UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

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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-fifth session, organized by electronic means, from May 3 to 7, 2021, agreed that the Test Guidelines for Pea (*Pisum sativum* L.) (document TG/7/10 Rev. 2) be partially revised for characteristics 58 "Resistance to *Fusarium oxysporum* f. sp. *pisi*", 59 "Resistance to *Erysiphe pisi* Syd." and 60 "Resistance to *Ascochyta pisi* – Race C" (see document TWV/55/16 "Report", Annex III).

3. The following changes are proposed:

(a) Revision of Characteristic 58 "Resistance to *Fusarium oxysporum* f. sp. *pisi*";
(b) Revision of explanation Ad. 58 "Resistance to *Fusarium oxysporum* f. sp. *pisi*" 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".

4. The proposed changes to are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

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

Current wording

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
58. **(+)**	**VG**	**Resistance to *Fusarium oxysporum* f. sp. *pisi***	**Résistance *à Fusarium oxysporum* f. sp. *pisi***	**Resistenz gegen *Fusarium oxysporum* f. sp. *pisi***	**Resistencia a *Fusarium oxysporum* f. sp. *pisi***		
QL		absent	absente	fehlend	ausente	Bartavelle	1
		present	présente	vorhanden	presente	New Era, Nina	9

Proposed new wording

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

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

Current wording

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

1.	Pathogen	*Fusarium oxysporum* f. sp. *pisi* (race 1)
2.	Quarantine status	no
3.	Host species	Pea – *Pisum sativum* L.
4.	Source of inoculum	GEVES[1] (FR), INIA[2] (ES) or SASA[3] (GB)
5.	Isolate	*Fusarium oxysporum* f. sp. *pisi* race 1 strain MATREF 04-02-01-01 (the test protocol has been validated with this isolate/race)
6.	Establishment isolate identity	genetically defined pea controls (See ISF website: http://www.worldseed.org)

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

7.	Establishment pathogenicity	Test on susceptible plants
8.	Multiplication inoculum	
8.1	Multiplication medium	Multiplication on agar medium: malt Agar or PDA for example
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	Shelflife/viability inoculum	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.
9.	Format of the test	
9.1	Number of plants per genotype	At least 20 plants and 5 non inoculated plants per variety.
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 negative 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 cutted and not caused by *F. oxysporum* infection.
10.	Inoculation	
10.1	Preparation inoculum	For agar plates, remove hyphen fragments by filtering solution through muslin. For liquid medium, filter through muslin.
10.2	Quantification inoculum	10^6 spores/ml
10.3	Plant stage at inoculation	seeds or 2 weeks old seedlings (2-3 node stage).

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10.4	Inoculation method	For seeds: sowing in contaminated substrate (soil based substrate), 750 ml of suspension of spores at 10^6 sp/ml for 5 l of substrate. For 2 weeks seedlings: Sowing in a mix of vermiculite + soil or soil based substrate Cut the apical 2/3 of the roots with scissors, 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	susceptible: Class 2: Range from most of the plant wilted/dried but still alive, to plants brown and dead with stem collapsed. resistant: Class 0: No symptoms or equivalent to negative control, 1 or 2 wilted/dried lower leaves and slight reduction in growth compared to negative control of same variety are acceptable. Class 1: Range from a few chlorotic or wilted/dried leaves not present on, or more than on the negative control, up to many leaves with symptoms of senescence or wilting, some leaf drop, upper part of the plant still green and growing.

		 
Class 0 resistant	Class 1 resistant	Class 2 susceptible

		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 agressivity of the test conditions.
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls.
12.	Interpretation of data in terms of UPOV characteristic states	
	absent [1]	susceptible
	present [9]	resistant
13.	Critical control points	Each lab has to define the best method of inoculation in its lab depending on controls results. Inoculation by sowing in contaminated soil can in some cases lead to germination problems. No conclusion can be done in this case, and the test should be repeated.

Proposed new wording

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

1.	Pathogen	*Fusarium oxysporum* f. sp. *pisi* (race 1)
2.	Quarantine status	No
3.	Host species	Pea – *Pisum sativum* L.
4.	Source of inoculum	GEVES[4] (FR), INIA[5] (ES) or SASA[6] (GB)
5.	Isolate	*Fusarium oxysporum* f. sp. *pisi* 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[7]: = MAT/REF 04-02-01-01[4]
6.	Establishment isolate identity	genetically defined pea controls See ISF website https://www.worldseed.org/our-work/plant-health/differential-hosts/ Version July 2019

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

	Race			
Differential hosts	**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 Worldseed.org website.

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

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[7] Harmores 2 CPVO project: 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	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.~~ 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 ~~negative~~ 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 *F. oxysporum* infection.
10.	Inoculation	
10.1	Preparation inoculum	~~For agar plates, remove hyphen fragments by filtering solution through muslin.~~ 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. For liquid medium, filter through muslin to remove large hyphal fragments.
10.2	Quantification inoculum	10^6 spores/ml
10.3	Plant stage at inoculation	seeds or 2 weeks old seedlings (2-3 nodes stage).
10.4	Inoculation method	For seeds: sowing in contaminated substrate (soil-based substrate), 750 ml of suspension of spores at 10^6 sp/ml for 5 l of substrate. For 2 weeks seedlings: Sowing in a mix of vermiculite + soil or soil-based substrate Cut the apical 2/3 of the roots ~~with scissors~~, 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	~~resistant:~~ Class 0: No symptoms or equivalent to non-inoculated ~~negative~~ control, 1 or 2 senesced (wilted/dried) lower leaves and slight reduction in growth compared to non-inoculated ~~negative~~ control of same variety are acceptable. Class 1: Range from a few chlorotic or wilted/~~dried~~/senesced leaves not present on, or more than on the ~~negative~~ non-inoculated control, up to many leaves with symptoms of senescence or wilting, some leaf drop, upper part of the plant still green and growing. ~~susceptible~~. Class 2: Range from most of the plant wilted~~/dried~~ or senesced but still alive, to plants brown and dead with stem collapsed. Classes 0 and 1 are generally resistant. Class 2 is generally susceptible. General remark: 1 or 2 senescent (wilted/dried) lower leaves and slight reduction in growth compared to non-inoculated control plants of same variety are acceptable.

Class 0
~~resistant~~

Class 1
~~resistant~~

Class 2
~~susceptible~~

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

		~~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 agressivity of the test conditions.~~

11.3	Validation of test	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). New Era expresses weak symptoms and variations can occur in these weak symptoms depending on the aggressivity of the test conditions. Susceptible: lower level of resistance than New Era (Bartavelle is highly susceptible) Resistant: same or higher level of resistance than New Era (Nina is highly resistant)
12.	Interpretation of data in terms of UPOV characteristic states	
	absent [1]	Susceptible
	present [9]	Resistant
13.	Critical control points	Each lab has to define the best method of inoculation in its lab depending on controls results. Inoculation by sowing in contaminated soil can in some cases lead to germination problems, particularly if the humidity of the soil is too high during the test. No conclusion can be done in this case, and the test should be repeated.

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

Current wording

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
59. **(+)**	**VG**	**Resistance to *Erysiphe pisi* Syd.**	**Résistance à *Erysiphe pisi* Syd.**	**Resistenz gegen *Erysiphe pisi* Syd.**	**Resistencia a *Erysiphe pisi* Syd.**		
QL		absent	absente	fehlend	ausente	Cabro	1
		present	présente	vorhanden	presente	Stratford, Vivaldi	9

Proposed new wording

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

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

Current wording

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

Resistant and Susceptible varieties
Cabro (susceptible = resistance absent (1))
Stratford, Vivaldi (resistant = resistance present (9))

Isolates and isolate identity
No isolates are maintained as infection is natural. There are no known races.

Genetic background
Two recessive genes confer resistance: er1 and er2

er1 er2 = resistant
Er1 Er2 = susceptible

Er1 er2 = susceptible
er1 Er2 = susceptible

Assessment of disease
Infected foliage surfaces are white and powdery. Tissue beneath the infected areas may turn purplish followed by the production of black fruiting structures. Badly infected tissue remains soft and fails to dry out naturally.

In resistant plants, infection is absent or localized in very small patches (pustules). Infestation may overtake resistant plants during senescence.

Proposed new wording

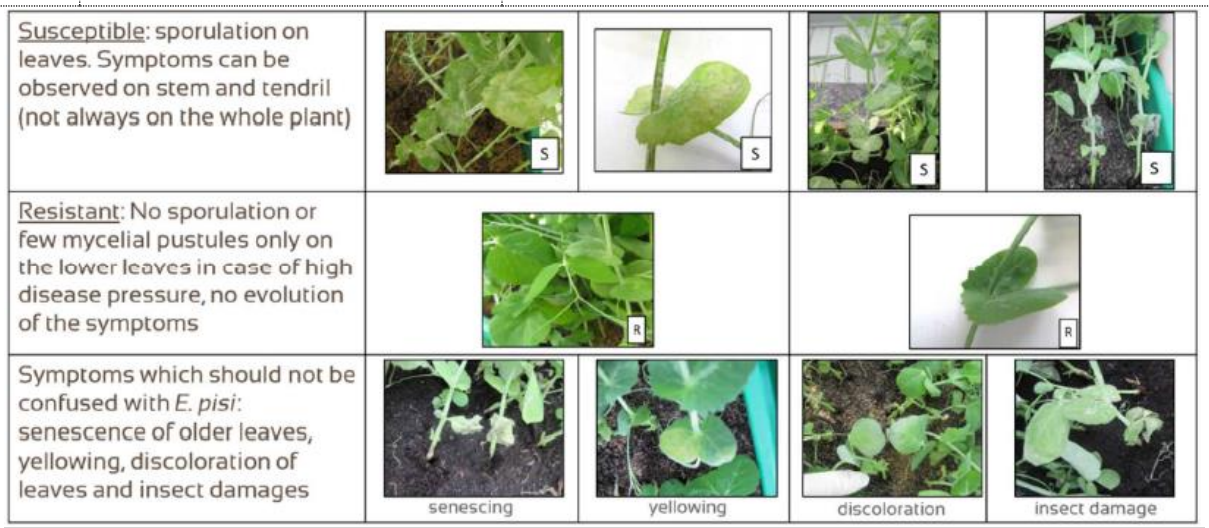
Ad. 59: Resistance to *Erysiphe pisi* Syd. ~~(Powdery Mildew)~~

1.	Pathogen	Powdery mildew – *Erysiphe pisi*
2.	Quarantine status	No
3.	Host species	Pea – *Pisum sativum* L
4.	Source of inoculum	GEVES[8] (FR)
5.	Isolate	*Erysiphe pisi* e.g. Reference strain validated in an inter laboratory test[9] isolate 2430 =MAT/REF/ 04-17-01[8]
6.	Establishment isolate identity	Validation by use specific EryF/EryR primers to validate the species of *Erysiphe* (use ITS primers from Attanayake et al, 2010[10].)
7.	Establishment pathogenicity	use susceptible variety (e.g. Aladin, Cabree or Ottoman)
8.	Multiplication inoculum	
8.1	Multiplication medium	Living plant
8.2	Multiplication variety	See 7
8.3	Plant stage at inoculation	See 10.3
8.4	Inoculation medium	
8.5	Inoculation method	See 10.4
8.6	Harvest of inoculum	For spraying by washing off with demineralized water For dry sprinkling by detaching leaves of a susceptible host plant
8.7	Check of harvested inoculum	Visual check for presence of sporulation
8.8	Shelf life/viability inoculum	1-2 hours
9.	Format of the test	
9.1	Number of plants per genotype	20 plants
9.2	Number of replicates	-
9.3	Control varieties	Susceptible: For vegetable crops: Cabree For agricultural crops: Aladin, Ottoman Resistant: For vegetable crop: Ema, Sugar Bon, Vivaldi, Stratagem (JI2302), For agricultural crop: Alezan
9.4	Test design	Exclude non-inoculated control plants of the same sample as it is impossible to place them exactly the same conditions (due to risk of contamination)
9.5	Test facility	green house or climatic room
9.6	Temperature	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. In some conditions it has been observed that increasing the day temperature up to 27°C allowed a good sporulation on the susceptible control or multiplication variety.
9.7	Light	at least 12h per day
9.8	Season	
9.9	Special measures	
10.	Inoculation	
10.1	Preparation inoculum	By spraying: Washing off from leaves by vigorous shaking in a closed container containing water. Sieve the suspension through muslin cloth. By sprinkling: Selection of leaves with strong sporulation.

[8] GEVES; matref@geves.fr

[9] Harmores 2 CPVO project: https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf

10.2	Quantification inoculum	By spraying: Counting spores; spores density should be 1x105 to 1x106 spores/mL By sprinkling: An estimated proportion of one diseased plant (with a strong sporulation) can be used to inoculate 10 plants.
10.3	Plant stage at inoculation	3-4 leaf stage
10.4	Inoculation method	By spraying: Spraying of the suspension of spores on leaves By sprinkling of the spores from the susceptible control plants used for multiplication: 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	Between 14-21 dpi, when sporulation is well expressed on the susceptible control.
11.	Observations	
11.1	Method	Visual
11.2	Observation scale	



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. Susceptible: sporulation on leaves. These symptoms can be observed on stem and tendril (not always on the whole plant). Resistant: 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	Absent (susceptible) [1] Present (resistant) [9]
13.	Critical control points	Watering for plant growth on the substrate (no spraying) to avoid washing the spores off the surface of the leaves. It is not possible to revive frozen spores. This pathogen is an obligate biotroph and cannot survive outside a living plant.

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”

Current wording

Ad. 60: Resistance to *Ascochyta pisi*, Race C

1.	Pathogen	*Ascochyta pisi*
2.	Quarantine status	no
3.	Host species	Pea – *Pisum sativum* L.
4.	Source of inoculum	GEVES[11] (FR) or SASA[12] (GB)
5.	Isolate	*Ascochyta pisi* race C strain 21A.13. (the test protocol has been validated with this isolate)[13].
6.	Establishment isolate identity	genetically defined pea controls (Physiological races of *A. pisi* and differentials, adapted from Gallais et Bannerot, 1992)

Physiological race (Dr Hubbeling) Strain	C 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

7.	Establishment pathogenicity	test on susceptible plants
8.	Multiplication inoculum	
8.1	Multiplication medium	V8 agar or Mathur medium or Potato Dextrose Agar or a synthetic medium.
8.4	Inoculation medium	water, option: add Tween 80 (wetting agent to aid dispersal of spores, e.g. 0.4%)
8.6	Harvest of inoculum	see 10.1
8.7	Check of harvested inoculum	see 10.2
8.8	Shelflife/viability inoculum	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 plants and 5 non inoculated plants per variety
9.2	Number of replicates	-
9.3	Control varieties	
	Susceptible	Crecerelle, Kelvedon Wonder
	Resistant	Nina and Madonna or Rondo
9.4	Test design	-
9.5	Test facility	climate 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 hyphen fragments by straining solution through muslin

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[13] Harmores 2 CPVO project

10.2	Quantification inoculum	10^6 spores/mL (to adapt depending 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 on all parts of the plant or serious symptoms surrounding the stem Madonna, Nina and Rondo will be resistant controls; varieties with same level of resistance as Madonna/Rondo and/or Nina will be interpreted as resistant. Crecerelle and Kelvedon Wonder will be susceptible controls, varieties with a lower level of resistance than Nina as well as Madonna/Rondo will be interpreted as susceptible.

Class 0:

Class 1:

Class 2: 	Details on Class 2: 
Class 3 : 	Details on Class 3 

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	
	absent [1] present [9]	susceptible (classes 2 and 3) resistant (classes 0 and 1)
13.	Critical control points	-

Proposed new wording

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

1.	Pathogen	*Ascochyta pisi*
2.	Quarantine status	No
3.	Host species	Pea – *Pisum sativum* L.
4.	Source of inoculum	GEVES[14] (FR) or SASA[15] (UK)
5.	Isolate	*Ascochyta pisi* race C ~~strain 21A.13.~~ ~~The test protocol has been validated in a European CPVO co-funded project[16] with this isolate.~~ E.g.: Reference strain validated in an inter-laboratory test[17] - strain 21A.13. = MAT/REF/ 04-17-01[14]
6.	Establishment isolate identity	Genetically defined on Pea controls ~~(Physiological races of *A. pisi* and differentials, adapted from Gallais et Bannerot, 1992)~~ see ISF website https://www.worldseed.org/our-work/plant-health/differential-hosts/ Version July 2019

~~Physiological race (Dr Hubbeling)~~ ~~Strain~~	~~C~~ ~~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 Worldseed.org website.

7.	Establishment pathogenicity	Test on susceptible plants
8.	Multiplication inoculum	
8.1	Multiplication medium	V8 agar or Mathur medium or Potato Dextrose Agar or a synthetic medium.

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[16] ~~Harmores 2 CPVO project: (https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf~~

[17] Harmores 2 CPVO project: https://cpvo.europa.eu/sites/default/files/documents/vem15_7_b_harmores_2_final_report.pdf

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	~~4/8h~~ 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 inoculated 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 hyph~~en~~al fragments by straining solution through muslin.
10.2	Quantification inoculum	10^6 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 ~~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.~~ ~~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	Susceptible: Crecerelle or Kelvedon Wonder are susceptible controls. Varieties with a lower level of resistance than Madonna or Rondo will be interpreted as susceptible. Resistant: 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. absent ..... [1] susceptible ~~(classes 2 and 3)~~ present ..... [9] resistant ~~(classes 0 and 1)~~
13.	Critical control points	-

[End of document]