fewer oospores were added. Information on the segregation of avirulence and virulence in crosses was then obtained by subculturing the progeny isolates and determining their virulence phenotypes using a differential series of resistant cultivars (Table 1).

TABLE 1. The differential series of lettuce cultivars resistant to *Bremia lactucae* used in this study[a]

Primary series[b]		Secondary series[c]	
Cultivar/line	*Dm* gene	Cultivar/line	*Dm* gene (or R-factor)[d]
Lednicky	1	Blondine	1 + 13
UCDM2	2	Mildura	1 + 3
Dandie	3	Amplus	2 + 4
T57/R4	4	Liba	1 + 2
Valmaine/Valverde	5/8[e]	Kordaat	1 + 3 + 4
Sabine	6	Avondefiance	5/8 + 6
Mesa	7 + 13	Salinas/Calmar	5/8 + 7 + 13
UCDM10	10	Sucrine	5/8 + 10
Capitan	11[f]	Fila	2 + 11
Hilde × *Lactuca serriola*	11[f]	Gelber Winterkonig	4 + 13 + 14
Empire Pennlake	13	Vanguard/Winterhaven	7 + 10 + 13
UCDM14	14	Kinemontepas	10 + 13 + 16
PIVT1309	15	Saffier	1 + 3 + 7 + 16
LSE 18	16	Diana	1 + 3 + 7 + 5/8
Cobham Green[g]	None	Hilde	(R12)[d]
		Mariska	(R18)[d]

[a]As determined by Hulbert and Michelmore (11); B. Farrara and R. W. Michelmore, *unpublished*.

[b]Cultivars/lines with well-characterized downy mildew resistance genes (*Dm*). UCDM lines were developed at UC Davis.

[c]Cultivars/lines with combinations of well-characterized resistance genes used to confirm conclusions from the primary series and lines with incompletely characterized R-factors[d].

[d]Resistance factors (R-factors) have been used when the resistance in a cultivar has not been fully characterized in terms of *Dm* genes.

[e]*Dm*5 and *Dm*8 are the same gene (11).

[f]Two cultivars are used because interactions with *Dm*11 are sometimes difficult to score.

[g]Susceptible check.

RESULTS

Virulence phenotypes and sexual compatibility types of Californian isolates. Only seven virulence phenotypes were detected from a total of 118 Californian isolates of *B. lactucae* collected in this survey (Table 2). All isolates except one (C85T1) could be classified into one of three distinct pathotypes. A pathotype is defined as a group of isolates with identical or near-identical virulence phenotypes; such isolates may, however, differ in other untested characteristics. Each Californian pathotype differed from the others at a minimum of four avirulence loci. All pathotype III isolates had identical virulence phenotypes. Pathotype II isolates varied in their response to *Dm*11. Pathotype I isolates varied in their response to *Dm*3 and *Dm*15; this may represent further variation within the Californian population as

discussed later. Sexual compatibility type was absolutely correlated with pathotype (Table 2).

All three pathotypes were each identified from several widely separated regions within California. Pathotype I isolates were collected from Kern, Monterey (two growing areas), San Luis Obispo, Ventura (two areas), and Imperial counties in California, and from Yuma (Arizona); pathotype II from Monterey (two areas), San Luis Obispo (two areas), Santa Barbara (two areas), Ventura (two areas) and Imperial counties, and from Yuma; and pathotype III from Monterey (two areas), Santa Barbara, Ventura, and Imperial counties. Growing areas within counties were at least 30 miles apart, and within areas, isolates were collected from different commercial fields. Isolate C85T1 was collected near Davis. Pathotype II was found in all years of the study; pathotype I was absent in 1986 (only 20 isolates were collected, from only three sites) and pathotype III isolates were not identified until 1983.

Some of the later isolates (from single plants) that had not been single-spored had virulence phenotypes unlike any of the pathotypes. Such isolates sporulated profusely on cultivars with *Dm*2, *Dm*3, *Dm*5/8, *Dm*6, *Dm*7, R12, *Dm*13, or *Dm*14, but sporulation on T57R4 (*Dm*4), UCDM10 (*Dm*10), Capitan (*Dm*11), Hilde × *L. serriola* (*Dm*11), and LSE18 (*Dm*16) varied from profuse to only a few spores being visible on one or two cotyledons of the cultivar. This indicated that the isolates were mixtures of pathotypes II and III. The hypothesis was confirmed for three isolates (of 16 with this phenotype, from three growing areas) by independently subculturing conidia from T57R4 (susceptible to pathotype III but not pathotype II) and UCDM10 (susceptible to pathotype II but not pathotype III) and then inoculating the two resultant lines onto the differential series of cultivars. All lines taken from T57R4 had the virulence phenotype of pathotype III and all lines from UCDM10 were pathotype II.

Isolates of each pathotype were also inoculated onto seedlings of *L. serriola* L., a common weed in lettuce production areas. Seed samples from four collections of *L. serriola* from the Salinas Valley, Monterey County, were supplied by Dr. E. J. Ryder (USDA, Salinas, CA). The Californian isolates were capable of colonizing these wild relatives of *L. sativa*. Asexual generation time was 6 or 7 days and sporulation was profuse.

Genetic analysis of avirulence in Californian isolates of *B. lactucae*. In this paper, only the segregation of avirulence is described. The genetics of avirulence in *B. lactucae* has already been studied in detail (20,22–24). Virulence is normally conferred by the presence of homozygous recessive alleles (*aa*) in the pathogen (20,22,24) and in this study did not segregate in the progeny of crosses between two isolates virulent to a particular resistance gene. There was no evidence of inhibitor loci (22) in Californian isolates that inhibited avirulence alleles from European isolates. The European isolates used in the present study were known to be sexually competent, and their genotypes had previously been determined (Table 3; 22–24). Therefore, only small numbers of progeny needed to be analyzed from each cross to determine whether a particular avirulence locus was segregating. Segregation of avirulence to a particular resistance gene implied that an isolate was heterozygous at that locus. Genetics of avirulence at the *A*2, *A*4, *A*5/8, *A*11, *A*15, and *A*16 loci is described for isolates of pathotype I (the *A*3 locus could not be studied as isolates avirulent to *Dm*3 were lost due to a freezer breakdown), at the *A*1, *A*4, *A*15, and *A*16 loci for pathotype II and the *A*1, *A*10, *A*11, and *A*15 loci for pathotype III (Table 4). Segregation of avirulence to R18 (Mariska) could not be studied as seed of this cultivar was unavailable until late in the investigation. At least two isolates of each pathotype were studied to sample potential variation within a pathotype.

Large numbers of oospores were formed in all crosses between European and Californian isolates of opposite compatibility type. Sexual progeny were readily obtained, however, only in crosses involving pathotypes II or III. The segregation of avirulence in these crosses (Table 4) indicated the genotype of pathotype II isolates to be: *A*1*a*1, *A*4*a*4, *A*15*a*15, and *A*16*a*16. The genotype of pathotype III is: *A*1*A*1, *A*10*A*10, and *A*15*A*15. Interactions between cultivars with *Dm*11 and progeny of crosses with pathotype III isolates were difficult to score as compatible or incompatible because of delayed sporulation and host necrosis; therefore, the genotype of pathotype III at the *A*11 locus is unclear. The complete genotypes are given in Table 5.

Although crosses involving pathotype I formed as many oospores in host tissue as did fertile crosses, in most cases progeny were rarely or never obtained. When progeny were recovered, they frequently exhibited very sparse and delayed sporulation when compared with the parental isolates. Sporulation of such isolates usually declined during cycles of subculturing, and isolates were often lost before the third cycle of culture; consequently, it was impossible to determine the virulence phenotypes of these isolates. The following crosses resulted in the recovery of no fit progeny isolates, even though oospores were formed and the crosses were repeated several times: C8M40 (pathotype I) × pathotype II isolates (five isolates); C8586 (pathotype I) × pathotype II isolates (two isolates); C8586 × S1 (European); C8586 × CG1 (European); C83M40 × AM (Australian); C8586 × AM; C83M40 × JP1 (Japanese); C8586 × JP1. Crosses between C83M40 and the European isolates S1 and CG1 each resulted in only one fit progeny isolate being recovered. One progeny isolate was also obtained from a cross between C8586 and C83M24.

Crosses between isolates of pathotypes I and III, however, did produce several normally sporulating progeny, as did a cross between the pathotype I isolate C83R4 and the European isolate IM25/P11. Analysis of the virulence phenotypes of these progeny indicated that pathotype I is heterozygous at avirulence loci *A*2, *A*4, *A*5/8, and *A*11, and homozygous at the *A*16 locus. Segregation data for alleles at the *A*2 and *A*4 loci, however, were complex. In the cross C8584 (pathotype I) × C83M47 (pathotype III) all progeny isolates were virulent on UCDM2 (*Dm*2), although C8584 was avirulent, and all but one were avirulent on T57R4 (*Dm*4). In contrast, most progeny isolates of the cross C83M40 (pathotype I) × C83M47 were avirulent on UCDM2 and virulent on T57R4. These results may indicate additional variation within pathotype I, possibly for genes affecting oospore germination or isolate fitness. If such genes were linked to the *A*2 and *A*4 loci in isolates heterozygous at these loci, this could explain such differences in segregation ratios. Larger progeny sizes would be needed to study these differences further.

Genetic analysis of isolate C85T1 was limited as only seven progeny were obtained when the cross was first made, and the

TABLE 2. Virulence phenotypes of California isolates of *Bremia lactucae*

Host resistance gene or factor	Pathotype			
	I	II	III	C85T1
*Dm*1	+[a]	*	*	*
*Dm*2	*	+	+	+
*Dm*3	*/+[b]	+	+	+
*Dm*4	*	*	+	*
*Dm*5/8	*	+	+	+
*Dm*6	+	+	+	+
*Dm*7	+	+	+	+
*Dm*10	+	+	*	*
*Dm*11	*	+[c]	*	*
*Dm*13	+	+	+	+
*Dm*14	+	+	+	+
*Dm*15	*/+[d]	*	*	+
*Dm*16	*	*	+	*
R12	+	+	+	+
R18	*[e]	*[e]	+[e]	*
None (Cobham Green)	+	+	+	+
Sexual compatibility type	B_1	B_2	B_2	B_1
Number of isolates	14	62	41	1

[a] +: Compatible interaction; *: incompatible interaction.
[b] Seven pathotype I isolates were virulent to *Dm*3, seven were avirulent.
[c] Sporulation of pathotype II isolates on cultivars with *Dm*11 was variable.
[d] Six pathotype I isolates were virulent to *Dm*15, five were avirulent, and the virulence of three was undetermined.
[e] Only one isolate of each pathotype was tested on R18.

isolate was later lost. The isolate was heterozygous at the loci *A*4, *A*10, *A*11, and *A*16; it could have been homozygous or heterozygous at *A*1.

DISCUSSION

This study has demonstrated that the Californian population of *B. lactucae* is dominated by only three pathotypes. At least two of the pathotypes have probably existed in California for many years. Interpretation of past studies was difficult as the differential cultivars previously used could not always be readily defined in terms of currently characterized resistance genes; nevertheless, early survey data (T. W. Whitaker, *unpublished*) is consistent with the presence of pathotype I isolates since 1933. Other isolates with different virulence phenotypes, designated as races 1, 2, 4, and 5

TABLE 3. Virulence genotypes of European isolates of *Bremia lactucae*[a]

Isolate	SCT[d]	Avirulence loci[b,c] 1	2	4	5/8	10	11	15	16
19c	B_2	*aa*	*aa*	*aa*	*aa*	*aa*	*aabb*	*Aa*	*Aa*
Tv	B_1	*aa*	*aa*	*aa*	*aall*[e]	*aa*	*Aabb*	*Aa*	*aa*
NL6	B_1	*aa*	*aa*	*aa*	*aall*	*aa*	*aabb*	*AA*	*Aa*
SF5	B_1	*AA*	*aa*	*aa*	*Aall*	*aa*	*Aabb*	*aa*	*AA*
CS9	B_1	*aa*	*aa*	*aa*	*aall*	*aa*	*aabb*	*aa*	*A-*[f]
IM25/R7	B_1	*aa*	*AA*	*aa*	*aall*	*aa*	*aabb*	*aa*	*aa*
IM25/P11	B_2	*aa*	*AA*	*aa*	*aall*	*aa*	*aabb*	*aa*	*aa*

[a] As characterized previously (22,23) and by T. W. Ilott, S. H. Hulbert, and R. W. Michelmore (*unpublished data*).
[b] *A*: Dominant avirulence allele; *a*: recessive virulence allele.
[c] Genotypes at avirulence loci 3, 6, 7, 12, 13, 14, and 18 are not given as segregation at these loci in California × European crosses is not considered in this paper (see text).
[d] SCT: Sexual compatibility type.
[e] A dominant inhibitor of avirulence has been reported to be epistatic to *A*5 (23) but is not present in any isolate used in this study.
[f] Full genotype undetermined.

TABLE 4. Segregation of avirulence and virulence in crosses between isolates of *Bremia lactucae*

Cross	Avirulence loci 1		2		4		5/8		10		11		15		16	
	*	+[a]	*	+	*	+	*	+	*	+	*	+	*	+	*	+
Pathotype I × European																
C83R4 × IM25/P11	...[b]		NA[c]		5	1	2	4	···		4	2	NA		NA	
Pathotype II × European																
C83R5 × IM25/R7	6	5	···		5	6	···		···		···		NA		NA	
C82P24 × Tv	8	6	···		6	8	···		···		···		7	1	6	8
C82P24 × NL6	9	5	···		4	10	···		···		···		8	0[d]	12	2
C82P24 × SF5	46	0[d]	···		28	18	···		···		···		20	26	22	0[d]
C82P24 × CS9	NS[e]		···		12	11	···		···		···		NS		NS	
C85B8A × Tv	2	6	···		7	1	···		···		···		3	2	2	6
Pathotype III × European																
C83M46 × IM25/R7	6	0	···		···		···		6	0	–[f]		NA		NA	
C83M47 × Tv	7	0	···		···		···		7	0	–		7	0	···	
C83M47 × NL6	9	0	···		···		···		9	0	–		9	0	···	
C84M4 × Tv	9	0	···		···		···		9	0	–		9	0	···	
C85B8B × Tv	8	0	···		···		···		8	0	–		8	0	···	
C85B8B × NL6	4	0	···		···		···		4	0	–		4	0	···	
Pathotype I × Pathotype III																
C83M40 × C83M47	13	0	11	2[g]	1	12[g]	10	3	13	0	–		13	0	5	0
C85B4 × C83M47	14	0	0	14[g]	13	1[g]	6	8	14	0	–		14	0	7	0
C85B6 × C83M47	6	0	4	2	5	1	4	2	6	0	–		6	0	6	0
C85T1 × 19c	7	0	···		···	1	···		5	3	6	1	4	3	3	4

[a] *: Incompatible interaction (isolate avirulent); +: compatible interaction (isolate virulent).
[b] Not presented; Californian isolates were virulent against corresponding *Dm* gene, therefore assumed to be homozygous virulent (*aa*).
[c] Cultivars containing single *Dm* gene not available at time of testing.
[d] European isolate homozygous for avirulence allele (*AA*).
[e] Not studied.
[f] Interactions between cultivars with *Dm*11 and progeny of crosses with pathotype III isolates were difficult to score unambiguously as compatible or incompatible because of delayed sporulation and host necrosis; see text.
[g] See text.

TABLE 5. The virulence genotypes of Californian isolates of *Bremia lactucae*

	Avirulence locus[a] 1	2	3	4	5/8	6	7	10	11	13	14	15	16
Pathotype I	*aa*	*Aa*	*aa*[b]	*Aa*	*Aa*	*aa*	*aa*	*aa*	*Aa*	*aa*	*aa*	*aa*[b]	*AA*
Pathotype II	*Aa*	*aa*	*aa*	*Aa*	*aa*	*aa*	*aa*	*aa*	*aa*[b]	*aa*	*aa*	*Aa*	*Aa*
Pathotype III	*AA*	*aa*	*aa*	*aa*	*aa*	*aa*	*aa*	*AA*	?[b]	*aa*	*aa*	*AA*	*aa*
C85T1	?[b]	*aa*	*aa*	*Aa*	*aa*	*aa*	*aa*	*Aa*	*Aa*	*aa*	*aa*	*aa*	*Aa*

[a] R-factors 12 and 18 are incompletely characterized.
[b] See text.

(12,13), were also reported in the 1930s, but were not revealed in the present survey. These races cannot be equated with any of the current pathotypes and, therefore, may represent additional variation once present in the Californian population. Pathotype I isolates were probably predominant until pathotype II isolates, first identified in Texas as race 6 (26), appeared in California in 1971 on Calmar (5). Pathotype III was not detected until 1983, its origin is unclear but cannot be from crosses or processes of asexual variation involving the preexisting pathotypes as it is avirulent (*AA*) on cultivars with *Dm*10, whereas pathotypes I and II are both virulent (and hence *aa*); pathotype III is also homozygous at the avirulent locus *A*1, whereas pathotype I is virulent (*a*1*a*1).

The structure of the Californian population of *B. lactucae* is clearly different from the European population. A great diversity of virulence phenotypes has been identified in European surveys, including some involving fewer isolates than the present study (1,25,29), and others concentrated on one agricultural region or single fields (1,3,9). Although particular disease outbreaks can be dominated by a single virulence phenotype (3,6), this dominance does not extend over several years and to widely separated production areas. It is thought that the sexual cycle, which is important in the epidemiology of *B. lactucae* in Sweden (9) and almost certainly occurs elsewhere in Europe, is responsible for these variations in virulence phenotype. The concept of distinct pathogen races or pathotypes is inappropriate in such sexually reproducing populations. In contrast, the Californian population consists of very few virulence phenotypes, a situation similar to that in Australia where one phenotype is predominant throughout the continent (28). Mechanisms of variation operating in Europe therefore seem not to occur extensively in California; in particular, the sexual cycle does not appear to be widespread. Virulence loci in *B. lactucae* are almost always unlinked (23; T. W. Ilott and R. W. Michelmore, *unpublished*); therefore, sexual reproduction would be expected to generate isolates with many different virulence phenotypes. The limited number of virulence phenotypes identified in this survey is therefore strong evidence that the sexual cycle is not important in the epidemiology of the pathogen in California, despite the presence of two sexual compatibility types. Furthermore, sexual compatibility type is not associated with virulence phenotype in sexually reproducing populations of *B. lactucae* (8,21), whereas, in California, pathotype and sexual compatibility type are perfectly correlated.

The lack of virulence phenotypes apparently generated by sexual recombination may, however, have been due to the recent occurrence of the B_2 sexual compatibility type in California. Before 1971, B_2 isolates may have been rare or absent from California, and recombinant isolates produced by infrequent sexual reproduction since then might not have been detected in our survey. As pathotype I isolates are avirulent on Calmar and Salinas, the predominant summer cultivars grown in California over the past 20 yr, opportunities for interaction between B_1 and B_2 isolates may have been limited, although both can infect wild *Lactuca* spp. and domestically grown lettuce. Nevertheless, no recombinant isolates were collected from sites where both pathotype I and pathotype II were present. The infertility of laboratory crosses also suggested that pathotypes I and II are unlikely to be interfertile in nature. The few progeny isolates produced by pairings between these pathotypes sporulated poorly and declined in fitness when subcultured. Such isolates would probably not survive in the field. Therefore, although genetic analysis of avirulence indicated that pathotype I isolates virulent on *Dm*3 and *Dm*15 could have resulted from crosses between pathotypes I and II, this appears unlikely, especially as other virulence phenotypes that would have been generated were not found.

Laboratory crosses between pathotype I and pathotype III did produce viable progeny. As pathotype III has only recently been identified in California, and the distribution of pathotype I is restricted by the predominance of cultivars with *Dm*5/8, opportunities for sexual reproduction may have been restricted to wild hosts. Isolate C85T1, however, could have resulted from sexual recombination between pathotypes I and III. Analysis of other genetic markers (particularly restriction fragment-length polymorphisms, currently being developed for *B. lactucae*; R. W. Michelmore et al, *unpublished*) will test this suggestion. Future isolate surveys may detect sexual progeny of these pathotypes.

There is little evidence that asexual mechanisms of variation have been important in the Californian population of *B. lactucae*. Pathotype I, despite many years of asexual existence, is heterozygous at several avirulence loci, including *A*5/8. Somatic recombination in pathotype I isolates could therefore have resulted in isolates virulent against *Dm*5/8, but this apparently did not occur as Calmar (*Dm*5/8, *Dm*7, and *Dm*13) remained free from downy mildew for more than 10 yr until pathotype II appeared in California, despite being the predominant cultivar during that time. Variations in virulence phenotype between isolates classified as pathotype I were found, however. Pathotype I isolates virulent on *Dm*3 or *Dm*15 may be products of asexual variation, although as these resistance genes have never been commercially deployed in California, selection for such variants seems unlikely. The different phenotypes within pathotype I may have been selected for following a period when greater variation was present in the Californian population (12,13), and therefore pathotype I possibly should not be considered as a single homogeneous population. Molecular markers will again be useful in resolving the origin of this diversity.

There may also be minor variation within pathotype II. Whereas most interactions were easily scored as compatible or incompatible, those involving pathotype II isolates and cultivars expressing *Dm*11 were more difficult to interpret. In these interactions sporulation was delayed (1–3 days later) and was less profuse than on susceptible host cultivars. Sporulation intensity varied from isolate to isolate, but consistent differences between pathotype II isolates could not be demonstrated. It may be that Capitan and Hilde × *L. serriola* carry modifier genes that affect the development of otherwise compatible or incompatible interactions with certain isolates.

Pathotype II isolates also sporulated sparsely, with associated necrosis, on LSE 18 (*Dm*16); however, interactions between those isolates and Kinemontepas (*Dm*10, *Dm*13, and *Dm*16) were completely incompatible. Avirulence to LSE 18 and Kinemontepas cosegregated among progeny from crosses between pathotype II isolates and isolates virulent on both cultivars. As avirulence genes are rarely linked (23; T. W. Ilott and R. W. Michelmore, *unpublished*), this suggested that pathotype II isolates contain a gene for avirulence to *Dm*16, and that genes modifying interactions between these isolates and cultivars with *Dm*16 may be present in both pathogen and host.

Oospores are considered to be important in the epidemiology of *B. lactucae* in Europe (9), and in other downy mildews (27), as a means of survival between crops. In California, however, sexual reproduction is unlikely to be significant in this way. As all three pathotypes can infect weedy *Lactuca* species, these hosts may act as reservoirs of downy mildew. Domestically grown lettuce could also be important. In addition, commercial lettuce is grown all year in California. Although seasonal crops are in widely separated growing areas, the absence of geographic differences in virulence phenotype indicates that there are not several isolated populations of the fungus and that transmission between crops is occurring by means of asexual spores.

The results of this survey cannot be used for analyses of population genetics, such as linkage disequilibria of virulence genes, which may be useful in planning strategies for cultivar deployment. Such analyses are only valid when they consider a large, sexually reproducing pathogen population from a homogeneous host (30). This is clearly not the case in the Californian population of *B. lactucae*. The absence of isolates carrying virulence to both *Dm*1 and *Dm*5/8, for example, is probably only due to the lack of sexual reproduction among Californian isolates. Based on data from European surveys and genetic studies, strong selection against combinations of virulence alleles not detected in this study would not be expected. Nevertheless, the survey does suggest that a combination of *Dm*1 and *Dm*5/8 would be useful in cultivar development, as it confers

resistance to all three pathotypes present in California. A current breeding objective is the introduction of *Dm*1, *Dm*4, and *Dm*5/8 into suitable cultivars (R. W. Michelmore et al, *unpublished*). In Europe, such a combination of resistance genes would not be expected to remain effective for long. In California, it might prove more durable, given the limited variation in the Californian population of *B. lactucae*. Two mutations would be required to create a virulent isolate asexually. Sexual reproduction between pathotypes I and III, however, may render this combination of resistance genes ineffective; although genetic analyses of avirulence in these pathotypes indicated that at least two sexual generations on a cultivar carrying none of these *Dm* genes would be necessary to generate an isolate virulent on all three resistance genes. A cultivar containing *Dm*1, *Dm*4, and *Dm*5/8 may, therefore, provide at least transient control of lettuce downy mildew in California.

LITERATURE CITED

1. Cornford, C. A., and Pitt, D. 1985. The nature of virulence in isolates of *Bremia lactucae* from South Devon. Plant Pathol. 34:38-40.
2. Crute, I. R. 1981. The host specificity of Peronosporaceous fungi and the genetics of the relationship between host and parasite. Pages 237-250 in: The Downy Mildews. D. M. Spencer, ed. Academic Press, New York.
3. Crute, I. R. 1986. The geographical distribution and frequency of virulence determinants of *Bremia lactucae*: Relationships between genetic control and host selection. Pages 193-212 in: Populations of Plant Pathogens: Their Dynamics and Genetics. M. S. Wolfe and C. E. Caten, eds. Blackwell Scientific Publications, Oxford.
4. Crute, I. R., and Johnson, A. G. 1976. The genetic relationship between races of *Bremia lactucae* and cultivars of *Lactuca sativa*. Ann. Appl. Biol. 83:125-137.
5. Crute, I. R., and Johnson, A. G. 1976. Breeding for resistance to lettuce downy mildew, *Bremia lactucae*. Ann. Appl. Biol. 84:287-290.
6. Dixon, G. R., and Wright, I. R. 1978. Frequency and geographical distribution of specific virulence factors in *Bremia lactucae* populations in England from 1973 to 1975. Ann. Appl. Biol. 88:287-294.
7. Flor, H. H. 1956. The complementary genetic systems in flax and flax rust. Adv. Genet. 8:29-54.
8. Gustafsson, M., Arhammer, M., and Gustavsson, I. 1983. Linkage between virulence genes, compatibility types and sexual recombination in the Swedish population of *Bremia lactucae*. Phytopathol. Z. 108:341-354.
9. Gustafsson, M., Liljeroth, E., and Gustavsson, I. 1985. Pathogenic variation and sexual reproduction in Swedish populations of *Bremia lactucae*. Theor. Appl. Genet. 70:643-649.
10. Hewitt, E. J. 1952. Sand and Water Culture Methods Used in the Study of Plant Nutrition. Technical Communication of the Commonwealth Bureau of Horticulture and Plantation Crops. East Malling, Maidstone, Kent.
11. Hulbert, S. H., and Michelmore, R. W. 1985. Linkage analysis of genes for resistance to downy mildew (*Bremia lactucae*) in lettuce (*Lactuca sativa*). Theor. Appl. Genet. 70:520-528.
12. Jagger, I. C., and Chandler, N. 1933. Physiologic forms of *Bremia lactucae* on lettuce. (Abstr.) Phytopathology 23:18-19.
13. Jagger, I. C., and Whitaker, T. W. 1940. The inheritance of immunity from mildew (*Bremia lactucae*) in lettuce. Phytopathology 30:427-433.
14. Johnson, A. G., Crute, I. R., and Gordon, P. L. 1977. The genetics of race specific resistance in lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). Ann. Appl. Biol. 86:87-103.
15. Johnson, A. G., Laxton, S. A., Crute, I. R., Gordon, P. L., and Norwood, J. M. 1978. Further work on the genetics of race specific resistance in lettuce (*Lactuca sativa*) to downy mildew (*Bremia lactucae*). Ann. Appl. Biol. 89:257-264.
16. Lebeda, A. 1981. Population genetics of lettuce downy mildew (*Bremia lactucae*). Phytopathol. Z. 101:228-239.
17. Mason, P. A. 1973. Studies on the biology of *Bremia lactucae* Regel. Ph.D. thesis. Cambridge University.
18. Michelmore, R. W., and Crute, I. R. 1982. A method for determining the virulence phenotype of isolates of *Bremia lactucae*. Trans. Br. Mycol. Soc. 79:542-546.
19. Michelmore, R. W., and Ingram, D. S. 1980. Heterothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 75S:47-56.
20. Michelmore, R. W., and Ingram, D. S. 1981. Recovery of progeny following sexual reproduction of *Bremia lactucae*. Trans. Br. Mycol. Soc. 77:131-137.
21. Michelmore, R. W., and Ingram, D. S. 1982. Secondary homothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 78:1-9.
22. Michelmore, R. W., Norwood, J., Ingram, D. S., Crute, I. R., and Nicholson, P. 1984. The inheritance of virulence in *Bremia lactucae* to match resistance factors 3, 4, 5, 6, 8, 9, 10 and 11 in lettuce (*Lactuca sativa*). Plant Pathol. 33:301-305.
23. Norwood, J. M., and Crute, I. R. 1984. The genetic control and expression of specificity in *Bremia lactucae* (lettuce downy mildew). Plant Pathol. 33:385-400.
24. Norwood, J. M., Michelmore, R. W., Crute, I. R., and Ingram, D. S. 1983. The inheritance of specific virulence in *Bremia lactucae* (downy mildew) to match resistance factors 1, 2, 4, 6 and 11 in *Lactuca sativa* (lettuce). Plant Pathol. 32:177-186.
25. Osara, K., and Crute, I. R. 1981. Variation for specific virulence in the Finnish *Bremia lactucae* population. Ann. Agric. Fenn. 20:198-209.
26. Sleeth, B., and Leeper, P. W. 1966. Mildew resistant lettuce susceptible to a new physiologic race of *Bremia lactucae* in south Texas. Plant Dis. Rep. 50:460.
27. Spencer, D. M., ed. 1981. The Downy Mildews. Academic Press, New York.
28. Trimboli, D. S., and Crute, I. R. 1984. The specific virulence characteristics of *Bremia lactucae* (lettuce downy mildew) in Australia. Aust. Plant Pathol. 12:58-60.
29. Wellving, A., and Crute, I. R. 1978. The virulence characteristics of *Bremia lactucae* populations present in Sweden from 1971 to 1976. Ann. Appl. Biol. 89:251-256.
30. Wolfe, M. S., and Knott, D. R. 1982. Populations of plant pathogens: Some constraints on analysis of variation in pathogenicity. Plant Pathol. 31:79-90.