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PARTIAL REVISION OF THE TEST GUIDELINES FOR SPINACH

Document prepared by an expert from the Netherlands

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1.   The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Spinach (document TG/55/7 Rev. 5).

2.   The Technical Working Party for Vegetables (TWV), at its fifty-second session, held in Beijing, China, from September 17 to 21, 2018, considered a proposal for a partial revision of the Test Guidelines for Spinach (*Spinacea oleracea* L.) on the basis of documents TG/55/7 Rev. 5 and TWV/52/6 "Partial Revision of the Test Guidelines for Spinach" and proposed the following revisions to the Test Guidelines for Spinach (see document TWV/52/20 "Report", paragraphs 71 and 72):

   (a)   Revision of Characteristic 18 "Resistance to *Peronospora farinosa* f. sp. *spinaciae*":
      (i)   Addition of "Race Pfs: 17", including example varieties;
      (ii)   Revision of explanation Ad. 18 in Chapter 8.2 "Explanations for individual characteristics".
      (iii)   Addition of "Race Pfs: 17" to Chapter TQ 7.3 "Other information"

3.   The proposed changes are presented below in grey highlight and <u>underline</u> (insertion) and ~~strikethrough~~ (deletion).

Proposed change to Characteristic 18 "Resistance to *Peronospora farinosa* f. sp. *spinaciae*"

		English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
18. (+)	VG	**Resistance to *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**	**Résistance à *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**	**Resistenz gegen *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**	**Resistencia a *Peronospora farinosa* f. sp. *spinaciae* (Pfs)**		
		[18.1 to 18.10]	[18.1 à 18.10]	[18.1 bis 18.10]	[18.1 a 18.10]		
		-------------------------	-------------------------	-------------------------	-------------------------	----------------------------	--------
18.11		**Race Pfs: 12**	**Race Pfs: 12**	**Pathotyp Pfs: 12**	**Raza Pfs: 12**		
	QL	absent	absente	fehlend	ausente	Boeing, Campania	1
		present	présente	vorhanden	presente	Finch, Pigeon, Red Kitten, Zebu	9
		-------------------------	-------------------------	-------------------------	-------------------------	----------------------------	--------
18.12		**Race Pfs: 13**	**Race Pfs: 13**	**Pathotyp Pfs: 13**	**Raza Pfs: 13**		
	QL	absent	absente	fehlend	ausente	Campania	1
		present	présente	vorhanden	presente	Boeing, Lion	9
		-------------------------	-------------------------	-------------------------	-------------------------	----------------------------	--------
18.13		**Race Pfs: 14**	**Race Pfs: 14**	**Pathotyp Pfs: 14**	**Raza Pfs: 14**		
	QL	absent	absente	fehlend	ausente	Campania, Pigeon	1
		present	présente	vorhanden	presente	Califlay, Lion	9
		-------------------------	-------------------------	-------------------------	-------------------------	----------------------------	--------
18.14		**Race Pfs: 15**	**Race Pfs: 15**	**Pathotyp Pfs: 15**	**Raza Pfs: 15**		
	QL	absent	absente	fehlend	ausente	Caladonia	1
		present	présente	vorhanden	presente	Pigeon	9
		-------------------------	-------------------------	-------------------------	-------------------------	----------------------------	--------
18.15		**Race Pfs: 16**	**Race Pfs: 16**	**Pathotyp Pfs: 16**	**Raza Pfs: 16**		
	QL	absent	absente	fehlend	ausente	Meerkat	1
		present	présente	vorhanden	presente	Caladonia	9
		-------------------------	-------------------------	-------------------------	-------------------------	----------------------------	--------
<u>18.16</u>		<u>**Race Pfs: 17**</u>	<u>**Race Pfs: 17**</u>	<u>**Pathotyp Pfs: 17**</u>	<u>**Raza Pfs: 17**</u>		
	<u>QL</u>	<u>absent</u>	<u>absente</u>	<u>fehlend</u>	<u>ausente</u>	<u>Pigeon</u>	<u>1</u>
		<u>present</u>	<u>présente</u>	<u>vorhanden</u>	<u>presente</u>	<u>Hydrus</u>	<u>9</u>

Current explanation

Ad. 18: Resistance to *Peronospora farinosa* f. sp. *spinaciae*

<u>Maintenance of races</u>

Type of medium:	Living host plants, obtainable from:
	    Naktuinbouw
	    P.O. Box 40
	    NL-2370 AA Roelofarendsveen
	    Netherlands
	    www.naktuinbouw.com
	or plant material with spores stored at -20° C for a maximum of one year

<u>Execution of test</u>

Growth stage of plants:	First cotyledons/leaf, eleven-day-old plants
Temperature:	15°C during day/12°C during night
Light:	15 hours per day, after emergence
Growing method:	In soil in pots or trays in a glasshouse or growth chamber
Method of inoculation:	Sporulating leaves, taken from host plants that were infected seven days before, are thoroughly rinsed with sterile tap water (maximum 150 ml water per 224 plants). The spore suspension is filtered through cheesecloth and sprayed on test plants until the inoculum covers the leaves but does not run off. 150 ml of suspension is enough for up to 3 x 224 plants. Spore density should be 20,000 to 100,000 conidia/ml water. The spore suspension should be used fresh.
Remarks:	Spinach downy mildew is wind-borne. Sporulating plants should be kept in closed containers or isolated chambers to prevent any cross-contamination. Resistant controls are needed in each multiplication and in each test to ensure the race identity. Light and humidity conditions during seedling development and incubation are critical. Optimal humidity of approximately 80-90% RH allows plant growth and fungal growth; strong light inhibits spore germination and infection. The test should be carried out in wintertime with protection against direct sunshine. After inoculation, the plants should remain under plastic for three days. After this time, the plastic should be slightly raised during the daytime.

<u>Duration of test</u>

- Multiplication	harvest spores 7 days after inoculation
- Sowing to inoculation:	11 days
- Inoculation to reading:	10 days

<u>Number of plants tested</u>   at least 20 plants

Evaluation of infection:	Resistance is usually complete; sometimes necrotic spots are visible as a result of infection. Susceptible plants show varying degrees of sporulation. Sporulation is visible as a grey covering on leaves, starting on the more humid abaxial side.

Differential varieties to identify races

Races Pfs: 1-8 and 10-16 of *Peronospora farinosa* f. sp. *spinaciae* are defined with a standard set of "differential varieties" according to the following table:

Differential variety	Pfs: 1	Pfs: 2	Pfs: 3	Pfs: 4	Pfs: 5	Pfs: 6	Pfs: 7	Pfs: 8	Pfs: 10	Pfs: 11	Pfs: 12	Pfs: 13	Pfs: 14	Pfs: 15	Pfs: 16
Viroflay	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Resistoflay	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S
Califlay	R	S	R	S	R	S	S	R	S	R	R	S	R	S	R
Clermont	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S
Campania	R	R	R	R	R	S	R	S	S	R	S	S	S	R	R
Boeing	R	R	R	R	R	R	R	S	S	R	S	R	S	R	R
Lion	R	R	R	R	R	R	R	R	S	R	R	R	R	R	R
Lazio	R	R	R	R	R	R	R	R	R	S	S	S	S	R	S
Whale	R	R	R	R	R	R	R	R	S	R	R	S	R	S	R
Pigeon	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S
Caladonia	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R
Meerkat	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S

Legend: R= resistance present; S = resistance absent, susceptible

Proposed new explanation

<u>Maintenance of races</u>

Type of medium: Living host plants, obtainable from:
     Naktuinbouw
     P.O. Box 40
     NL-2370 AA Roelofarendsveen
     Netherlands
     www.naktuinbouw.com
or plant material with spores stored at -20° C for a maximum of one year

<u>Execution of test</u>

Growth stage of plants: First cotyledons/leaf, eleven-day-old plants

Temperature: 15°C during day/12°C during night

Light: 15 hours per day, after emergence

Growing method: In soil in pots or trays in a glasshouse or growth chamber

Method of inoculation: Sporulating leaves, taken from host plants that were infected seven days before, are thoroughly rinsed with sterile tap water (maximum 150 ml water per 224 plants). The spore suspension is filtered through cheesecloth and sprayed on test plants until the inoculum covers the leaves but does not run off. 150 ml of suspension is enough for up to 3 x 224 plants. Spore density should be 20,000 to 100,000 conidia/ml water. The spore suspension should be used fresh.

Remarks: Spinach downy mildew is wind-borne. Sporulating plants should be kept in closed containers or isolated chambers to prevent any cross-contamination. Resistant controls are needed in each multiplication and in each test to ensure the race identity.

Light and humidity conditions during seedling development and incubation are critical. Optimal humidity of approximately 80-90% RH allows plant growth and fungal growth; strong light inhibits spore germination and infection.

The test should be carried out in wintertime with protection against direct sunshine. After inoculation, the plants should remain under plastic for three days. After this time, the plastic should be slightly raised during the daytime.

<u>Duration of test</u>

- Multiplication   harvest spores 7 days after inoculation
- Sowing to inoculation: 11 days
- Inoculation to reading: 10 days

<u>Number of plants tested</u>   at least 20 plants

Evaluation of infection — Resistance is usually complete; sometimes necrotic spots are visible as a result of infection. Some varieties may have a slightly lower level of resistance. In the table this is indicated by (R), showing for example a slight tip sporulation.
Susceptible plants show varying degrees of sporulation. Sporulation is visible as a grey covering on leaves, starting on the more humid abaxial side.

Differential varieties to identify races
Disease resistance reactions of spinach downy mildew races on differentials of the International Working Group on *Peronospora* (IWGP)

Races Pfs: 1-8 and 10-16 17 of *Peronospora farinosa* f. sp. *spinaciae* are defined with a standard set of "differential varieties" differentials according to the following table:

Differentials and type isolates are available at Naktuinbouw:

Naktuinbouw
P.O. Box 40
NL-2370 AA Roelofarendsveen
Netherlands
www.naktuinbouw.com

Differential variety/ Near Isogenic Lines (NIL)	Race - Pfs:															
	1	2	3	4	5	6	7	8	10	11	12	13	14	15	16	17
Viroflay	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NIL 5	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S
NIL 3	R	S	R	S	R	S	S	R	S	R	R	S	R	S	R	S
NIL 4	R	R	R	R	S	S	S	S	S	S	S	S	S	R	S	S
NIL 6	R	S	R	R	R	S	R	S	S	R	S	(R)	S	R	R	S
NIL 1	R	R	R	R	R	R	R	S	S	R	S	R	S	R	R	S
Whale	R	R	R	(R)	R	(R)	(R)	R	(R)	R	R	S	R	(R)	R	S
Pigeon	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S
Caladonia	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S
Meerkat	R	R	R	R	R	R	R	R	R	R	R	R	R	R	S	(R)
Hydrus	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

Legend: S = susceptible, R or (R) = resistant

(table available under http://www.worldseed.org)

Proposal to add "Race Pfs: 17" to TQ 7.3 "Other information"

"7.3   Other information

[…]

"(b)   Resistance to pests and diseases (specify)

"(i)   Resistance to *Peronospora farinosa* f. sp. *spinaciae*

Race Pfs: 1	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 2	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 3	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 4	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 5	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 6	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 7	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 8	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 10	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 11	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 12	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 13	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 14	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 15	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 16	[ ] absent	[ ] present	[ ] not tested
Race Pfs: 17	[ ] absent	[ ] present	[ ] not tested

[…]"

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