

Guidelines for Spinach Downy Mildew: *Peronospora effusa*

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Host: *Spinacia oleracea* L.

Pathogen: *Peronospora effusa* (Pe)

Background: In recent years 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. Until recently, four races of spinach downy mildew were known to exist in the USA and EU. The name 'race 5' was introduced for the new strains that overcame resistance of cultivars formerly considered resistant to the disease. In time, a second new race was identified and called 'race 6'. However, comparison between strains of the races 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.

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. Based on the current system for downy mildew race classification using genetic information provided by spinach breeders, a differential set of spinach lines, cultivars and F1 hybrids was developed for identifying races of the spinach downy mildew pathogen. Using this set, new downy mildew isolates from all over the world were tested at Naktuinbouw.

Extensive tests showed that 3 new isolates of Pfs had developed in different parts of the world. To avoid further confusion, an agreement was reached to identify known races 1, 2, 3 and 4 as Pfs1, Pfs2, Pfs3 and Pfs4 using the internationally accepted notation Pfs for *Peronospora farinose* f. sp. *spinaciae* (= *P. Effusa*). The relatively new race, Pfs5, found in the USA and the EU was formerly called race 5 in the US and race 6 in Europe. Pfs6, found in the USA, was formerly US race 6. Pfs7, found in the EU, had formerly been named race 5 in the EU. The cooperators established that some F1 spinach hybrids were resistant to one or more of the identified races, allowing growers to choose (in cooperation with seed companies), resistant cultivars for their growing areas. Today, the naming of spinach Downy mildew has been updated to *Peronospora effusa* and the corresponding code designated as Pe: 1 - 17.

The International Working Group on *Peronospora* (IWGP) 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 with naming new races. 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 Jan deVisser (JandeVisser@popvriendseeds.nl).

By 2018, 17 races of Pe had been described, with races 4 through 17 identified since 1990 (Irish et al., 2008). Although major (qualitative) and minor (quantitative) gene resistance to the various races had been identified in spinach, major gene resistance was most widely used. Characterization of Pe races has been based on qualitative disease reactions of isolates on a set

of host differentials. The previous set of spinach differentials (see **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. 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 *Pe* (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 until now. Both 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 <i>Peronospora effusa</i> | | | | | | | | | | | | | | | | |
|---------------------------|-----------------------------------|---|---|-----|---|-----|-----|---|---|----|----|----|----|----|----|----|-----|
| | 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 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |

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

[#] The differential response of Boeing to these known races of Pfs 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 farinosa* f. sp. *spinaciae*.

| Differential | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 9 | 10 | 11 | 12 |
|--------------|----------|-------|-------|-------|-------|-------|-------|--------|-----------|---------|--------|
| Race | Viroflay | NIL 5 | NIL 3 | NIL 4 | NIL 6 | NIL 1 | NIL 2 | Pigeon | Caladonia | Meerkat | Hydrus |
| Pe: 1 | + | - | - | - | - | - | - | - | - | - | - |
| Pe: 2 | + | - | + | - | + | - | - | - | - | - | - |
| Pe: 12 | + | + | - | + | + | + | + | - | - | - | - |
| Pe: 13 | + | + | + | + | (-) | - | + | - | - | - | - |
| Pe: 14 | + | + | - | + | + | + | + | + | - | - | - |
| Pe: 15 | + | + | + | - | - | - | - | - | + | - | - |
| Pe: 16 | + | + | - | + | - | - | + | + | - | + | - |
| Pe: 17 | + | + | + | + | + | + | + | + | + | (-) | - |
| Pe: 18 | + | + | + | + | - | - | + | + | + | + | - |
| Pe: 19 | + | + | - | + | + | + | + | + | - | + | + |

The following is a summary protocol for screening for disease resistance to the downy mildew pathogen. More detailed accounts of the protocol 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.

Spinach is typically planted in a commercial potting mix in 100 cm x 50 cm flats. Twelve differentials (see **Table 1 & 2** for the spinach differentials currently used, and reactions of these differentials to the known races of Pe) are grown per tray with approximately 15 plants of a given differential per row. Plants grown under fluorescent lights are fertilized once a week after seedling emergence with Peter's commercial fertilizer (13-13-13). Seedlings are inoculated with a sporangial suspension (2.5×10^5 sporangia/ml) of the appropriate isolate of the pathogen at the first true-leaf stage, when the true leaves are approximately 2 cm long (**Figure 1**). Inoculum is applied using an air-brush paint sprayer with compressed air (**Figure 2**). Between 10 and 20 ml of inoculum is used to inoculate approximately 150 seedlings per flat.



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



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

The inoculated plants are placed in a dew chamber maintained at 18-20°C with 100% relative humidity for a 24 h period. The plants are then moved to a growth chamber maintained at 18-20°C with a 12 h photoperiod for 6 days. After the 6 days, the plants are returned to the dew chamber for 24 h to induce sporulation of Pe, and the seedlings are scored for reaction to the inoculated isolate.

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**). 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 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.

Pitfalls and trouble-shooting

1. When examining field isolates of Pe, samples should be kept cool (<20°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 of Pe, the inoculum should be sprayed onto the surface of one or two water agar plates. The lids should be replaced and the plates 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 Pe isolate is viable, 10 to 50% of the spores should have germinated within 24 h.



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.

3. Extreme care and very good sterile technique should be used to avoid cross-contamination when working with multiple strains of Pe. Each strain should be handled separately, at a different time, and seedlings inoculated with different strains 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 Pe and verifying resistance claims of specific spinach cultivars. For details on the UPOV guidelines, contact Diederik Smilde at Naktuinbouw in the Netherlands (see their contact information below).

Ordering seeds of spinach differential lines:

Seeds of each of the differential lines listed in Table 1 can be ordered from the USDA GRIN (Germplasm Resources Information Network). 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.

To set up an account, go to <https://npgsweb.ars-grin.gov/gringlobal/search> and select 'New user' at the top of the opening page and follow instructions to create a profile and establish an account.

To order seeds, go to <https://npgsweb.ars-grin.gov/gringlobal/search> and log in to your USDA

GRIN account. Type in 'spinach Downy mildew differentials' 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 seeds per differential can be ordered at no charge, as long as there is adequate seed in supply. The USDA National Plant Germplasm System in which the GRIN database is housed may not always have adequate seed of all the differentials listed to provide a full set of differentials.

Note: A limited supply of 50 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.

If you have difficulties ordering seeds, contact us at cppsi@ucdavis.edu for assistance.

Seeds of the spinach NILs and differential lines in Table 2 can be ordered from **Dr. Jim Correll** (jcorrell@uark.edu or tel: 479-575-2710).

NOTE 1: *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 *Peronospora effusa*: Reference strains of contemporary races of *Peronospora farinosa* f. sp. *spinaciae* known to occur in the US can be ordered from **Dr. Jim Correll** (jcorrell@uark.edu or tel: 479-575-2710).

NOTE 1: *Please contact Dr. Correll (jcorrell@uark.edu or tel: 479-575-2710) to determine availability of particular races of interest, and for details on paying for the order. The estimated cost for ordering strains of specific races of *P. effusa* is \$400 to \$500/strain, depending on the prevalence of the races requested and/or the difficulty of increasing inoculum of the particular strain(s)/race(s) requested.*

NOTE 2: *People ordering strains of contemporary US races must provide proof of appropriate USDA APHIS permit for those races. Dr. Correll may not be able to provide cultures of all known races.*

Feedback: Please contact us at www.cppsi.ucdavis.edu for 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.

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

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