

# Technical Working Party for Vegetables

TWV/56/18

## Fifty-Sixth Session

Virtual meeting, April 18 to 22, 2022

Original: English

Date: March 17, 2022

## PARTIAL REVISION OF THE TEST GUIDELINES FOR MELON

*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 Melon (document TG/104/5 Rev. 2).
2. The Technical Working Party for Vegetables (TWV), at its fifty-fifth session, hosted by Turkey and organized by electronic means, from May 3 to 7, 2022, agreed that the Test Guidelines for Melon (*Cucumis melo* L.) (document TG/104/5 Rev. 2) be partially revised for Characteristics 69 “Resistance to *Fusarium oxysporum* f. sp. *melonis*” and 70 “Resistance to *Podosphaera xanthii* (*Sphaerotheca fuliginea*) (Powdery mildew)” (see document TWV/54/9 “Report”, Annex III).
3. The following changes are proposed:
  - (a) Revision of Characteristics 69.1 to 69.4 “Resistances to *Fusarium oxysporum* f. sp. *melonis* (Fom) - races 0, 1, 2, and 1.2”;
  - (b) Revision of explanation Ad. 69 “Resistances to *Fusarium oxysporum* f. sp. *melonis* (Fom) - races 0, 1, 2, and 1.2” in Chapter 8.2 “Explanations for individual characteristics”;
  - (c) Revision of Characteristics 70.1 to 70.5 “Resistances to *Podosphaera xanthii* (Px) - races 1, 2, 3, 5, 3.5”;
  - (d) Revision of explanation Ads. 70.1 to 70.3, 71 “Resistances to *Podosphaera xanthii* (Px), Resistance to *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*), race 1 (Powdery mildew) Gc (Ec)” 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 Characteristics 69.1 to 69.4 “Resistances to *Fusarium oxysporum* f. sp. *melonis* (Fom) - races 0, 1, 2, and 1.2”

*Current wording*

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
<b>69. VG</b>	<b>Resistance to <i>Fusarium oxysporum</i> f. sp. <i>melonis</i></b>	<b>Résistance à <i>Fusarium oxysporum</i> f. sp. <i>melonis</i></b>	<b>Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>Melonis</i></b>	<b>Resistencia al <i>Fusarium oxysporum</i> f. sp. <i>melonis</i></b>		
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<b>69.1 (*) (+)</b>	<b>Race 0</b>	<b>Race 0</b>	<b>Pathotyp 0</b>	<b>Raza 0</b>		
<b>QL</b>	absent	absente	fehlend	ausente	Jaune Canari 2	1
	present	présente	vorhanden	presente	Jador, Védreantais	9
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<b>69.2 (*) (+)</b>	<b>Race 1</b>	<b>Race 1</b>	<b>Pathotyp 1</b>	<b>Raza 1</b>		
<b>QL</b>	absent	absente	fehlend	ausente	Jaune Canari 2, Védreantais	1
	present	présente	vorhanden	presente	Arapaho, Jador, Rubbens	9
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<b>69.3 (*) (+)</b>	<b>Race 2</b>	<b>Race 2</b>	<b>Pathotyp 2</b>	<b>Raza 2</b>		
<b>QL</b>	absent	absente	fehlend	ausente	Arapaho, Jaune Canari 2, Rubbens	1
	present	présente	vorhanden	presente	Anasta, Cléo, Jador, Védreantais	9
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<b>69.4 (*) (+)</b>	<b>Race 1.2</b>	<b>Race 1.2</b>	<b>Pathotyp 1.2</b>	<b>Raza 1.2</b>		
<b>QN</b>	susceptible	sensible	anfällig	susceptible	Jaune Canari 2, Védreantais, Virgos	1
	moderately resistant	moyennement résistant	mäßig resistent	moderadamente resistente	Lunasol	2
	highly resistant	hautement résistant	hochresistent	altamente resistente	Dinero, Isabelle	3

## Proposed new wording

	English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
69. A	VG	Resistance to <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> (Fom)	Résistance à <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> (Fom)	Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> (Fom)	Resistencia al <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> (Fom)	
69.1		Race 0 (Fom: 0)	Race 0	Pathotyp 0	Raza 0	
(*)						
(+)						
QL	absent	absente	fehlend	ausente	Jaune Canari 2 Atos, Charentais T	1
	present	présente	vorhanden	presente	Cadence, Charentais Fom-2, Dibango, Jador, Jubilo, Karakal, Védtrantais	9
69.2		Race 1 (Fom: 1)	Race 1	Pathotyp 1	Raza 1	
(*)						
(+)						
QL	absent	absente	fehlend	ausente	Jaune Canari 2 Atos, Charentais T, Védtrantais	1
	present	présente	vorhanden	presente	Arapaho, Jador, Rubbens Cadence, Charentais Fom-2, Dibango, Jubilo, Karakal	9
69.3		Race 2 (Fom: 2)	Race 2	Pathotyp 2	Raza 2	
(*)						
(+)						
QL	absent	absente	fehlend	ausente	Arapaho, Jaune Canari 2, Rubbens Atos, Dibango, Marianna	1
	present	présente	vorhanden	presente	Anasta, Cléo, Jador, Cadence, Charentais Fom-1, Jubilo, Karakal, Védtrantais	9
69. B	VG	Intermediate resistance to <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> Race 1.2 (Fom: 1.2)	Resistance modérée à <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> Race 1.2 (Fom: 1.2)	Mäßige Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> Pathotyp 1.2 (Fom: 1.2)	Resistencia moderada a <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> Raza 1.2 (Fom: 1.2)	
(+)						
QN	absent	absente	fehlend	ausente	Graffio, Prity, Virgos	1
	present	présente	vorhanden	presente	Isabelle, Kyriel, Lunasol, Meliance, Piboule	9

Proposed revision of explanation Ad. 69 “Resistances to *Fusarium oxysporum* f. sp. *melonis* (Fom) - races 0, 1, 2, and 1.2” in Chapter 8.2 “Explanations for individual characteristics”

*Current wording*

Ads. 69.1 - 69.3: Resistance to *Fusarium oxysporum* f. sp. *melonis*, races 0, 1 and 2 (Fom)

1.	Pathogen	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i>
2.	Quarantine status	no
3.	Host species	<i>Cucumis melo</i>
4.	Source of inoculum	GEVES (FR), Naktuinbouw (NL)
5.	Isolate	Fom: 0, Fom: 1, Fom: 2
6.	Establishment isolate identity	use differential varieties:

	Gene	Race 0	Race 1	Race 2
Charentais T		S	S	S
Védrantais	<i>Fom-1</i>	R	S	R
Charentais Fom-2	<i>Fom-2</i>	R	R	S
Isabelle, Jador		R	R	R

7.	Establishment pathogenicity	use susceptible melon varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	on agar medium – e.g. Potato Dextrose Agar
8.2	Multiplication variety	-
8.3	Plant stage at inoculation	-
8.4	Inoculation medium	on liquid medium
8.5	Inoculation method	-
8.6	Harvest of inoculum	-
8.7	Check of harvested inoculum	-
8.8	Shelflife/viability inoculum	-
9.	Format of the test	
9.1	Number of plants per genotype	at least 20
9.2	Number of replicates	e.g. 3
9.3	Control varieties	Jaune Canari 2 (susceptible) Védrantais, Arapaho, Rubbens, Anasta, Cleo (resistant, depending on the considered race)

	Gene	Race 0	Race 1	Race 2
Jaune Canari 2		S	S	S
Védrantais	<i>Fom-1</i>	R	S	R
Arapaho, Rubbens	<i>Fom-2</i>	R	R	S
Anasta, Cleo		R	R	R

9.4	Test design	-
9.5	Test facility	glasshouse or climatic room
9.6	Temperature	18-25°C
9.7	Light	12h
9.8	Season	all seasons
9.9	Special measures	optional: shading (no direct sunlight during 12 h after inoculation)
10.	Inoculation	
10.1	Preparation inoculum	aerated culture 7-10 days, eg. Czapek Dox broth some isolates need filtration or centrifugation resuspend the pelleted spores in demineralized water
10.2	Quantification inoculum	spore count; adjust to 10 <sup>6</sup> -10 <sup>7</sup> per mL
10.3	Plant stage at inoculation	cotyledon expanded

10.4	Inoculation method	soaking of the root system in a suspension of liquid medium of fungus at least 30 sec - 5 min
10.5	First observation	7 days post inoculation
10.6	Second observation	14 -20 days post inoculation
10.7	Final observations	20 days post inoculation
11.	Observations	
11.1	Method	visual, comparative
11.2	Observation scale	
	[1] absent	Growth retardation in combination with yellowing or wilting cotyledons (useful for judging the severity of the attack), possible internal vessel browning, death of plant.
	[9] present	no symptoms
11.3	Validation of test	on standards
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	QL
13.	Critical control points	For Race 1.2 the modified protocol on the next page should be used.

Ad. 69.4: Resistance to *Fusarium oxysporum* f. sp. *melonis*, race 1.2 (Fom)

1.	Pathogen	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i>
2.	Quarantine status	no
3.	Host species	<i>Cucumis melo</i>
4.	Source of inoculum	GEVES (FR), Naktuinbouw (NL)
5.	Isolate	Fom: 1.2 (moderately aggressive): TST strain
6.	Establishment isolate identity	use differential varieties: Védrantais, Virgos (susceptible) Lunasol (moderately resistant) Dinero, Isabelle (highly resistant)
7.	Establishment pathogenicity	use susceptible melon varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	on agar medium e.g. Potato Dextrose Agar
8.2	Multiplication variety	-
8.3	Plant stage at inoculation	-
8.4	Inoculation medium	on liquid medium
8.5	Inoculation method	-
8.6	Harvest of inoculum	-
8.7	Check of harvested inoculum	-
8.8	Shelflife/viability inoculum	-
9.	Format of the test	
9.1	Number of plants per genotype	at least 30
9.2	Number of replicates	e.g. 3
9.3	Control varieties	
	[1] susceptible	Védrantais, Virgos
	[2] moderately resistant	Lunasol (the lowest accepted level)
	[3] highly resistant	Dinero, Isabelle, Jador
9.4	Test design	-
9.5	Test facility	glasshouse or climatic room
9.6	Temperature	18-25°C

9.7	Light	at least 12h
9.8	Season	All seasons in a climatic room / in a greenhouse: be aware of the strong environmental effect: winter could be too severe and summer could be too mild.
9.9	Special measures	optional shading (no direct sunlight during 12 h after inoculation)
10.	Inoculation	
10.1	Preparation inoculum	aerated culture 7-10 d old – e.g.: Czapek Dox broth
10.2	Quantification inoculum	spore count; adjust to $2 \cdot 10^4$ - $10^5$ per ml
10.3	Plant stage at inoculation	cotyledons expanded
10.4	Inoculation method	soaking of the trays in spore suspension; 700 ml for a tray with 25 - 30 plants, plants are not uprooted
10.5	First observation	7 - 14 days post inoculation
10.6	Second observation	14 - 21 days post inoculation
10.7	Final observations	21- 28 days post inoculation
11.	Observations	
11.1	Method	visual, comparative
11.2	Observation scale	symptoms:
	[1] susceptible	Védrañtais: growth retardation, yellow cotyledons, drying, possible internal vessel browning, death of the plant
	[2] moderately resistant	Symptoms may be present, but the level of expression must be distinctly lower than the susceptible control variety. = the lowest level of resistance is defined by the behavior of Lunasol
	[3] highly resistant	Symptoms may be present, but the level of expression must be lower than the moderately control variety Lunasol.
11.3	Validation of test	on standards; Lunasol is intermediate and will show a percentage of diseased plants (quantitative evaluation)
11.4	Off-types	calibrate with Lunasol
12.	Interpretation of data in terms of UPOV characteristic states	QN
13.	Critical control points	A moderately aggressive type of Fom: 1.2 should be used as this is likely to show the difference between the presence and absence of resistance most clearly. There are two types of <i>Fusarium oxysporum</i> f. sp. <i>melonis</i> , Fom:1.2, viz. Fom: 1.2y which is a yellowing type with yellowing symptoms on leaves and another type and Fom: 1.2w which is a wilt type with wilting symptoms on leaves.

## Proposed new wording

Ads. 69 A: 69.1 - 69.3: Resistance to *Fusarium oxysporum* f. sp. *melonis*, races 0, 1 and 2 (Fom: 0, Fom: 1, Fom: 2)

1.	Pathogen	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i> <u>races 0, 1, and 2</u>
2.	Quarantine status	No
3.	Host species	<u>Melon</u> - <i>Cucumis melo</i>
4.	Source of inoculum	GEVES (FR) <sup>1</sup> <u>for races 0 and 1</u> Naktuinbouw (NL) <u>for race 2</u>
5.	Isolate	<u>Fom: 0, Fom: 1, Fom: 2</u> e.g. Reference strain validated in an inter-laboratory test <sup>2</sup> <u>Fom:0</u> - <u>Strain MLZ</u> = MAT/REF/04-07-01-03-02 <sup>1</sup>  <u>Fom: 1</u> - <u>Strain FOM 26</u> = MAT/REF/04-07-01-01 <sup>1</sup>  <u>Fom: 2</u> - <u>Strain F185</u>
6.	Establishment isolate identity	<u>use differential varieties:</u> Test on differential hosts (potentially including Durango, see 13.). The most recent table is available through ISF at <a href="https://www.worldseed.org/our-work/plant-health/differential-hosts/">https://www.worldseed.org/our-work/plant-health/differential-hosts/</a> <u>Situation July 2019</u>

Differential host	Gene present	Fom: 0*	Fom: 1*	Fom: 2*	Fom: 1.2*
Charantais T*	-	S	S	S	S
Védrantais*, Doublon*	<i>Fom-1</i>	HR	S	HR	S
Charantais Fom-2*, CM17187*	<i>Fom-2</i>	HR	HR	S	S
Isabelle*	<i>Polygenic?</i>	HR	HR	HR	IR

S = susceptible; HR = highly resistant; IR = intermediate

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

Courtesy of Worldseed.org website

	<u>Gene</u>	<u>Race 0</u>	<u>Race 1</u>	<u>Race 2</u>
<u>Charentais T</u>		<u>S</u>	<u>S</u>	<u>S</u>
<u>Védrantais</u>	<u>Fom-1</u>	<u>R</u>	<u>S</u>	<u>R</u>
<u>Charentais Fom-2</u>	<u>Fom-2</u>	<u>R</u>	<u>R</u>	<u>S</u>
<u>Isabelle, Jador</u>		<u>R</u>	<u>R</u>	<u>R</u>

7.	Establishment pathogenicity	use susceptible melon varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	on agar medium – e.g., Potato Dextrose Agar, <u>Malt agar at 20°C to 25°C</u>
8.2	Multiplication variety	-
8.3	Plant stage at inoculation	-
8.4	<u>Inoculation medium</u>	<u>on liquid medium</u>
8.5	Inoculation method	-

<sup>1</sup> [matref@geves.fr](mailto:matref@geves.fr)

<sup>2</sup> Harmores 3 CPVO project ([https://cpvo.europa.eu/sites/default/files/documents/report\\_harmores\\_3\\_final\\_meeting\\_v0\\_0.pdf](https://cpvo.europa.eu/sites/default/files/documents/report_harmores_3_final_meeting_v0_0.pdf))



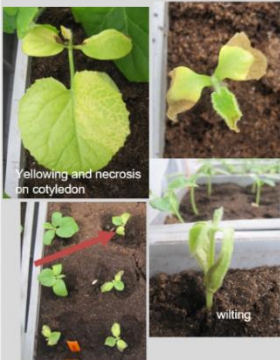
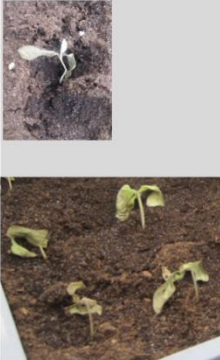
8.6	Harvest of inoculum	7-10 day-old culture
8.7	Check of harvested inoculum	-
8.8	Shelf life /viability inoculum	Between 4 to 8 h, keep cool to prevent spore germination
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 at least 30 plants, it is important to have at least 5 non-inoculated plants per genotype to be able to judge growth reduction
9.2	Number of replicates	At least e.g. 3 replicates
9.3	Control varieties	Jaune Canari 2 (susceptible) Védrantais, Arapaho, Rubbens, Anasta, Cleo (resistant, depending on the considered race)

	Gene	Race 0	Race 1	Race 2
Jaune Canari 2		S	S	S
Védrantais	Fom-1	R	S	R
Arapaho, Rubbens	Fom-2	R	R	S
Anasta, Cleo		R	R	R

9.3.1	Control varieties for the test with race 0	Susceptible: Charentais T Resistant: Védrantais, Charentais Fom-2
9.3.2	Control varieties for the test with race 1	Susceptible: Charentais T, Védrantais Resistant: Charentais Fom-2
9.3.3	Control varieties for the test with race 2	Susceptible: Marianna (less susceptible than Charentais Fom-2, Charentais T) Resistant: Charentais Fom-1
9.4	Test design	3 replicates of 10 plants to allow statistical analysis (in different trays) and at least 5 non-inoculated plants per genotype
9.5	Test facility	glasshouse or climatic room
9.6	Temperature	18-25 24°C
9.7	Light	At least 12h
9.8	Season	all seasons
9.9	Special measures	optional: shading (no direct sunlight during 12 h after inoculation) Recommend having really 18°C at night and not above 25°C during the day.
10.	Inoculation	
10.1	Preparation inoculum	aerated culture 7-10 days, eg. Czapek-Dox broth some isolates need filtration or centrifugation resuspend the pelleted spores in demineralized water Scrape spore cultures with water from agar medium (see 8.1) or optional multiplication on liquid medium (e.g. Messiaen (1991) synthetic liquid medium, sucrose 50g/L, on permanent agitator-shaker or aerated Czapek-Dox culture medium for 5-7 days at room temperature). Remark: Beware of toxin productions by some isolates (see remark under 13.)
10.2	Quantification inoculum	spore count; adjust to $10^6$ – $10^7$ per mL $4 \times 10^5$ to $1 \times 10^6$ sp/mL
10.3	Plant stage at inoculation	cotyledon expanded
10.4	Inoculation method	soaking of the root system in a suspension of liquid medium of fungus at least 30 sec – 5 min Plant at the inoculation stage are harvested carefully, roots and hypocotyls are immersed in spore suspension for 2-15 min; trimming of roots is an option; transplant in trays.
10.5	First observation	7 days post inoculation



10.6	<del>Second observation</del>	<del>14–20 days post inoculation</del>
10.7	Final observations	<del>20 days post inoculation</del> 1 <sup>st</sup> notation: symptoms on susceptible control at classes 2 and 3 with a strong proportion at 3. A second notation can be necessary to re-evaluate some unclear varieties
11.	Observations	
11.1	Method	Visual <u>observation</u> , <u>comparative</u>
11.2	Observation scale	
	<del>[1] absent</del>	<del>Growth retardation in combination with yellowing or wilting cotyledons (useful for judging the severity of the attack), possible internal vessel browning, death of plant.</del>
	<del>[0] present</del>	<del>no symptoms</del>

Mock	Class 0	Class 1	Class 2	Class 3
At least 5 plants	Healthy plant: no symptoms of yellowing and wilting, could be some growth reduction due to inoculation stress compared to mock. Sometimes in the mock we can observed some yellowing, different from the symptoms of <i>Fusarium</i>	Light symptoms of yellowing/wilting	typical symptoms : yellowing, wilting and necrosis, stunting (growth stopped)	Death of plant (Dead)
				

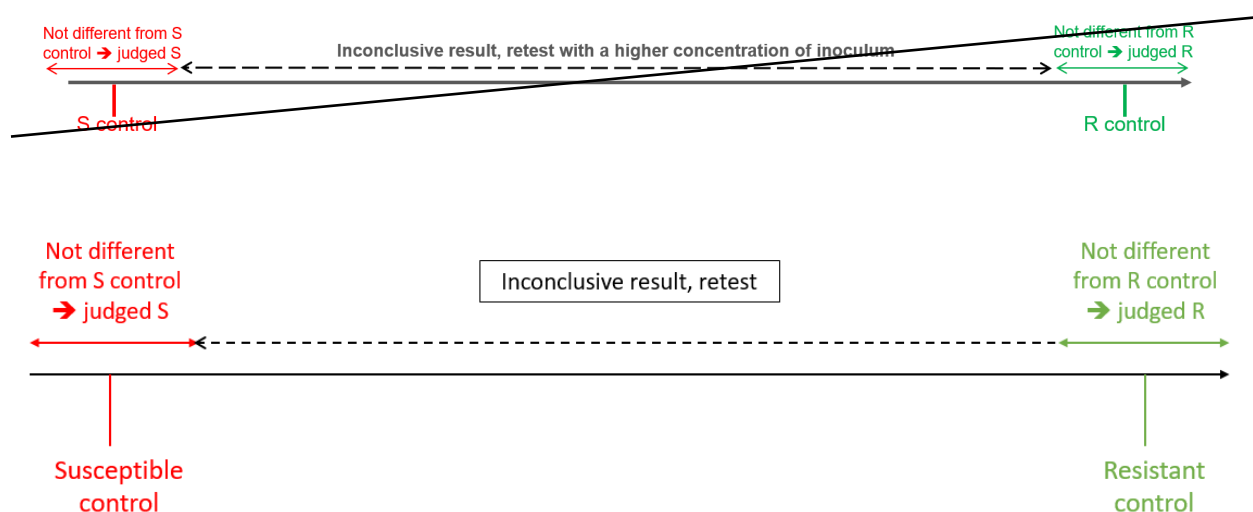
Other symptoms of vein clearing could be difficult to judge, it is advised to make a later notation to observe the evolution of this symptom over the time.



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

11.3	Validation of test	<del>on standards</del> Validation on controls. Controls expected response: <u>Resistant:</u> Plants at classes 0 and 1, sometimes very few plants at classes 2 or 3 <u>Susceptible:</u> Plants at classes 2 and 3
11.4	Off-types	-

12.	Interpretation of data in terms of UPOV characteristic states	<p><u>QL</u></p> <p>In case of varieties with a response between the susceptible and the resistant control, repeat the test.</p> <p>In case of confirmation of the result, the variety will be judged heterogeneous.</p> <p>In case of unclear results, retest or test in another lab.</p>
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13.	Critical control points	<p>For Race 1.2 the modified protocol on the next page should be used.</p> <p>For race 2, a differential with Fom-3 gene (e.g. Durango) could be added, to validate the capacity of the isolate to partially attack this variety.</p> <p>In the case of inoculum increased in Messiaen (1991) synthetic liquid medium, on permanent agitator-shaker, inoculum can be used after 5 to 7 days. For race 0 and 1, dilution 1/12 is recommended, while it must not be less than 1/20 for race 2. At a lower dilution (higher concentration of the medium), it has been observed that toxins released in the medium by the race 2 can cause some yellowing of melon plants, even if they are resistant. Alternatively, spores can be “washed” by resuspending a mass of spores collected on a Millipore filter with vacuum force.</p>
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Ad 69 B 69.4: Resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 (Fom: 1.2)







1.	Pathogen	<i>Fusarium oxysporum</i> f. sp. <i>melonis</i> race 1.2 (Fom: 1.2)
2.	Quarantine status	No
3.	Host species	Melon - <i>Cucumis melo</i> L.
4.	Source of inoculum	GEVES (FR) <sup>3</sup> , Naktuinbouw (NL)
5.	Isolate	<p>Fom: 1.2 (moderately aggressive): TST strain e.g. Reference strain validated in an inter-laboratory test<sup>4</sup></p> <p>Fom: 1.2</p> <p>- Strain TST</p> <p>= MAT/REF/04-07-01-04<sup>3</sup></p>

<sup>3</sup> [matref@geves.fr](mailto:matref@geves.fr)

<sup>4</sup> Harmores 3 CPVO project ([https://cpvo.europa.eu/sites/default/files/documents/report\\_harmores\\_3\\_final\\_meeting\\_v0\\_0.pdf](https://cpvo.europa.eu/sites/default/files/documents/report_harmores_3_final_meeting_v0_0.pdf))

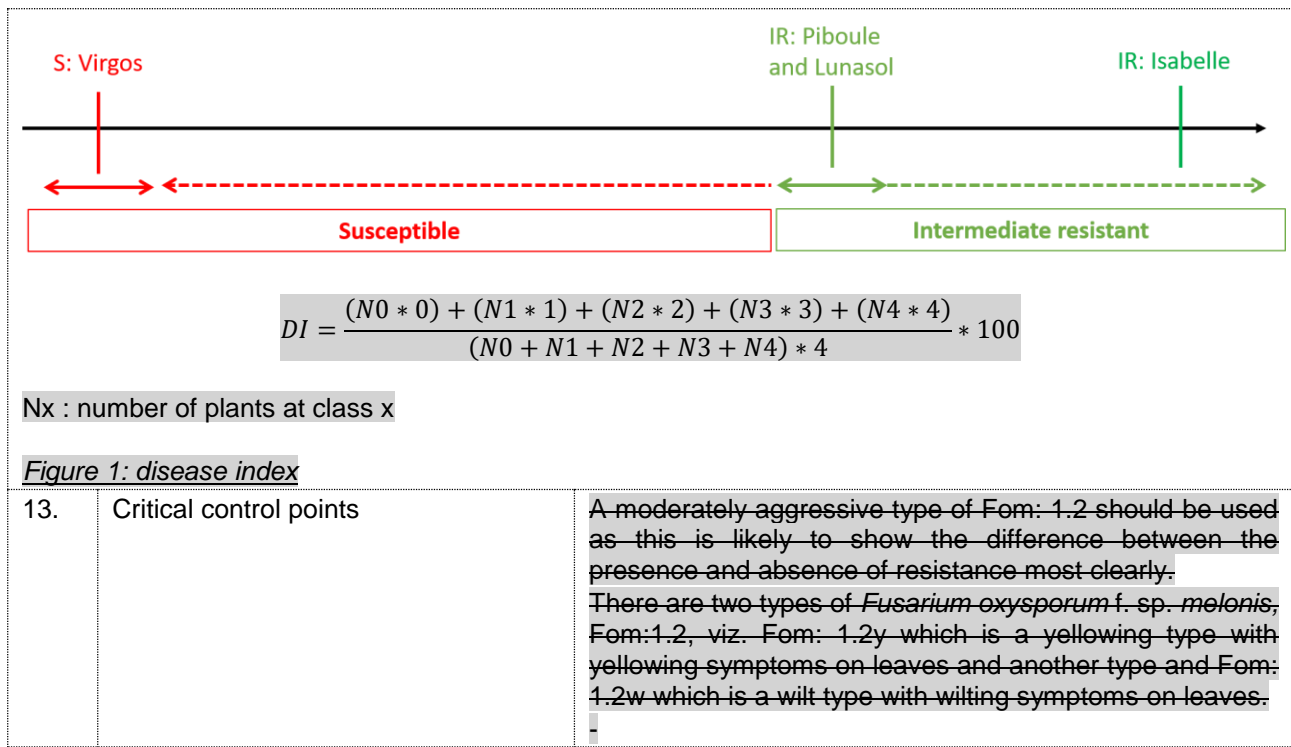
6.	Establishment isolate identity	<del>use differential varieties:</del> Védraçais, Virgos (susceptible) Lunasol (moderately resistant) Dinero, Isabelle (highly resistant) Test on differentials (potentially including Durango, see 13.). The most recent table is available through ISF at <a href="https://www.worldseed.org/our-work/plant-health/differential-hosts/">https://www.worldseed.org/our-work/plant-health/differential-hosts/</a> <u>Situation July 2019</u>																																	
		<table> <tr> <th>Differential host</th><th>Gene present</th><th>Fom: 0*</th><th>Fom: 1*</th><th>Fom: 2*</th><th>Fom: 1.2*</th></tr> <tr> <td>Charantais T*</td><td>-</td><td>S</td><td>S</td><td>S</td><td>S</td></tr> <tr> <td>Védraçais*, Doublon*</td><td><i>Fom-1</i></td><td>HR</td><td>S</td><td>HR</td><td>S</td></tr> <tr> <td>Charantais Fom-2*, CM17187*</td><td><i>Fom-2</i></td><td>HR</td><td>HR</td><td>S</td><td>S</td></tr> <tr> <td>Isabelle*</td><td><i>Polygenic?</i></td><td>HR</td><td>HR</td><td>HR</td><td>IR</td></tr> </table> <p>S = susceptible; HR = highly resistant; IR = intermediate</p> <p>*differential hosts and isolates that are used by the seed sector</p> <p style="text-align: right;"><u>Courtesy of Worldseed.org website</u></p>				Differential host	Gene present	Fom: 0*	Fom: 1*	Fom: 2*	Fom: 1.2*	Charantais T*	-	S	S	S	S	Védraçais*, Doublon*	<i>Fom-1</i>	HR	S	HR	S	Charantais Fom-2*, CM17187*	<i>Fom-2</i>	HR	HR	S	S	Isabelle*	<i>Polygenic?</i>	HR	HR	HR	IR
Differential host	Gene present	Fom: 0*	Fom: 1*	Fom: 2*	Fom: 1.2*																														
Charantais T*	-	S	S	S	S																														
Védraçais*, Doublon*	<i>Fom-1</i>	HR	S	HR	S																														
Charantais Fom-2*, CM17187*	<i>Fom-2</i>	HR	HR	S	S																														
Isabelle*	<i>Polygenic?</i>	HR	HR	HR	IR																														
7.	Establishment pathogenicity	use susceptible melon varieties																																	
8.	Multiplication inoculum																																		
8.1	Multiplication medium	on agar medium e.g. Potato Dextrose Agar, <u>Sabouraud</u> , at 20°C to 25°C																																	
8.2	Multiplication variety	-																																	
8.3	Plant stage at inoculation	-																																	
8.4	<del>Inoculation medium</del>	<del>on liquid medium</del>																																	
8.5	Inoculation method	-																																	
8.6	Harvest of inoculum	<u>7-10 day-old culture</u>																																	
8.7	Check of harvested inoculum	-																																	
8.8	Shelf life/viability inoculum	-																																	
9.	Format of the test																																		
9.1	Number of plants per genotype	<u>at least 30</u> <u>30 plants per variety plus 5 non-inoculated controls</u>																																	
9.2	Number of replicates	<u>e.g. At least 3 (in different trays)</u>																																	
9.3	Control varieties	<u>Susceptible: Virgos</u> <u>Intermediate resistant: Piboule and Lunasol and Isabelle (Isabelle is expected to have a lower disease index than Piboule and Lunasol).</u> <u>Piboule and Lunasol are both needed to illustrate the lower level to intermediate resistance. They are representative of different genetic constructions which don't have necessarily the same behavior according the tests, and the labs.</u>																																	
	<del>[1] susceptible</del>	<del>Védraçais, Virgos</del>																																	
	<del>[2] moderately resistant</del>	<del>Lunasol (the lowest accepted level)</del>																																	
	<del>[3] highly resistant</del>	<del>Dinero, Isabelle, Jador</del>																																	
9.4	Test design	<u>3 replicates of 10 plants to allow statistical analysis (in different trays) and at least 5 non-inoculated plants per genotype.</u>																																	
9.5	Test facility	glasshouse or climatic room																																	
9.6	Temperature	<u>18-25 24°C</u>																																	
9.7	Light	at least 12h																																	

9.8	Season	All seasons in a climatic room / in a greenhouse: be aware of the strong environmental effect: winter could be too severe and summer could be too mild.
9.9	Special measures	optional shading (no direct sunlight during 12 h after inoculation)
10.	Inoculation	
10.1	Preparation inoculum	<del>aerated culture 7-10 d old — e.g.: Czapek-Dox broth</del> Scrape cultures with water on agar medium (see 8.1) or optional multiplication on liquid medium (e.g. Potato Dextrose Broth (PDB), Czapek-Dox culture medium for 7 days at room temperature and darkness or Messiaen (1991) synthetic liquid medium, sucrose 50g/L, on permanent agitator-shaker, at room-temperature, inoculum can be used after 5 to 7 days)
10.2	Quantification inoculum	<del>spore count; adjust to <math>2 \cdot 10^4</math>—<math>10^5</math> per ml</del> $1 \times 10^5$ - $1 \times 10^6$ sp/mL, depending on inoculation method (see 10.4) and lab conditions
10.3	Plant stage at inoculation	cotyledons expanded, first leaf emerging
10.4	Inoculation method	<del>soaking of the trays in spore suspension; 700 ml for a tray with 25 – 30 plants, plants are not uprooted</del> Two methods can be used for inoculation. - Absorption: Absorption of a suspension of spores, e.g. 700mL of a suspension at $1 \cdot 10^5$ sp/mL for 50 plants in a tray 30 cm*30 cm. - Injection: Injection of a suspension of spores into the soil at the base of the plant, e.g. 5mL at $10^6$ sp/mL per plant.
10.5	First observation	<del>7– 14 days post inoculation</del>
10.6	Second observation	<del>14 – 21 days post inoculation</del>
10.7	Final observations	<del>21– 28 days post inoculation</del> 1st notation: symptoms on susceptible control at least at class 3 [generally 10-21 dpi]. A second notation can be necessary to reevaluate some unclear varieties.
11.	Observations	
11.1	Method	Visual observation, comparative
11.2	Observation scale	symptoms:
	[1] susceptible	<del>Védrantais: growth retardation, yellow cotyledons, drying, possible internal vessel browning, death of the plant</del>
	[2] moderately resistant	<del>Symptoms may be present, but the level of expression must be distinctly lower than the susceptible control variety. = the lowest level of resistance is defined by the behavior of Lunasol</del>
	[3] highly resistant	<del>Symptoms may be present, but the level of expression must be lower than the moderately control variety Lunasol.</del>

Non-inoculated plants	Class 0	Class 1	Class 2	Class 3	Class 4
Varieties must be compared to the non-inoculated plants.	Healthy plant, the whole plant is green or at the same level than the mock. Just a light yellowing can be accepted on the mock	Light level of symptoms, light yellowing on cotyledons and/or leaves without necrosis	Moderate level of symptoms, yellowing on cotyledon and/or leaves, starting of necrosis and wilting but not extended	Severe symptoms of yellowing and/or wilting on cotyledons and/or leaves with extended necrosis	Dead plant, no green leaf part or hypocotyl is dry
					

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

11.3	Validation of test	<p>Validation on controls. Controls expected response:</p> <ul style="list-style-type: none"> <li>- <b>Intermediate Resistant:</b> Maximum of plants at classes 0 and 1, with few plants in the other classes. Low level of disease index generally below 40%. A difference of disease index is generally observed between Piboule and Lunasol compared to Isabelle</li> <li>- <b>Susceptible:</b> Plants at classes 3 and 4, and in some cases few plants at class 2. Very high disease index above 80%.</li> </ul>
11.4	Off-types	<p>calibrate with Lunasol</p> <ul style="list-style-type: none"> <li>-</li> </ul>
12.	Interpretation of data in terms of UPOV characteristic states	<p><b>QN</b></p> <p>Interpretation of varieties depending on controls (figure 1)  Note 1 = Intermediate resistance absent = susceptibility  Note 9 = Intermediate resistance present</p> <p>Quantitative analysis is based on the disease index (DI) and the distribution of plants per class compared to the controls.</p> <p>The varieties statistically not different from one of the intermediate resistant controls or with a lower disease index have to be judged as intermediate resistant.</p> <p>The varieties between the susceptible and the intermediate resistant controls have to be judged as susceptible (not resistant enough to be considered intermediate resistant).  If not clear results, the use of statistic is highly suggested.</p>



Proposed revision of Characteristics 70.1 to 70.5 “Resistances to *Podosphaera xanthii* (Px) - races 1, 2, 3, 5, 3.5”

*Current wording*

70.	VG	Resistance to <i>Podosphaera xanthii</i> ( <i>Sphaerotheca fuliginea</i> ) (Powdery mildew)	Résistance à <i>Podosphaera xanthii</i> ( <i>Sphaerotheca fuliginea</i> ) (oidium)	Resistenz gegen <i>Podosphaera xanthii</i> ( <i>Sphaerotheca fuliginea</i> ) (Echter Mehltau)	Resistencia a <i>Podosphaera xanthii</i> ( <i>Sphaerotheca fuliginea</i> ) (Oidio)	
		-----	-----	-----	-----	-----
70.1		<b>Race 1</b>	<b>Race 1</b>	<b>Pathotyp 1</b>	<b>Raza 1</b>	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Jaune Canari 2, Védramtais 1
		moderately resistant	moyennement résistant	mäßig resistent	moderadamente resistente	Escrito 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Anasta,Cézanne, 3
		-----	-----	-----	-----	-----
70.2		<b>Race 2</b>	<b>Race 2</b>	<b>Pathotyp 2</b>	<b>Raza 2</b>	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Galoubet, Védramtais 1
		moderately resistant	moyennement résistant	mäßig resistent	moderadamente resistente	Escrito, Pendragon 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Anasta, Cézanne 3
		-----	-----	-----	-----	-----
70.3		<b>Race 3</b>	<b>Race 3</b>	<b>Pathotyp 3</b>	<b>Raza 3</b>	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Védramtais 1
		moderately resistant	moyennement résistant	mäßig resistent	moderadamente resistente	Nettuno 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Batista, Godiva 3
		-----	-----	-----	-----	-----
70.4		<b>Race 5</b>	<b>Race 5</b>	<b>Pathotyp 5</b>	<b>Raza 5</b>	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Védramtais 1
		moderately resistant	moyennement résistant	mäßig resistent	moderadamente resistente	Hugo, Pendragon 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Arapaho 3
		-----	-----	-----	-----	-----
70.5		<b>Race 3-5</b>	<b>Race 3-5</b>	<b>Pathotyp 3-5</b>	<b>Raza 3-5</b>	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Védramtais 1
		moderately resistant	moyennement résistant	mäßig resistent	moderadamente resistente	Cisco 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	90625 3



Proposed new wording

70.	VG	Resistance to <i>Podosphaera xanthii</i> (Px) (ex <i>Sphaerotheca</i> <i>fuliginea</i> ) (Powdery mildew)	Résistance à <i>Podosphaera xanthii</i> (Px) (ex <i>Sphaerotheca</i> <i>fuliginea</i> ) (oïdium)	Resistenz gegen <i>Podosphaera xanthii</i> (Px) (ex <i>Sphaerotheca</i> <i>fuliginea</i> ) (Echter Mehltau)	Resistencia a <i>Podosphaera xanthii</i> (Px) (ex <i>Sphaerotheca</i> <i>fuliginea</i> ) (Oidio)	
70.1		Race 1 (Px: 1)	Race 1 (Px: 1)	Pathotyp 1 (Px: 1)	Raza 1 (Px: 1)	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Jaune Canari 2, Védrantais 1
		moderately intermediate resistant	moyennement résistant à un niveau intermédiaire	mäßig mittel resistant	moderadamente resistente resistencia intermedia	Escrito 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Anasta, Cézanne Arum 3
70.2		Race 2 (Px: 2)	Race 2 (Px: 2)	Pathotyp 2 (Px: 2)	Raza 2 (Px: 2)	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Galoubet, Védrantais 1
		moderately intermediate resistant	moyennement résistant à un niveau intermédiaire	mäßig mittel resistant	moderadamente resistente resistencia intermedia	Escrito, Pendragon 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Anasta, Cézanne Arum 3
70.3		Race 3 (Px: 3)	Race 3 (Px: 3)	Pathotyp 3 (Px: 3)	Raza 3 (Px: 3)	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Védrantais 1
		moderately intermediate resistant	moyennement résistant à un niveau intermédiaire	mäßig mittel resistant	moderadamente resistente resistencia intermedia	Nettuno Arago, Durango 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Batista, Godiva Arum 3
70.4		Race 5 (Px: 5)	Race 5 (Px: 5)	Pathotyp 5 (Px: 5)	Raza 5 (Px: 5)	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Védrantais 1
		moderately intermediate resistant	moyennement résistant à un niveau intermédiaire	mäßig mittel resistant	moderadamente resistente resistencia intermedia	Hugo, Pendragon Arago, Durango 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	Arapaho Arum 3
70.5		Race 3-5 (Px: 3.5)	Race 3-5 (Px: 3.5)	Pathotyp 3-5 (Px: 3.5)	Raza 3-5 (Px: 3.5)	
(+)						
QN		susceptible	sensible	anfällig	susceptible	Védrantais 1
		moderately intermediate resistant	moyennement résistant à un niveau intermédiaire	mäßig mittel resistant	moderadamente resistente resistencia intermedia	Ciseo Arago, Durango 2
		highly resistant	hautement résistant	hochresistent	altamente resistente	90625 Arum 3



Revision of explanation Ads. 70.1 to 70.3, 71 “Resistances to *Podosphaera xanthii* (Px), Resistance to *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*), race 1 (Powdery mildew) Gc (Ec)” in Chapter 8.2 “Explanations for individual characteristics”

### Current wording

Ads. 70.1 to 70.3: Resistance to *Podosphaera xanthii* (*Sphaerotheca fuliginea*) (Powdery mildew) Px (St)

Ad. 71: Resistance to *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*), race 1 (Powdery mildew) Gc (Ec)

1.	Pathogen	Powdery mildew: <i>Podosphaera xanthii</i> ( <i>Sphaerotheca fuliginea</i> ) races 1, 2, 3, 5 and 3-5 <i>Golovinomyces cichoracearum</i> ( <i>Erysiphe cichoracearum</i> ) race 1
2.	Quarantine status	no
3.	Host species	<i>Cucumis melo</i>
4.	Source of inoculum	GEVES (FR)
5.	Isolate	Px: races 1, 2, 3, 5 and 3-5; Gc: race 1
6.	Establishment isolate identity	on differentials:

	Powdery Mildew					
	<i>Podosphaera xanthii</i> ( <i>Sphaerotheca fuliginea</i> )					<i>Golovinomyces cichoracearum</i> ( <i>Erysiphe cichoracearum</i> )
	race 1	race 2	race 3	race 5	race 3-5	race 1
Védrantais	S	S	S	S	S	S
Nantais Oblong	S	S	S	S	S	R
PMR 45	R	S	S	S	S	S
Edisto 47, WMR 29	R	R	R	S	S	S
PI 124112, 90625	R	R	R	R	R	R
PMR 5	R	R	S	R	S	R
PI 414723	R	R	IR	R	R/ IR	R

Legend: S susceptible (high sporulation); R resistant (low sporulation), IR (moderately resistant)

7.	Establishment pathogenicity	use susceptible melon varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	detached cotyledon in Petri-dish on 0.35 – 0.5% Agar, 1-2% mannitol, possible add of 1% sucrose
8.2	Multiplication variety	susceptible varieties
8.3	Plant stage at inoculation	young, unfolded cotyledon; decontaminated with e.g. 0.05% mercuric chloride or 3 to 5% bleach (NaClO + NaCl)
8.4	Inoculation medium	air
8.5	Inoculation method	scatter conidia on the cotyledons transferred by blowing
8.6	Harvest of inoculum	use cotyledons with strong sporulation
8.7	Check of harvested inoculum	check presence of spores
8.8	Shelf life/viability inoculum	on cotyledon, 17-23°C, under very low light intensity; maximum storage time is 15 days, after the inoculation <u>Remark:</u> In case of longer term preservation, inoculate locally with a few spores, store at 14°C/12h low light per day
9.	Format of the test	
9.1	Number of plants per genotype	at least 16 plants
9.2	Number of replicates	e.g. 3

9.3	Control varieties	
-----	-------------------	--

Powdery Mildew						
	<i>Podosphaera xanthii</i>					<i>Golovinomyces cichoracearum</i>
	race 1	race 2	race 3	race 5	race 3-5	race 1
susceptible	Jaune Canari 2, Védraçais	Galoubet, Védraçais	Védraçais	Védraçais	Védraçais	Védraçais
moderately resistant	Escrito	Escrito, Pendragon	Nettuno	Hugo, Pendragon	Cisco	Anasta
highly resistant	Anasta, Cézanne	Anasta, Cézanne	Batista, Godiva	Arapaho	90625	Heliobel

9.4	Test design	leaf discs placed on 0.4% agar with 1- 4% mannitol and possible add of 0.003% benzimidazol
9.5	Test facility	climatic room
9.6	Temperature	20-24°C
9.7	Light	12 to 24h darkness after inoculation
9.8	Season	-
9.9	Special measures	Inoculation tower needed for even distribution of dry spores.
10.	Inoculation	
10.1	Preparation inoculum	-
10.2	Quantification inoculum	-
10.3	Plant stage at inoculation	<u>Routine method</u> : leaf disks, 2 cm in diameter, from young plants. <u>Complementary method</u> , if necessary: young plants
10.4	Inoculation method	<u>Routine method</u> : on leaf disks: inoculation tower needed for even distribution of dry spores. <u>Complementary method</u> : take spores from a cotyledon covered with conidia and deposit them on a leaf or blow the spores from a cotyledon.
10.5	First observation	8-10 days post inoculation
10.6	Second observation	-
10.7	Final observations	11-12 days post inoculation
11.	Observations	-
11.1	Method	visual
11.2	Observation scale	
	[1] susceptible	medium or intense sporulation all over the leaf disc surface
	[2] intermediate	weak sporulation all over the surface or isolated colonies on more than 10% of the surface
	[3] resistant	isolated colonies on less than 10% of the surface or no sporulation
11.3	Validation of test	on controls
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	QN
13.	Critical control points	-

## Proposed new wording

Ads. 70.1 to 70.3 5: Resistance to *Podosphaera xanthii* (Px) (~~ex~~ *Sphaerotheca fuliginea*) (Powdery mildew) Px (Sf) races 1, 2, 3, 5, 3.5 (Px: 1, 2, 3, 5, 3.5)

Ad. 71: Resistance to *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*), race 1 (Powdery mildew) Gc (Ec)

1.	Pathogen	Powdery mildew: <i>Podosphaera xanthii</i> ( <del>ex</del> <i>Sphaerotheca fuliginea</i> ) races 1, 2, 3, 5 and 3.5 <i>Golovinomyces cichoracearum</i> ( <del>ex</del> <i>Erysiphe cichoracearum</i> ) race 1 <del>Only Podosphaera xanthii was validated in Harmores 3 project.</del>
2.	Quarantine status	No
3.	Host species	Melon - <i>Cucumis melo</i> L.
4.	Source of inoculum	GEVES (FR) <sup>5</sup>
5.	Isolate	Px: races 1, 2, 3, 5 and 3.5; e.g. Reference strain validated in an inter-laboratory test <sup>6</sup> Px: 1 - Strain Sm 3 = MAT/REF/04-07-03-01 <sup>5</sup> Px: 2 - Strain S87-7 = MAT/REF/04-07-03-02 <sup>5</sup> Px: 3 - Strain 00Sm39 = MAT/REF/04-07-03-04-02 <sup>5</sup> Px: 5 - Strain 98Sm65 = MAT/REF/04-07-03-03-01-02 <sup>5</sup> Px: 3.5 - Strain 04Sm2 = MAT/REF/04-07-03-05-01 <sup>5</sup>  Gc: 1 - Strain GEVES = MAT/REF/04-07-02-01 <sup>5</sup>
6.	Establishment isolate identity	on differentials (table1)

	Powdery Mildew					
	<i>Podosphaera xanthii</i> ( <i>Sphaerotheca fuliginea</i> )					<i>Golovinomyces cichoracearum</i> ( <i>Erysiphe cichoracearum</i> )
	race 1	race 2	race 3	race 5	race 3-5	race 1
Védraçais	S	S	S	S	S	S
Nantais Oblong	S	S	S	S	S	R
PMR 45	R	S	S	S	S	S
Edisto 47, WMR 29	R	R	R	S	S	S
PI 124112, 90625	R	R	R	R	R	R
PMR 5	R	R	S	R	S	R
PI 414723	R	R	IR	R	R/IR	R

Legend: S – susceptible (high sporulation); R – resistant (low sporulation), IR (moderately resistant)

<sup>5</sup> [matref@geves.fr](mailto:matref@geves.fr)

<sup>6</sup> Harmores 3 CPVO project ([https://cpvo.europa.eu/sites/default/files/documents/report\\_harmores\\_3\\_final\\_meeting\\_v0\\_0.pdf](https://cpvo.europa.eu/sites/default/files/documents/report_harmores_3_final_meeting_v0_0.pdf))

Table 1:

Races of *Podosphaera xanthii* (Px) and *Golovinomyces cichoracearum* (Gc), J. McCreight and M. Pitrat

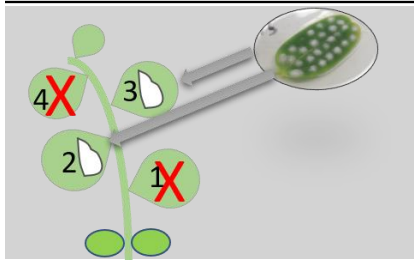
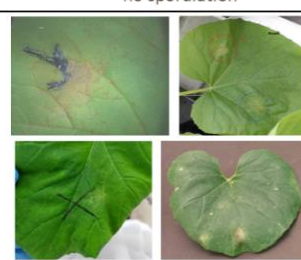



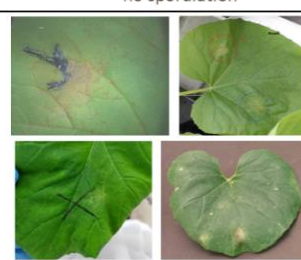



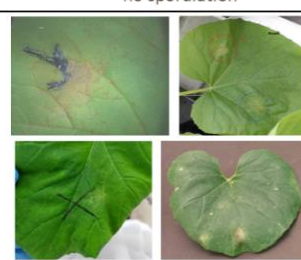




		<i>Podosphaera xanthii</i>						<i>Golovinomyces cichoracearum</i>	
	Race 0	Race 1	Race 2	Race 3	Race 4	Race 5	Race 3.5	Race 0	Race 1
Iran H	S	S	S	S	S	S	S	S	S
Védrantais	R	S	S	S	S	S	S	R	S
PMR45	R	R	S	S	S	S	S	R	S
WMR29	R	R	R	R	S	S	S	R	S
Edisto 47	R	R	R	R	R	S	S	R	S
MR-1, PI124112	R	R	R	R	R	R	R	R	R
PMR5	R	R	R	S	S	R	S	R	R
Nantais Oblong	R	S	S	S	S	S	S	R	R

7.	Establishment pathogenicity	use susceptible melon varieties								
8.	Multiplication inoculum									
8.1	Multiplication medium	<del>detached cotyledon in Petri-dish on 0.35 — 0.5% Agar,</del> 1-2% mannitol, possible add of 1% sucrose Melon plantlets								
8.2	Multiplication variety	<del>susceptible varieties</del> Susceptible variety, for example Védra <span style="text-decoration: underline;">ntais</span> . For higher isolates like 3.5 or 5, a variety with broken resistance is recommended to keep the isolate pure.								
8.3	Plant stage at inoculation	<del>young, unfolded cotyledon; decontaminated with e.g. 0.05% mercuric chloride or 3 to 5% bleach (NaClO + NaCl)</del> Cotyledon								
<del>8.4</del>	<del>Inoculation medium</del>	Air								
8.5	Inoculation method	<del>scatter conidia on the cotyledons transferred by blowing</del> Sowing in substrate, for example soil or disinfected peat inside a closed mini glasshouse. When the cotyledons have expanded, remove them from the plant. Disinfect the cotyledons by soaking them for 3 minutes in a mercuric chloride solution (0.05%) or in sodium hypochlorite solution. Rinse them with sterilized water. Dry the cotyledons with sterile paper towel, then place them in Petri dishes with the following medium: <table><tr><td>Sucrose</td><td>10g</td></tr><tr><td>Mannitol</td><td>20g</td></tr><tr><td>Agar</td><td>5g</td></tr><tr><td>Distilled water</td><td>1 liter</td></tr></table> <del>Scatter conidia on the cotyledons and blow them or deposit conidia at the surface of cotyledons. Incubate the inoculated cotyledons in Petri dishes for example at 23°C during 14 hours in the light and at 18°C during 10 hours in the dark or 17°C permanently under very low light intensity. 9 to 11 days after the inoculation, the cotyledons will be covered with conidia and can be used as an inoculum.</del>	Sucrose	10g	Mannitol	20g	Agar	5g	Distilled water	1 liter
Sucrose	10g									
Mannitol	20g									
Agar	5g									
Distilled water	1 liter									
8.6	Harvest of inoculum	<del>use cotyledons with strong sporulation</del> Sporulation on cotyledons								
8.7	Check of harvested inoculum	<del>check presence of spores</del>								
8.8	Shelf life /viability inoculum	<del>on cotyledon, 17-23°C, under very low light intensity; maximum storage time is 15 days, after the inoculation</del> Remark: In case of longer term preservation, inoculate locally with a few spores, store at 14°C/12h low light per day Maximum 1 to 1.5 months after the inoculation.								

9.	Format of the test	
9.1	Number of plants per genotype	at least 16 plants At least 20 plants per variety and controls, 5 plants for other differentials.
9.2	Number of replicates	e.g. 3 -
9.3	Control varieties	

	Powdery Mildew					
	<i>Podosphaera xanthii</i>					<i>Golovinomyces cichoracearum</i>
	race 1	race 2	race 3	race 5	race 3-5	race 1
Susceptible	Jaune Canari 2, Védrentais	Galoubet, Védrentais	Védrentais	Védrentais	Védrentais	Védrentais
moderately resistant	Escrito	Escrito, Pendragon	Nettuno	Hugo, Pendragon	Cisco	Anasta
highly resistant	Anasta, Cézanne	Anasta, Cézanne	Batista, Godiva	Arapaho	90625	Heliobel

		<p><u>For <i>Podosphaera xanthii</i> (Px) race 1:</u></p> <ul style="list-style-type: none"> <li>• Susceptible: Védrentais</li> <li>• Intermediate resistant: Escrito</li> <li>• Resistant: Arum</li> </ul> <p><u>For <i>Podosphaera xanthii</i> (Px) race 2:</u></p> <ul style="list-style-type: none"> <li>• Susceptible: Védrentais</li> <li>• Intermediate resistant: Escrito, Pendragon</li> <li>• Resistant: Arum</li> </ul> <p><u>For <i>Podosphaera xanthii</i> (Px) races 3, 5, 3.5:</u></p> <ul style="list-style-type: none"> <li>• Susceptible: Védrentais</li> <li>• Intermediate resistant: Arago, Durango</li> <li>• Resistant: Arum</li> </ul> <p><u>For <i>Golovinomyces cichoracearum</i> (Gc) race 1:</u></p> <ul style="list-style-type: none"> <li>• Susceptible: Escrito, Score, Védrentais</li> <li>• Intermediate resistant: Flores, Anasta</li> <li>• Resistant: Cézanne, Heliobel, Théo</li> </ul>
9.4	Test design	<p>leaf discs placed on 0.4% agar with 1–4% mannitol and possible add of 0.003% benzimidazole</p> <p>Include differentials to validate the race (at least 5 plants per differentials) and compare the level of sporulation.</p>
9.5	Test facility	<p>climatic room</p> <p>Climatic chamber or greenhouse</p>
9.6	Temperature	20-24°C
9.7	Light	<p>12 to 24h darkness after inoculation</p> <p>At least 12 hours</p>
9.8	Season	-
9.9	Special measures	Inoculation tower needed for even distribution of dry spores.
10.	Inoculation	
10.1	Preparation inoculum	-
10.2	Quantification inoculum	-

10.3	Plant stage at inoculation	<p><del>Routine method:</del> leaf disks, 2 cm in diameter, from young plants.</p> <p><del>Complementary method, if necessary:</del> young plants</p> <p>Whole plants at 3-4 true leaf fully expanded stage. Inoculation on the leaves 2 and 3 indicated on the diagram below.</p>  <p>Courtesy of GEVES-SNES in the framework of CPVO Harmores project.</p>								
10.4	Inoculation method	<p><del>Routine method:</del> on leaf disks: inoculation tower needed for even distribution of dry spores.</p> <p><del>Complementary method:</del> take spores from a cotyledon covered with conidia and deposit them on a leaf or blow the spores from a cotyledon.</p> <p>Take spores from a cotyledon already covered with conidia and deposit them on a leaf. Different isolates can be tested on the same plant (or the same leaf) if the local deposit is well separated from each other and if a mark indicates the place of the deposit.</p>								
10.5	First observation	8-10 days post inoculation								
10.6	Second observation	-								
10.7	Final observations	11-12 days post inoculation								
		The date of notation should be chosen based on expected symptoms on the three controls. Sporulation should be well expressed on the susceptible control.								
11.	Observations									
11.1	Method	Visual observation of sporulation								
11.2	Observation scale									
	[1] susceptible	medium or intense sporulation all over the leaf disc surface								
	[2] intermediate	weak sporulation all over the surface or isolated colonies on more than 10% of the surface								
	[3] resistant	isolated colonies on less than 10% of the surface or no sporulation								
<table><tr><th>Class 1: No development of the fungus (no mycelium or dead mycelium) or no sporulation</th><th>Class 3: weak sporulation</th><th>Class 5: moderate sporulation</th><th>Class 9: strong sporulation</th></tr><tr><td></td><td></td><td></td><td></td></tr></table>			Class 1: No development of the fungus (no mycelium or dead mycelium) or no sporulation	Class 3: weak sporulation	Class 5: moderate sporulation	Class 9: strong sporulation				
Class 1: No development of the fungus (no mycelium or dead mycelium) or no sporulation	Class 3: weak sporulation	Class 5: moderate sporulation	Class 9: strong sporulation							
										
 <p>Example of contamination by environment on the susceptible control, test not validated</p>										
Courtesy of GEVES-SNES in the framework of CPVO Harmores project										

11.3	Validation of test	<p>on controls</p> <p><u>Validation on controls.</u></p> <p><u>Additional information for expected responses of <i>Podosphaera xanthii</i> controls</u></p> <p><u>Resistant:</u></p> <ul style="list-style-type: none"> <li>Plants at class 1, or most of the plants at class 1 and few plants at class 3 (very low disease index).</li> <li>Plants at class 3 but in this case the susceptible control should be all at class 9.</li> <li>No plants at classes 5 or 9.</li> </ul> <p><u>Intermediate Resistant:</u></p> <ul style="list-style-type: none"> <li>Between the resistant and the susceptible control.</li> <li>Generally, plants at classes 3 and 5.</li> </ul> <p><u>Susceptible:</u></p> <ul style="list-style-type: none"> <li>Plants at class 9, or most of the plants at class 9 and few plants at class 5 (high disease index).</li> <li>Few plants at class 3 but in this case the resistant controls should be all at class 1 and the intermediate resistant control at classes 3 and 1.</li> <li>No plants at class 1.</li> </ul>
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	<p><b>QN</b></p> <p><u>Interpretation of varieties depending on controls (figure 1)</u></p> <p>Note 1 = Resistance absent = susceptibility Note 2 = Intermediate resistance present Note 3 = Resistance present</p> <p><u>Quantitative analysis is based on the disease index and the distribution of plants per class compared to the controls.</u></p> <p><u>Additional information for <i>Podosphaera xanthii</i> controls:</u> The varieties between the intermediate resistant and the resistant control have to be judged as intermediate resistant (because they are not resistant enough to be considered resistant). The varieties between the susceptible and the intermediate resistant control have to be judged as susceptible (because they are not resistant enough to be considered intermediate resistant).</p> <div style="border: 1px solid black; padding: 5px; margin-top: 10px;"> <math display="block">DI = \frac{(N1 * 0) + (N3 * 1) + (N5 * 2) + (N9 * 3)}{(N1 + N3 + N5 + N9) * 3} * 100</math> </div> <p>Nx: Number of plants at class X</p>

**Figure 1: disease index**

13.	Critical control points	To avoid cross contamination, it is advised to not produce inoculum of different races in the same room.
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