Guidelines for Identification of Races of Lettuce Downy Mildew using Differential Resistant Varieties

(Version 2.0, October 2023)

Authors: Phyllis Himmel, Nicki Phillips
Revisions: Phyllis Himmel, Kelley Clark

Host: *Lactuca sativa*
Pathogen: *Bremia lactucae*

Background: Lettuce downy mildew is the most important foliar pathogen of lettuce worldwide. *Bremia lactucae* is the causal agent that infects all lettuce types at all growth stages. Infection and spread of this disease are favored by the cool and humid environments occurring in lettuce production regions. Yield losses can occur under all levels of disease pressure because lettuce is usually consumed as a fresh product. When grown in arid climates, levels of disease and crop loss may vary widely depending on the prevailing weather conditions.

Bremia lactucae is an obligate parasite belonging to the oomycete group of organisms. This is a complex organism consisting of multiple races (pathotypes) that occur wherever lettuce is grown. Races can be identified by the pattern of virulence across differentiating host sets of lettuce genotypes containing known resistance genes (*Dm*). Many of the current races in nature have developed resistance to some of the fungicides used to control this disease. In addition, two mating types, B1 and B2 have been identified and are needed for sexual reproduction and the development of oospores. Oospores are readily produced following artificial inoculation of seedling cotyledons, are sometimes formed in nature and can persist in dead tissue left as debris after harvest.

Damp and cool conditions are required for infection and symptom development. The wind-disseminated sporangia germinate on the leaf surface and penetrate epidermal cells to infect lettuce leaves. Infection can take place in 3 – 4 hours if free moisture is present on leaves. Young plants will initially develop light green to chlorotic angular spots visible on both the upper and lower leaf surfaces (Fig. 1). On red varieties, the initial spots may appear more grayish and water-soaked. White fluffy mycelial growth predominantly on the leaf underside follows 2 – 3 weeks after infection (Fig. 2). Older lesions will turn brown and necrotic due to secondary infections. On rare occasions, infection of very young seedlings results in systemic infection (Fig. 3), causing dark discoloration of stem tissue.

Fig. 1. Light green to yellow angular spots on upper leaf surface.

Fig. 2. White fluffy mycelial growth on leaf underside.

Fig. 3. Dark discoloration of stem tissue in systemic infection.

The most effective management of downy mildew is in the use of resistant cultivars and the application of fungicides such as Metalaxyl after infection and before the development of disease. Drip irrigation reduces leaf wetness and humidity and can reduce disease severity but does not prevent disease. Over 50 sources of resistance are known and an increasing number of resistance genes have been deployed in commercial lettuce varieties.

Races of *B. lactucae*: New races of *B. lactucae* develop in response to the deployment of disease resistant lettuce cultivars and today, many races exist as the fungus continues to evolve to overcome commercial sources of resistance. **Reliable information about resistance in relation to the many races of *B. lactucae* is essential for a successful and durable disease control strategy.** For that reason, the International Bremia Evaluation Board was formed to facilitate the accurate identification of new races.

International Bremia Evaluation Board (IBEB) – a joint initiative: https://worldseed.org/our-work/disease-resistance/other-initiatives/ibeb/

IBEB is a joint initiative of lettuce breeding companies in USA, France and the Netherlands, University of California-Davis, the Dutch inspection service (Naktuinbouw) and the French National Seed Station (GEVES). IBEB's mission is to identify new races of *B. lactucae* that pose a significant threat to the American and European lettuce industry and promote the use of standardized race names in communications with growers.

To this end, IBEB utilizes a well-defined and internationally agreed system of race naming. Genetic studies and companies in IBEB provide information on the genetic background of lettuce varieties. UC-Davis, Naktuinbouw and GEVES serve as independent testing facilities to facilitate the process of identifying new races of *B. lactucae*. Information about the evolution of the pathogen in response to resistance in lettuce varieties is updated regularly.

Because there is little movement of *B. lactucae* between the USA and Europe, race nomination specific to each continent is done by regional IBEB committees: IBEB-US for the USA, and IBEB-EU for Europe. A global coordinating body (IBEB-G) coordinates the use of a common set of host differentials, common test protocols, and communications on *B. lactucae* races. IBEB-G also advises on scientific denomination of new genes for resistance to *B. lactucae*.

IBEB decides on the composition of a set of host differential lines with distinct diagnostic major resistance genes and on the naming of new races of *Bremia lactucae* (Table 1). The current core set of 19 differential resistant cultivars is designated set D; additional cultivars may also be included. A binary coding system for the description of reaction patterns is used and denominated. All isolates have the prefix "BI:" followed by a space and the race number: European races have the suffix "EU" and American isolates have the suffix "US". Note that with this nomenclature for American races the prefix "CA" is replaced by the suffix "US" and Roman numerals are no longer being used. The numbering of races for the US and EU is independent; contemporary race numbers are currently less than 10 for the US and over 30 for the EU. When a race is no longer detected in the field the number is retired from evaluation of resistant varieties; this has occurred for BI:1 to 15EU and BI: 1 to 6US. Archived records of BI races can be found on the IBEB webpage.

Table 1. Reactions of Bremia races BI: 7-9US and BI: 16-40EU to the IBEB D set of differentials

	GreenTowers	Dandie	R4T57D	UC Dm14	NunDm15	CGDm16	Colorado	FrRsal-1	Argelès	RYZ 2164	RYZ910457	Bedford	Balesta	Bartoli	Design	Kibrille	Fenston	Bataille	RYZ20007	Set	D sextet code
		Dm3	Dm4	Dm14	Dm15	Dm16	Dm18	Rsal-1	R38	Dm24/38	R52	R53	R54	R55	R56	Dm11,R57	R65	R59	Dm11,R58		
ID	0	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18		
Sextet value		1	2	4	8	16	32	1	2	4	8	16	32	1	2	4	8	16	32		
BI: 7US	+	+	-	+	+	+	+	-	-	-	-		-	-		-	-	-	-	D	61-.-.
BI: 8US	+	+	+	+	+	+	+	-	-	-	-		-	-	+	-	-	+	-	D	63-.-18
BI: 9US	+	+	-	+	+	+	+	+	-	-	+	(-)	-	-	+	-	-	-	-	D	61-09-02
BI: 16EU	+	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	D	19-00-00
BI: 17EU	+	+	-	+	+	-	+	+	-	-	-	(+)	-	-	-	-	-	-	-	D	45-17-00
BI: 18EU	+	-	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	D	50-00-00
BI: 20EU	+	+	+	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	D	51-00-00
BI: 21EU	+	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-	D	27-01-00
BI: 22EU	+	-	+	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-	D	46-32-00
BI: 23EU	+	+	+	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-	-	D	19-02-00
BI: 24EU	+	-	+	-	-	+	+	-	+	-	-	-	-	-	(-)	-	(-)	(-)	-	D	50-02-00
BI: 25EU	+	-	+	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	D	50-01-00
BI: 26EU	+	+	+	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	D	51-03-00
BI: 27EU	+	+	+	+	+	-	+	-	+	+	-	-	+	-	-	-	-	-	-	D	47-38-00
BI: 29EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	D	62-07-00
BI: 30EU	+	-	+	+	+	-	+	-	+	+	-	-	-	-	+	-	-	-	-	D	46-06-02
BI: 31EU	+	+	+	+	-	-	+	-	-	+	+	-	-	-	+	-	-	-	-	D	39-12-02
BI: 32EU	+	+	+	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	D	27-00-04
BI: 33EU	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	-	-	-	D	62-07-06
BI: 34EU	+	-	+	+	-	+	+	+	+	+	+	-	-	+	(-)	-	-	-	-	D	54-15-01
BI: 35EU	+	-	+	+	+	+	+	+	+	+	+	-	-	-	+	+	-	-	-	D	62-15-06
BI: 36EU	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	-	D	55-15-01
BI: 37EU	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	+	-	-	D	46-15-14
BI: 38EU	+	-	+	+	+	-	+	+	+	+	+	-	-	-	+	+	-	-	+	D	46-15-38
BI: 39EU	+	+	+	+	-	+	+	+	+	+	+	-	-	+	-	-	-	-	+	D	55-15-33
BI: 40EU	+	-	+	+	+	+	+	+	+	+	+	+	-	+	(-)	-	-	-	-	D	62-31-01

Inoculation and evaluations for resistance and susceptibility: *B. lactucae* can be maintained as sporulated cultures on lettuce cotyledons stored at -20°C for up to 6 months and longer at -80°C. **To increase inoculum,** 6 to 8 day old seedlings of the appropriate susceptible variety are spray inoculated (Fig. 4) with the collected spores from frozen cultures or seedlings infected with the targeted race. Infected cotyledons are collected (Fig. 5) and shaken in distilled water (Fig. 6) to make suspensions of spores. Spores are filtered through cheesecloth, then resuspended in distilled water (Fig. 7). After inoculation, incubate in a growth chamber or incubator at 15°C with 100% humidity and 16 hours of light /day. Sporulation will occur approximately 7 days after inoculation. Once the culture starts sporulating, there is a 3-day window of use for optimal germination and infection. **For test preparation and evaluation**, inoculated seedlings are collected and prepared as described above and the concentration is adjusted to 1 X 10^4 or 1 X 10^5 spores ml^{-1}. For best infection rates, use spore suspensions as soon as possible after preparation.

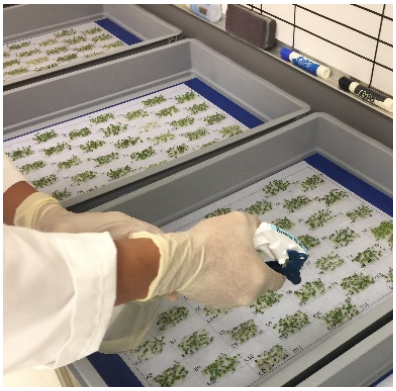


Fig. 4. Spray inoculate to runoff.

Fig. 5. Collection of sporulating inoculum on seedlings.

Fig. 6. Suspend and shake in distilled water.

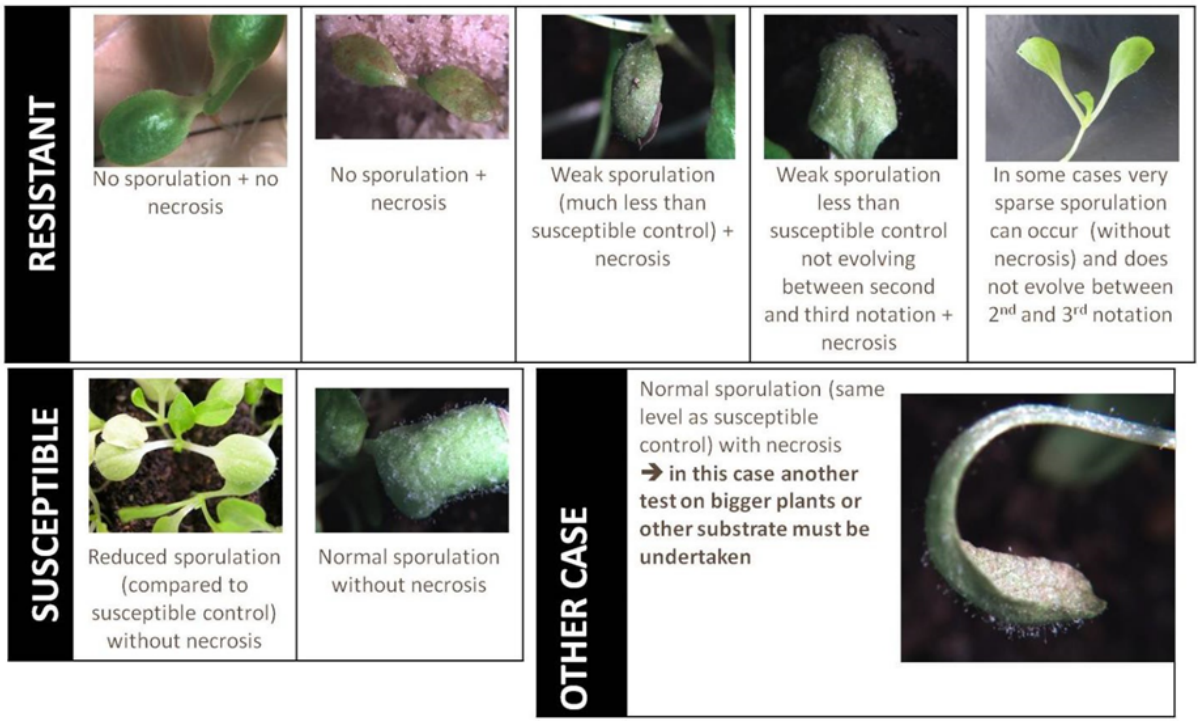
Test 3 reps of 10 to 30 seedlings per variety (depending on the size of the test container). Germinate seeds of targeted varieties on sterile, premoistened (with Hewitt's or half strength Hoagland's solution) filter or blotter paper fit into covered, divided clear plastic boxes or in covered tubs. Five to seven days after sowing (Fig. 8), spray inoculate cotyledons to runoff with the above-prepared sporangial suspension (Fig. 5).

Fig. 7. Filter and suspend in distilled water.

Fig. 8. Inoculate 5 – 7 days after sowing.

Inoculum and seedling preparations are all carried out under sterile conditions in a laminar flow hood. Each tested race should be handled and incubated separately at different times. Incubate covered boxes of inoculated seedlings as described above in inoculum preparation. Evaluate the test 7, 11 and 15 days after inoculation. The IBEB observation scale below is followed for the interpretation of resistance and susceptibility.

IBEB Observation scale for the interpretation of *B. lactucae* reaction phenotypes on lettuce seedlings



For more information on *B. lactucae*, occurrence of this race in California and downy mildew resistance in lettuce, visit the University of California Bremia and Michelmore lab websites: https://bremia.ucdavis.edu and https://michelmorelab.ucdavis.edu . When evaluating the response of the differentiating hosts to a race in question, look for a characteristic pattern of resistant and susceptible reactions (Fig. 9) compared to documented responses (Table 1).

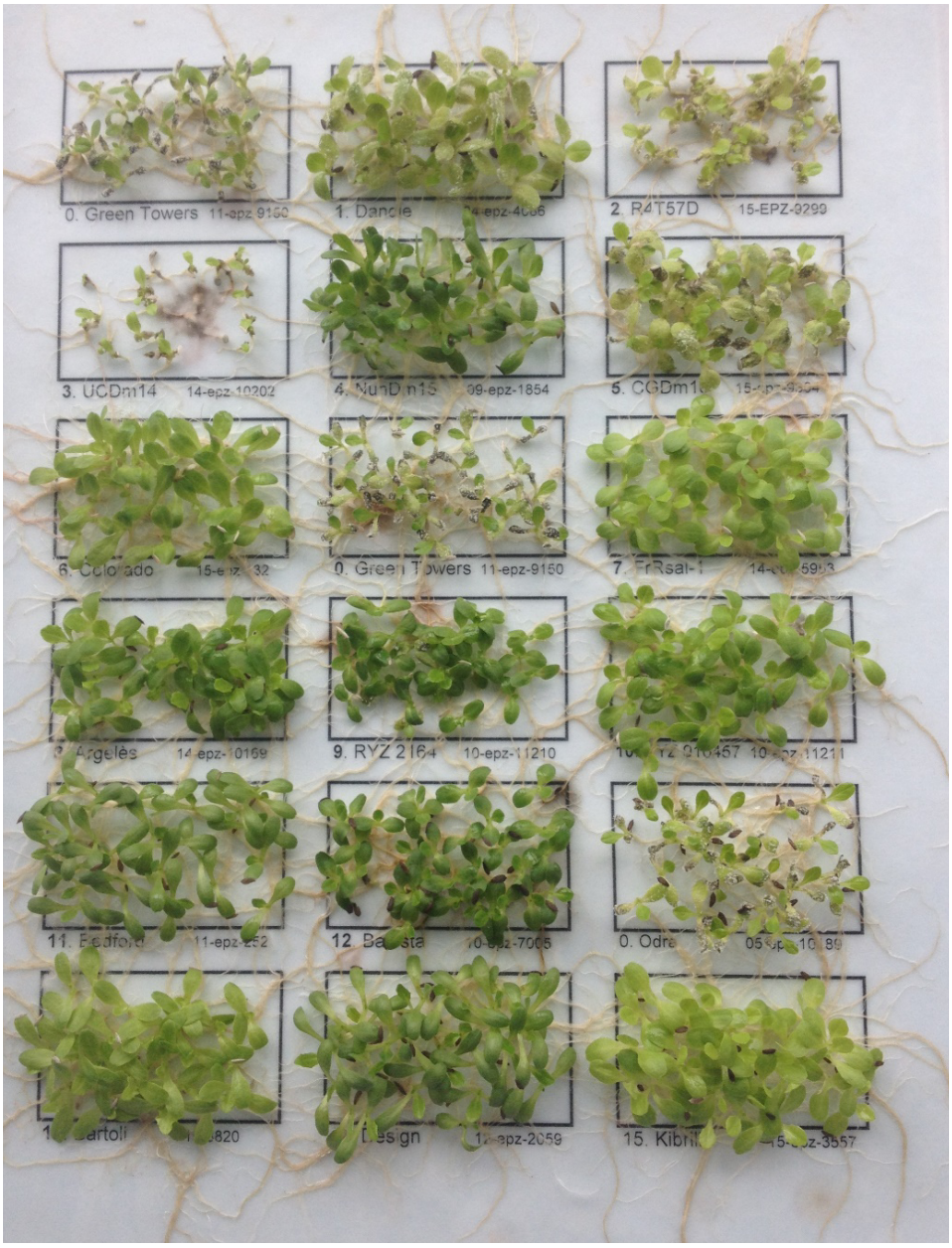


Fig. 9. Pattern of differentiating host response to the targeted *B. lactucae*

The UPOV (Union for the Protection of Varieties in the EU has similar guidelines for characterizing races of *B. lactucae* and verifying resistance claims of specific lettuce varieties. For more details on the UPOV guidelines go to http://www.upov.int/test_guidelines or contact Diederik Smilde at Naktuinbouw in the Netherlands, d.smilde@naktuinbouw.nl for more information.

GEVES MATREF (Variety registration, Seed certification and Reference materials) also has guidelines for characterizing race of *B. lactucae* and verifying resistance claims of specific lettuce varieties. For more details on the GEVES MATREF guidelines go to https://www.geves.fr/tools/reference-material-matref/

Contacts for ordering seeds of differentiating hosts and *B. lactucae* races:

Contact Kacie Wynne, IBEB-US representative at k.wynne@rijkzwaan.com or Diederik Smilde, IBEB-EU representative at d.smilde@naktuinbouw.nl for seeds of the set of differential resistant varieties.

Contact Richard Michelmore at rwmichelmore@ucdavis.edu to request and arrange to receive reference races of *B. lactucae*.

If you have any questions or comments, please contact CSSPI Director, Kelley Clark at kjclark@ucdavis.edu

Liability waiver: The CPPSI Collaboration for Plant Pathogen Strain Identification, USDA NPGS/GRIN, APS, ASTA, and all other associated members and participating organizations or companies have done their best to provide information that is up-to-date and published in refereed journals and, therefore, no liability for the use of this information is accepted. The inoculation protocol described in this document has been demonstrated to be effective at identifying races of lettuce downy mildew and resistance traits of lettuce varieties.

Selected Literature:

Ilott, T. W., Hulbert, S. H., and Michelmore, R. W. 1989. Genetic analysis of the gene-for-gene interaction between lettuce (*Lactuca sativa*) and *Bremia lactucae*.

International Bremia Evaluation Board (IBEB) – International Seed Federation. www.worldseed.org

Koike, S. T., Gladders, P. and Paulus, A. O. 2007. Downy Mildew, 302-304 In Vegetable Diseases, A Color Handbook, Academic Press 488 pages.

Parra, L., Maisonneuve, B., Lebeda, A., Schut, J., Christopoulou, M., Jeuken, M., McHale, L., Truco, M-J, Crute, I. and Michelmore, R. 2016. Rationalization of genes for resistance to Bremia lactucae in lettuce. Euphytica August 2016, Volume 210, Issue 3, pp 309–326.

Sargent, J. A., Tommerup, I. C. and Ingram, D. S. 1972. The penetration of a susceptible lettuce variety by the downy mildew fungus *Bremia lactucae* Regel.

Smilde, D. and van Dijk-Veldhuizen, A. 2015. A streamlined differential set for *Bremia lactucae.* In: Proceedings of Eucarpia meeting on Leafy Vegetables, 14-17 April 2015, Murcia, Spain.

Su, H., van Bruggen, A. H. C., Subbarao, K. V. and Sherm, H. 2004. Sporulation of *Bremia lactucae* affected by temperature, relative humidity and wind in controlled conditions. Phytopathology 94:396-401.

University of California Integrated Pest Management Program. 2012 Lettuce Downy Mildew. www.ipm.ucdavis.edu

Wu, B. M., van Bruggen, A. H. C. and Subbarao, K. V. 2017. Downy Mildew, 32 – 35 In Compendium of lettuce Diseases and Pests, Second Edition, APS Press 165 pages.