

Technical Committee

TC/58/23

**Fifty-Eighth Session**  
**Geneva, October 24 and 25, 2022****Original:** English  
**Date:** October 5, 2022**PARTIAL REVISION OF THE TEST GUIDELINES FOR PEA***Document prepared by an expert from France**Disclaimer: this document does not represent UPOV policies or guidance*

1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Pea (document TG/7/10 Rev. 2).
2. The Technical Working Party for Vegetables (TWV), at its fifty-sixth session<sup>1</sup>, considered a proposal for a partial revision of the Test Guidelines for Pea (*Pisum sativum* L.) on the basis of documents TG/7/10 Rev. 2 and TWV/56/17 "Partial revision of the Test Guidelines for Pea" and proposed the following changes (see document TWV/56/22 "Report", paragraph 92):
  - (a) Revision of Characteristic 58 "Resistance to *Fusarium oxysporum* f. sp. *pisii* Race 1";
  - (b) Revision of explanation Ad. 58 "Resistance to *Fusarium oxysporum* f. sp. *pisii* Race 1" in Chapter 8.2 "Explanations for individual characteristics";
  - (c) Revision of Characteristic 59 "Resistance to *Erysiphe pisi* Syd.";
  - (d) Revision of explanation Ad. 59 "Resistance to *Erysiphe pisi* Syd." in Chapter 8.2 "Explanations for individual characteristics";
  - (e) Revision of explanation Ad. 60 "Resistance to *Ascochyta pisi*, Race C (Ascochyta Leaf and Pod Spot)" in Chapter 8.2 "Explanations for individual characteristics".
3. The proposed changes to are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

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<sup>1</sup> organized by electronic means, from April 18 to 22, 2022

Proposed revision of Characteristic 58 "Resistance to *Fusarium oxysporum* f. sp. *pisi*."

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
<b>58. VG (+)</b>	<b>Resistance to <i>Fusarium oxysporum</i> f. sp. <i>pisi</i></b> <b>Race 1</b>	<b>Résistance à <i>Fusarium oxysporum</i> f. sp. <i>pisi</i></b> <b>Race 1</b>	<b>Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>pisi</i></b> <b>Pathotyp 1</b>	<b>Resistencia a <i>Fusarium oxysporum</i> f. sp. <i>pisi</i></b> <b>Raza 1</b>		
<b>QL</b>	absent	absente	fehlend	ausente	<u>Aviron</u> , Bartavelle, <u>Curling</u> , <u>Digit</u>	1
	present	présente	vorhanden	presente	<u>Astronaute</u> , <u>Bingo</u> , <u>Foudre</u> , <u>Kristoff</u> , <u>Namrata</u> , New Era, Nina, <u>Roitelet</u>	9

Proposed revision of explanation Ad. 58 “Resistance to *Fusarium oxysporum* f. sp. *pisi* Race 1” in Chapter 8.2 “Explanations for individual characteristics”

Ad. 58: Resistance to *Fusarium oxysporum* f. sp. *pisi* Race 1 (Near wilt)

1.	Pathogen	<i>Fusarium oxysporum</i> f. sp. <i>pisi</i> (race 1)
2.	Quarantine status	No
3.	Host species	Pea – <i>Pisum sativum</i> L.
4.	Source of inoculum	GEVES <sup>2</sup> (FR), INIA <sup>3</sup> (ES) or SASA <sup>4</sup> (GB)
5.	Isolate	<i>Fusarium oxysporum</i> f. sp. <i>pisi</i> race 1 strain MATREF 04-02-01-01 (the test protocol has been validated with this isolate/race.) E.g. Reference strain validated in an inter laboratory test <sup>5</sup> : = MAT/REF 04-02-01-01 <sup>2</sup>
6.	Establishment isolate identity	genetically defined pea controls See ISF website <a href="https://www.worldseed.org/our-work/plant-health/differential-hosts/">https://www.worldseed.org/our-work/plant-health/differential-hosts/</a> Version July 2019

<b>Differentials host susceptible:</b>	M410, Bartavelle, Little Marvel
<b>resistant:</b>	New Era, Mini 93, Dark Skin Perfection, Vantage, WSU 23, New Season, WSU 31, 74SN5, Sundance II, Grant

Differential hosts	Race			
	Fop: 1*	Fop: 2	Fop: 5	Fop: 6
Little Marvel, M410*	S	S	S	S
Dark Skin Perfection, Vantage*	HR	S	S	S
Mini*	S	HR	S	S
New Era, Mini 93*	HR	HR	S	S
Sundance II*	HR	S	HR	S
Grant*	HR	S	S	HR
New Season	HR	HR*	S	HR
WSU 23*	HR	HR	HR	HR
WSU 28*	HR	S	HR	HR
WSU 31, 74SN5*	HR	HR	HR	HR

S = susceptible; HR = highly resistant; HR\* reaction may vary with isolate

\*differential hosts and isolates that are used by the seed sector

Courtesy of International Seed Federation.

7.	Establishment pathogenicity	Test on susceptible plants
8.	Multiplication inoculum	
8.1	Multiplication medium	Multiplication on agar medium: malt Agar or PDA for example

<sup>2</sup> [matref@geves.fr](mailto:matref@geves.fr)

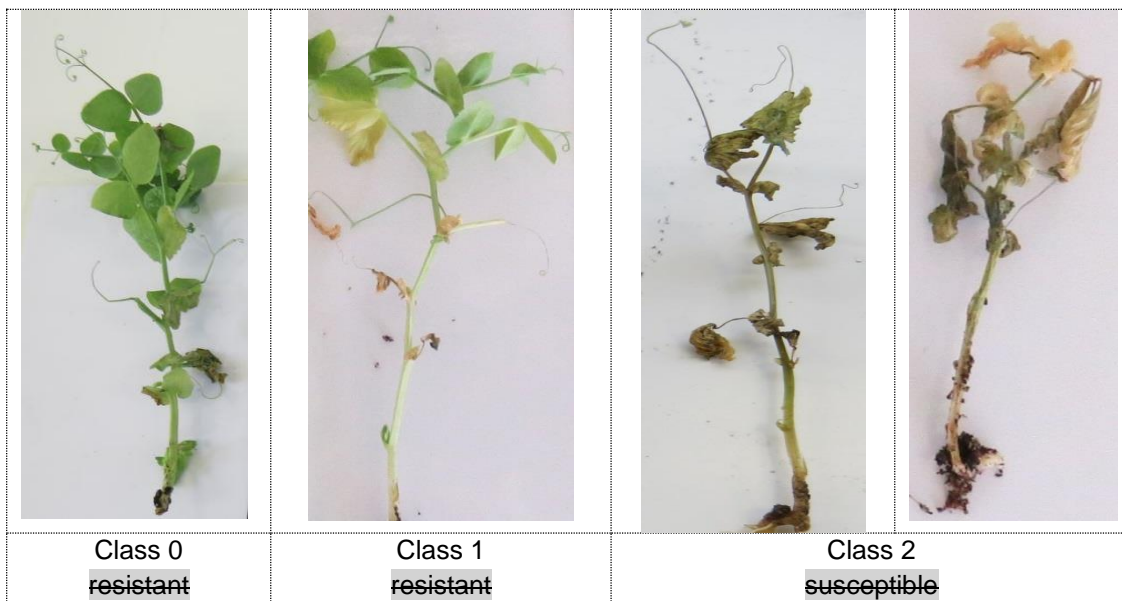
<sup>3</sup> [resistencias@inia.es](mailto:resistencias@inia.es)

<sup>4</sup> [Marian.McEwan@sasa.gov.scot](mailto:Marian.McEwan@sasa.gov.scot)

<sup>5</sup> Harmores 2 CPVO project: [https://cpvo.europa.eu/sites/default/files/documents/vem15\\_7\\_b\\_harmores\\_2\\_final\\_report.pdf](https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf)

8.4	Inoculation medium	Multiplication on agar medium: water for scraping agar plates. Multiplication on liquid medium: Potato Dextrose Broth, Kerrs broth or Czapek-Dox (3 to 7 days old aerated culture) for example.
8.6	Harvest of inoculum	see 10.1
8.7	Check of harvested inoculum	see 10.2
8.8	Shelf life/viability inoculum	<del>Between 4 and 8 hours, keep cool to prevent germination of spores. Viability of spores should be more than 3 years if stored at -20°C.</del> The spores can be stored more than 3 years at -20°C.
9.	Format of the test	
9.1	Number of plants per genotype	At least 20 inoculated plants and 5 non inoculated plants per variety per genotype to be able to judge growth reduction.
9.2	Number of replicates	-
9.3	Control varieties	Susceptible controls: Bartavelle Resistant controls: New Era and Nina
9.5	Test facility	Climate room or greenhouse.
9.6	Temperature	20-25°C
9.7	Light	12 hours or longer
9.9	Special measures	It is important to compare the inoculated plants with the <del>negative</del> non inoculated control plants of the same sample. This allows interpretation of symptoms of root rot, senescence or 'wilting' caused by the stress of having roots cut and not symptoms caused by <i>F. oxysporum</i> infection.
10.	Inoculation	
10.1	Preparation inoculum	<del>For agar plates, remove hyphen fragments by filtering solution through muslin.</del> Initial fungal growth on agar plates (Malt or PDA). This is then used as liquid medium inoculum after removing hyphal fragments by filtering solution through muslin cloth. For liquid medium, filter through muslin cloth to remove large hyphal fragments.
10.2	Quantification inoculum	10 <sup>6</sup> spores/ml
10.3	Plant stage at inoculation	seeds or 2 weeks old seedlings (2-3 nodes stage).
10.4	Inoculation method	<u>For seeds:</u> sowing in contaminated substrate (soil-based substrate), 750 ml of suspension of spores at 10 <sup>6</sup> sp/ml for 5 l of substrate. <u>For 2 weeks seedlings:</u> Sowing in a mix of vermiculite + soil or soil-based substrate Cut the apical 2/3 of the roots <del>with scissors</del> , dip the root of the seedling in the spores suspension for 1 to 5 minutes and transplant in clean soil based substrate in a new tray.
10.7	Final observations	28 days post-inoculation.
11.	Observations	
11.1	Method	Visual

11.2	Observation scale	<p><b>resistant:</b>  <b>Class 0:</b> No symptoms or equivalent to <b>non-inoculated negative control</b>, 1 or 2 <b>senesced</b> (wilted/dried) lower leaves and slight reduction in growth compared to <b>non-inoculated negative control</b> of same variety are acceptable.  <b>Class 1:</b> Range from a few chlorotic or wilted/dried/senesced leaves not present on, or more than on the <b>negative non-inoculated control</b>, up to many leaves with symptoms of senescence or wilting, some leaf drop, upper part of the plant still green and growing.</p> <p><b>susceptible:</b>  <b>Class 2:</b> Range from most of the plant wilted/dried or senesced but still alive, to plants brown and dead with stem collapsed.</p> <p><b>Classes 0 and 1 are generally resistant. Class 2 is generally susceptible.</b></p>
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Courtesy of GEVES-SNES in the framework of CPVO Harmores project.

		<p>Varieties with the same or higher level of resistance as New Era will be interpreted as resistant. Varieties with a lower level of resistance than New Era will be interpreted as susceptible. Nina will be highly resistant, Bartavelle will be highly susceptible. New Era expresses weak symptoms and variation can occur in these weak symptoms depending on the aggressivity of the test conditions.</p>
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11.3	Validation of test	<p><u>Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls (distribution of plants per symptoms classes, eventually completed by a disease index).</u></p> <p><u>New Era expresses weak symptoms and variations can occur in these weak symptoms depending on the aggressiveness of the test conditions.</u></p> <p><u>Susceptible: lower level of resistance than New Era (Bartavelle is highly susceptible)</u></p> <p><u>Resistant: same or higher level of resistance than New Era (Nina is highly resistant)</u></p>
12.	Interpretation of data in terms of UPOV characteristic states	
	absent [1]	Susceptible
	present [9]	Resistant
13.	Critical control points	<p>Each lab has to define the best method of inoculation in its lab depending on controls results.</p> <p>Inoculation by sowing in contaminated soil can in some cases lead to germination problems, <u>particularly if the humidity of the soil is too high during the test.</u> No conclusion can be done in this case, and the test should be repeated.</p>

Proposal to revise Characteristic 59 “Resistance to *Erysiphe pisi* Syd.”*Proposed new wording*

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
<b>59.</b>	<b>VG</b>	<b>Resistance to <i>Erysiphe pisi</i> Syd.</b>	<b>Résistance à <i>Erysiphe pisi</i> Syd.</b>	<b>Resistenz gegen <i>Erysiphe pisi</i> Syd.</b>	<b>Resistencia a <i>Erysiphe pisi</i> Syd.</b>	
<b>(+)</b>						
<b>QL</b>	absent	absente	fehlend	ausente	<u>Cabro, Aladin, Astronaute, Aviron, Cabree, Dexter, Ottoman</u>	1
	present	présente	vorhanden	presente	<u>Stratford, Alezan, Boogie, Ema, LG Amigo, Stratagem (JI2302), Sugar Bon, Vivaldi,</u>	9

Proposed revision of explanation Ad. 59 “Resistance to *Erysiphe pisi* Syd.” in Chapter 8.2 “Explanations for individual characteristics”































Ad. 59: Resistance to *Erysiphe pisi* Syd. (Powdery Mildew)

1.	Pathogen	<u>Powdery mildew – <i>Erysiphe pisi</i></u>
2.	Quarantine status	<u>No</u>
3.	Host species	<u>Pea – <i>Pisum sativum</i> L</u>
4.	Source of inoculum	<u>GEVES<sup>6</sup> (FR)</u>
5.	Isolate	<u><i>Erysiphe pisi</i></u> <u>e.g. Reference strain validated in an inter laboratory test<sup>7</sup></u> <u>isolate 2430</u> <u>=MAT/REF/ 04-17-01<sup>6</sup></u>
6.	Establishment isolate identity	<u>Validation by use specific EryF/EryR primers to validate the species of <i>Erysiphe</i> (use ITS primers from Attanayake et al, 2010<sup>8</sup>.)</u>
7.	Establishment pathogenicity	<u>use susceptible variety (e.g. Aladin, Cabree or Ottoman)</u>
8.	Multiplication inoculum	
8.1	Multiplication medium	<u>Living plant</u>
8.2	Multiplication variety	<u>See 7</u>
8.3	Plant stage at inoculation	<u>See 10.3</u>
8.4	Inoculation medium	
8.5	Inoculation method	<u>See 10.4</u>
8.6	Harvest of inoculum	<u>For spraying by washing off with demineralized water</u> <u>For dry sprinkling by detaching leaves of a susceptible host plant</u>
8.7	Check of harvested inoculum	<u>Visual check for presence of sporulation</u>
8.8	Shelf life/viability inoculum	<u>1-2 hours</u>
9.	Format of the test	
9.1	Number of plants per genotype	<u>20 plants</u>
9.2	Number of replicates	<u>-</u>
9.3	Control varieties	<u>Susceptible:</u> <u>For vegetable crops: Cabree</u> <u>For agricultural crops: Aladin, Ottoman</u> <u>Resistant:</u> <u>For vegetable crop: Ema, Sugar Bon, Vivaldi, Stratagem (JI2302).</u> <u>For agricultural crop: Alezan</u>
9.4	Test design	<u>Exclude non-inoculated control plants of the same sample as it is impossible to place them exactly in the same conditions (due to risk of contamination)</u>
9.5	Test facility	<u>green house or climatic room</u>
9.6	Temperature	<u>It is advised to perform the test at 20°C, but depending on laboratory conditions, test can be performed at temperature as high as 25°C. It is advised not to go below 18°C.</u>
9.7	Light	<u>at least 12h per day</u>
9.8	Season	
9.9	Special measures	
10.	Inoculation	
10.1	Preparation inoculum	<u>By spraying:</u> <u>Washing off from leaves by vigorous shaking in a closed container containing water. Sieve the suspension through muslin cloth.</u> <u>By sprinkling:</u> <u>Selection of leaves with strong sporulation.</u>

<sup>6</sup> GEVES; matref@geves.fr

<sup>7</sup> Harmores 2 CPVO project: [https://cpvo.europa.eu/sites/default/files/documents/vem15\\_7\\_b\\_harmores\\_2\\_final\\_report.pdf](https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf)



10.2	Quantification inoculum	<u>By spraying:</u> Counting spores; spores density should be $1 \times 10^5$ to $1 \times 10^6$ spores/mL <u>By sprinkling:</u> An estimated proportion of one diseased plant (with a strong sporulation) can be used to inoculate 10 plants.															
10.3	Plant stage at inoculation	<u>3-4 leaf stage</u>															
10.4	Inoculation method	<u>By spraying:</u> Spraying of the suspension of spores on leaves <u>By sprinkling of the spores from the susceptible control plants used for multiplication:</u> To detach the spores for inoculation, the multiplication control plants are shaken above the tray of tested plants.															
10.5	First observation																
10.6	Second observation																
10.7	Final observations	<u>Between 14-21 dpi, when sporulation is well expressed on the susceptible control.</u>															
11.	Observations																
11.1	Method	<u>Visual</u>															
11.2	Observation scale	<table border="1"> <tr> <td><u>Susceptible:</u> sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant)</td> <td></td> <td></td> <td></td> <td></td> </tr> <tr> <td><u>Resistant:</u> No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms</td> <td colspan="2"></td> <td colspan="2"></td> </tr> <tr> <td>Symptoms which should not be confused with <i>E. pisi</i>: senescence of older leaves, yellowing, discoloration of leaves and insect damages</td> <td> senescing</td> <td> yellowing</td> <td> discoloration</td> <td> insect damage</td> </tr> </table>	<u>Susceptible:</u> sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant)					<u>Resistant:</u> No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms					Symptoms which should not be confused with <i>E. pisi</i> : senescence of older leaves, yellowing, discoloration of leaves and insect damages	 senescing	 yellowing	 discoloration	 insect damage
<u>Susceptible:</u> sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant)																	
<u>Resistant:</u> No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms																	
Symptoms which should not be confused with <i>E. pisi</i> : senescence of older leaves, yellowing, discoloration of leaves and insect damages	 senescing	 yellowing	 discoloration	 insect damage													
<u>Courtesy of GEVES-SNES in the framework of CPVO Harmores project.</u>																	
11.3	Validation of test	<u>Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.</u> <u>Susceptible:</u> sporulation on leaves. These symptoms can be observed on stem and tendril (not always on the whole plant). <u>Resistant:</u> No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms															
11.4	Off-types	-															
12.	Interpretation of data in terms of UPOV characteristic states	<u>Absent (susceptible) [1]</u> <u>Present (resistant) [9]</u>															
13.	Critical control points	<u>Watering for plant growth on the substrate (no spraying) to avoid washing the spores off the surface of the leaves.</u> <u>It is not possible to revive frozen spores. This pathogen is an obligate biotroph and cannot survive outside a living plant.</u>															

Proposed revision of explanation Ad. 60 "Resistance to *Ascochyta pisi*, Race C (*Ascochyta* Leaf and Pod Spot)" in Chapter 8.2 "Explanations for individual characteristics"

Ad. 60: Resistance to *Ascochyta pisi*, Race C (*Ascochyta* Leaf and Pod Spot)

1.	Pathogen	<i>Ascochyta pisi</i>
2.	Quarantine status	No
3.	Host species	Pea – <i>Pisum sativum</i> L.
4.	Source of inoculum	GEVES <sup>9</sup> (FR) or SASA <sup>10</sup> (UK)
5.	Isolate	<i>Ascochyta pisi</i> race C strain 21A.13. The test protocol has been validated in a European CPVO co-funded project <sup>11</sup> with this isolate. E.g.: Reference strain validated in an inter-laboratory test <sup>12</sup> - strain 21A.13. = MAT/REF/ 04-17-01 <sup>9</sup>
6.	Establishment isolate identity	Genetically defined on Pea controls ( <i>Physiological races of A. pisi</i> and differentials, adapted from Gallais et Bannerot, 1992) see ISF website <a href="https://www.worldseed.org/our-work/plant-health/differential-hosts/">https://www.worldseed.org/our-work/plant-health/differential-hosts/</a> Version July 2019

Physiological race (Dr Hubbeling)	C
Strain	Tézier 21A.13
Gullivert	S
Rondo	R
Finale	R
Kelvedon Wonder	S
Dark Skin Perfection	S
Arabal, Cobri, Starcovert, Sucovert, Vitalis	S

R = resistant; S = susceptible

Differential hosts	Races	D	-	-	-	C	B	E
	Strains	N°1	Several isolates	N°4	N°14	Tézier* 21A.13	-	-
Arabal, Cobri*, Starcovert, Sucovert, Vitalis		S	S	S	S	S	S	S
Dark Skin Perfection*		S	S	S	S	S	HR	S
Kelvedon Wonder*		HR	S	S	S	S	HR	HR
Finale*		HR	HR	S	S	HR	-	-
Rondo*		HR	HR	S	S	HR	HR	S
Gullivert*		HR	HR	HR	HR	S	HR	HR

S = susceptible; HR = highly resistant

\*differential hosts and isolates that are used by the seed sector

Courtesy of International Seed Federation.

7.	Establishment pathogenicity	Test on susceptible plants
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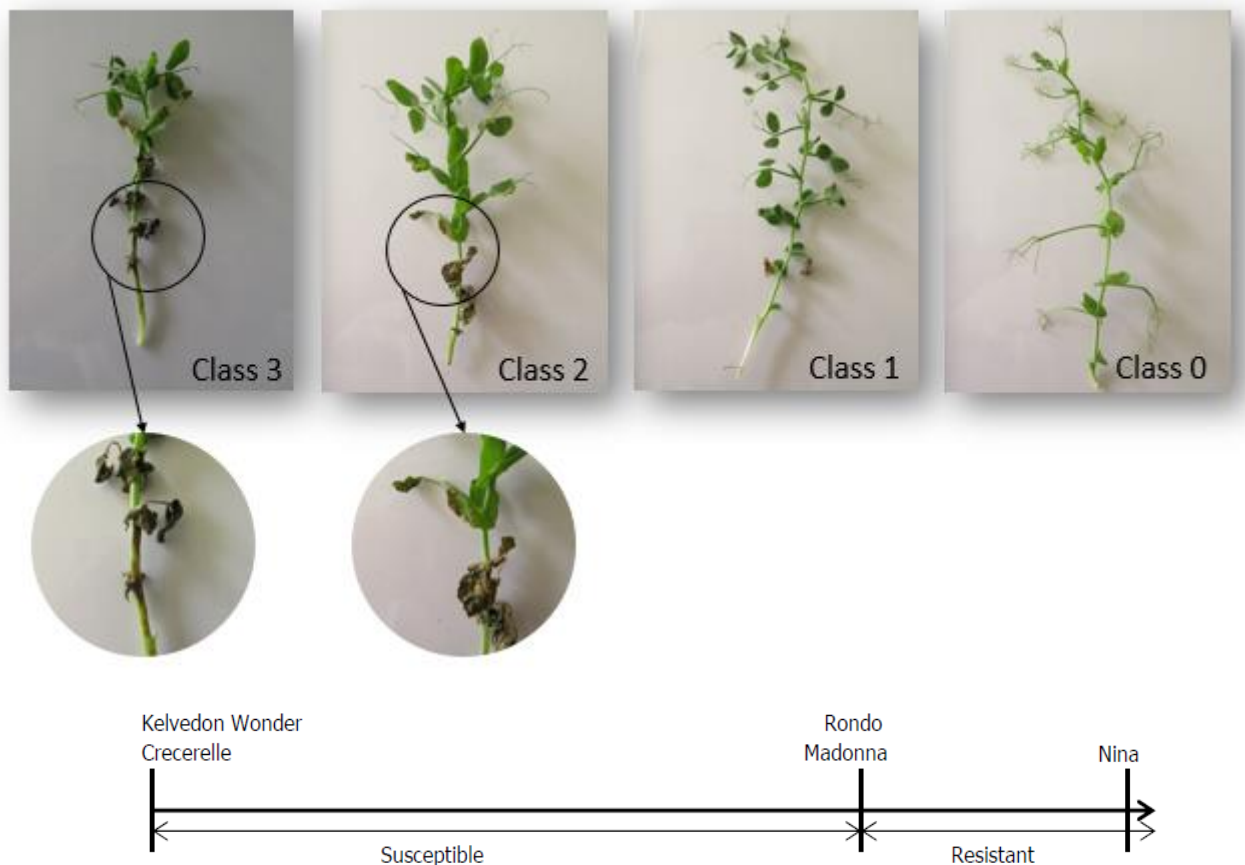
<sup>9</sup> [matref@geves.fr](mailto:matref@geves.fr)

<sup>10</sup> [Marian.McEwan@sasa.gov.scot](mailto:Marian.McEwan@sasa.gov.scot)

<sup>11</sup> Harmores 2 CPVO project: [https://cpvo.europa.eu/sites/default/files/documents/vem15\\_7\\_b\\_harmores\\_2\\_final\\_report.pdf](https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf)

<sup>12</sup> Harmores 2 CPVO project: [https://cpvo.europa.eu/sites/default/files/documents/vem15\\_7\\_b\\_harmores\\_2\\_final\\_report.pdf](https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf)

8.	Multiplication inoculum	
8.1	Multiplication medium	V8 agar or Mathur medium or Potato Dextrose Agar or a synthetic medium.
8.2	Multiplication variety	-
8.3	Plant stage at inoculation	-
8.4	Inoculation medium	water, option: add Tween 80 (wetting agent to aid dispersal of spores, e.g. 0.4%)
8.5	Inoculation method	-
8.6	Harvest of inoculum	See 10.1
8.7	Check of harvested inoculum	See 10.2
8.8	Shelf life/viability inoculum	<del>4/8h</del> Between 4 and 8 hours, keep cool to prevent spores' germination
9.	Format of the test	
9.1	Number of plants per genotype	At least 20 <u>inoculated</u> plants and 5 non-inoculated plants per variety.
9.2	Number of replicates	-
9.3	Control varieties	Susceptible controls: Crecerelle, Kelvedon Wonder Resistant controls: Madonna or Rondo (lower resistance thresholds) and Nina (higher resistance control)
9.4	Test design	-
9.5	Test facility	Climatic room or greenhouse.
9.6	Temperature	20°C
9.7	Light	12 hours or longer
9.8	Season	-
9.9	Special measures	High humidity or watering by spraying 2 or 3 times per day.
10.	Inoculation	
10.1	Preparation inoculum	Remove hyphenaal fragments by straining solution through muslin cloth.
10.2	Quantification inoculum	10 <sup>6</sup> spores/mL (to adapt depending on conditions of tests).
10.3	Plant stage at inoculation	2 weeks old seedlings (i.e. 2-3 node stage).
10.4	Inoculation method	Spraying on green leaves without surface moisture.
10.5	First observation	
10.6	Second observation	
10.7	Final observations	10-18 days post-inoculation.
11.	Observations	
11.1	Method	Visual
11.2	Observation scale	Class 0: no symptoms Class 1: few small superficial necrosis Class 2: bigger darker and deep necrosis Class 3: necrosis at each level of the plant or serious symptoms surrounding the stem  <del>Madonna, Rondo, and Nina are resistant controls. Varieties with the same or higher level of resistance than Madonna or Rondo will be interpreted as resistant.</del> Crecerelle or Kelvedon Wonder are susceptible controls. Varieties with a lower level of resistance than Nina as well as Madonna or Rondo will be interpreted as susceptible.



Courtesy of GEVES-SNES in the framework of CPVO Harmores project.

11.3	Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.
11.4	Off-types	
12.	Interpretation of data in terms of UPOV characteristic states	<p><b>Susceptible:</b> Crecerelle or Kelvedon Wonder are susceptible controls. Varieties with a lower level of resistance than Madonna or Rondo will be interpreted as susceptible.</p> <p><b>Resistant:</b> Madonna, Rondo, and Nina are resistant controls. Varieties with the same or higher level of resistance than Madonna or Rondo will be interpreted as resistant.</p> <p>absent ..... [1] susceptible (classes 2 and 3) present ..... [9] resistant (classes 0 and 1)</p>
13.	Critical control points	-