Guidelines for Identification of Spinach Downy Mildew (*Peronospora effusa*) Races on Differential Spinach Hosts

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Host: Spinach (*Spinacia oleracea* L.)
Pathogen: *Peronospora effusa*, formerly *Peronospora farinosa* f. sp. *spinaciae*

Background: Spinach downy mildew is caused by the obligate, oomycete pathogen *Peronospora effusa*, formerly known as *Peronospora farinosa* f. sp. *spinaciae*. Spinach growers in the European Union (EU) and the USA have been confronted with outbreaks of downy mildew on spinach cultivars that, in the past, were resistant to this disease. Investigations showed that the cultivars grown had not changed, so it was concluded that the pathogen had overcome resistance in these cultivars through formation of new isolates or races. Since the early 2000s, the number of new *P. effusa* races identified has substantially increased (**Figure 1**).

Comparison between isolates in the USA and EU showed that US races 5 and 6 were not identical to EU races 5 and 6, resulting in a confusing situation. Confusion and uncertainty developed among growers and seed companies. Thus, seed companies from the Netherlands set up a cooperative effort with Naktuinbouw (General Netherlands Inspection Service for Horticulture) and researchers from the University of Arkansas (Jim Correll) and the University of California (Steven Koike) to address this problem. A system was developed for race classification the spinach downy mildew pathogen using a differential set of spinach cultivars and F1 hybrids. Using this set, new downy mildew isolates from all over the world were tested at Naktuinbouw.

The International Working Group on *Peronospora effusa* (IWGP) has developed guidelines for the naming of spinach downy mildew races and monitors outbreaks of downy mildew and the development of new strains. If appropriate, new race names are approved in a systematic manner to avoid confusion. Cultures of new races will be made available via Naktuinbouw in the Netherlands for seed companies willing to test their breeding material against the new races. Researchers from all over the world are invited to join this initiative and use the differential host set to identify new isolates in their regions. For more information, contact Diederik Smilde (d.smilde@naktuinbouw.nl), Jim Correll (jcorrell@uark.edu), or the International Working Group *Peronospora* (IWGP) chairperson Anne Konigs (a.konigs@rijkzwaan.nl).

Characterization of *P. effusa* races has been based on qualitative disease reactions of isolates on a set of host differentials. The previous set of spinach differentials (**Table 1**) included commercial hybrids as well as open-pollinated cultivars and breeding lines. However, the nature of some of the differential hosts has been problematic as seeds of hybrids are produced using proprietary inbred lines, which may become unavailable when companies stop producing particular hybrids.

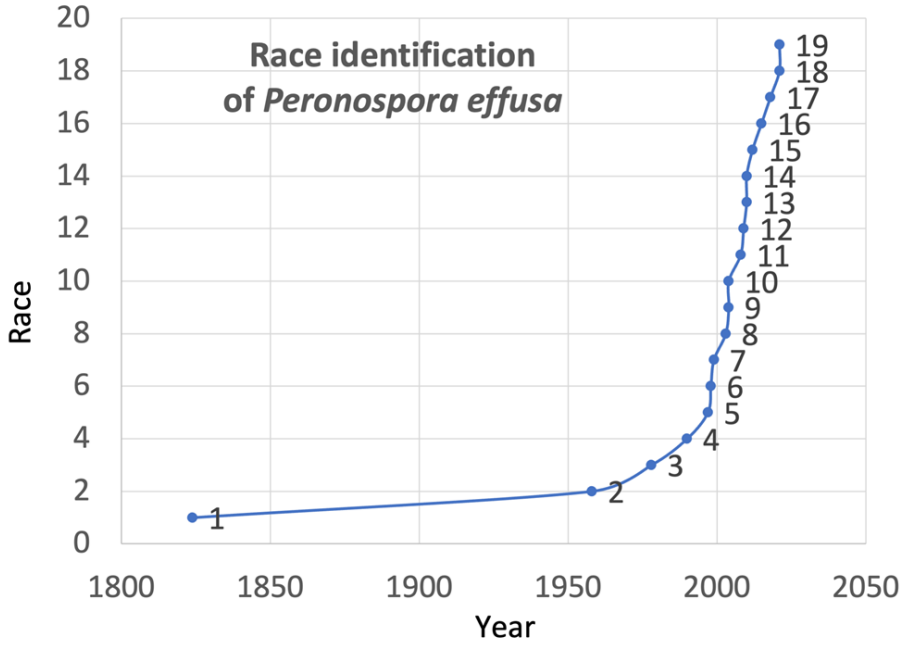

Figure 1. Emergence of new *P. effusa* races over time, indicating the exponential increase in new races identified post 2000.

As a result, a project was initiated at the University of Arkansas to develop open-pollinated, near-isogenic lines (NILs) with single resistance loci in a common susceptible genetic background for race identification of *P. effusa* (Irish et al., 2008). These NILs have been used to elucidate the genetic basis of resistance, develop molecular markers linked to resistance loci, and have been used to develop a new set of differentials (**Table 2**).

In 2021, two new races of *P. effusa* were published by the IWGP on the basis of a worldwide evaluation of isolates from growers' fields and trap nurseries. Isolate SP1924 found in Europe, is denominated as race Pe: 18. Isolate UA202001E, found in the USA, is denominated as race Pe: 19. As seen in **Table 2**, Race Pe: 18 is able to infect the differentials NIL2, 3, 4, 5, Pigeon, Caladonia, and Meerkat. Pe: 18 has been found in the US in 2015 to 2018, not in 2019 and 2020. And in Europe it has been found more often in the last 3 years. Race Pe: 19 is able to infect the differentials NIL1, 2, 4, 5, 6, Pigeon, Meerkat and Hydrus. Pe: 19 has been reported only from the USA. Both new races pose a significant threat to the spinach industry in all parts of the world, and resistance to these new races is important.

Table 1. Disease reactions of commercial spinach differentials for determining the race identification of isolates of the spinach downy mildew pathogen, *Peronospora effusa*.

	Race of *Peronospora effusa*[1]																
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
Viroflay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Resistoflay	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Califlay	-	+	-	+	-	+	+	-	-	+	-	-	+	-	+	-	+
Clermont	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+
Boeing #	-	-	-	-	-	-	-	+	-	+	-	+	-	+	-	-	+
Lion	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
Lazio	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+
Whale	-	-	-	(-)	-	(-)	(-)	-	-	+	-	+	+	-	+	-	+
Pigeon	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+
Caladonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+
Meerkat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	(-)
Hydrus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

[1]Races of the downy mildew pathogen as of June 2018.

The differential response of Boeing to these known races of Pe is the same as that of Avenger, the name assigned to this cultivar in the USA.

\+ = Susceptible reaction = sporulation observed on cotyledons in the differential seedling test.

\- = Resistant reaction = no sporulation observed on cotyledons in the differential seedling test. (-) = Reduced level of infection, often referred to as field resistance = sparse sporulation on the tips of cotyledons in the differential seedling test.

+/- = Variability in the number of resistant and susceptible plants observed.

nt = Not (yet) tested.

Table 2. Disease reactions of commercial and NIL spinach differentials for determining the race identification of isolates of the spinach downy mildew pathogen, *Peronospora effusa.*

Reaction patterns of *Peronospora effusa* (Pe) races																			
Differentials		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
V	Viroflay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1	NIL 5	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	NIL 3	-	+	-	+	-	+	+	-	-	+	-	-	+	-	+	-	+	+	-
3	NIL 4	-	-	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
4	NIL 6	-	+	-	-	-	+	-	+	+	+	-	+	-	+	-	-	+	-	+
5	NIL 1	-	-	-	-	-	-	-	+	-	+	-	+	(-)	+	-	-	+	-	+
6	NIL 2	-	-	-	-	-	-	-	-	-	-	+	+	+	+	-	+	+	+	+
7	NIL 9	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
8	Caladonia	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-
9	Meerkat	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	(-)	+	+
10	Hydrus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

Update 1 May 2022: Pigeon is replaced by NIL 9 with Pe: 15 = susceptible

The following is a summary protocol for screening for disease resistance to the downy mildew pathogen. More detailed protocols have been published (see references below). Others have effectively used a similar protocol with minor differences in temperature, number of days for incubation, plant age, etc.

1. Spinach is typically planted in a commercial potting mix in 100 cm x 50 cm flats.

2. Eleven differentials (**Table 2**) are grown per tray with approximately 20 plants of a given differential per row.

3. Plants grown under fluorescent lights are fertilized once a week after seedling emergence with Peter's commercial fertilizer (13-13-13).

4. Three-week-old seedlings (first true-leaf stage) are inoculated with a sporangial suspension (2.5 x 10^5 sporangia/ml) of the appropriate isolate of the pathogen (**Figure 1**). Inoculum is applied using an air-brush paint sprayer with compressed air (**Figure 2**).

5. The inoculated plants are placed in a dew chamber maintained at 18-20^{o}C with 100% relative humidity for a 24 h period. The plants are then moved to a growth chamber maintained at 18-20^{o}C with a 12 h photoperiod for 6 days.

6. After the 6 days, the plants are returned to the dew chamber for 24 h to induce sporulation of *P. effusa* sporangia, and the seedlings are scored for reaction to the inoculated isolate.

7. Plants are scored as resistant or susceptible based on symptoms of chlorosis (**Figure 3**) and sporulation of the downy mildew pathogen on the cotyledons (**Figure 4**) and true leaves (**Figure 5**).

8. Plants exhibiting any evidence of chlorosis and sporulation are considered susceptible. In a reliable test, a resistant line will have >95% of the plants resistant to the isolate, and the susceptible differential (Viroflay) will have >95% susceptible plants in the test.

Figure 1. Seedlings of spinach differential cultivars ready for inoculation with an isolate of the downy mildew pathogen.

Figure 2. Inoculating seedlings of spinach differential cultivars with *Peronospora effusa spores* using an air-brush paint sprayer with compressed air.

Figure 3. Chlorosis on a cotyledon of a spinach seedling inoculated with an isolate of *Peronospora effusa.*

Figure 4. Sporulation of *Peronospora effusa* on a cotyledon of an inoculated spinach seedling, representing a susceptible reaction to that race of the pathogen.

Figure 5. Sporulation of *Peronospora* effusa on a true leaf of an inoculated spinach seedling, representing a susceptible reaction to that race of the pathogen.

Figure 6. Germinating sporangia of *Peronospora effusa* on a plate of water agar 24 hours after spraying a sporangial suspension onto the agar, as a test of viability of the pathogen isolate.

Pitfalls and trouble-shooting:

1. When examining field isolates of *P. effusa*, samples should be kept cool (<20^{O}C) in containers or in bags with high humidity but no free moisture. The spores should be washed off the plants into cold water and the water kept cold until the spore suspension is sprayed onto spinach plants.

2. When inoculating an isolate on differential host plants, the inoculum should also be sprayed onto the surface of one or two water agar plates. The plates should be incubated in the dew chamber along with the inoculated plants. Spore germination should be checked using a microscope after 24 h (**Figure 6**). If the isolate is viable, 10 to 50% of the spores should have germinated within 24 h.

3. Extreme care and very good sterile technique should be used to avoid cross-contamination when working with multiple isolates of *P. effusa*. Each isolate should be handled separately, at a different time, and seedlings inoculated with different isolates should be kept in different growth chambers or incubators in different rooms.

4. Intermediate levels of infection (>5% but <95%) suggests the isolate being examined may be a mixture of more than one race, or the material being examined may be segregating for resistance.

The UPOV (Union for the Protection of Varieties) in the EU has similar guidelines for characterizing races of *P. effusa* and verifying resistance claims of specific spinach cultivars.

Ordering seeds of spinach differential lines:
Seeds of nine differential spinach cultivars (from Table 1) including Viroflay, Resistoflay, Califlay, Clermont, Campania, Boeing, Lion, Lazio and Whale can be ordered from the USDA GRIN (Germplasm Resources Information Network: https://www.ars-grin.gov/). You may search the USDA GRIN database without logging in, but cannot order seeds until you create an account and log in to the database.

Type in '**CPPSI***' in the search window. Select the differential hosts to order. Select the cart button at the top of the page to generate an order form. Select 'submit' to place your order.

A limited supply of 100 seeds per differential can be ordered at no charge, as long as there is adequate seed in supply. The NPGS may not always have adequate seed of all the differentials listed above to provide a full set of differentials.

Seeds of the spinach NILs and differential lines (from Table 2) can be ordered from Jim Correll (jcorrell@uark.edu or at 479-575-2710). A limited supply of 100 seeds per differential can be ordered at no charge, as long as there is adequate seed in supply.

Ordering strains of contemporary US races of *P. effusa*:
Reference strains of contemporary races of *P. effusa* known to occur in the US can be ordered from Jim Correll (jcorrell@uark.edu or at 479-575-2710).

NOTE: People ordering isolates of contemporary US races must provide proof of appropriate USDA APHIS permit for those races. Jim Correll may not be able to provide isolates of all known races. Have seedlings ready for inoculation when isolates of *P. effusa* are requested.

Please contact Kelley Clark at kjclark@ucdavis.edu if you have issues acquiring these materials.

People/programs with expertise on spinach downy mildew races and host differentials:

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Feedback

Please contact Kelley Clark at kjclark@ucdavis.edu about inquiries on how to participate and support CPPSI, provide feedback on new strains identified and views on the inoculation protocols, differential hosts, or any related matter is welcomed.

Liability waiver

The Collaboration for Plant Pathogen Strain Identification (CPPSI), 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 the spinach downy mildew and resistance traits of spinach cultivars.

References:

1. Bentley, T. C. 2006. Development of a near-isogenic line, scar marker evaluation, and storage procedure assessment for downy mildew of spinach. M.S. thesis, University of Arkansas, Fayetteville, AR.
2. Brandenberger, L. P., Correll, J. C., and Morelock, T. E. 1991. Nomenclature of the downy mildew fungus on spinach. Mycotaxon 41:157-160.
3. Brandenberger, L. P., Correll, J. C., Morelock, T. E., and McNew, R. W. 1991. Identification and cultivar reactions to a new race (race 4) of *Peronospora farinosa* f. sp. *spinaciae* on spinach in the United States. Plant Dis. 75:630-634.
4. Brandenberger, L. P., Correll, J. C., Morelock, T. E., and McNew, R. W. 1994. Characterization of resistance of spinach to white rust (*Albugo occidentalis*) and downy mildew (*Peronospora farinosa* f. sp. *spinaciae*). Phytopathology 84:431-437.
5. Brandenberger, L. P., Morelock, T. E., and Correll, J. C. 1992. Evaluation of spinach germplasm for resistance to a new race (race 4) of *Peronospora farinosa* f. sp. *spinaciae*. HortSci. 27:1118-1119.
6. Choi, Y. J., Hong, S. B, and Shin, H. D. 2007. Re-consideration of *Peronospora farinosa* infecting *Spinacia oleraceae* as a distinct species, *Peronospora effusa*. Mycol. Res. 111:381-391
7. Correll, J. 2018. Denomination of Pfs: 17, a new race of downy mildew in spinach. Salinas Valley Agriculture. April 16, 2018. http://ucanr.edu/blogs/blogcore/postdetail.cfm?postnum=26906.
8. Correll, J. C., Bluhm, B. H., Feng, C., Lamour, K., du Toit, L. J., and Koike, S.T. 2011. Spinach: Better management of downy mildew and white rust through genomics. Europ. J. Plant Pathol. Special Issue (*accepted with revisions*).
9. Correll, J. and Koike, S. 2017. Race diversity and the biology of the spinach downy mildew pathogen. CLGRB Annual Report April 1, 2016 to March 31, 2017.
10. Correll, J. C., Koike, S. T., Brandenberger, L. P., Black, M. C., and Morelock, T. E. 1990. A new race of downy mildew threatens spinach. Calif. Agric. 44:14-15.
11. Correll, J. C., Morelock, T. E., Black, M. C., Koike, S. T., Brandenberger, L. P., and Dainello, F. J. 1994. Economically important diseases of spinach. Plant Dis. 78:653-660.
12. Eenink, A. H. 1974. Linkage in *Spinacia oleracea* L. between the locus for resistance to *Peronospora spinaciae* Laub. and the locus for tolerance for cucumber virus 1. Euphytica 23:485-487.
13. Eenink, A. H., 1976. Linkage in *Spinacia oleracea* L. of two race-specific genes for resistance to downy mildew *Peronospora farinosa* f. sp. *spinaciae* Byford. Euphytica 25:713- 715.
14. Holton, M. 1991. Inheritance of resistance to downy mildew (*Peronospora farinosa* f. sp. *spinaciae*) in spinach (*Spinacia oleracea* L.). Ph.D. dissertation, University of California, Davis, CA.
15. Irish, B. 2004. New races of the downy mildew pathogen of spinach, identification of molecular markers for disease resistance, and molecular characterization of diversity in spinach germplasm. Ph.D. dissertation, University of Arkansas, Fayetteville, AR.
16. Irish, B. M., Correll, J. C., Feng, C., Bentley, T., and de los Reyes, B. G. 2008. Characterization of a resistance locus (*Pfs-1*) to the spinach downy mildew pathogen (*Peronospora farinose* f. sp. *spinaciae*) and the development of a molecular marker linked to Pfs-1. Phytopathology 98:894-900.
17. Irish, B. M., Correll, J. C., Koike, S. T., and Morelock, T. E. 2007. Three new races of the spinach downy mildew pathogen identified by a modified set of spinach differentials. Plant Dis. 91:1392-1396.

18. Irish, B. M., Correll, J. C., Koike, S. T., Schafer, J., and Morelock, T. E. 2003. Identification and cultivar reaction to three new races of the spinach downy mildew pathogen (*Peronospora farinosa* f. sp. *spinaciae*), from the United States and Europe. Plant Dis. 87:567-572.
19. Irish, B. M., Correll, J. C., Raid, R. N., and Morelock, T. E 2004. First report of *Peronospora farinosa* f. sp. *spinaciae* (race 5) of spinach in Florida. Plant Dis. 88:84.
20. Jones, R. K., and Dainello, F. J. 1982. Occurrence of race 3 of *Peronospora effusa* on spinach in Texas and identification of sources of resistance. Plant Dis. 66:1078-1079.
21. Lorenzini, G., and Nali, C. 1994. A new race (race 4) of spinach downy mildew in Italy. Plant Dis. 78:208.
22. Lyon, R., Correll, J., Feng, C., Bluhm, B., Shrestha, S., Shi, A. and Lamour, K. 2016. Population structure of *Peronospora effusa* in the southwestern United States. PLoS ONE 11(2): e0148385.doi:10.1371/journal.pone.0148385.
23. Nali, C. 1998. A novel threat for spinach in Italy: A new race of downy mildew. Adv. Hortic. Sci. 12:179-182.
24. Satou, M., Nishi, K, Kubota, M., Fukami, M., Tsuji, H., and Van Ettekoven, K. 2006. Appearance of race 5 of spinach downy mildew fungus, *Peronospora farinosa* f. sp. *spinaciae*, in Japan. J. Gen. Plant Pathol. 72:193-194.
25. Satou, M., Sugiura, T., Ohsaki, R., Honda, N., Horiuchi, S., and Yamauchi, N. 2002. A new race of spinach downy mildew in Japan. J. Gen. Plant Pathol. 68:49-51.
26. Shimazaki, Y. 1990. Appearance of a new race 4 of downy mildew on spinach. Ann. Phytopathol. Soc. Jpn. 56:95.
27. Smith, P. G. 1950. Downy mildew immunity in spinach. Phytopathology 40:65-68.
28. Smith, P. G., Webb, R. E., Millett, A. M., and Luhn, C. H. 1961. Downy mildew on spinach. Calif. Agric. 15:5.
29. Smith, P. G., Webb, R. E., and Luhn, C. H. 1962. Immunity to race 2 of spinach downy mildew. Phytopathology 52:597-599.
30. Zink, F. W., and Smith, P. G. 1958. A second physiologic race of spinach downy mildew. Plant Dis. Rep. 42:818.15.