Proc. Fla. State Hort. Soc. 103:140-142. 1990.

FIELD EVALUATION OF CRISPHEAD AND BUTTERHEAD LETTUCE FOR DOWNY MILDEW RESISTANCE AND CHARACTERIZATION OF VIRULENCE PHENOTYPE

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Additional key words. Bremia lactucae, disease resistance, *Lactuca sativa*, pathotype.

Abstract. **Down mildew of lettuce (*Lactuca sativa* L.), caused by *Bremia lactucae* Regel, recently has been a serious problem in the Everglades Agricultural Area. Forty crisphead and 10 butterhead cultivars and breeding lines were planted in commercial fields naturally infected with downy mildew to identify potential sources of resistance. All crisphead types were susceptible. Butterhead types 'Everglades' and 'Mantilla' were found to be completely resistant whereas breeding line 70880 was partially resistant. Several isolates of the fungus were collected for virulence phenotype determinations. Sporulation generally was not observed on tester lines containing *Dm* resistant genes 1, 11, and 15. This information will be very useful for developing genetic control strategies of downy mildew in Florida.**

Lettuce downy mildew epidemics during the 1989 and 1990 growing season have been quite devastating, causing extensive losses in the Everglades Agricultural Area of south Florida (10,11). Cool temperatures and extended periods of high relative humidities occur in this region during late fall to early spring. These extended dew periods result in long periods of leave wetness which are associated with rapid downy mildew development (1). Extensive sporulation and associated necrosis occurs on lettuce leaves resulting in a decrease of marketable yields. Subsequently, there has been deterioration in postharvest storage and transit.

Recommendations for controlling downy mildew have primarily emphasized fungicide applications and resistant cultivars (1,3,13). Maneb, an ethylene bisdithiocarbamate (EBDC) fungicides, was used for managing downy mildew for many years because of its effectiveness, low cost, and compatibility with other pesticides. However, in fall of 1989, manufacturers voluntarily withdrew the registration of this fungicide for use on lettuce because of the public's concern about ethylene thiourea residues. Ethylene thiourea is a degradation product of EBDC fungicides and has been linked with tumor production in laboratory animals (11).

When first introduced, metalaxyl, a systemic fungicide specific for controlling fungi in the Oomycetes, was superior to EBDC's for controlling downy mildew (3). However, field trials during 1989 and 1990, and laboratory tests have demonstrated the presence of a metalaxyl-insensitive strain in Florida (12). Insensitivity has been reported in California (13) and Europe (3). Because of a section 18 exemption, the only other available fungicide beside copper for managing downy mildew is fosetyl-Al. Development of insensitivity to this fungicide is a distinct possibility.

Sporadic outbreaks of downy mildew have taken place over the years in the Everglades Agricultural Area. Although little information about races of *B. lactucae* exists in Florida, race changes probably have occurred since previously resistant lettuce cultivars became susceptible (5,6). During 1989-1990 most of the commercially grown butterhead, crisphead, romaine, and other leafy lettuce types in Florida were susceptible to downy mildew.

B. lactucae has exhibited extensive variation in virulence. Many resistant genes have been identified but rendered ineffective by variability in the pathogen population. Currently, 13 single dominant resistance genes (*Dm*) in the host and their complementary avirulence genes in the pathogen have been described (3,4,13). By using this 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 the compatible (sporulation and/or necrosis) or incompatible (no sporulation or necrosis) reaction of an isolate of *B. lactucae* on the host tester set. In California, four pathotypes of *B. lactucae* have been identified by virulence phenotype determinations (13).

The objectives of the present study were to: 1) identify potential sources of downy mildew resistance in butterhead and crisphead lettuce types, and 2) determine the virulence phenotype of the pathogen.

Materials and Method

Field evaluation. Forty crisphead and 10 butterhead cultivars and breeding lines were direct-seeded in commerical fields in double-row raised beds on 3 foot centers on 2 and 13 Oct. 1989, respectively. Fields were fertilized according to soil test recommendations. Lettuce was thinned at the four-leaf stage, providing a 12- and 9-inch in row spacing for the crisphead and butterhead type, respectively. Experimental units consisted of single 30-ft sections of bed. Crisphead lettuce plots containing breeding lines and cultivars were not replicated. However, breeding lines and cultivar of the 10 butterhead types were arranged in a randomized complete block design with 4 replications. Downy mildew severity ratings were recorded for the crisphead and butterhead trials on 13 and 16 Dec., 1989, respectively. Downy mildew was visually estimated using the

Florida Agricultural Experiment Station Journal Series No. N-00303. The authors thank South Bay Growers, Inc. for their assistance in conducting these field studies. The use of trade or chemical names in this publication does not imply endorsement nor criticism of similar ones not mentioned by the University of Florida-Institute of Food and Agricultural Sciences.

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Horsfall-Barratt rating scale (7) to assess disease severity on second leaf from soil surface from ten randomly selected plants per experimental unit.

Virulence phenotype determination. Isolates of *B. lactucae* were obtained during the 1989-1990 growing season from several naturally infested lettuce fields in the Everglades Agricultural Area. Using the technique of Michelmore and Crute (9), sporangia of *B. lactucae* of each isolate were inoculated individually onto a differential series of 15 lettuce lines at the seedling stage (Table 3). These inoculated seedlings, contained in a clear plastic compartmentalized utility boxes, were placed in a 15C incubator with a 16 hr. photoperiod. Seedlings were scored for the presence or absence of sporulation and necrosis approximately 7, 12, and 17 days after inoculation.

Results and Discussion

Environmental conditions were optimal for disease development. Downy mildew sporulated profusely on lettuce providing high inoculum levels in and surrounding the experiments.

Table 1. Ranking of the response of crisphead lettuce breeding lines and cultivars to downy mildew infection.

Breeding line/Cultivar	Disease severity[z]
FL70051	25.2
FL70689-3	24.6
FL70688-1	22.8
FL70307	22.3
FL70030	21.6
FL70296	20.8
FL70297	19.9
FL70298	18.3
FL70066	18.2
FL60972	17.7
FL70074	17.6
FL70580	16.9
FL70049	16.4
FL60673	16.1
FL70689-2	16.0
FL70294	14.1
FL70083	13.6
FL70090	13.4
FL70110	12.2
Florical Early	12.1
FL70189	12.1
FL70300	11.7
FL70040	11.1
FL70124	11.1
FL33062	10.7
FL70077	10.4
FL60890	10.0
FL70584	9.4
Ithaca	9.3
FL70665-8	9.1
FL70579	9.0
Raleigh	8.8
FL70877	8.7
FL70688-2	8.6
South Bay	8.5
FL70600	7.0
FL70227	6.8
FL70878	6.8
FL70299	4.8
FL70689-4	4.6

[z]Percentage of leaf area with sporulating lesions observed on second leaf from the soil surface as determined by the Horsfall-Barratt Rating System.

All the crisphead breeding lines and cultivars tested were susceptible to downy mildew (Table 1). Although disease severities ranged from 4.6 to 25.2%, no line or cultivar was found to be completely resistant. Sporulating lesions were often observed deep within the lettuce head. Consequently, the harvested heads of most of these lines and cultivars were classified as unmarketable.

The butterhead trial contained downy mildew severities ranging from 0 to 33.8% (Table 2). Two butterhead cultivars, Everglades and Mantilia, were completely resistant; sporulation was absent. One breeding line, 70880, had partial resistance with only a few sporulating lesions.

Of 4 isolates of *B. lactucae* tested on the lettuce lines containing the 13 *Dm* resistant genes, sporulation was completely absent on *Dm*1 and *Dm*15 (Table 3). Three of the four isolates on *Dm*4 and *Dm*10 had delayed or sparse sporulation, or sporulation present on only one or a few seedlings. This observation for 2 of the isolates was similar on *Dm*11 and *Dm*16.

A pathotype is defined as a group of isolates with identical or near identical virulence phenotype (8). Three of the Florida isolates of downy mildew tentatively resemble the pathotype IV described in California based on the response to the differential series of resistant cultivars (13). The sparse sporulation on lines with *Dm* 4, 10, or 16 is characteristic of the unstable heterokaryotic nature of CA pathotype IV isolate. However, one isolate was similar to pathotype III.

Breeding line 70880 is the cross 'Montello' x 'Brazil 48'. Everglades is a cross of the cultivars Great Lakes x Fulton x Gallega x Dark Green Boston x Trocadero. 'Mantilia' is a commercial butterhead type containing downy mildew resistant genes which confer resistance to Dutch races NL 1-5 + 7. Resistance to each NL isolate may be due to one or several *Dm* genes (2). Without a konwledge of its pedigree and a careful genetic analysis, it is impossible to know which genes are effective in 'Mantilia'.

Based on the response of the differential series of resistant cultivars, it is possible that Mantilia' might contain at least *Dm*1 or *Dm*11 resistant genes. Incorporating one and/or both of these *Dm* genes into Florida adapted cultivars would confer resistance to Pathotype IV as well as II and III. The *Dm*15 gene also could be used to incorporate re-

Table 2. Response of several butterhead breeding lines and cultivars to downy mildew infection.

Breeding line/Cultivar	Disease severity[y]
70682-1	33.8
Florida Buttercrisp	12.3
Everglades	0.0
70292	19.4
70880	1.9
Florida 202	8.8
70881	15.9
Dark Green Boston	11.9
Orfeo	11.3
Mantilla	0.0
FLSD (P≤0.05)[z]	7.6

[z]FLSD = Fisher's Least Significant Difference.
[y]Percentage of leaf area with sporulating lesions observed on second leaf from the soil surface as determined by the Horsfall-Barratt Rating System.

Table 3. Reaction of four isolates of *Bremia lactucae* on Dm resistant genes.

Cultivar	Dm genes	Reaction of isolates[z] ISO1	ISO2	ISO3	ISO4
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	+	+	(+)	+
Empire or Pennlake	13	+	+	+	+
UCDM 14	14	+	+	+	+
PIVT1309	15	–	–	–	–
LSE 18	16	+	+	*	+
Cogham Green	0	+	+	+	+

[z] – = no sporulation
+ = profuse sporulation
(+) = sparse sporulation
* = 1 to 5 plants of about 50 with sporulation

sistance but is from an accession of *Lactuca serriola* (4). Using a commercial cultivar vs an accession or breeding line would facilitate the transfer of desirable horticultural traits. Breeders are presently attempting to incorporate resistance to pathotype II and III into lettuce cultivars adapted to Florida conditions since IV can break down into these components (13).

Although only a few isolates of *B. lactucae* have been tested so far in Florida, it is possible that a mixture of more than one pathotype might exist. In California, Pathotypes III and IV represented 31% and 63%, respectively, of numerous isolates collected during 1987 to 1989 (13). Isolates of *B. lactucae* collected throughout the Everglades Agricultural Area during 1989-1990 downy mildew epidemic are currently being tested for virulence phenotype to further characterize the Pathotype populations in Florida. This information will be useful in the development of future downy mildew control strategies.

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Proc. Fla. State Hort. Soc. 103:142-148. 1990.

FACTORS AFFECTING DEVELOPMENT OF FUSARIUM CROWN ROT OF TOMATO

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Additional index words. Calcium amendments, light duration, inoculum concentration, spore germination, temperature.

Abstract. **Fusarium crown rot of tomato (*Lycopersicon esculentum* Mill.) caused by *Fusarium oxysporum* Schlecht. f. sp. *radicis-lycopersici* Jarvis and Shoemaker, occurs frequently in the sandy soils of Florida, especially in the southwestern district. Several experiments were carried out under controlled conditions in growth rooms and chambers, and in the field to determine the effect of various factors on disease development. Disease incidence and severity were less on 1 and 2 week old plants than on 3 to 6 week old plants in the greenhouse. The optimum light duration for disease occurrence in growth chambers was 12 hours daily, and the optimum temperature was 68F. In general, disease development decreased with decreasing spore concentration in a growth room. A concentration of 12.5 or 24 million microspores/ml of inoculum produced consistent results with a high percentage of severely diseased plants, whereas lower concentrations produced slight symptoms, making disease evaluations difficult. Increasing rates of $CaCO_3$ in growth room studies increased the pH and soluble Ca content of the medium and decreased disease incidence and severity. Increasing rates of $CaSO_4$ in growth room studies increased the soluble Ca content of the medium more than $CaCO_3$, but did not affect pH of the medium or disease development. Adjusting the pH upward with $NaHCO_3$ greatly alleviated crown rot**

Florida Agricultural Experimental Station Journal Series No. N-00271.