

Results of Ringtest ISF WG DRT Fom: 2 resistance in melon, conducted in spring and summer 2022

Diederik Smilde, Naktuinbouw, November 2023

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1. Introduction
 2. Results
 3. Discussion
 4. Proposals
 5. Conclusions
- Table 1-5
Annex 1-5
-

1. Introduction

Context

Currently, in the Melon *Fusarium oxysporum* f.sp. *melonis* (Fom) ISF table and CPVO protocol for race 2, only two classes exist: S and HR. But in practice the phenotype is sometimes difficult to assign to either S or HR. A ringtest is needed to improve and harmonise methods and interpretations in different laboratories.

Race specific resistance genes Fom-2 and Fom-1 confer resistance to races 0 and 1, and races 0 and 2, respectively (Risser et al. 1976). Another gene, Fom-3, controls resistance to races 0 and 2 in cultivar Perlita-FR (Zink and Gubler 1985). Oumouloud (2010) has found evidence for the presence of a recessive resistance gene, designated fom-4, in Spanish cantaloupe cv. Tortuga, effective for Fom: 0 and Fom: 2.

Goals of ISF project, defined at the start.

- Define intermediate control and validate a protocol that reliably differentiates between S, IR and HR levels for Fom:2 in Melon
- Identify protocol factors (temperature, photoperiod (?), isolate) which influence expression of resistance to Fom: 2.
- Decide which control variety with intermediate resistance expression should be added to official protocols.
- Agree on what should be the official claim (S/IR/HR?) in presence of the intermediate type of resistance, and define rules for use in DUS.

Test design

- Ringtest with **14 laboratories**
- 3 repetitions of 10 seeds for each treatment (variety x isolate x condition)
- 5 non-inoculated plants for each treatment
- 2 Isolates:
 - **I1** (SP, aggressive)

- I2 (US, mild)
- 3 Conditions
 - 'Cold' = 23C°/18°C with 12 hours light
 - 'Standard' = 23C°/18°C with 16 hours light (current testing conditions)
 - 'Warm' = 24C°/24°C with 16 hours light

Note: Each condition will be tested in at least 9 labs, to reduce the workload without losing a lot of statistical power.

Test method

The test method described in the CPVO protocol was applied. The plants were tested by dipping roots in a suspension with 10⁶ spores per ml. The final observation was done in week 3 after inoculation.

Observation scale

Scale	CPVO-TP/104/2-Rev.2
0	Healthy plant: no symptoms of yellowing and wilting, could be some growth reduction
1	Light symptoms of yellowing and wilting
2	Typical symptoms: yellowing, wilting and necrosis, stunting (growth stopped)
3	Death of plant (Dead)

Judgments Three judgments were given within for the three replicates in one lab, isolate and condition and variety. For the analysis of the judgment, the three judgments were translated into one single judgment to replace each set of three replicated judgments. This translation is tabulated in Annex 2 and 3. In addition to this, the repartition of plants over observation classes was used to evaluate heterogeneity (HG). This two-step procedure for judgments was necessary because heterogeneity judgements on 10 plants are less reliable than heterogeneity judgments on 30 plants

Disease index

The disease index is equivalent to the normalized average score of the individual plants. The normalization transforms the 0-3 scale into an arbitrary 0-1 scale.

2. Results

Controls

The susceptible control variety Marianna and the differential Charentais-Fom-2 were consistently judged S and the disease index was 100%. The differential Védreantais and the resistant control Charentais-Fom-1 were consistently judged R, and the disease index was 0%. Therefore, we may consider all tests as validated.

Intermediate phenotypes

Perlita has a phenotype that may be interpreted as intermediate resistance. HMC-1 appears to be on the more resistant side and Ducral, San Marcos and Doral on the more susceptible side. All intermediate phenotypes give rise to some degree of discordance between labs. This is true when we calculate the discordance with or without taking into account heterogeneity, and also on the basis of disease index (Table 2 and 3).

Heterogeneous judgments

Most of the heterogeneous judgments were found by only three labs (E, I, M). There may be technical difficulties associated with this, that should be considered in the individual lab. The overall frequency of HG judgment appears to be limited. This means that this data set provides a good basis for discussion and conclusion.

Aggressive isolate

The intermediate phenotypes on the more resistant side depend a bit on the isolate: with the mild isolate, their resistance may be overestimated, with the aggressive isolate the resistance may be underestimated, depending on what isolate is present in a field. The differentiation of the two isolates is more pronounced with Ducral, San Marcos and Doral than with Perlita (Table 2, 3 and 5).

Warm condition

The warm condition promoted the resistance in Perlita and HMC-1, but promoted susceptibility in Ducral (Table 2, 3, and 5). San Marcos and Doral. This effect of the warm condition was more pronounced with the aggressive isolate I1 (SP).

For example, in Table 3 complete concordance (no discordance) between the individual labs on the basis of DI can be found for the tests with Isolate I1 in the warm condition, while for the normal and cold condition some discordance was found (11-22%) with isolate I1. With isolate I2 the discordance was much higher. For some varieties the discordance was even 100%, reflecting the fact that for example Ducral is judged mainly R with Isolate I2.

This observation is a strong argument in favour of the use of Perlita as the lower border of resistance.

Statistics

Pathostat (<https://www.geves.fr/tools/pathostat/>) produces an interpretation (R/IR/S) by comparing to the distribution of symptoms of the variety with the distribution of symptoms of the IR control variety, taking into account whether the level of susceptibility is lower or higher than the control. (Also, when all plants are in the two extreme classes, Pathostat will judge HG (%) when there are too many off-types, as defined by CPVO.) In Table 4, Perlita is used as the lower border of IR in Pathostat, with Védreantais and Marianna as the R and S controls, respectively. (Charentais Fom-1 is prescribed as S control by Pathostat, but was not used in all temperature treatments due to seed shortage. Védreantais is a good replacement)

In the 'warm' tests with Isolate I1, all labs draw the same conclusion for all varieties, except for HMC-1. For HMC-1, Pathostat concludes IR in 5 labs and R in 5 other labs (5IR/5R).

In the 'warm' tests with Isolate I2, there is discordance between labs for HMC-1 (1IR/9R), Ducral (3IR/6R), San Marcos (5S/5R), and Doral (5S/2IR/2R).

Table 5 is repeating the DI data in Table 3 in a format that is suitable for comparing with Table 4. A more comprehensive comparison is given in Annex 4.

3. Discussion

Can we draw an additional borderline between HR and IR?

This question was posed at the beginning of the project. The data suggest that this is not possible, because the HR/IR judgments are discordant between labs in the six combinations of isolate and condition that we have tested. This is true for the judgments given by trained observers, for judgments based on comparing disease index, and also for judgments based on pathostat, even in the conditions

(warm) and with the isolate (I1) with the most harmonised results. In contrast, there is concordance for IR/S judgments in the warm condition for I1.

Advantages of statistical approach

Tables 4 and 5 show the advantage of Pathostat decisions over a more straightforward approach, using the DI of Perlita as a cut-off point. For I1 there is no difference in the conclusions on the borderline between IR and S, but for I2 there are remarkable differences.

Pathostat decisions about the borderline between IR and S are different from the decisions based on DI only. The statistical model leads to a better evaluation of the concordance between of labs.

In theory, the statistical power will improve with higher plant numbers. So the borderline between IR and S may be more robust with more plants in each experiment. However, higher plant numbers may also reveal flaws in the statistical model. This is beyond the scope of this ringtest.

4. Proposals

To obtain consistent and harmonised results, it is proposed to use:

- I1 (SP, aggressive) as the standard isolate
- the warm condition 24C°/24°C with 16 hours light
 - the standard condition 23C°/18°C appears to be a little bit less reliable
- Perlita as IR/R control that distinguishes between S and IR/R.
 - As a consequence of this proposal, Ducral, San Marcos and Doral will be classified as Susceptible, in spite of a low level of resistance being present in these varieties under certain circumstances.
- Two classes for DUS: resistance present and resistance absent. The level of resistance will not be relevant.
 - “Resistance present” in DUS may correspond with IR and/or R in the market.
- Three classes for market communication.
 - A test with more than 30 plants may be advisable, in order to distinguish IR and HR.

5. Conclusions

The following conclusions were discussed and agreed in an online meeting of the participants on 9 March 2023.

- Consistent results between labs with 2 resistance levels
- Inconsistent results between labs with 3 resistance levels
- Perlita is needed/suitable to define the border between S and IR/R
- Perlita does not provide decisive information to separate IR and HR

- There is no need to adapt the differential host table on the ISF website because Perlita is not a differential.
- There is a need to adapt the UPOV guideline/CPVO protocol, adding Perlita as a resistant control and explaining how to use Perlita for validation of the experiment, and for interpretation of the observation obtained from varieties that are different from susceptible and resistant control varieties. A proposal for a new wording of the UPOV protocol can be found in Annex 5.

Tables and Annexes:

Table 1. Varieties, codes and expected performance with Fom: 2

- ringtest codes were used to prevent observers bias (repetitions were hidden)
- codes of Ducral, Doral and San Marcos are revealed here
- the original plan contained also Durango under code #05, but unfortunately a wrong seed sample has been used.
- reference data can be found in Harmores3 and in Annex 1 (isolate R = SP)

This Report	Ringtest Code	Variety	Expectation in proposal	Updated expectation	Code in Harmores3	Confirmed in Harmores3
1	#01	Charentais Fom-1	HR	HR		R
2	VEDRA	Védrantais	HR	HR		R
3	#08	HMC-1	IR	IR	-	
4	#06	Perlita	IR	IR	-	
5	#07	Ducral	IR		Harmo-19C	no
6	#04	San Marcos	IR	S	-	
7	#02	Doral	IR	S	Harmo-19E/Bayer-E	
8	MARIA	Marianna	S			S
9	#03	Charentais Fom-2	S			S
10	#05	unknown			-	

Table 2. Judgments Resistant (R/IR) Susceptible (S) and Heterogeneous (HG) for isolates I1 and I2, conditions Cold, Standard and Warm, and 10 varieties. Annex 2 and 3 show the translation of triplicate to single judgments for R/IR and S. Additionally, repartitions were used for HG. Judgments are counted, with and without HG.

	Lab A	Lab-C	Lab-D	Lab-E	Lab-F	Lab-G	Lab-H	Lab-I	Lab-J	Lab-K	Lab-L	Lab-M	Lab-N	Judgments				
														with HG			without HG	
I1 (SP)														R/IR	HG	S	R/IR	S
Cold														R/IR	HG	S	R/IR	S
Charentais Fom-1	R/IR	R/IR		R/IR			R/IR	R/IR				R/IR		6	0	0	7	0
Védrantais	R/IR	HG		R/IR			R/IR	R/IR				R/IR		5	1	0	7	0
HMC-1	R/IR	S		HG			R/IR	HG				S		2	2	2	4	3
Perlita	HG	S		S			S	HG				HG		0	3	3	2	5
Ducral	S	S		S			S	HG				S		0	1	5	0	7
San Marcos	S	S		S			S	HG				S		0	1	5	1	6
Doral	S	S		S			S	S				S		0	0	6	0	7
Marianna	S	S		S			S	S				S		0	0	6	0	7
Charentais Fom-2	S	S		S			S	S				S		0	0	6	0	7
#05	S	S		S			S	S				S		0	0	6	0	7
Standard														R/IR	HG	S	R/IR	S
Charentais Fom-1			R/IR		R/IR	R/IR		R/IR	R/IR		HG	R/IR		6	1.00	0	7	0
Védrantais			R/IR	R/IR	R/IR	R/IR		HG	R/IR		HG	R/IR	R/IR	7	2	0	9	0
HMC-1			R/IR	HG	R/IR	R/IR		HG	R/IR		S	S	R/IR	5	2	2	5	4
Perlita			R/IR	S	R/IR	R/IR		HG	R/IR		S	S	R/IR	5	1	3	5	4
Ducral			S	S	S	S		HG	R/IR		S	S	HG	1	2	6	1	8
San Marcos			S	S	S	S		S	R/IR		S	S	S	1	0	8	1	8
Doral			S	S	S	S		S	S		S	S	S	0	0	9	0	9
Marianna			S	S	S	S		S	S		S	S	S	0	0	9	0	9
Charentais Fom-2			S	S	S	S		S	S		S	S	S	0	0	9	0	9
#05			S	S	S	S		S	S		S	S	S	0	0	9	0	8
Warm														R/IR	HG	S	R/IR	S
Védrantais	R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	10	0	0	10	0
HMC-1	R/IR	R/IR	HG		R/IR	R/IR	R/IR		R/IR	R/IR	S		R/IR	8	1	1	9	1
Perlita	S	R/IR	HG		R/IR	R/IR	R/IR		R/IR	R/IR	S		R/IR	7	1	2	7	3
Ducral	S	HG	S		S	R/IR	S		R/IR	S	S		S	2	1	7	2	8
San Marcos	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10
Doral	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10
Marianna	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10
Charentais Fom-2	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10
#05	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10
I2 (US)														R/IR	HG	S	R/IR	S
Cold														R/IR	HG	S	R/IR	S
Charentais Fom-1	R/IR	R/IR		R/IR			R/IR	R/IR		R/IR		R/IR		7	0	0	7	0
Védrantais	R/IR	R/IR		R/IR			R/IR	HG		R/IR		R/IR		6	1	0	7	0
HMC-1	R/IR	R/IR		HG			R/IR	HG		R/IR		R/IR		5	2	0	7	0
Perlita	R/IR	R/IR		HG			HG	S		R/IR		S		3	2	2	5	2
Ducral	R/IR	R/IR		R/IR			R/IR	HG		R/IR		R/IR		6	1	0	7	0
San Marcos	S	R/IR		HG			R/IR	HG		R/IR		S		3	2	2	4	3
Doral	S	R/IR		HG			R/IR	HG		R/IR		HG		3	3	1	5	2
Marianna	S	S		S			S	S		S		S		0	0	7	0	7
Charentais Fom-2	S	S		S			S	S		S		S		0	0	7	0	7
#05	S	S		S			S	S		S		S		0	0	6	0	6
Standard														R/IR	HG	S	R/IR	S
Charentais Fom-1			R/IR		R/IR	R/IR		R/IR		R/IR		R/IR		6	0	0	6	0
Védrantais			R/IR	HG	R/IR	R/IR		R/IR	R/IR		R/IR	HG	R/IR	7	2	0	9	0
HMC-1			R/IR	R/IR	R/IR	R/IR		R/IR	R/IR		R/IR	HG	R/IR	8	1	0	8	1
Perlita			R/IR	HG	R/IR	R/IR		S	R/IR		R/IR	HG	R/IR	6	2	1	7	2
Ducral			R/IR	R/IR	R/IR	R/IR		R/IR	R/IR		R/IR	HG	R/IR	8	1	0	9	0
San Marcos			R/IR	HG	S	R/IR		R/IR	R/IR		R/IR	R/IR	HG	6	2	1	7	2
Doral			S	S	S	R/IR		R/IR	R/IR		R/IR	S	R/IR	5	0	4	5	4
Marianna			S	S	S	S		S	S		S	S	S	0	0	9	0	9
Charentais Fom-2			S	S	S	S		S	S		S	S	S	0	0	9	0	9
#05			S	S	S	S		S	S		S	S	S	0	0	9	0	9
Warm														R/IR	HG	S	R/IR	S
Védrantais	R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	10	0	0	10	0
HMC-1	HG	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	9	1	0	10	0
Perlita	S	R/IR	HG		R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	8	1	1	9	1
Ducral	HG	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	9	1	0	10	0
San Marcos	S	S	HG		R/IR	R/IR	R/IR		R/IR	R/IR	R/IR		R/IR	7	1	2	8	2
Doral	S	R/IR	HG		HG	R/IR	S		R/IR	R/IR	R/IR		R/IR	6	2	2	6	4
Marianna	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10
Charentais Fom-2	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10
#05	S	S	S		S	S	S		S	S	S		S	0	0	10	0	10

Table 3. Average disease index (DI) for the ten varieties in the ring test for each isolate and treatment and laboratory. Conclusions are based on the average DI of all labs, using Perlita as the border between R/IR and S. The same decision rule was applied to the DI of individual labs. Discarding lab conclusions are highlighted in yellow. The frequency of discordance among labs is given as a percentage.

	Lab-A	Lab-C	Lab-D	Lab-E	Lab-F	Lab-G	Lab-H	Lab-I	Lab-J	Lab-K	Lab-L	Lab-M	Lab-N	Average	decision if Perlita = R	Discordance		
																based on DI	Judgm. with HG	Judgm. without HG
I1 (SP)																		
Cold																		
Charentais Fom-1	0.03	0.15		0.04			0.38	0.07				0.10		0.11	R/IR	-	-	-
Védrantais	0.01	0.23		0.10			0.49	0.11						0.13	R/IR	-	-	-
HMC-1	0.12	0.85		0.47			0.70	0.47				0.63		0.46	R/IR	0.14	0.33	0.43
Perlita	0.24	0.86		0.88			0.84	0.51		0.58	0.56	0.91		0.64	R/IR	-	0.50	0.71
Ducral	0.92	1.00		0.95			1.00	0.63		0.82	0.91	0.91		0.89	S	-	0.17	-
San Marcos	1.00	0.96		1.00			1.00	0.21		0.94	0.90	0.90		0.86	S	0.14	0.17	0.14
Doral	1.00	0.99		1.00			0.97	0.94		0.97	0.95	0.95		0.97	S	-	-	-
Marianna	1.00	1.00		1.00			1.00	0.93		0.99	0.99	0.91		0.97	S	-	-	-
Charentais Fom-2	1.00	1.00		1.00			1.00	1.00		0.98	1.00	1.00		1.00	S	-	-	-
#05	1.00	1.00		0.93			1.00	1.00		0.96	1.00	1.00		0.99	S	-	-	-
Standard																		
Charentais Fom-1					0.02	0.31					0.34			0.11	R/IR	-	-	-
Védrantais				0.11		0.33		0.22			0.27	0.25		0.13	R/IR	-	-	-
HMC-1			0.03	0.71	0.04	0.60		0.31	0.06		0.88	0.62		0.36	R/IR	0.22	0.22	0.44
Perlita			0.23	0.98	0.06	0.48		0.70	0.50		0.80	0.82	0.04	0.51	R/IR	-	0.33	0.44
Ducral			0.77	1.00	0.97	0.73		0.78	0.20		0.99	0.78	0.61	0.76	S	0.22	0.33	0.11
San Marcos			0.92	1.00	1.00	1.00		1.00	0.34		1.00	0.88	0.87	0.89	S	0.11	0.11	0.11
Doral			0.97	1.00	1.00	0.96		0.97	0.57		1.00	0.70	1.00	0.91	S	0.11	-	-
Marianna			0.94	1.00	0.93	0.98		0.99	0.67		1.00	0.96	1.00	0.94	S	-	-	-
Charentais Fom-2			0.96	1.00	0.95	1.00		1.00	0.81		1.00	0.98	1.00	0.97	S	-	-	-
#05			1.00	1.00	0.93	0.98		1.00	0.89		1.00	1.00	1.00	0.97	S	-	-	-
Warm																		
Védrantais	0.06	0.07	0.02			0.26	0.02				0.07			0.02	R/IR	-	-	-
HMC-1	0.18	0.11	0.38		0.01	0.29	0.12		0.01	0.04	0.77			0.07	R/IR	0.20	0.10	0.10
Perlita	0.78	0.24	0.61		0.08	0.42	0.41		0.27	0.66	0.83			0.10	R/IR	-	0.20	0.30
Ducral	0.97	0.76	1.00		0.86	0.66	0.79		0.33	0.78	0.98			0.44	R/IR	-	0.30	0.20
San Marcos	0.97	1.00	1.00		1.00	0.92	0.98		1.00	0.93	1.00			0.94	R/IR	-	-	-
Doral	1.00	1.00	1.00		1.00	0.96	1.00		0.98	1.00	1.00			1.00	R/IR	-	-	-
Marianna	1.00	1.00	1.00		0.87	0.94	0.96		0.97	0.99	1.00			0.98	R/IR	-	-	-
Charentais Fom-2	1.00	1.00	1.00		0.92	0.94	0.97		0.96	0.98	1.00			0.98	R/IR	-	-	-
#05	0.99	1.00	0.96		0.98	0.99	1.00		1.00	0.97	1.00			1.00	R/IR	-	-	-
I2 (US)																		
Cold																		
Charentais Fom-1	0.01	0.04		0.07			-	-				0.10		0.03	R/IR	-	-	-
Védrantais	-	0.03		0.01			0.11	0.48						0.09	R/IR	-	-	-
HMC-1	0.20	0.02		0.34			0.10	0.62			0.27			0.22	R/IR	0.14	-	-
Perlita	0.68	0.19		0.14			0.49	0.93		0.07	0.60			0.44	R/IR	-	0.71	0.71
Ducral	0.14	0.06		0.13			0.23	0.24		-	0.33			0.17	S	1.00	1.00	1.00
San Marcos	0.94	0.33		0.58			0.26	0.35		-	0.68			0.45	S	0.43	0.71	0.57
Doral	0.91	0.46		0.68			0.69	0.44		0.03	0.26			0.50	S	0.43	0.86	0.71
Marianna	1.00	0.95		0.97			1.00	0.98		0.99	0.99			0.90	S	-	-	-
Charentais Fom-2	1.00	1.00		1.00			1.00	0.89		1.00	1.00			0.98	S	0.14	-	-
#05	1.00	0.92		1.00			1.00	0.83		0.98	0.98			0.95	S	0.17	-	-
Standard																		
Charentais Fom-1				-		0.27								0.04	R/IR	-	-	-
Védrantais				0.15		0.27				0.01		0.24		0.07	R/IR	-	-	-
HMC-1				0.09	0.03	0.46			0.02		0.44			0.12	R/IR	0.11	-	0.11
Perlita			0.03	0.42	0.03	0.48		0.56	0.12		0.08	0.44		0.24	R/IR	-	0.11	0.22
Ducral			0.10	0.10	0.36	0.57		-	-	-	0.20			0.15	S	0.56	1.00	1.00
San Marcos			0.43	0.66	0.63	0.30		0.10			-		0.17	0.25	S	0.56	0.89	0.78
Doral			0.70	0.84	0.64	0.50		-	-		0.10	0.56	0.07	0.38	S	0.22	0.56	0.56
Marianna			1.00	0.97	1.00	0.86		0.85	0.88		1.00	0.89	1.00	0.94	S	-	-	-
Charentais Fom-2			0.97	0.99	1.00	1.00		0.99	0.98		1.00	0.98	1.00	0.99	S	-	-	-
#05			1.00	1.00	1.00	1.00		1.00	1.00		1.00	1.00	1.00	1.00	S	-	-	-
Warm																		
Védrantais	0.16	-				0.22	0.01							0.02	R/IR	-	-	-
HMC-1	0.58	0.02				0.22	0.01							0.08	R/IR	-	-	-
Perlita	0.93	0.06	0.14			0.39	0.14			0.34				0.03	R/IR	-	0.10	0.10
Ducral	0.46	0.07	0.17			0.10			0.01	-	0.06			0.09	S	0.40	1.00	1.00
San Marcos	1.00	0.86	0.40		0.14	0.12	0.51		-	0.02	-			0.01	S	0.30	0.80	0.80
Doral	1.00	0.11	0.78		0.52	0.18	0.67			0.03	0.24			0.05	S	0.20	0.80	0.60
Marianna	1.00	1.00	1.00		0.99	0.71	1.00		0.91	0.84	1.00			1.00	R/IR	-	-	-
Charentais Fom-2	1.00	0.92	1.00		1.00	0.93	1.00		1.00	0.96	0.93			1.00	R/IR	-	-	-
#05	1.00	1.00	1.00		0.88	0.96	1.00		1.00	0.97	1.00			1.00	R/IR	-	-	-

Table 4. Analysis for isolate I1 and I2 in warm condition in Pathostat, with control varieties Védrantais “R”, Perlita “IR - lower bound” and Marianna “S”. Pathostat interpretations are R (green), IR (orange), S (yellow) or heterogeneous (%).

Isolate I1 (warm condition)	Lab A	Lab-C	Lab-D	Lab-F	Lab-G	Lab-H	Lab-J	Lab-K	Lab-L	Lab-N
Védrantais (Resistant)	R	R	R	R	R	R	R	R	R	R
HMC-1	IR	R	IR	R	R	IR	R	IR	IR	R
Perlita (Intermediate)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Ducral	S	S	S	S	S	S	S	S	S	S
San Marcos	S	S	S	S	S	S	S	S	S	S
Doral	S	S	S	S	S	S	S	S	S	S
Marianna (Susceptible)	S	S	S	S	S	S	S	S	S	S
Charentais Fom-2	S	S	S	S	S	S	S	S	S	S
#05	S	S	S	S	S	S	S	S	S	S
Isolate I2 (warm condition)	Lab A	Lab-C	Lab-D	Lab-F	Lab-G	Lab-H	Lab-J	Lab-K	Lab-L	Lab-N
Védrantais (Resistant)	R	R	R	R	R	R	R	R	R	R
HMC-1	IR	R	R	R	R	R	R	R	R	R
Perlita (Intermediate)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Ducral	IR	IR	IR	R	R	R	R	R	R	R
San Marcos	S	S	S	S	R	S	R	R	R	R
Doral	S	IR	S	S	R	S	R	IR	S	R
Marianna (Susceptible)	S	S	S	S	S	S	S	S	S	S
Charentais Fom-2	S	%	S	S	S	S	S	S	S	S
#05	S	S	S	%	S	S	S	S	S	S

Table 5. Disease index (DI) for isolate I1 and I2 in warm condition. Green = DI lower than Perlita, yellow = DI higher than Perlita, white: DI the same as Perlita (accuracy: two decimals)

Isolate I1 (warm condition)	Lab A	Lab-C	Lab-D	Lab-F	Lab-G	Lab-H	Lab-J	Lab-K	Lab-L	Lab-N
Védrantais (Resistant)	0.06	0.07	0.02	0.00	0.26	0.02	0.00	0.00	0.07	0.02
HMC-1	0.18	0.11	0.38	0.01	0.29	0.12	0.01	0.04	0.77	0.07
Perlita (Intermediate)	0.78	0.24	0.61	0.08	0.42	0.41	0.27	0.66	0.83	0.10
Ducral	0.97	0.76	1.00	0.86	0.66	0.79	0.33	0.78	0.98	0.44
San Marcos	0.97	1.00	1.00	1.00	0.92	0.98	1.00	0.93	1.00	0.94
Doral	1.00	1.00	1.00	1.00	0.96	1.00	0.98	1.00	1.00	1.00
Marianna (Susceptible)	1.00	1.00	1.00	0.87	0.94	0.96	0.97	0.99	1.00	0.98
Charentais Fom-2	1.00	1.00	1.00	0.92	0.94	0.97	0.96	0.98	1.00	0.98
#05	0.99	1.00	0.96	0.98	0.99	1.00	1.00	0.97	1.00	1.00

Isolate I2 (warm condition)	Lab A	Lab-C	Lab-D	Lab-F	Lab-G	Lab-H	Lab-J	Lab-K	Lab-L	Lab-N
Védrantais (Resistant)	0.16	0.00	0.00	0.00	0.22	0.01	0.00	0.00	0.00	0.02
HMC-1	0.58	0.02	0.00	0.00	0.22	0.01	0.00	0.00	0.00	0.00
Perlita (Intermediate)	0.93	0.06	0.14	0.00	0.39	0.14	0.00	0.34	0.00	0.03
Ducral	0.46	0.07	0.17	0.00	0.10	0.00	0.01	0.00	0.06	0.00
San Marcos	1.00	0.86	0.40	0.14	0.12	0.51	0.00	0.02	0.00	0.01
Doral	1.00	0.11	0.78	0.52	0.18	0.67	0.00	0.03	0.24	0.05
Marianna (Susceptible)	1.00	1.00	1.00	0.99	0.71	1.00	0.91	0.84	1.00	1.00
Charentais Fom-2	1.00	0.92	1.00	1.00	0.93	1.00	1.00	0.96	0.93	1.00
#05	1.00	1.00	1.00	0.89	0.96	1.00	1.00	0.97	1.00	1.00

Table 6. Disease index (DI) per variety for each isolate and condition.
Green = DI lower than Perlita, yellow = DI higher than Perlita

Variety	I1 (SP)			I2 (US)			Average
	Cold	Standard	Warm	Cold	Standard	Warm	
Charentais Fom-1	0.11	0.11		0.03	0.04		0.07
Védrantais	0.13	0.13	0.05	0.09	0.07	0.04	0.08
HMC-1	0.46	0.36	0.20	0.22	0.12	0.08	0.23
Perlita	0.64	0.51	0.44	0.44	0.24	0.20	0.40
Ducral	0.89	0.76	0.76	0.17	0.15	0.09	0.46
San Marcos	0.86	0.89	0.97	0.45	0.25	0.31	0.62
Doral	0.97	0.91	0.99	0.50	0.38	0.36	0.68
Marianna	0.97	0.94	0.97	0.97	0.94	0.95	0.96
Charentais Fom-2	1.00	0.97	0.98	0.98	0.99	0.97	0.98
#05	0.99	0.97	0.99	0.95	1.00	0.98	0.98

Annex 1- Data from Harmores3 (2019), for comparison with ringtest ISF 2022

Fom: 2, strain L (K, MATREF 4-7-1-2, more aggressive)																		
Variety	uncoded control	ISF 2022	Soaking root and transplanting											Injection	Expected	Repartition	Discordance	Confirmed
			lab 3	lab 4	lab 5	lab 6	lab 8	lab 10	lab 11	lab 12	lab 14	lab 18	lab 16					
Solemio	*		S	S	S	S	S	S	S	S	S	S	S	S	S	S	0%	S
Solemio			S	S	S	S	S	S	S	S	S	S	S	S	IR	S	8%	S
Marianna	*		S	HG	S	S	S	S	S	S	S	S	S	S	S	S	8%	S
Marianna		x	S	HG	HG	S	S	S	S	S	S	S	S	IR	S	S	23%	S
Charentais Fom-2		x	S	HG	HG	S	S	S	S	S	S	S	S	S	S	S	17%	S
Harmo2018-1			S	HG	S	S	S	HG	S	S	S	IR	S	IR	C	91%		
Harmo-19D			IR	HG	S	S	S	S	S	HG	S	IR	IR	IR	C	73%		
Charentais Fom-1	*	x	R	R	R	R	R	HG	R	R	R	R	R	R	R	R	8%	R
Charentais Fom-1		x	R	R	R	R	R	HG	R	R	R	R	R	IR	R	R	17%	R
Kiros			R	R	R	R	R	R	R	R	R	R	R	R	R	R	0%	R
MR-1			R	R	R	R	R	R	R	R	R	R	R	IR	R	R	9%	R
Vedrantais		x	R	R	R	R	R	R	R	R	R	R	R	R	R	R	0%	R
Harmo-19C		x	HG	R	HG	S	IR=S	HG	S	R	S	R	IR	R	C	73%		
Harmo-19E			HG								HG			R		100%		

*uncoded controls

Legend: S: susceptible; IR: intermediate resistant; R: resistant; HG: heterogeneous

Repartition = C (continuum) indicates a need for repeating the test

Fom: 2, strain R – M, F185, less aggressive

Fom: 2, strain R – M, F185, less aggressive																		
Variety	uncoded control	ISF 2022	Soaking root and transplanting											Injection	Expected	Repartition	Discordance	Confirmed
			lab 3	lab 4	lab 5	lab 6	lab 8	lab 10	lab 11	lab 12	lab 14	lab 18	lab 2					
Solemio	*		S	S	S	S	S	S	S	S	S	S	S	S	S	S	0%	S
Solemio			S	S	S	S	S	S	S	S	S	S	IR	S	S	S	9%	S
Marianna	*		S	S	S	S	S	S	S	S	S	S	S	S	S	S	0%	S
Marianna		x	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0%	S
Charentais Fom-2		x	S	S	S	S	S	S	S	S	S	S	S	S	S	S	0%	S
Harmo2018-1			S	S	S	S	S	S	S	S	S	S	IR	S	IR	C	91%	
Harmo-19D			S	S	HG	S	S	S	S	IR	S	IR	IR	IR	C	73%		
Charentais Fom-1	*	x	R	R	R	R	R	HG	R	R	IR	R	R	R	R	R	18%	R
Charentais Fom-1		x	R	R	R	R	R	R	R	R	HG	R	R	R	R	R	9%	R
Kiros			R	R	R	R	R	HG	R	R	S	R	R	R	R	R	18%	R
MR-1			IR	IR	R	R	R	R	R	R	IR	R	R	R	R	R	27%	R
Vedrantais		x	R	IR	R	R	R	R	R	R	IR	R	R	R	R	R	18%	R
Harmo-19C		x	HG	S	S	S	S	S	S	HG	S	R	R	R	C	82%		

Note: Harmo-19C was expected R in Harmores3, but is expected S in ISF 2022

Annex 2 – Translation of judgment triplets into single judgments. The triplets were obtained from a one variety, isolate and condition in one laboratory. The single judgments were used for evaluating the results in Annex 3.

Legend: R = Resistant, S = Susceptible, I = Intermediate, % = heterogeneous, ? = undecided
* = possibly heterogeneous

Note: Some labs have used IS for judging.

Triplet of judgments	R/IR				S			
	3R	3IR	mixed R-IR	mix RRS	mix RSS	mix S-	mix SS-	3S
	Ra	Rb	Rc	RRS	RSS	Sa	Sb	Sc
%%%			4					
%IR?%			1					
???			4					
??R			2					
?IRIR			1					
?R?			1					
?RR			2					
?SS							1	
IRIR?			1					
IRIRIR		20						
IRIRR?			1					
R%%			1					
R*RR*	1							
R?R			1					
RIR?			2					
RIRIR			18					
RIRIR?			1					
RR	1							
RR%			4					
RR?	3							
RRIR			16					
RRR	130							
RRR*	1							
S% S?								1
S%%						4		
S%S?						1		
S??						2		
SIR%						1		
SIR?						1		
SIRIR						5		
SISIR						1		
SISIS						1		
SRR				6				
SS								5
SS%							3	
SS?							7	
SSIR							9	
SSIR?							1	
SSR					8			
SSS								219
	136	20	61	6	8	16	21	225

Annex 3 – Evaluation of single judgments per isolate, condition and variety for all labs together.

Single judgment:	R/IR							S				Total	R/IR	S	%S	
	3R	3IR	mixed R-IR	mix RRS	mix RSS	mix S-	mix SS-	3S								
3 judgments in same lab:																
I1 (SP)	39	10	26	2	4	9	14	143	247							
Cold	11	3	7		3	2	4	40	70							
Charentais Fom-1	5		2						7	7	0	0%				
Védrantais	4	1	2						7	7	0	0%				
HMC-1	2	1	1		2		1		7	4	3	43%				
Perlita		1	1		1	1	1	2	7	2	5	71%				
Ducral						1	1	5	7	0	7	100%				
San Marcos			1					6	7	1	6	86%				
Doral								7	7	0	7	100%				
Marianna								7	7	0	7	100%				
Charentais Fom-2								7	7	0	7	100%				
#05								6	7	0	7	100%				
Standard	14	2	10	2	1	3	8	47	87	R/IR	S	%S				
Charentais Fom-1	5	1	1						7	7	0	0%				
Védrantais	5		2	2					9	9	0	0%				
HMC-1	2	1	2			1	1	2	9	5	4	44%				
Perlita	2		3		1		1	2	9	5	4	44%				
Ducral			1			2	2	4	9	1	8	89%				
San Marcos			1				2	6	9	1	8	89%				
Doral								9	9	0	9	100%				
Marianna							1	8	9	0	9	100%				
Charentais Fom-2								9	9	0	9	100%				
#05							1	7	8	0	8	100%				
Warm	14	5	9			4	2	56	90							
Védrantais	8		2						10	10	0	0%				
HMC-1	5		4					1	10	9	1	10%				
Perlita	1	3	3			1	1	1	10	7	3	30%				
Ducral		2				3	1	4	10	2	8	80%				
San Marcos								10	10	0	10	100%				
Doral								10	10	0	10	100%				
Marianna								10	10	0	10	100%				
Charentais Fom-2								10	10	0	10	100%				
#05								10	10	0	10	100%				
I2 (US)	97	10	35	4	4	7	7	82	246							
Cold	23	5	12	2	1	2	3	21	69							
Charentais Fom-1	7								7	7	0	0%				
Védrantais	6		1						7	7	0	0%				
HMC-1	2	1	3	1					7	7	0	0%				
Perlita	2	2	1		1	1			7	5	2	29%				
Ducral	4		3						7	7	0	0%				
San Marcos	1		3			1		2	7	4	3	43%				
Doral	1	2	1	1			1	1	7	5	2	29%				
Marianna								7	7	0	7	100%				
Charentais Fom-2							1	6	7	0	7	100%				
#05							1	5	6	0	6	100%				
Standard	34	3	12	2	3	2	2	29	87							
Charentais Fom-1	5		1						6	6	0	0%				
Védrantais	8			1					9	9	0	0%				
HMC-1	7		1		1				9	8	1	11%				
Perlita	4		3		1	1			9	7	2	22%				
Ducral	4	1	3	1					9	9	0	0%				
San Marcos	3	1	3			1		1	9	7	2	22%				
Doral	3	1	1		1		1	2	9	5	4	44%				
Marianna							1	8	9	0	9	100%				
Charentais Fom-2								9	9	0	9	100%				
#05								9	9	0	9	100%				
Warm	40	2	11			3	2	32	90							
Védrantais	9		1						10	10	0	0%				
HMC-1	8		2						10	10	0	0%				
Perlita	5	1	3					1	10	9	1	10%				
Ducral	8		2						10	10	0	0%				
San Marcos	6		2			1	1	1	10	8	2	20%				
Doral	4	1	1			2		2	10	6	4	40%				
Marianna							1	9	10	0	10	100%				
Charentais Fom-2								10	10	0	10	100%				
#05							1	9	10	0	10	100%				
	136	20	61	6	8	16	21	225	493							

The table is expanded for separate labs in Table 3.

Each variety was judged in three replications per lab. Judgment triplets were translated into two classes as in Annex 2.

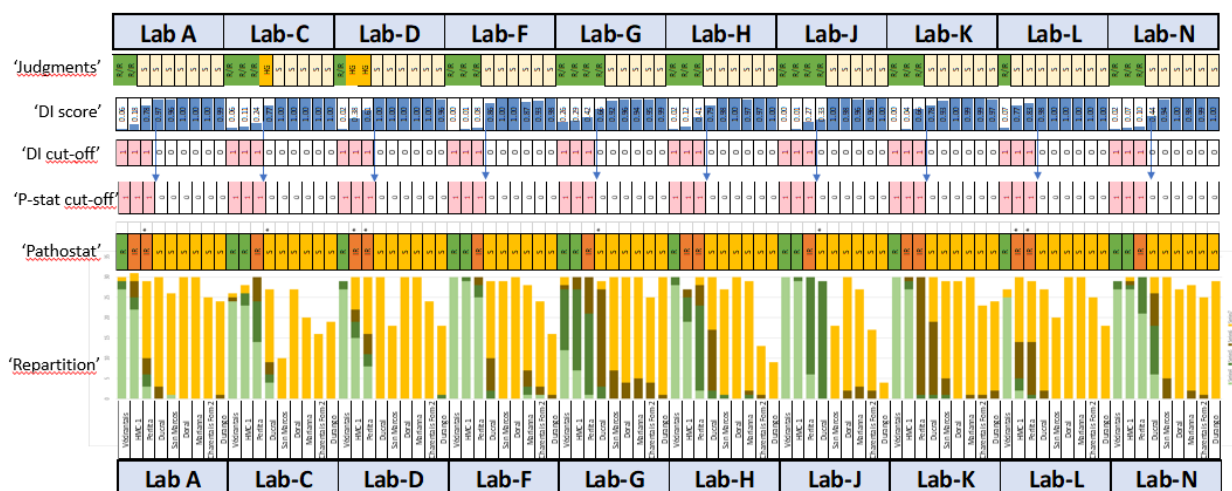
The single judgements are counted, and the percentage susceptible judgments is calculated in columns on the right.

The single judgment counts are repeated in Table 3

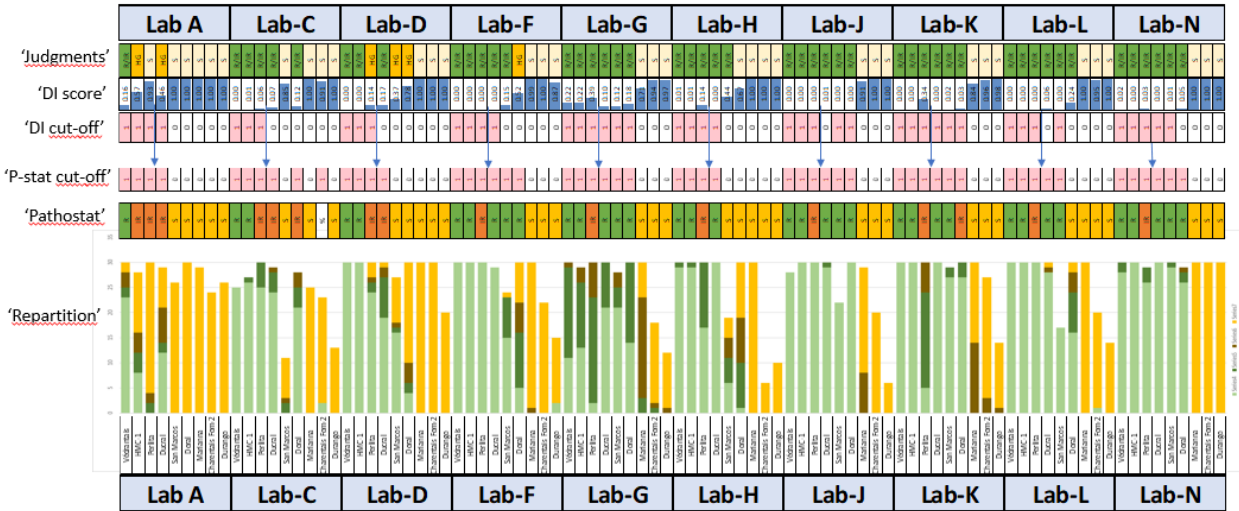
Annex 4. Comparison of results from 10 Labs (A-N)

- ‘Judgments’ - by the observers in each lab, separately for three repetitions of 10 plants, and integrated to a single judgment as explained in annex 2 and 3.
- ‘DI score’ - averaged over 30 plants
- ‘DI cut-off’, based on the comparison of ‘DI score’ of the variety with the DI score of Perlita as the lower border of IR,
- ‘P-stat cut-off’, based on the Pathostat decisions,
- ‘Pathostat’ – decisions with Perlita as IR lower bound, Védrantais as R, Marianna as S control.
- ‘Repartition’ – stacked bar graph of the DI scores
- Variety names

Fom: 2 ISF ringtest in 10 Labs in Warm Condition with Isolate I1



Fom: 2 ISF ringtest in 10 Labs in Warm Condition with Isolate I2



Annex 5. Proposal for a draft updated UPOV protocol for the characteristic Fom.

Note: UPOV TWV was from 1 to 5 May 2023. This proposal was written in March 2023 as a draft for UPOV and as far as possible corrected according to the discussion at the TWV. But the final document of the TWV can be slightly different.

Ad 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> <u>races 0, 1, and 2</u>
2.	Quarantine status	No
3.	Host species	Melon - <i>Cucumis melo</i>
4.	Source of inoculum	e.g. GEVES (FR) ¹
5.	Isolate	e.g., Reference strain validated in an inter-laboratory test ^{2, 3} Fom: 2 - Strain F185
6.	Establishment isolate identity	The most recent table is available through ISF at https://www.worldseed.org/our-work/plant-health/differential-hosts/Situation July 2019

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

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

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

Courtesy of Worldseed.org website



7.	Establishment pathogenicity	use susceptible melon varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	on agar medium – e.g., Potato Dextrose Agar, Malt agar at 20°C to 25°C
8.2	Multiplication variety	-
8.3	Plant stage at inoculation	-
8.5	Inoculation method	-
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 or keep cool to prevent spore germination

¹ matref@geves.fr

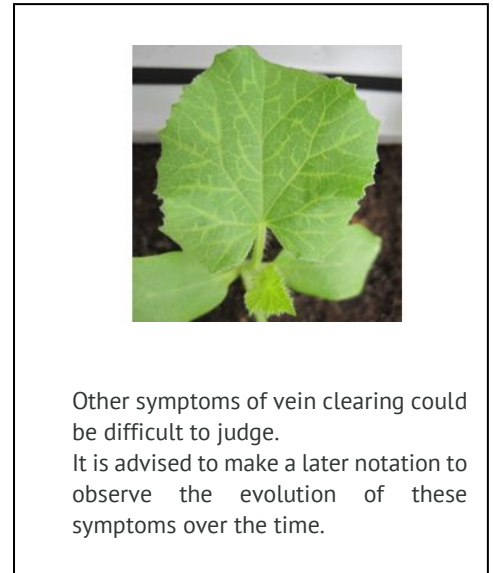
² Harmores 3 CPVO project (https://cpvo.europa.eu/sites/default/files/documents/report_harmores_3_final_meeting_v0_0.pdf)

³ ISF EG DRT Fom: 2 resistance in Melon – [Link to include – not yet available](#)

9.	Format of the test	
9.1	Number of plants per genotype	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 (3 x10)
9.3	Control varieties	
<u>9.3.3</u>	Control varieties for race 2	Resistance absent: Marianna Resistance present: Perlita, Charentais Fom-1, Védrantais
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	- Fom: 2: 24°C
9.7	Light	- Fom: 2: 16h
9.9	Special measures	
10.	Inoculation	
10.1	Preparation inoculum	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). <i>Remark:</i> Beware of toxin productions by some isolates (see remark under 13.)
10.2	Quantification inoculum	4x10 ⁵ to 1x10 ⁶ sp /mL
10.3	Plant stage at inoculation	cotyledon expanded
10.4	Inoculation method	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	1 st notation: symptoms on Resistance absent (susceptible) control at classes 2 and 3 with a strong proportion at class 3
10.6	Second observation	A second notation can be necessary to re-evaluate some unclear varieties
11.	Observations	
11.1	Method	Visual <u>observation</u>
11.2	Observation scale	

Mock	Class 0	Class 1
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
		

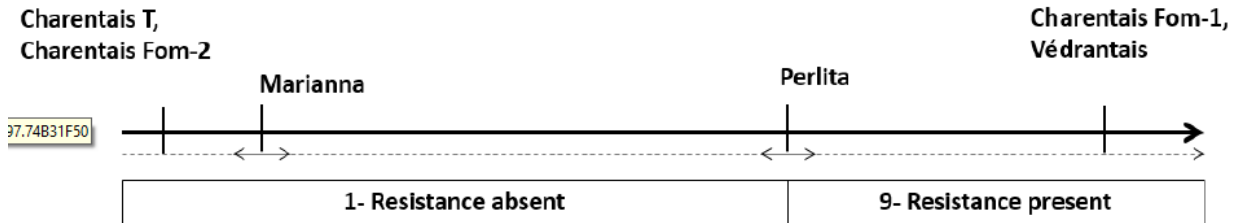
Class 2	Class 3
typical symptoms : yellowing, wilting and necrosis, stunting (growth stopped)	Death of plant (Dead)



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

11.3	Validation of test	<p>Validation on controls. In case of the Fom: 2 test Controls expected response:</p> <ul style="list-style-type: none"> • Susceptible controls, with UPOV characteristic state 'Resistance absent', should have most of the plants in observation classes 2 and 3, and few or no plants in observation classes 0 or 1. <ul style="list-style-type: none"> ○ Marianna, the susceptible control is less susceptible than Charentais Fom-2, Charentais T • Resistant controls should have most of the plants in observation classes 0 and 1, and few or no plants in observation classes 2 or 3. <ul style="list-style-type: none"> ○ Perlita, the lower threshold resistance control should have at least some plants in observation class 1, 2 or 3. It has to be less resistant than Charentais Fom-1, Védrentais.
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	<p>In case of varieties with a response between the susceptible (resistance absent) and the resistant control, repeat the test. In case of confirmation of the result, the variety will be judged heterogeneous. In case of unclear results, retest or test in another lab.</p>

Resistance to Fom: 2



13.	Critical control points	<p>For race 2, the control Perlita, with the Fom-3 gene, allows 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.</p> <p>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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