# Insensitivity to Metalaxyl in California Populations of *Bremia lactucae* and Resistance of California Lettuce Cultivars to Downy Mildew

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## ABSTRACT

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Ninety-seven California isolates of *Bremia lactucae* were characterized for virulence phenotype, metalaxyl insensitivity, and sexual compatibility type from 1987 to 1989. Seventy-eight lettuce cultivars and 85 lettuce accessions were screened for resistance to lettuce downy mildew caused by the three major California pathotypes of *B. lactucae*. Of the 97 *B. lactucae* isolates, two were California pathotype IA, four were pathotype II, 30 were pathotype III, and 61 seemed to be pathotype IV. Several pathotype IV isolates were separated easily into derivatives having virulence phenotypes identical to either pathotype II or pathotype III. Two isolates derived from pathotype IV isolates, however, had virulence phenotypes that deviated from virulence phenotypes previously reported for California. When assayed for fungicide insensitivity, the two pathotype IA isolates were sensitive to metalaxyl, while the four pathotype II isolates, 73% of the pathotype III isolates, and 82% of the pathotype IV isolates were insensitive to metalaxyl. When pathotype IV isolates were separated into pathotype II and pathotype III components, both derivatives expressed the same sensitivity to metalaxyl as their pathotype IV progenitor. Of the 175 lettuce accessions tested, 114 were resistant to at least one California pathotype, with 51, 12, and 82 being resistant to at least pathotype IA, II, or III, respectively. In addition, 11 accessions were resistant to all three pathotypes. Of the 21 crisphead cultivars that were resistant to at least pathotype III, five may be adapted for the coastal valleys where downy mildew can be severe. Combining genetic and chemical strategies may provide effective control of downy mildew in California if metalaxyl-insensitive pathotype II derivatives do not cause epidemics in the field.

*Additional keywords:* Disease control, disease resistance, *Lactuca sativa*, Ridomil 2E.

The interaction between lettuce, *Lactuca sativa* L., and the downy mildew fungus, *Bremia lactucae* Regel, is one of the best characterized gene-for-gene relationships (6,8,13,16,19,20). Thirteen single dominant resistance genes (*Dm*) clustered into four linkage groups in the host and complementary avirulence genes in the pathogen have been described to date. *B. lactucae* is a heterothallic, diploid Oomycete fungus (14). Genetic studies have shown avirulence alleles (*Avr*) to be dominant to virulence alleles (*avr*). Recent collections of California populations of *B. lactucae* have been characterized for virulence phenotype, sexual compatibility type, and molecular markers (11,12). Based on these data, the California isolates have been classified into pathotypes I, II, III, and IV. Each pathotype seems to be asexually propagated; there was no evidence of sexual reproduction in California. Pathotype IV seems to have resulted from the somatic fusion of pathotypes II and III (11).

Lettuce downy mildew can result in extensive losses in lettuce production and quality. These losses are caused by decreases in marketable yield before harvest and deterioration in quality during postharvest transit and storage. The five counties encompassing the coastal valleys of California account for about 40% of the lettuce crop in the United States (1,18). Epidemics of downy mildew can be severe during much of the spring and summer growing seasons in the coastal valleys, especially when cool temperatures and high humidity occur. Methods to control downy mildew include cultural practices to minimize canopy humidity, the growth of resistant cultivars, and application of fungicides. Effective, though transient, control of downy mildew in California and elsewhere has been achieved by each of the latter two methods.

At least 13 major genes have been reported that confer resistance to downy mildew as well as several genetically uncharacterized sources of resistance (6,8–10,14). Since the mid-1960s, *Dm5/8* has been present in the majority of coastal crisphead cultivars, the predominant lettuce type grown in California. *Dm5/8* remained effective in California against downy mildew until a new strain rendered this resistance ineffective in the early 1970s (6). Following the breakdown in resistance conferred by *Dm5/8*, chemical control using manganese- and copper-based compounds resulted in an intermediate but unreliable level of control. Effective chemical control of lettuce downy mildew in California was first achieved in 1983 when metalaxyl (as Ridomil 2E, Ciba-Geigy

Corp.) became available for foliar application. In 1987, however, metalaxyl apparently failed to control downy mildew in some fields in the coastal valleys. Insensitivity to metalaxyl has also been reported in *B. lactucae* in Europe (7) and related Oomycete pathogens (*Phytophthora infestans* (Mont.) de Bary, *Peronospora* Corda. spp. and *Pseudoperonospora cubensis* (Berk. & M. A. Curtis) Rostovzev) during the early 1980s (2,17). While metalaxyl has been initially highly effective, the target organisms have frequently developed insensitivity when exposed to metalaxyl alone.

A strategy integrating chemical applications and genetic resistance has been utilized effectively in England to control lettuce downy mildew (7). Applications of metalaxyl on lettuce in England began in 1978 and initially provided excellent control of downy mildew. In 1983, control failure attributable to metalaxyl insensitivity in *B. lactucae* was confirmed in one lettuce district, and by 1985, insensitive isolates had been collected from all the major lettuce-growing regions (7). Metalaxyl insensitivity was initially restricted to isolates of one virulence phenotype. Growers were advised to use lettuce cultivars containing *Dm11*, which was effective against the metalaxyl-insensitive isolates, and to apply metalaxyl, mixed with the protectant fungicide mancozeb, to control all metalaxyl-sensitive isolates. Even though potential for sexual recombination exists and variation for virulence phenotype is extensive in England (7), control failures attributable to isolates that were both metalaxyl-insensitive and virulent on *Dm11* were undetected 4 yr after the integrated control strategy was implemented along with recommendations to reduce the likelihood of generating such isolates (3,4). Metalaxyl-insensitive isolates that are virulent on *Dm11* have now been detected; however, applications of metalaxyl (in combination with a protectant fungicide) onto cultivars containing *Dm6* and *Dm11* continue to be effective (4).

In California, the variability for virulence phenotype in *B. lactucae* is restricted to a few asexually propagated pathotypes, and there is no evidence of sexual reproduction (11,12). Therefore, a strategy combining genetic and chemical control of downy mildew may be both effective and durable in California. This study was undertaken to determine the extent of metalaxyl insensitivity among the populations of downy mildew in the major lettuce-growing regions of California (3) and to determine the resistance of lettuce cultivars to the California pathotypes. This information is being used to assess a strategy of combining genetic and chemical components to control downy mildew in California.

## MATERIALS AND METHODS

Procedures for isolate maintenance and determination of virulence phenotype and sexual compatibility type were as described previously (12,14), except where noted. An inoculum density of $1–3 \times 10^5$ conidia per milliliter was used for most experiments.

**Collection and maintenance of isolates.** Isolates of *B. lactucae* were obtained during 1987, 1988, and 1989 from five regions of California (Santa Maria and Salinas valleys, and Ventura, Fresno, and Tulare counties). Each isolate, derived from one or a few infected lettuce leaves, was collected by the authors or by cooperators who mailed samples to the authors. Previous analyses had shown that collections from the same field nearly always had the same pathotype; therefore, for this survey collections from the same field were considered as a single isolate. The cultivar Dark Green Boston (Asgrow Seed Co., San Juan Bautista, CA; synonym Cobham Green), which has no known genes for resistance to downy mildew, was used for initial isolation (as detached cotyledons) and routine maintenance (as intact seedlings) of all isolates, except where noted.

**Determination of virulence phenotype.** Each isolate was inoculated individually onto 7-day-old seedlings of a differential series of 17 lines (Table 1) planted into clear plastic compartmented boxes (Corth Plastics, Santa Clara, CA). The seedlings were scored for the presence of asexual sporulation and host necrosis approximately 7, 12, and 17 days after inoculation. The virulence phenotype of each isolate was compared with the virulence phenotypes of the California pathotypes reported by Ilott et al (12; Table 2). Sporulation on each differential line was usually either profuse by 7–12 days after inoculation (compatible reaction) or totally absent (incompatible reaction). Profuse sporulation on a differential line indicated that the isolate did not express the avirulence (*Avr*) allele(s) matching the corresponding *Dm* allele(s) in the plant. Exceptions, delayed or sparse sporulation, are discussed later.

**Determination of sexual compatibility type.** The sexual compatibility type (SCT) of each isolate was determined by matings with isolates of known SCT. Conidia of each field isolate were mixed separately at equal spore densities with conidia of isolates of each of the two SCT, $B_1$ and $B_2$; each mixture was incubated by placing drops of conidial suspension onto moistened, detached cotyledons of cultivar Cobham Green in culture dishes. For most isolates, a third inoculation containing only conidia from the field isolate was made to test for homothallism (15). Field isolates forming oospores in combination with the $B_1$ type isolate were designated SCT $B_2$ and vice versa.

**Determination of metalaxyl sensitivity in *B. lactucae*.** Cobham Green seedlings were grown in sealed plastic GA-7 boxes (Magenta Corp., Chicago, IL) on blotter paper moistened with nutrient solution containing 0, 1, 25, or 100 μg of metalaxyl per milliliter, supplied as Ridomil 25WP or Ridomil 2E (Ciba-Geigy Corp.). The presence of asexual sporulation and host necrosis was assessed approximately 7, 12, and 17 days following inoculation.

**Determination of resistance to downy mildew in lettuce.** Seed of advanced breeding lines (tested on a confidential basis) and of cultivars were donated by seed company, university, and USDA cooperators or were retrieved from the University of California, Davis (UCD) seed collection. Seed of wild *Lactuca* species and experimental accessions were from the UCD seed collection. Sets of three clear plastic boxes, 18 compartments each (12), were sown with 30–50 seeds per compartment, 17 accessions per box, plus Cobham Green as a positive control. Seven days after planting, each box was inoculated with one of three isolates representing the three major California pathotypes, IA, II, or III (11,12; Table 2). The presence of sporulation and host necrosis was assessed approximately 7, 12, and 17 days following inoculation. At the same time, the virulence phenotype of each standard isolate was verified using the differential series of lines (Table 1). Each lettuce accession was tested against an isolate of each pathotype at least twice.

## RESULTS

**1987, 1988, and 1989 survey of California isolates of *Bremia lactucae*.** The 16 isolates characterized in 1987 were collected from

TABLE 1. Differential series of lettuce lines resistant to downy mildew used in this study

| Line | *Dm* gene[a] |
|---|---|
| Dark Green Boston | None known |
| Lednicky | *1* |
| UCDM2 | *2* |
| Dandie | *3* + ?[b] |
| R4/T57 | *4* |
| Valmaine | *5/8* |
| Sabine | *6* |
| LSE57/15 | *7* + ? |
| UCDM10 | *10* |
| Sucrine | *5/8* + *10* |
| Capitan | *11* |
| H × LS | *11* |
| Hilde | R12[c] |
| Empire | *13* |
| UCDM14 | *14* |
| PIVT1309 | *15* |
| LSE18 | *16* |

[a] Farrara et al, 1987 (6).
[b] ? = may contain an additional, rarely effective factor.
[c] R-factor = incompletely characterized genetic resistance factor.

two growing regions: Salinas, 14; and Santa Maria, 2. The 59 isolates characterized in 1988 were collected from four regions: Salinas, 29; Santa Maria, 25; Ventura County, 4; and Tulare County, 1, with most, 45, being collected during May and June 1988. The 22 isolates characterized in 1989 were collected from Salinas, 5; Santa Maria, 9; Ventura, 5; and Fresno, 3.

*Virulence phenotypes and sexual compatibility types.* From the virulence phenotype and sexual compatibility type (SCT) data, all but two of the field isolates tested could be classified as one of the pathotypes reported previously (11,12; Table 2). All of the isolates tested were heterothallic; the two pathotype IA isolates were $B_1$ and the rest were $B_2$. Of the 16 isolates collected in 1987, two were pathotype IA, 10 were pathotype III, and the remaining four seemed to be either pathotype IV or a mixture of pathotype II and pathotype III. Of the 59 isolates collected in 1988, 21 were pathotype III. During initial characterization of the other 38 isolates, sporulation on lines containing *Dm10* was usually delayed, sparse, and present on only one or a few seedlings, indicating that they were either pathotype IV or a mixture of pathotypes II and III. A similar delayed sporulation was also observed for one of these 38 isolates (C88T49, discussed later) on seedlings of the differential line PIVT1309, which contains *Dm15*. Of the 22 isolates detected in 1989, 18 were either pathotype IV or a mixture of pathotypes II and III. The remaining four isolates were collected from cultivars known to be resistant to pathotype III and were all pathotype II.

Several of the isolates that seemed to be either pathotype IV or mixtures of pathotypes II and III were characterized further by subculturing them onto seedlings of lines containing *Dm* genes, which would separate mixtures into pathotype II and pathotype III components. When conidia from these isolates (except C88T42, discussed later) were harvested from seedlings of the line R4/T57 (*Dm4*) and maintained on this line for several asexual generations, the derived isolates had a virulence phenotype identical to pathotype III. When conidia from these same isolates (except C88T42 and C88T49) were harvested from, and then maintained on, seedlings of lines containing *Dm10* (UCDM10 or Sucrine), the derived isolates had a virulence phenotype identical to pathotype II. The virulence phenotype of these derivatives was stable even after a number of consecutive asexual generations on seedlings of Cobham Green, which has no known *Dm* genes.

Two types of experiments were conducted to distinguish between the breakdown of an unstable heterokaryotic pathotype IV isolate and a mixture of pathotype II and pathotype III isolates. First, mixtures of pathotypes II and III were made experimentally with varying proportions of conidia of each pathotype. Even when conidia of pathotype II represented a rare component (0.2% or 1%) of the mixture, the virulence phenotype observed (slightly delayed yet profuse sporulation on one or a few seedlings of lines containing *Dm10*) did not resemble the delayed, sparse sporulation and associated necrosis usually observed with the complex field isolates. Second, eight isolates were derived from single conidia of one complex isolate, C88T3, using the method described previously (15). The virulence phenotype of all eight single-spore-derived (SSD) isolates was identical to that of C88T3; they initially produced infrequent, sparse sporulation on seedlings of lines containing *Dm4*, *Dm10*, or *Dm16*. Derivatives of one of these SSD isolates were generated by subculturing independently from R4T57 (*Dm4*) and UCDM10 (*Dm10*); these derivatives had virulence phenotypes identical to pathotype III and pathotype II, respectively. Therefore, many, or all, of the complex isolates characterized during this study were probably pathotype IV, which seems to be an unstable heterokaryon composed of nuclei derived from pathotypes II and III; this is consistent with the conclusions based on previous analyses of pathotype IV isolates using molecular markers (11).

Two isolates, C88T49 and C88T42, and their derivatives exhibited virulence phenotypes novel to California. Isolate C88T49 sporulated on PIVT1309 (*Dm15*); a derivative, C88T49/15, was maintained on PIVT1309. When retested, this derivative had a virulence phenotype identical to pathotype II plus virulence on *Dm15* and *Dm16* (Table 2). This virulence phenotype was stable in the absence of selection on *Dm15*; C88T49/15 sporulated profusely on PIVT1309 (*Dm15*) and LSE18 (*Dm16*) after culture for four consecutive asexual generations on seedlings of Cobham Green. Isolate C88T42 and its derivatives were different than any isolate described above. In contrast to other complex isolates, C88T42 exhibited profuse sporulation on seedlings of all lines containing *Dm10* by 11 days after inoculation. A derivative, C88T42/10, was generated by maintenance on seedlings of UCDM10; this isolate was virulent on *Dm10* and avirulent on *Dm16*, as expected for a pathotype II component. Unlike other derivatives generated on UCDM10, however, C88T42/10 was also virulent on *Dm4*. Another derivative, C88T42/4, generated by maintenance on seedlings of R4/T57, was virulent on *Dm4* and apparently avirulent on *Dm10* and *Dm16*, as expected for the pathotype III component; however, when C88T42/4 was maintained on seedlings in the presence of metalaxyl, its derivative

TABLE 2. Virulence phenotypes of California pathotypes and variant isolates of *Bremia lactucae*

| Pathotype or isolate | Reaction to *Dm* gene (or R-factor)[a] | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *1* | *2* | *3* | *4* | *5/8* | *6* | *7* | *10* | *11* | R12 | *13* | *14* | *15* | *16* |
| IA[b] | + | − | + | − | − | + | + | + | − | + | + | + | − | − |
| IB[b] | + | − | + | − | − | + | + | + | − | + | + | + | + | − |
| II[b] | − | + | + | (−) | + | + | + | + | (−) | + | + | + | − | − |
| III[b] | − | + | + | + | + | + | + | − | − | + | + | + | − | + |
| IV[b] | − | + | + | (−) | + | + | + | (−) | − | + | + | + | − | (−) |
| (IV)[c] | − | + | + | + | + | + | + | (+) | − | + | + | + | − | + |
| (IV)/10[d] | − | + | + | (−) | + | + | + | + | (−) | + | + | + | − | (−) |
| (IV)/4[d] | − | + | + | + | + | + | + | − | − | + | + | + | − | + |
| C88T42 | − | + | + | + | + | + | + | + | − | + | + | + | − | + |
| C88T42/4[d] | − | + | + | + | + | + | + | − | − | + | + | + | − | (−) |
| C88T42/4R[d] | − | + | + | + | + | + | + | + | − | + | + | + | − | (−) |
| C88T42/10[d] | − | + | + | + | + | + | + | + | − | + | + | + | − | (−) |
| C88T49/15[d] | − | + | + | − | + | + | + | + | (−) | + | + | + | + | + |
| C85T1[b] | − | + | + | − | + | + | + | − | − | + | + | + | + | − |

[a] + = compatible reaction, profuse sporulation; − = incompatible reaction, no sporulation; (−) = incompatible reaction, sparse sporulation associated with necrosis; and (+) = intermediate reaction, mixture of +, −, and (−) reactions. R-factor = incompletely characterized genetic resistance factor.

[b] As described by Hulbert and Michelmore (9) and Ilott et al (10).

[c] (IV) = Pathotype IV isolates described in this paper. Differences observed between pathotype IV isolates scored at different times was probably attributable to differences in inoculum density; see text.

[d] Laboratory derivatives of pathotype (IV) isolates: /4 = maintained on lettuce line, R4/T57 (*Dm4*); /4R = maintained on R4/T57 (*Dm4*) and metalaxyl (1 μg/ml); /10 = maintained on lettuce line, UCDM10 (*Dm*10); and /15 = maintained on lettuce accession, PIVT1309 (*Dm*15).

(C88T42/4R) continued to be avirulent on *Dm16* but now was virulent on *Dm10*. Therefore, the derivatives of the complex isolate C88T42 have novel virulence phenotypes and are distinguished from the previously characterized California pathotypes by their reactions on *Dm4*, *Dm10*, and *Dm16* (Table 2).

*Metalaxyl sensitivity.* Metalaxyl insensitivity was detected in the majority of the isolates collected during 1987, 1988, and 1989 (Table 3). Thirty-five of the 36 isolates collected in the Santa Maria growing region were insensitive to metalaxyl. In the Salinas growing region, 33 of the 46 isolates which were pathotype III or pathotype IV, were insensitive to metalaxyl; both of the pathotype IA isolates were sensitive to metalaxyl. The four isolates collected during 1988 from Ventura County and one isolate from Tulare County were sensitive to metalaxyl; however, in 1989 five isolates collected in Ventura County and three isolates from Fresno County were insensitive to metalaxyl. Fungicide application records, when available, indicated that metalaxyl-insensitive isolates originated from fields with and without preceding applications of Ridomil 2E. Derivatives of several pathotype IV isolates that had been maintained on *Dm4*- or *Dm10*-containing lines, retained the metalaxyl response observed with the progenitor field isolate; both metalaxyl-insensitive pathotype II or pathotype III isolates were derived from pathotype IV isolates in the laboratory. Four metalaxyl-insensitive pathotype II isolates were collected from the field during 1989 from cultivars known to be resistant to pathotype III.

The level of insensitivity to metalaxyl differed between California and English isolates; under laboratory conditions, an insensitive English isolate could tolerate higher levels of metalaxyl than insensitive California isolates. One microgram of metalaxyl was sufficient to prevent sporulation of metalaxyl-sensitive isolates. All of the California metalaxyl-insensitive field isolates sporulated as quickly (about 7 days after inoculation) and as profusely on seedlings of Cobham Green grown in the presence of 1 μg of metalaxyl per milliliter as on seedlings grown in the absence of metalaxyl. Sporulation of California metalaxyl-insensitive isolates on seedlings grown on nutrient solution containing 100 μg of metalaxyl per milliliter, however, was usually sparse or absent 7 to 8 days after inoculation although it was profuse 11 to 19 days after inoculation. When the California metalaxyl-insensitive isolates were maintained on seedlings grown in the presence of 1 or 100 μg of metalaxyl per milliliter for several asexual generations, they continued to exhibit delayed and sparse sporulation when inoculated onto seedlings grown in the presence of 100 μg of metalaxyl per milliliter. In contrast, an English metalaxyl-insensitive isolate, B87A/84 (5), sporulated quickly (within 7 days after inoculation) and profusely on seedlings grown in the presence of 100 μg of metalaxyl per milliliter and could be routinely maintained under these conditions in our laboratory. Therefore, while the metalaxyl insensitivity of the California isolates was sufficient to cause a failure of control in the field, they were more sensitive to metalaxyl than the English metalaxyl-insensitive isolate under laboratory conditions.

TABLE 3. Metalaxyl sensitivity and California pathotype of isolates of *Bremia lactucae* collected in California during 1987–1989

| California pathotype[a] | Number of isolates | | | | | |
|---|---|---|---|---|---|---|
| | Salinas | | Santa Maria | | Other | |
| | S[b] | I[c] | S | I | S | I |
| IA | 2 | 0 | 0 | 0 | 0 | 0 |
| II | 0 | 3 | 0 | 1 | 0 | 0 |
| III | 7 | 16 | 0 | 6 | 1 | 0 |
| IV | 6 | 14 | 1 | 28 | 4 | 8 |
| Totals | 15 | 33 | 1 | 35 | 5 | 8 |

[a] As described by Hulbert and Michelmore (9) and Ilott et al (10).
[b] S = Sensitive to metalaxyl; I = Insensitive to metalaxyl.
[c] Applications of Ridomil 2E in fields from which metalaxyl-insensitive isolates were collected: 15% treated with Ridomil 2E, 25% not treated with Ridomil 2E, 60% records of fungicide treatments unavailable.

**Survey of lettuce germ plasm for resistance to downy mildew.** A total of 175 lettuce accessions were tested, including 78 lettuce cultivars (Table 4) and 68 advanced breeding lines, incorporating lines from 11 commercial breeding companies. Resistance to at least one of the pathotypes tested (pathotypes IA, II, and III) was observed in 51, 12, and 82 accessions, respectively. Eleven accessions were resistant to all three pathotypes tested. Because metalaxyl insensitivity has been observed in pathotype II and pathotype III isolates, plant resistance to these two pathotypes was of particular interest. Four breeding lines and three cultivars were resistant to at least pathotype II. Forty-five breeding lines and 24 cultivars were resistant to at least pathotype III. In addition, two crisphead breeding lines, two butterhead cultivars, and one new crisphead cultivar were resistant to all three California pathotypes used in this study. According to seed producers' recommendations, four of 21 crisphead cultivars with resistance to pathotype III and one new crisphead cultivar with resistance to all three California pathotypes may be adapted to production during some part of the growing season in the coastal valleys of California where downy mildew can be severe.

## DISCUSSION

Metalaxyl insensitivity in *B. lactucae* has spread throughout the major coastal California lettuce growing regions since the failure of metalaxyl to control lettuce downy mildew was first reported in 1987. Insensitivity developed 4 yr after metalaxyl became available for foliar application on lettuce in California. About 68% of the isolates collected during 1987, 1988, and 1989 in Salinas for this survey and 97% of those collected in Santa Maria were insensitive to metalaxyl. These values may overestimate the actual prevalence of metalaxyl insensitivity because isolates were not collected at random and isolates from fields where control had failed may be overrepresented. However, fungicide application records were available for 40% of the fields from which isolates were collected; 15% of the metalaxyl-insensitive isolates were collected from fields treated with metalaxyl and 25% from fields not treated with metalaxyl. Therefore, metalaxyl-insensitive isolates were prevalent regardless of whether the field had been treated with metalaxyl.

The relative frequencies of the California pathotypes of *B. lactucae* have changed dramatically over the last 7 yr. Data are now available from isolate surveys during each of three recent time periods in California: 59 isolates from 1982 to 1984, 58 isolates during 1985–1986 (12), and 97 isolates during 1987, 1988, and 1989 reported here. Pathotype I declined from 20% of the isolates during 1982–1984 to 2% during 1987–1989. Because the most popular crisphead cultivars grown since the mid-1960s are resistant to pathotype I due to *Dm5/8*, there has been strong selection pressure against this pathotype. No variants of pathotype I that are virulent on cultivars containing *Dm5/8* have been detected, despite the pathogen apparently being heterozygous at the critical locus (*Avr5/8 avr5/8*; 12). Pathotype II, which was first detected in the mid-1970s and is virulent on all the popular crisphead cultivars, was detected only as a laboratory derivative of pathotype IV during 1987 and 1988 and in the field only from cultivars known to be resistant to pathotype III in 1989; this is in contrast to 1982–1984 when it represented the predominant pathotype (65% of the isolates). This may be because pathotype II is less prolific than pathotype III and pathotype IV; under laboratory conditions, pathotype II clearly sporulates less profusely than pathotype III on seedlings of Cobham Green, which contains no known downy mildew resistance genes, and on other cultivars on which both are virulent (*unpublished observations*). Because susceptible host material is not limiting in commercial lettuce fields, pure populations of pathotype II still may be present at a low frequency. Pathotype III, first detected in 1983, and pathotype IV, first detected in 1984, have become the predominant pathotypes in California; they represented 31% and 63%, respectively, of the 97 isolates collected during 1987–1989. The population structure will continue to change as new cultivars with resistance to these pathotypes are introduced.

The development of metalaxyl insensitivity and the shift in virulence phenotype apparently have occurred independently in *B. lactucae* in California. Selection of a successful metalaxyl-insensitive mutant would most likely occur within the predominant phenotype present during metalaxyl applications. In England (7), the first metalaxyl-insensitive isolates had the same virulence phenotype as the most common phenotype among the metalaxyl-sensitive isolates collected that season. In California, metalaxyl-insensitivity was first confirmed in pathotype III and pathotype IV, the predominant pathotypes at the time. No independent pathotype II isolates were collected from the field during 1988; however, some pathotype II isolates that were derived from pathotype IV isolates in the laboratory and pathotype II isolates collected from the field during 1989 were insensitive to metalaxyl. Therefore, metalaxyl-insensitive pathotype II isolates are present in California; whether they are important under field conditions is unknown.

The variant virulence phenotypes detected in this study were minor deviations from phenotypes observed in previous studies. This supports the conclusion based on previous surveys and data from molecular markers that California isolates can be grouped into four asexually propagated pathotypes. The California pathotypes differ from each other at several unlinked avirulence loci (12,13); it is unlikely that sexual recombination would have resulted in only the variant virulence phenotypes reported here. Therefore, the novel phenotypes detected in this survey were probably generated by asexual mechanisms.

Many of the isolates seemed to be pathotype IV, which is an unstable heterokaryon containing nuclei derived from pathotypes II and III. These isolates initially expressed partially compatible reactions (delayed and sparse sporulation) on *Dm4*, *Dm10*, and *Dm16*, and most of those tested could be separated easily into pathotype II and pathotype III components by culturing on selective host genotypes; both of the derived components had the virulence reactions expected with these three *Dm* genes. The avirulent reactions on *Dm4*, *Dm10*, and *Dm16*, previously reported for pathotype IV (11), were shown to be caused by the lower initial inoculum densities ($1–5 \times 10^4$ conidia per milliliter) and shorter observation periods than used in the present study (*unpublished data*).

Heterokaryons may provide mechanisms for generating asexual variation in California. Each of the four variant phenotypes

TABLE 4. Resistance of lettuce cultivars to California pathotypes of *Bremia lactucae*

| Cultivar[a] | Source[b] | Leaf type[c] | Reaction[d] to California pathotype | | |
|---|---|---|---|---|---|
| | | | IA | II | III |
| Amaral 400 | RS | C | S | S | S |
| Autumn Gold | BC,PY | C | S | S | S |
| Avoncrisp | (UC) | C | R | S | S |
| Bella | FM | B | S | S | S |
| Bix | AS | C | R | S | S |
| Black Seeded Simpson | (UC) | L | S | S | S |
| Blanco | RS | C | S | S | S |
| Bounty | PS | C | R | S | S |
| Bullseye*Su | PS | C | R | R | R |
| Buttercrunch | RS | B | S | S | S |
| Cal K-60 | MS | C | R | S | S |
| Calicel | UC | C | R | S | S |
| Capitan** | (UC) | B | R | IR | IR |
| Chaparral | SS | C | R | S | S |
| Classic | AS | C | S | S | S |
| Climax | SS | C | S | S | S |
| Coolguard** | AS | C | S | S | R |
| Corsica | RS | R | S | S | S |
| Crispy | AS | C | S | S | S |
| Dark Green Boston | (UC) | B | S | S | S |
| Delmar | MS | C | R | S | S |
| Diplomat | RS | C | S | S | S |
| Domingos 42** | BC | C | S | S | R |
| Domingos 43** | RS | C | S | S | R |
| El Toro*Sp | HM | C | S | S | R |
| Empire | (UC) | C | S | S | S |
| Esmeralda** | RS | B | R | R | R |
| Excell | RS | C | S | S | S |
| Fame | AS | C | S | S | S |
| Fanfare | FM | L | S | S | S |
| Floribibb | UF | B | R | S | S |
| Floricos-83 | UF | R | R | S | S |
| FM-8248*Sp | FM | C | R | S | R |
| Gabilan** | BC | C | S | S | R |
| Grande | RS | C | S | S | S |
| Greenfield | HM | C | IS | S | S |
| La Jolla | RS | C | S | S | S |
| Mantilia** | NK | B | R | IS | IR |
| Marquette | BC | C | S | S | S |
| Merit 3186 | SS | C | S | S | S |
| Mondian | NK | B | R | IS | IS |
| Montemar | FM | C | IR | S | S |
| Mor 109** | RS | C | S | S | R |
| Orfeo | RS | B | S | S | S |
| Pacific | BC | C | R | S | S |
| Palmetto** | RS | C | S | S | R |
| Palo Verde** | RS | C | S | S | R |
| Parris Island | CV,FM | R | S | S | S |
| Pennlake | (UC) | C | S | S | S |
| Pinnacle | BC | C | S | S | S |
| Prizehead | FM | L | S | S | S |
| Pybas 101 | PY | C | R | S | S |
| Pybas 102 | PY | C | R | S | S |
| Raleigh | UF | C | S | S | S |
| Red Coach 74** | RS | C | S | S | IR |
| Royal Green | RS | L | S | S | S |
| Royal Red | RS | L | S | S | S |
| Salinas | FM | C | R | S | S |
| Saltan | BC | C | R | S | S |
| Salverde | FM | C | R | S | S |
| Sea Green*Sp | AS,BC,BO,FM | C | S | S | R |
| Seamist | CV | C | R | S | S |
| Sierra | BO | C | S | S | S |
| Snowbird** | FM | C | S | S | R |
| South Bay | UF | C | S | S | S |
| Tall Guzmaine | UF | R | S | S | S |
| Target*Su | PS | C | R | IS | R |
| Valley Queen** | BC | C | S | S | IR |
| Valmaine | (UC) | R | R | S | S |
| Valor** | RS | C | S | S | R |
| Vancrisp** | RS | C | S | S | R |
| Vanguard** | BO | C | S | S | R |
| Vanguard 75** | BO | C | S | S | R |
| Vanmax** | FM | C | S | S | R |
| Viva II | RS | C | S | S | S |
| Waldmann's Green | CV,RS,FM | L | S | S | S |
| Winter Supreme | RS | C | S | S | SR |
| Winterhaven** | BO,RS | C | S | S | R |

[a] ** = resistant to at least pathotype III. According to seed producer's recommendations, *Sp = may be useful for spring harvest period in coastal valleys of California, and *Su = may be useful for summer harvest period in coastal valleys of California.

[b] AS = Asgrow, HM = Harris Moran, SS = Sunseeds, BC = Bruce Church, NK = Northrup King, UC = UC Line, BO = Brinker Orsetti, PS = Petoseed, (UC) = UC seed collection, CV = Central Valley Seeds, PY = Pybas Seed, UF = University of Florida, FM = Ferry Morse, and RS = Royal Sluis/Hortinnova Research.

[c] B = Butterhead, C = Crisphead, L = Green or Red Leaf, and R = Romaine/Cos.

[d] S = susceptible, R = resistant, IS = intermediate susceptible (sparse sporulation), IR = intermediate resistant (sparse sporulation associated with tissue breakdown, or profuse on less than 10% of seedlings), and SR = heterogeneous reaction (profuse sporulation on 10–25% of seedlings).

reported here (metalaxyl-insensitive pathotype III; laboratory-derived, metalaxyl-insensitive pathotype II; C88T42 [*Dm4* virulent, metalaxyl-insensitive pathotype II?] and C88T49/15 [*Dm15* and *Dm16* virulent, metalaxyl-sensitive pathotype II?]) could have been the result of separate mutations at four independent loci (metalaxyl insensitivity, *Avr4*, *Avr15*, and *Avr16*). Alternatively, if pathotype IV isolates act as transient somatic hybrids, there would be the possibility of karyogamy and asexual recombination between genomes of pathotypes II and III. These variants and each of the California pathotypes are now being analyzed using molecular markers to determine their relationships and the mechanisms of variation.

The genetic basis for metalaxyl insensitivity in California isolates is unknown. In genetic studies of *B. lactucae* (5) and *P. infestans* (21) in England, metalaxyl insensitivity was determined at one locus expressing incomplete dominance (designated "*P*" for phenylamide fungicide insensitivity in *B. lactucae*). In both genetic studies, $F_1$ sexual progeny (heterozygous for insensitivity) were intermediate to the parents in their sensitivity to high levels of metalaxyl. In the studies of *B. lactucae* in England, the $F_1$ isolates exhibited an intermediate latent period when compared to the insensitive and sensitive parents. Asexual cultures of the insensitive parent also expressed the intermediate phenotype following multiple asexual generations in the absence of metalaxyl but returned to the fully insensitive phenotype after one cycle of selection on metalaxyl. The field isolates used in the English studies may therefore have been heterokaryons of homozygous (*PP*) and heterozygous (*Pp*) insensitive nuclei (5). The phenotype of one of the English isolates used in the genetic studies was confirmed in this laboratory; isolate B87A/84 showed no delay in sporulation in the presence of high levels of metalaxyl (100 μg/ml). In contrast, sporulation of the California isolates was delayed in the presence of 100 μg of metalaxyl per milliliter. Even after repeated selection on seedlings grown on either 1 or 100 μg of metalaxyl per milliliter, the California isolates expressed only partial insensitivity to 100 μg of metalaxyl per milliliter. There are several alternative explanations for these differences between the English and California isolates: the California isolates may be heterozygous (*Pp*); the California isolates may be a balanced heterokaryon (*PP plus Pp* nuclei; if *P* is linked in cis to a recessive deleterious mutation, *PP* nuclei would not accumulate to high levels); or California isolates may have a different mutation at the same or different locus to that in the English isolate conferring a different level or mechanism of insensitivity to metalaxyl. Sexual crosses are being made to investigate these alternatives.

The screening of lettuce cultivars for resistance to the California pathotypes of lettuce downy mildew was undertaken for two reasons. Initial observations in 1987 indicated that metalaxyl insensitivity may have been restricted to pathotype III; therefore, cultivars resistant to pathotype III used in conjunction with Ridomil 2E might have provided effective control. In addition, the California lettuce cultivars had not been characterized for resistance to *B. lactucae* since the California isolates had been classified into four pathotypes (11,12). A desirable crisphead cultivar, the predominant lettuce type grown in California, would be resistant to all the California pathotypes and adapted to the coastal growing regions where downy mildew can be a problem in the spring and summer. To date, only one new crisphead cultivar, Bullseye, and several advanced breeding lines may satisfy these criteria. Several crisphead cultivars were resistant to at least pathotype III (Table 4); however, most were winter plant types and not adapted to the coastal valleys. Only five are reportedly adapted to some part of the spring and summer growing seasons in the coastal valleys. Three of these (El Toro, FM8248, and Sea Green) are reportedly adapted for the spring harvest period and do not currently occupy large acreages. Another new cultivar, Target, was resistant to both pathotype IA and pathotype III and is reportedly adapted to the coastal valleys during the summer harvest period. Any cultivar that is resistant to either pathotype II or pathotype III should be resistant to pathotype IV under field conditions; partially compatible reactions were obtained with *Dm4*, *Dm10*, and *Dm16* in the laboratory only when extremely high inoculum densities were used under optimal conditions for infection. Pathotype II isolates are not as vigorous as pathotype III isolates in the laboratory; therefore, if metalaxyl-insensitive isolates of pathotype II do not cause severe disease under field conditions, the use of Sea Green, FM8248, El Toro, or Target when combined with applications of Ridomil 2E, may give at least partial control of downy mildew. Metalaxyl insensitivity has been detected in field isolates of pathotype II, however, and cultivars with resistance to both pathotypes II and III in conjunction with applications of Ridomil 2E may be needed for complete control. The effectiveness of growing cultivars resistant to pathotype III with foliar applications of metalaxyl (as Ridomil 2E) is now being tested in the field.

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