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

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ABSTRACT


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 B1 SCT, and all pathotype II and III isolates had the B2 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.

Additional 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and other incompletely characterized resistance factors also exist (11,14,15; B. Farrara and R. W. Michelmore, unpublished).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 Dm7, Dm5,8, and Dm13 (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
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).

_ Bur. latae_. 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 × 10⁶ 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 μE m⁻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-cotyledon isolates were obtained 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-cotyledon 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

<p>| TABLE 1. The differential series of lettuce cultivars resistant to <em>Bremia latae</em>. used in this study. |
|-----------------------------------|-----------------------------------|-----------------------------------|-----------------------------------|</p>
<table>
<thead>
<tr>
<th>Primary series#</th>
<th>Secondary series#</th>
<th>Dm gene</th>
<th>Cultivar/line</th>
<th>Dm gene (or R-factor)</th>
</tr>
</thead>
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<tr>
<td>Lednicky 1</td>
<td>Blondine</td>
<td>1</td>
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<tr>
<td>UCDM2</td>
<td>Mildura</td>
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<td>+13</td>
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<tr>
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<td>Amplus</td>
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<td>+14</td>
<td></td>
</tr>
<tr>
<td>T57/ R4</td>
<td>Liba</td>
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<td></td>
</tr>
<tr>
<td>Valmair/Valverde 5/8</td>
<td>Kordaat</td>
<td>5</td>
<td>+11</td>
<td></td>
</tr>
<tr>
<td>Sable 6</td>
<td>Avondale</td>
<td>6</td>
<td>+13</td>
<td></td>
</tr>
<tr>
<td>Mesa 7 + 13</td>
<td>Salinas/Calmar</td>
<td>7</td>
<td>+13</td>
<td></td>
</tr>
<tr>
<td>UCDM10</td>
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<td></td>
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<tr>
<td>Capitan 11</td>
<td>Fila</td>
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<td></td>
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<tr>
<td>Hilde × L. latae</td>
<td>Gerber Winterkonig</td>
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<tr>
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<td>+13</td>
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<td>Kinemontepas</td>
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<td>LSE 18</td>
<td>Diana</td>
<td>18</td>
<td>+13</td>
<td></td>
</tr>
<tr>
<td>Cobham Green</td>
<td>None</td>
<td></td>
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</tr>
</tbody>
</table>

# As determined by Hubert and Michelmore (11); B. Farrara and R. W. Michelmore, unpublished.
# Cultivars/lines with well-characterized downy mildew resistance genes (Dm) were developed at UC Davis.
# Cultivars/lines with combinations of well-characterized resistance genes used to confirm conclusions from the primary series and lines with incompletely characterized R-factors.
# Resistance factors (R-factors) have been used when the resistance in a cultivar has not been fully characterized in terms of Dm genes.
# Dm5 and Dm8 are the same gene (11).
# Two cultivars are used because interactions with Dm11 are sometimes difficult to score.
# Susceptible check.

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 × 300 × 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. _Bur. latae_. is predominantly heterothallic, with two sexual compatibility types designated B₁ and B₂ (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 B₁, another with a B₂ isolate, and a third with no further isolate (to test for homothallism). After a 1-h incubation in the growth room, the cotyledons were examined using a dissecting microscope (40X) for the presence of oospores. Isolates forming oospores in combination with the B₁ compatibility type isolate were designated B₁ and vice versa. No homoathallic 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 10⁶ 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).

RESULTS

Virulence phenotypes and sexual compatibility types of Californian isolates. Only seven virulence phenotypes were detected from a total of 118 Californian isolates of _Bur. latae_. 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 Dm11. Pathotype I isolates varied in their response to Dm3 and Dm15; 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 3 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 Dm2, Dm3, Dm5/8, Dm6, Dm7, R12, Dm13, or Dm14, but sporulation on T57R4 (Dm4), UCDM10 (Dm10), Capitan (Dm11), Hilde × L. serriola (Dm11), and LSE18 (Dm16) 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 California 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 virulence in Californian isolates of B. lactucae. In this paper, only the segregation of virulence is described. The genetics of virulence in B. lactucae has already been studied in detail (20, 22, 24, 28, 29), Virulence is normally conferred by the presence of homologous 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 virulence 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). Therefore, only small numbers of progeny needed to be analyzed from each cross to determine whether a particular virulence locus was segregating. Segregation of virulence to a particular resistance gene implied that an isolate was heterozygous at that locus. Genetics of virulence at the A2, A4, A5/8, A11, A15, and A16 loci is described for isolates of pathotype I (the A3 locus could not be studied as isolates virulent to Dm3 were lost due to a freezer breakdown), at the A1, A4, A15, and A16 loci for pathotype II and the A1, A10, A11, and A15 loci for pathotype III (Table 4). Segregation of virulence to R18 (Mariska) could not be studied as seed of this cultivar was unavailable until the investigation. At least two isolates of each pathotype were studied to sample potential variation within a pathotype.

Large numbers of ooosporides 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 virulence in these crosses (Table 4) indicated that the genotype of pathotype I II isolates to be 1: A12, A44A1, A15A15, and A16A16. The genotype of pathotype III is: A12A12, A10A10, and A15A15. Interactions between cultivars with Dm11 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 Dm11 locus is unclear. The complete genotypes are given in Table 5.

Although crosses involving pathotype I formed as many ooosporides 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 was 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; C85M40 (pathotype I) × pathotype II isolates (two isolates); C85M6 (pathotype I) × pathotype II isolates (two isolates); C85M8 × S1 (European); C85M6 × C1 (European); C85M40 × AM (Australian); C85M6 × A; C85M40 × JP1 (Japanese); C85M6 × JP1. Crosses between C85M40 and the European isolates S1 and C1 each resulted in only one fit progeny isolate being recovered. One progeny isolate was also obtained from a cross between C85M8 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 A2, A4, A5/8, and A11, and homozgous at the A16 locus. Segregation data for alleles at the A2 and A4 loci, however, were complex. In the cross C85M40 (pathotype I) × C83M47 (pathotype III) all progeny isolates were virulent on UCDM12 (Dm2), although C85M40 was avirulent, and all but one were avirulent on T57R4 (Dm4). 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 ooosporide germination or isolate fitness. If such genes were linked to the A2 and A4 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 B. lactucae |
|--------------------------|--------------------------|--------------------------|
| Host resistance gene or factor | Pathotype               |
|                           | I   | II  | III | C85T1 |
| Dm12                     | *   | *   | +   | *    |
| Dm2                      | +   | *   | +   | +    |
| Dm3                      | *   | +   | +   | +    |
| Dm5/8                    | *   | +   | +   | +    |
| Dm7                      | +   | *   | +   | *    |
| Dm10                     | +   | +   | *   | *    |
| Dm11                     | +   | *   | +   | *    |
| Dm13                     | +   | +   | *   | *    |
| Dm14                     | +   | *   | +   | *    |
| Dm15                     | +   | +   | *   | *    |
| Dm16                     | +   | +   | *   | *    |
| R12                      | +   | *   | +   | *    |
| R18                      | +   | *   | +   | *    |
| None (Cobham Green)      | +   | +   | +   | +    |

Sexual compatibility type: B1, B2, B3, B4

Number of isolates: 14, 62, 41

*+*: Compatible interaction; *−*: incompatible interaction.

Seven pathotype I isolates were virulent to Dm3, seven were avirulent.

Sporulation of pathotype II isolates on cultivars with Dm11 was variable.

Six pathotype I isolates were virulent to Dm15, five were avirulent, and the virulence of three was undetermined.

Only one isolate of each pathotype was tested on R18.
isolate was later lost. The isolate was heterozygous at the loci A4, A10, A11, and A16; it could have been homozygous or heterozygous at A1.

**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>
<thead>
<tr>
<th>Isolate</th>
<th>SCT</th>
<th>Avirulence loci</th>
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<tr>
<td></td>
<td></td>
<td>1   2   4   5/8 10 11 15 16</td>
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<tr>
<td>19c</td>
<td>B1</td>
<td>aa  aa  aa  aa  aa  aa  aa  aa</td>
</tr>
<tr>
<td>Tv</td>
<td>B1</td>
<td>aa  aa  aa  aa  aa  aa  aa  aa</td>
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<tr>
<td>NL6</td>
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<td>aa  aa  aa  aa  aa  aa  aa  aa</td>
</tr>
<tr>
<td>SF5</td>
<td>B1</td>
<td>AA  aa  aa  aa  aa  aa  aa  aa</td>
</tr>
<tr>
<td>CS9</td>
<td>B1</td>
<td>AA  AA  aa  aa  aa  aa  aa  aa</td>
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<tr>
<td>IM25/R7</td>
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<tr>
<td>IM25/P11</td>
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<td>aa  AA  AA  AA  AA  AA  AA  AA</td>
</tr>
</tbody>
</table>

*As characterized previously (22,23) and by T. W. Hilt, S. H. Hu, and R. W. Michelmore (unpublished data).

* A: Dominant avirulence allele; a: recessive virulence allele.

* 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).

*SCT: Sexual compatibility type.

* A dominant inhibitor of avirulence has been reported to be epistatic to A5 (23) but is not present in any isolate used in this study.

* Full genotype undetermined.

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

<table>
<thead>
<tr>
<th>Cross</th>
<th>Avirulence loci</th>
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<tbody>
<tr>
<td>Pathotype I × European</td>
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<tr>
<td>C83R4 × IM25/P11</td>
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</tr>
<tr>
<td>Pathotype II × European</td>
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<td>C83R5 × IM25/P11</td>
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<td>C83R2 × CS9</td>
<td></td>
</tr>
<tr>
<td>C85R8 × Tv</td>
<td></td>
</tr>
<tr>
<td>Pathotype III × European</td>
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</tr>
<tr>
<td>C83M46 × IM25/P11</td>
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<tr>
<td>C83M47 × IM25/P11</td>
<td></td>
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<td>C83M47 × Tv</td>
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<td>C83M47 × Tv</td>
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<tr>
<td>C83M40 × C83M47</td>
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<tr>
<td>C85B4 × C83M47</td>
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</tr>
<tr>
<td>C85B6 × C83M47</td>
<td></td>
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<tr>
<td>C85T1 × 19c</td>
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</tbody>
</table>

*Not presented; Californian isolates were virulent against corresponding Dm gene, therefore assumed to be homozygous virulent (aa).

* Cultivars containing single Dm gene not available at time of testing.

* European isolate homologous for avirulence allele (AA).

* Not studied.

* Interactions between cultivars with DmIII 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.

* See text.

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

<table>
<thead>
<tr>
<th>Avirulence locus</th>
<th>1</th>
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<th>4</th>
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* R-factors 12 and 18 are incompletely characterized.

* See text.
other genetic markers (particularly restriction fragment-length polymorphisms, currently being developed for \( B. \) \textit{laetarea}, R. W. Michelmore \textit{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 \( B. \) \textit{laetarea}. Pathotype I, despite many years of asexual existence, is heterozygous at several avirulence loci, including \( A_{5}/S \). Somatic recombination in pathotype \( I \) isolates could therefore have resulted in isolates virulent against \( D_{m5/8} \), but this apparently did not occur as \( C_{almar} (D_{m5/8}, D_{m7}, \) and \( D_{m13} \) remained free from downy mildew for more than 10 yr until pathotype \( I \) 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 \( D_{m3/} \) or \( D_{m15} \) may both be products of asexual variation, although as these resistance genes have never been commercially deployed in California, selection for such variants seems unlikely. The differences between 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 \( I \). Whereas most interactions were easily scored as compatible or incompatible, those involving pathotype \( I \) isolates and cultivars expressing \( D_{m11} \) 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 \( I \) isolates could not be demonstrated. It may be that Captain and Hilde \( t \) L. \textit{serioisolata} carry modifier genes that disturb the development of otherwise compatible or incompatible interactions with certain isolates.

Pathotype \( I \) isolates also sporulated sparsely, with associated necrosis, on \( LSE \) 18 \( (D_{m16}) \); however, interactions between those isolates and \( K \) \textit{cinemontes} \( (D_{m10}, D_{m13}, \) and \( D_{m16} \) were completely incompatible. Avirulence to \( LSE \) 18 and \( K \) \textit{cinemontes} cosegregated among progeny from crosses between pathotype \( I \) isolates and isolates virulent on both cultivars. As virulence genes are rarely linked \( (23; T. W. Iott and R. W. Michelmore, \textit{unpublished}) \), the interaction of these isolates and cultivars with \( D_{m16} \) may be present in both pathogen and host.

Osparos are considered to be important in the epidemiology of \( B. \) \textit{laetarea} 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 weakly \textit{laetarea} 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 heterogeneous host (30). This is clearly not the case in the Californian population of \( B. \) \textit{laetarea}. The absence of isolates carrying virulence to both \( D_{m1} \) and \( D_{m5/8} \), 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 \( D_{m1} \) and \( D_{m5/8} \) 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 Dm1, Dm4, and Dm5/8 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 Dm1, Dm4, and Dm5/8 may, therefore, provide at least transient control of lettuce downy mildew in California.

LITERATURE CITED


