Pathotyping of *Bremia lactucae* in Florida

L. E. DATNOFF, Associate Professor of Plant Pathology, R. T. NAGATA, Associate Professor of Horticulture, and R. N. RAID, Associate Professor of Plant Pathology, Departments of Plant Pathology and Horticulture, University of Florida, Institute of Food and Agricultural Sciences, Everglades Research and Education Center, P.O. Box 8003, Belle Glade 33430-8003

ABSTRACT

Datnoff, L. E., Nagata, R. T., and Raid, R. N. 1994. Pathotyping of *Bremia lactucae* in Florida. Plant Dis. 78:854-857.

Isolates of *Bremia lactucae* were obtained from infected lettuce coming from California into Florida for fresh market sale and preparation of fresh mixed salads and from naturally infected lettuce fields in Florida during 1991 and 1992. Using an established lettuce tester set containing the 13 *Dm* resistant genes, isolates of *B. lactucae* were grouped into distinct pathotypes based on presence or absence of or infrequent sporulation. Among all the isolates of *B. lactucae* tested, sporulation was absent on *Dm1*, *Dm11*, and *Dm15*. These isolates usually produced infrequent or sparse sporulation on seedlings of lines containing *Dm4*, *Dm10*, and *Dm16*. Based on this information, 72% of the isolates obtained from infected lettuce coming from California into Florida were pathotype IV. Of the isolates from Florida field-grown infected lettuce, 50% were determined to be pathotype IV and 28%, pathotype III. These data suggest that infected lettuce arriving from California may be an important source of primary inoculum for epidemics of downy mildew in Florida.

Additional keywords: *Lactuca sativa*

Downy mildew of lettuce (*Lactuca sativa* L.) caused by *Bremia lactucae* Regel has been the most serious foliar disease problem in the Everglades Agricultural Area (EAA) located near Lake Okeechobee in South Florida (12). Epidemics of this disease during the 1989–1990, 1990–1991, and 1991–1992 growing seasons caused extensive losses in marketable yields. Because of the cool temperatures and extended periods of high relative humidities during late fall and early spring, periods of leaf wetness are prolonged, resulting in extensive fungal sporulation and leaf necrosis. Consequently, lettuce marketable yields are decreased and deterioration occurs during postharvest storage and transit.

Outbreaks of downy mildew in the EAA were sporadic prior to the late 1980s and early 1990s. Although little information about races of *B. lactucae* existed in Florida before this time, race changes probably occurred, since previously resistant lettuce cultivars became susceptible (5,6).

Pathogenicity of *B. lactucae* has exhibited extensive variation in virulence (1). Many resistance genes have been identified but rendered ineffective by variability in the pathogen population. To date, 13 single dominant resistance genes (*Dm*) in the host and their complementary avirulence genes in the pathogen have been described (1,4,13). By using an established lettuce tester set containing the 13 *Dm* resistant genes, isolates of *B. lactucae* can be grouped into distinct pathotype(s) on the basis of their virulence phenotype (8). Virulence phenotype is inferred from the compatible (sporulation and/or necrosis) or incompatible (no sporulation or necrosis) reaction of an isolate of *B. lactucae* on the host tester set. This information is invaluable because once the pathotypes are determined, plant breeders know which resistant genes to incorporate into lettuce and plant pathologists can trace inoculum sources to determine the origins of epidemics prior to suggesting control strategies.

During the early 1980s, Florida processors needed to import lettuce from California to meet market supply obligations because lettuce is not available from early May to late November in the EAA. Recent observations (*unpublished*) revealed that California lettuce coming into Florida was infected with downy mildew. This represented a possible inoculum source, since imported lettuce overlaps with Florida production, which begins with plantings in mid-September and first harvest in late November.

In California, four major pathotypes of *B. lactucae* have been identified by virulence phenotype determinations (13). Only a few Florida isolates have been tested in recent years (3). The objective of this study was to characterize the predominant phenotypic populations of *B. lactucae* found in California lettuce shipments to Florida and in southern Florida field-grown lettuce. This information will be useful to determine if California lettuce is a potential inoculum source for Florida epidemics.

Florida Agricultural Experiment Station Journal Series No. R-03618.

Accepted for publication 6 June 1994.

MATERIALS AND METHODS

Eighteen isolates of *B. lactucae* were obtained from infected lettuce transported into the state from California for fresh market sale and preparation of fresh mixed salads for food service industries. These lettuce shipments originated from the Salinas to Santa Maria production areas. California-infected lettuce exhibiting profuse sporulating lesions was collected on the following sample dates in 1991: 22 and 30 August; 6, 13, 20, and 27 September; 11, 18, and 25 October; and 1 November. Eighteen isolates were also collected from eight naturally infected EAA lettuce fields in 1991 and 1992. All isolates, whether obtained from California or Florida lettuce, were derived from groups of lesions bulked together. Sporangia of each isolate were increased by inoculating lettuce cultivar Ithaca at the cotyledon stage. Approximately 7–10 days later, when profuse sporulation was observed, sporangia were harvested and used to determine virulence phenotype. Sporangia of *B. lactucae* of each isolate were inoculated onto a differential series of 14 lettuce lines (Table 1) at the seedling stage, using the method of Michelmore and Crute (10). These inoculated seedlings, contained in clear plastic, compartmentalized utility boxes, were placed in an incubator at 15 C with a 16-hr photoperiod supplemented by fluorescent lamps that provided an average PAR of 72.8 $\mu E \cdot s^{-1} \cdot m^{-2}$. Seedlings were scored for the presence or absence of sporulation and necrosis approximately 7, 12, and 17 days after inoculation. Isolates obtained from California lettuce and Florida-grown lettuce were characterized into their respective pathotypes based on pathotypes of *B. lactucae* found in California (13) (Table 2). The time interval involved in determining virulence phenotype for each individual isolate ranged from 4 to 6 wk.

RESULTS AND DISCUSSION

A total of 36 isolates of *B. lactucae* were collected and tested for virulence phenotype. Eighteen of these isolates represented samples collected from fresh market and processing lettuce with visible sporulation of *B. lactucae* coming into Florida from California during the

months of June to November 1991 (Table 3). The other eighteen isolates represented samples collected from diseased lettuce in eight different naturally infected fields from around the EAA during the months of December 1991 to May 1992 (Table 4).

Among the 36 isolates of *B. lactucae* tested on the lettuce lines containing the 13 *Dm* resistance genes, sporulation was generally absent on *Dm1, Dm11*, and *Dm15*. In addition, these isolates usually produced infrequent or sparse sporulation on seedlings of lines containing *Dm4, Dm10*, or *Dm16*. Infrequent or sparse sporulation on these *Dm* lines is characteristic of the unstable heterokaryotic nature of California pathotype IV isolate (13). Based on this information and on Table 2, which contains pathotype IV isolates and laboratory derivatives as described by Schettini et al (13), the 18 isolates collected from fresh market and processing lettuce transported from California to Florida were grouped as follows: 13 isolates (72%) were pathotype IV and five (28%) did not have a virulence phenotype of one of the four California pathotypes or could not be fully characterized because one or more of the tester lines died during the test (Table 3). Of the 18 isolates collected from the naturally infected lettuce fields in Florida, nine (50%) were pathotype IV, five (28%) were pathotype III, and four (22%) did not have a virulence phenotype of one of the four California pathotypes or could not be fully characterized because during the test one or more of the tester lines died (Table 4).

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

Cultivar	*Dm* genes[a]
Lednicky	*1*
UCDM 2	*2*
Dandie	*3*
R4 T57/E	*4*
Valmaine	*5/8*
Sabine	*6*
LSE 57/15	*7*
UCDM 10	*10*
Mondian	*11*
Hilde	*R12*[b]
Empire or Pennlake	*13*
UCDM 14	*14*
P1VT1309	*15*
LSE 18	*16*

[a] After Farrara et al (4).
[b] Resistance factor 12 has not been confirmed to be a single gene.

Discrepancies were seen in the virulence responses of some of the isolates even though they were given the same pathotype designation. For example, isolates 3 and 4 collected from commercial lettuce sources (Table 3) were designated as pathotype IV even though the reactions to *Dm7* were different. A couple of factors contributed to this decision. Seeds of LSE 57/15 used in this study had either poor or delayed germination at times, and only one or two seedlings would be viable from the 10 that were planted per cell. Although profuse sporulation would occur with associated necrosis on existing seedlings, initially sporulation appeared sparse or delayed. Consequently, reactions were typed as either (−) or (+) with several isolates. In addition, differences observed between pathotype IV isolates scored at different times were probably attributable to differences in inoculum densities (1–4 × 10^4 conidia per milliliter). Also, "novel" pathotypes were observed that did not fit any known virulence phenotype response. Nevertheless, the isolates that were grouped into known pathotypes (Table 3) found in lettuce shipped from California to Florida were similar to those isolates (Table 4) recovered from Florida field-grown lettuce.

At present, infected lettuce coming from California appears to be a potentially important primary source of inoculum for epidemics in Florida. However, another possible source of inoculum may be the overseasoning of oospores in the soil. Since only one mating type, B_2, so far has been reported in Florida (12), oospores probably are not produced to overseason during Florida's highly unfavorable summer months. Although the possibility of homothallic isolates exists (11), numerous infected lettuce leaves (about 100) collected from several fields during the 1992 epidemic were cleared and stained using the methods of Yuen and Lorbeer (14) and Marlatt et al (9), and oospores of *B. lactucae* were not observed. In addition, important weed hosts are not known to exist for survival of *B. lactucae* in Florida. These general observations and the aforementioned pathotype results suggest that infected lettuce arriving from California during Florida's off-season may be an important source of primary inoculum.

Two butterhead types grown in Florida recently were found to be completely resistant to downy mildew (2,3). It is possible that these butterhead types contained either one or a combination of *Dm1, Dm11*, and *Dm15* genes for resistance. Incorporating one and/or all of these *Dm* genes into Florida-adapted cultivars would confer resistance to pathotype IV as well as to pathotypes II and III, since avirulence is dominant and pathotype IV is a fusion of pathotypes II and III (7,13). This breeding strategy could be useful to Florida growers, since 78% of the Florida pathotypes appeared to be either III or IV.

Since "novel" isolates that overcome *Dm1* and *Dm15* have been reported to exist in California (13), and since *Dm11* provides only partial protection, this same scenario could also occur in Florida. This is especially true because some novel pathotypes were detected in

Table 2. Virulence phenotypes of California pathotypes of *Bremia lactucae*[a]

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

[a] From Schettini et al (13).
[b] + = Compatible reaction, profuse sporulation; − = incompatible reaction, no sporulation; (−) = incompatible reaction, sparse or infrequent sporulation associated with necrosis; and (+) = intermediate reaction, mixture of +, −, and (−) reactions. *R*-factor = incompletely characterized genetic resistance factor.
[c] D. Zungri and R. Michelmore, *personal communication*.
[d] Pathotype IV isolates as described by Schettini et al (13).
[e] Laboratory derivatives of pathotype IV isolates.

Table 3. Virulence phenotypes of *Bremia lactucae* collected from commercial lettuce sources[a]

Isolate	Reaction to *Dm* gene (or *R*-factor)[b]														PD[c]
	1	*2*	*3*	*4*	*5/8*	*6*	*7*[d]	*10*	*11*	*R12*	*13*	*14*	*15*	*16*	
ISO 1	−	+	+	(−)	+	(+)	(−)	−	−	+	+	(+)	−	−	IV
ISO 2	−	+	+	+	+	+	+	(−)	−	+	+	+	−	−	IV
ISO 3	−	+	+	(−)	+	+	(+)	−	−	+	+	+	−	−	IV
ISO 4	−	+	+	(−)	+	+	(−)	−	−	+	+	+	−	−	IV
ISO 5	−	+	+	−	+	+	(+)	−	−	+	+	+	−	−	IV
ISO 6	−	+	+	−	(+)	+	D	−	−	+	+	+	−	−	IV
ISO 7	−	+	+	+	+	+	(−)	−	−	(+)	+	(+)	−	−	IV
ISO 8	−	+	+	(−)	+	+	(+)	−	−	+	+	+	−	−	IV
ISO 9	−	+	+	(−)	+	+	(−)	−	−	+	+	+	−	(−)	IV
ISO 10	−	+	+	(−)	+	+	(−)	−	−	+	+	+	−	(−)	IV
ISO 11	−	−	−	−	−	−	−	−	−	−	−	−	−	−	?
ISO 12	−	+	+	−	+	+	(−)	−	−	+	+	+	−	−	IV
ISO 13	−	+	+	(−)	+	+	(−)	−	−	+	+	+	−	−	IV
ISO 14	−	+	+	(−)	+	+	+	+	−	+	+	+	−	(−)	IV
ISO 15	−	+	+	+	+	+	+	+	−	+	+	+	(−)	D	?
ISO 16	(−)	+	+	+	+	+	+	+	−	+	+	+	D	(+)	?
ISO 17	(+)	+	+	+	+	+	+	+	−	+	+	+	−	(−)	?
ISO 18	(+)	+	+	+	+	+	+	+	−	+	+	+	−	(−)	?

[a] Fresh market and processing lettuce infected with downy mildew coming from California into Florida.
[b] + = Compatible reaction, profuse sporulation; − = incompatible reaction, no sporulation; (−) = incompatible reaction, sparse or infrequent sporulation associated with necrosis; and (+) = intermediate reaction, mixture of +, −, and (−) reactions. *R*-factor = incompletely characterized genetic resistance factor. D = dead plants; ? = unknown.
[c] Pathotype determination.
[d] Seeds of LSE 57/15 had either poor or delayed germination at times, and only one or two seedlings would be viable from the 10 that were planted per cell. Although profuse sporulation would occur with associated necrosis on existing seedlings, initially sporulation appeared sparse or delayed. Consequently, reactions were typed as either (−) or (+) with several isolates. In addition, differences observed between pathotype IV isolates scored at different times were also probably attributable to differences in inoculum densities.

Table 4. Virulence phenotypes of *Bremia lactucae* collected from eight field locations in Florida

Isolate	Reaction to *Dm* gene (or *R*-factor)[a]														PD[b]
	1	*2*	*3*	*4*	*5/8*	*6*	*7*	*10*	*11*	*R12*	*13*	*14*	*15*	*16*	
ISO 1	−	+	+	+	+	+	+	−	−	+	+	+	−	+	III
ISO 2	−	+	+	+	+	+	+	−	−	+	+	+	−	+	III
ISO 3	−	+	+	+	+	+	+	−	−	+	+	+	−	(−)	IV
ISO 4	−	+	+	+	+	+	+	(−)	−	+	+	+	−	(−)	IV
ISO 5	−	+	+	+	+	+	+	+	−	+	+	+	−	+	IV
ISO 6	−	+	+	+	+	+	+	(−)	−	+	+	+	−	+	IV
ISO 7	−	+	+	(−)	+	+	+	+	−	+	+	+	−	+	III
ISO 8	−	+	+	+	+	+	+	−	−	+	+	+	−	(−)	IV
ISO 9	−	+	+	+	+	+	+	−	(−)	+	+	+	−	+	III
ISO 10	−	+	+	+	+	+	+	−	−	+	+	+	−	+	III
ISO 11	−	(−)	(−)	−	+	(−)	(−)	−	−	+	(−)	−	−	−	?
ISO 12	−	+	+	(−)	+	+	(−)	−	−	−	(−)	(−)	−	−	?
ISO 13	−	+	+	(−)	+	+	+	(−)	−	+	+	+	−	D	?
ISO 14	−	+	+	+	+	+	+	(−)	−	+	+	+	−	(−)	IV
ISO 15	−	+	+	+	+	+	+	(−)	−	+	+	+	−	(−)	IV
ISO 16	−	+	+	+	+	+	+	(−)	−	+	+	+	(−)	D	?
ISO 17	−	+	+	+	+	+	+	(−)	−	+	+	+	−	+	IV
ISO 18	−	+	+	+	+	+	+	(−)	−	+	+	+	−	(−)	IV

[a] + = Compatible reaction, profuse sporulation; − = incompatible reaction, no sporulation; (−) = incompatible reaction, sparse or infrequent sporulation associated with necrosis; and (+) = intermediate reaction, mixture of +, −, and (−) reactions. *R*-factor = incompletely characterized genetic resistance factor. D = dead plants; ? = unknown.
[b] Pathotype determination.

California-infected lettuce shipped to Florida and in Florida field-grown lettuce (Tables 3 and 4). It is extremely important to reduce or contain infectious lettuce material coming from California to Florida. Otherwise, any Florida breeding strategy will always be subservient to changes occurring in California. Sanitation efforts, such as burying infected lettuce debris from preparation of fresh mixed salads for food service industries far from field sites, are in effect to help contain the inoculum coming into Florida. These and other control efforts will be combined with breeding efforts to develop an integrated program for management of lettuce downy mildew.

ACKNOWLEDGMENTS

We thank South Bay Growers, Inc., for their assistance in conducting these studies, and J. Carroll, M. Robins, and E. Skiles, for their invaluable technical support. We also thank R. W. Michelmore for providing several of the lettuce tester lines for downy mildew resistance and technical expertise while these studies were being conducted.

LITERATURE CITED

1. Crute, I. R. 1987. The occurrence, characteristics, distribution, genetics, and control of a metalaxyl-resistant pathotype of *Bremia lactucae* in the United Kingdom. Plant Dis. 71:763-767.
2. Datnoff, L. E., Nagata, R. T., and Raid, R. N. 1991. Evaluation of butterhead lettuce for resistance to downy mildew. Biol. Cult. Control Plant Dis. 6:12.
3. Datnoff, L. E., Nagata, R. T., Raid, R. N., Schettini, T. M., and Michelmore, R. W. 1990. Field evaluation of crisphead and butterhead lettuce for downy mildew resistance and characterization of virulence phenotype. Proc. Fla. State Hortic. Soc. 103:140-142.
4. Farrara, F., Ilott, T. W., and Michelmore, R. W. 1987. Genetic analysis of factors for resistance to downy mildew (*Bremia lactucae*) in species of lettuce (*Lactuca sativa* and *L. serriola*). Plant Pathol. 36:499-514.

5. Guzman, V. L., and Zitter, T. A. 1983. Floricos 83: A cos lettuce cultivar resistant to two viruses, for Florida organic soils. Fla. Agric. Exp. Stn. Circ. S-305.
6. Guzman, V. L., and Zitter, T. A. 1984. Floribibb: A gourmet lettuce resistant to two viruses, for Florida organic soils. Fla. Agric. Exp. Stn. Circ. S-313.
7. Hulbert, S. H., and Michelmore, R. W. 1988. DNA restriction fragment length polymorphism and somatic variation in the lettuce downy mildew fungus, *Bremia lactucae*. Mol. Plant-Microbe Interact. 1:17-24.
8. 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.
9. Marlatt, R. B., Lewis, R. W., and McKittrick, R. T. 1963. Observing *Bremia lactucae* in lettuce. Plant Dis. Rep. 47:126-128.
10. 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.
11. Michelmore, R. W., and Ingram, D. S. 1982. Secondary homothallism in *Bremia lactucae*. Trans. Br. Mycol. Soc. 78:1-9.
12. Raid, R. N., and Datnoff, L. E. 1990. Loss of EBDC fungicides: Impact on control of downy mildew of lettuce. Plant Dis. 74:829-831.
13. Schettini, T. M., Legg, E. J., and Michelmore, R. W. 1991. Insensitivity to metalaxyl in California populations of *Bremia lactucae* and resistance of California lettuce cultivars to downy mildew. Phytopathology 81:64-70.
14. Yuen, J. E., and Lorbeer, J. W. 1987. Natural and experimental production of oospores of *Bremia lactucae* in lettuce in New York. Plant Dis. 71:63-64.