2019-2023

ISF Podosphaera xanthii project Melon

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ISF DRT WG

2019-2023

Project for validation of isolates and differentials for *Podosphaera xanthii* (Px) - Melon

Introduction:

Background:

Powdery mildew is one of the major diseases of melon worldwide. Two species are involved: *Podosphaera xanthii* and *Golovinomyces cichoracearum*. Several races have been identified for both species. This study will be focused only on *Podosphaera xanthii* (Px). From years of field test results, prevalent races in the US seem to be stable in their occurrence and distribution. They are thought to be races 1, 2, 5 and S (S being similar to race 3.5 in the EU). In Europe, a CASDAR project showed that a very high level of virulence profiles was observed on melon, mainly race 3.5 type, while on other cucurbits, mainly race 1 and 2 types were observed. The current differentials and race coding system are not evolutive and can in some case lead to confusions in naming. Therefore, more detailed discussion on these differential responses was needed to develop a core group of differentiating melon hosts appropriate for races that occur in the US and in the EU. Development of a core group of differentiating melon hosts is a crucial step towards understanding similarities and differences between EU and US races of melon powdery mildew. Differential sets have been described by researchers (Lebeda et al., Cucurbitaceae, Avignon 2008; Pitrat, E-phytia 2013), to identify races of Px. Based on this knowledge, the seed industry is willing to validate a differential set and reference isolates for Px on melon, to establish a manageable set of differentials that may be used internationally, and that is based on major resistance genes and races of melon Px that are relevant to the melon production industry, i.e., have an economic impact. A uniform and objective race coding and naming system is needed to communicate commercially relevant races to customers.

The ISF DRT WG CPM project proposes, therefore, to establish a race coding system with a differential set and reference isolates. They would be used worldwide for communication to customers and registration/protection purposes.

During a meeting of the ISF DRT SUB-Working group CPM on melon, publicly available data presented and discussed by Jim McCreight (USDA), Ales Lebeda (Palacky University) and Thierry Jaunet (ESA WG Disease Resistance, ISF DRT, HM.Clause) allowed the group to define candidates for differentials, criteria for reference isolates, and interlaboratory assay protocol. It was planned to validate these candidates with an interlaboratory test.

The interlaboratory test will be coordinated by Valerie Grimault (GEVES) and Sandrine Houdault (GEVES).

Participants:

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Different steps in this project:

1. Collection of isolates from different regions of world and short characterization

2. characterization of 25 different isolates on largest set of differential candidates

➔Definition of a set of differentials and pattern of interest

3. Ring test of validation with the restricted set of isolate and differentials.

4. Purification of isolates and confirmation ring test

5. Definition of a new pattern of different isolates and the two additional races Px: 6 and 7

6. Publication of the results

1. Collection of isolates from different regions of world and short characterization

Differential isolates were harvested by participants, from February 2018 to the end of 2018. The representation of geographic diversity was important. The following areas were mentioned than where isolates were to be collected: South and Central-Eastern Europe, Morocco, Senegal, Middle East- Israel, US, Brazil and in the case of Asia and central America from major production areas. The collection was done in the most representative areas from commercial resistant varieties around 3 to 5 isolates per region. Each provider of isolates did an initial characterisation on short set of differentials.

Table 1 shows the results of the initial characterization. It shows a majority of Px: 3.5 isolates on the reduced set of differentials.

Table 1. Results of initial characterisation

Isolate #	Name and Company	Collection Date	City/Country Collected	GPS Coordinates	Melon Variety	Crop Planting Date	Growth Stage*	Pesticide Applications	Disease Pressure**	Other Pests Present***		Response: Vedrantais	Response : PMR45	Response : PMR5	Response: Edisto47	Response: WMR29	Initial ID based on this system
Px1 -Sm3	MATREF											9	1	1	1	1	Px:1
Px2- S87.7	MATREF											9	9	1	1	1	Px:2
Px3- 00Sm39	MATREF											9	9	9	1	1	Px:3
Px3-5 - 04Sm2	MATREF											9	9	5	9	9	Px:3-5
Px5 - 98Sm65	MATREF											9	9	1	9	9	Px:5
RZ_ID470	RIJK ZWAAN	20/06/2018	Pak Chong (Thaïland)					Not in the last 14 days	high			9	9	9	9	9	Px:3-5
RZ_ID873	RIJK ZWAAN	20/06/2018	Zacapa (Guatemala)						low			9	9	9	9	9	Px:3-5
RZ_ID1154	RIJK ZWAAN	07/06/2018	El Ejido (Spain)						low			9	9	9	9	9	Px:3-5
RZ_ID1198	RIJK ZWAAN	07/06/2018	Licata (Italy)									9	9	9	1	1	Px:3
RZ_ID1296	RIJK ZWAAN	20/06/2018	Qingdao (China)					No treatment has been applied				9	1	1	1	1	Px:1
RZ_ID1330	RIJK ZWAAN	20/06/2018	Johannesburg (South Africa)						med			9	9	1	9	9	Px:5
RZ_ID1849	RIJK ZWAAN	12/10/2018	Torre-Pacheco (Spain)						med			9	9	9	9	9	Px:3-5
RZ_ID2101	RIJK ZWAAN	12/10/2018	Herencia (Spain)						high			9	9	9	9	9	Px:3-5
RZ_ID2163	RIJK ZWAAN	12/10/2018	Cinco Casas (Spain)						med			9	9	9	9	9	Px:3-5
RZ_ID2302	RIJK ZWAAN	12/10/2018	Graveson (France)									9	9	9	9	9	Px:3-5
MON18-1	Monsanto	30/05/2018	Spain-ElEjido	36,47/-2,42	SV7601MC		harvest		med			9	9	5	9	9	Px:3-5
MON18-2	Monsanto	20/06/2018	Spain-Murcia	37,92/-1,25	SV7601MC		harvest		med			9	9	5	9	9	Px:3-5
MON18-3	Monsanto	10/10/2018	Italy-Latina	41,50/12,76	9424		harvest		med			9	9	9	9	9	Px:3-5
Px MUR	Daniel BASF	15/06/2018	Spanish isolate 2018	Torrepacheco	Coliseo (BASF)	Late season	Fruit set	No	Medium	CYSDV, ToLCNDV		9	9	9	9	9	Px:3-5
Px ITA	Daniel BASF	30/05/2018	Italian isolate 2018	Venturina	SV2335 (Seminis)	Mid season	Fruit set	No	High	No		9	9	9	9	9	Px:3-5
SAK-SPA-18-1	Sakata	04/07/2018	Murcia/Spain				harvest		high			9	9	9	9	9	Px:3-5
SAK-SPA-18-2	Sakata	04/07/2018	Murcia/Spain				harvest		high			9	9	9	9	9	Px:3-5
SAK-SPA-18-3	Sakata	04/07/2018	Murcia/Spain				harvest		high			9	9	9	9	9	Px:3-5
SAK-SPA-18-4	Sakata	04/07/2018	Murcia/Spain				harvest		high			9	9	9	9	9	Px:3-5
SRY18-0057	HM.Clause	2018	Spain/Alicante									9	9	9	9	9	Px:3-5
SRY18-0043	HM.Clause	2018	Spain/Alicante									9	9	9	9	9	Px:3-5
Mel2381 - 18-0010	HM.Clause	2018	France/BDR									9	9	1	9	9	Px:5
Mel2381 - 18-0009	HM.Clause	2018	France/BDR		VED							9	9	9	9	9	Px:3-5
SRY-18-0105.1	HM.Clause	2018	France/Lot et Garonne									9	9	9	9	9	Px:3-5
DVS-S	Michelle Ma HM.Clause	43344	Davis, CA, USA									9	9	9	9	NT	Race S
SAK	Marco Bello Sakata Seed America	43435	Fort Myers, FL USA				vegetative		med	none		9	1	1	1	1	Px:1
Px Uber race 586	Craig Sandlin Syngenta Seeds	2007	Yolo county	38.63, -121.8	Unknown		Vegetative	None	High	None		9	9	9	9	9	Uber race
SalGH2018	Jim McCreight USDA, ARS	43525	Salinas (USA)	36.6725 N 121.6094								9	9	9	9	9	Race S
CM18041	Gautier	19/11/2018	Morocco				Harvest		High			9	9	9	9	9	Px:3-5
CM18044	Gautier	19/11/2018	Morocco				Harvest		High			9	9	9	9	9	Px:3-5
CM18043	Gautier	19/11/2018	Morocco				Harvest		High			9	9	9	9	9	Px:3-5
CM18045	Gautier	19/11/2018	Morocco				Harvest		High			9	9	9	9	9	Px:3-5
CM18042	Gautier	19/11/2018	Morocco				Harvest		High			9	9	9	9	9	Px:3-5

Discussion between the partners led to the selection of the most representative list of isolates from the different areas. The main selection criteria were 1) different geographical origins and 2) economic relevance. After discussion, a list of 19 candidate isolates (on the 38 isolates collected) was selected (in green in the table).

Due to the lack of isolates from areas of interest, harvesting and characterization were extended until September 2019. The aim is to obtain isolates from area like Brazil, Guatemala, la Mancha (Spain) and Portugal. ➔5 isolates were added at the end to obtain a list of 25 isolates (Table 2). Theses isolates were selected to be characterized in the next step ringtest.

Table 2 . Isolates selected

	Isolate #	Name and Company	Collection Date	City/Country Collected	Response: Vedrantais	Response : PMR45	Response : PMR5	Response: Edisto47	Response: WMR29	Initial ID based on this system
1	Px1 -Sm3	MATREF			9	1	1	1	1	Px:1
2	Px2- S87.7	MATREF			9	9	1	1	1	Px:2
3	Px3- 00Sm39	MATREF			9	9	9	1	1	Px:3
4	Px3-5 - 04Sm2	MATREF			9	9	5	9	9	Px:3-5
5	Px5 - 98Sm65	MATREF			9	9	1	9	9	Px:5
6	RZ_ID470	RIJK ZWAAN	20/06/2018	Pak Chong (Thaïland)	9	9	9	9	9	Px:3-5
7	RZ_ID873	RIJK ZWAAN	20/06/2018	Zacapa (Guatemala)	9	9	9	9	9	Px:3-5
8	RZ_ID1296	RIJK ZWAAN	20/06/2018	Qingdao (China)	9	1	1	1	1	Px:1
9	RZ_ID1330	RIJK ZWAAN	20/06/2018	Johannesburg (South Africa)	9	9	1	9	9	Px:5
10	MON18-1	Monsanto	30/05/2018	Spain-ElEjido	9	9	5	9	9	Px:3-5
11	Px ITA - venturia	Daniel BASF	30/05/2018	Italian isolate 2018	9	9	9	9	9	Px:3-5
12	SAK-SPA-18-1	Sakata	04/07/2018	Murcia/Spain	9	9	9	9	9	Px:3-5
13	SRY18-0043	HM.Clause	2018	Spain/Alicante (Murcia)	9	9	9	9	9	Px:3-5
14	SRY-18-0105.1	HM.Clause	2018	France/Lot et Garonne	9	9	9	9	9	Px:3-5
15	SAK	Marco Bello Sakata Seed America	43435	Fort Myers, FL USA	9	1	1	1	1	Px:1
16	Px Uber race 586	Craig Sandlin Syngenta Seeds	2007	Yolo county	9	9	9	9	9	Uber race
17	SalGH2018	Jim McCreight USDA, ARS	43525	Salinas (USA)	9	9	9	9	9	Race S
18	CM18043	Gautier	19/11/2018	Morocco	9	9	9	9	9	Px:3-5
19	Px ENVERO	BASF		Spain (La Mancha)	9	9	9	9	9	Px:3-5
20	SRY 18-109-2-Hm clause	HM.Clause		Brazil						
21	Isolat 22- Arum, basf	BASF		Murcia/Spain	9	9	9	9	9	Px:3-5
22	RZ_ID4578	RIJK ZWAAN	02/08/2019	Spain (La Mancha, cuidad Real)	9	9	9	9	9	
23	MON19-4	Monsanto	16/08/2019	Spain (La Mancha)	9	9	9	9	9	Px:3-5A
24	MON19-6	Monsanto	16/08/2019	Spain (La Mancha)	9	9	9	9	9	Px:3-5B
25	MEL 2381-18-27 - Hm clause	HM.Clause		Guatemala						

2. Characterization of 25 different isolates on largest set of differential candidates 2020-2021

The second step was a more complete characterization , by ringtest , of the isolates selected on the panel of differentials with the protocol on whole plant as defined in the project Harmores 3 and published by CPVO (protocol in annexe 1). The GEVES provided to each participant a sample of seeds of each variety's differential. The GEVES had multiplied all the isolates and sent it culture to each participant. The source of isolate was the same for each laboratory.

Meanwhile, some candidate differentials were multiplied by Rijk Zwaan and the purity and homogeneity were controlled genetically.

Table 4. Differentials candidate table used in this project

Variety	Origine seeds
Védrantais	MATREF
PMR 45	MATREF
PMR5	MATREF
Edisto47	MATREF
RIL 1 = RIL N°14 (Védrantais × 414723)	INRAE*
PI482420	MATREF*
RIL 4 =RIL N°44 (Védrantais × 414723)	INRAE *
SVI105	MONSANTO*
WMR29	MATREF
PI124112	MATREF
PI313970	MONSANTO
Ames 31282	MONSANTO
Arum	MATREF
Durango	MATREF
Arago	MATREF

For sending seeds to participating labs MUA's were prepared. ISF collected the signed Framework agreements.

The results of each participant were sent to GEVES with different delays (due to a pandemic period).

Table 5: Rawdata in September 2020

Variety	lab 5 - isolate A	lab 6 - isolate A	lab 7 - isolate B	lab 8 - isolate B	lab 2 - isolate C	lab 3 - isolate C	lab 7 - isolate C	lab 3 - isolate D	lab 6 - isolate D	lab 5 - isolate E	lab 2 - isolate F	lab 7 - isolate F	lab 2 - isolate G	lab 7 - isolate G	lab 7 - isolate H	lab 8 - isolate H	lab 5 - isolate I	lab 8 - isolate I	lab 3 - isolate J	lab 4 - isolate J	lab 8 - isolate J	lab 1 - isolate K	lab 4 - isolate K	lab 8 - isolate K	lab 1 - isolate M	lab 4 - isolate M	lab 8 - isolate M	lab 1 - isolate N	lab 4 - isolate N	lab 6 - isolate N	lab 3 - isolate O	lab 5 - isolate O	lab 2 - isolate P	lab 8 - isolate P	lab 5 - isolate Q	lab 6 - isolate Q	lab 4 - isolate R	lab 8 - isolate R	lab 1 - isolate S	lab 2 - isolate S	lab 3 - isolate S	lab 1 - isolate T	lab 7 - isolate T	lab 4 - isolate U	lab 6 - isolate U	lab 8 - isolate U	lab 3 - isolate V	lab 8 - isolate V	lab 5 - isolate W	lab 6 - isolate W	lab 1 - isolate X	lab 2 - isolate X	lab 8 - isolate Y
Vedrantais	88.9	100.0	100.0	83.3	97.2	96.7	100.0	100.0	100.0	100.0	94.4	100.0	100.0	100.0	100.0	97.2	91.7	94.4	100.0	100.0	81.8	100.0	100.0	80.6	100.0	100.0	80.6	100.0	#DIV/0!	100.0	91.7	83.3	97.2	93.9	100.0	100.0	100.0	69.7	100.0	100.0	0.0	100.0	100.0	11.1	8.3	69.4	0.0	96.7	72.7	95.8	100.0	100.0	83.3
PMR45	100.0	95.8	100.0	88.9	97.0	81.0	100.0	0.0	37.5	80.0	0.0	0.0	97.2	100.0	0.0	3.3	100.0	83.3	100.0	100.0	86.1	100.0	100.0	63.6	100.0	100.0	83.3	100.0	#DIV/0!	100.0	66.7	100.0	78.8	80.6	88.9	100.0	100.0	69.7	97.2	97.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	81.8	74.1	100.0	100.0	93.9	69.4
PMR5	77.8	95.8	83.3	83.3	33.3	66.7	37.5	0.0	0.0	72.2	0.0	0.0	50.0	66.7	0.0	2.8	8.3	8.3	16.7	0.0	27.8	88.9	100.0	75.0	91.7	100.0	77.8	86.1	#DIV/0!	95.8	66.7	75.0	30.6	47.2	100.0	100.0	100.0	63.6	97.2	44.4	100.0	100.0	66.7	0.0	0.0	0.0	0.0	61.1	66.7	45.8	100.0	52.8	0.0
Edisto47	100.0	100.0	100.0	77.8	100.0	61.1	100.0	0.0	0.0	55.6	0.0	0.0	100.0	100.0	0.0	0.0	2.8	5.6	83.3	100.0	88.9	97.2	100.0	77.8	100.0	109.1	75.0	25.0	0.0	50.0	13.9	86.1	0.0	0.0	36.4	54.2	100.0	77.8	100.0	97.2	88.9	100.0	100.0	0.0	0.0	0.0	0.0	80.6	75.8	100.0	100.0	100.0	63.9
RIL 1 =RIL N°14	50.0	4.2	33.3	55.6	88.9	100.0	100.0	33.3	0.0	50.0	91.7	100.0	58.3	33.3	0.0	50.0	0.0	24.2	33.3	5.6	60.6	63.9	0.0	45.5	69.4	33.3	63.6	100.0	#DIV/0!	75.0	33.3	0.0	30.6	52.8	100.0	91.7	0.0	24.2	66.7	36.1	11.1	100.0	66.7	0.0	0.0	15.2	0.0	96.3	100.0	75.0	100.0	72.2	66.7
PI482420	46.7	8.3	66.7	66.7	38.9	86.1	66.7	0.0	0.0	23.3	33.3	66.7	78.8	66.7	0.0	19.4	5.6	6.7	27.8	0.0	22.2	61.1	0.0	54.5	97.2	100.0	69.4	100.0	#DIV/0!	87.5	66.7	86.1	9.1	33.3	75.0	83.3	0.0	22.2	11.1	7.4	11.1	19.4	28.6	0.0	0.0	0.0	60.0	97.2	91.7	50.0	69.4	45.5	3.0
RIL 4 =RIL N°44	8.3	0.0	44.4	50.0	58.3	81.5	44.4	0.0	0.0	8.3	16.7	7.4	45.5	28.6	0.0	2.8	2.8	0.0	22.2	0.0	22.2	16.7	33.3	57.6	25.0	33.3	57.6	91.7	83.3	100.0	66.7	13.9	27.3	19.4	97.2	100.0	0.0	27.8	72.2	36.4	66.7	100.0	95.2	0.0	0.0	2.8	0.0	27.8	0.0	0.0	94.4	45.5	30.6
SVI105	0.0	0.0	33.3	3.7	15.2	7.4	0.0	15.2	0.0	0.0	12.1	3.0	30.3	6.7	0.0	3.0	0.0	0.0	0.0	0.0	8.3	30.6	11.1	3.7	47.2	19.4	39.4	61.1	0.0	0.0	33.3	0.0	5.6	0.0	0.0	0.0	0.0	2.8	3.0	2.8	18.2	33.3	16.7	16.7	0.0	0.0	5.6	0.0	45.5	0.0	0.0	47.2	61.1	0.0
WMR29	93.3	100.0	100.0	80.6	88.9	93.3	100.0	0.0	8.3	86.7	0.0	0.0	83.3	100.0	0.0	0.0	13.3	0.0	66.7	100.0	86.1	75.0	100.0	77.8	94.4	100.0	86.1	30.6	77.8	79.2	0.0	80.0	27.8	0.0	44.4	95.8	100.0	77.8	97.2	75.0	0.0	100.0	100.0	0.0	0.0	0.0	0.0	66.7	85.2	95.8	100.0	94.4	66.7
PI124112	0.0	33.3	100.0	38.9	41.7	22.2	15.2	0.0	0.0	0.0	13.9	9.1	66.7	26.7	0.0	0.0	0.0	0.0	0.0	11.1	3.0	22.2	100.0	47.2	80.6	100.0	66.7	16.7	#DIV/0!	0.0	8.3	13.3	0.0	0.0	0.0	4.2	100.0	41.7	58.3	69.4	73.3	83.3	100.0	0.0	0.0	0.0	0.0	83.3	0.0	4.2	100.0	97.2	2.8
PI313970	0.0	9.5	33.3	19.4	30.6	5.6	0.0	5.6	0.0	0.0	11.1	0.0	41.7	0.0	0.0	19.4	0.0	2.8	0.0	13.9	8.3	36.1	25.0	19.4	33.3	27.8	47.2	50.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	19.4	19.4	16.7	25.0	0.0	41.7	38.9	0.0	0.0	0.0	2.8	47.2	0.0	0.0	55.6	58.3	15.2
Ames 31282	88.9	100.0	100.0	80.6	94.4	100.0	100.0	38.9	100.0	100.0	97.2	100.0	100.0	100.0	100.0	84.8	77.8	86.1	66.7	100.0	78.8	100.0	100.0	75.0	100.0	100.0	72.2	97.2	100.0	100.0	22.2	91.7	86.1	91.7	83.3	100.0	97.2	63.9	100.0	97.2	0.0	100.0	100.0	0.0	0.0	77.8	0.0	91.7	69.4	100.0	100.0	97.2	66.7
Arum	0.0	0.0	76.7	69.4	13.9	33.3	28.6	0.0	0.0	0.0	0.0	14.3	44.4	100.0	0.0	0.0	0.0	2.8	0.0	0.0	0.0	25.0	0.0	22.2	94.4	100.0	83.3	25.0	0.0	8.3	77.8	38.1	0.0	0.0	0.0	8.3	0.0	8.3	27.8	0.0	33.3	52.8	0.0	0.0	0.0	2.8	0.0	100.0	13.3	12.5	38.9	0.0	0.0
Durango	50.0	14.3	61.9	66.7	100.0	100.0	100.0	0.0	0.0	22.2	0.0	0.0	51.9	33.3	0.0	0.0	0.0	25.0	0.0	16.7	33.3	83.3	33.3	66.7	75.0	33.3	66.7	100.0	#DIV/0!	100.0	66.7	19.0	51.5	50.0	90.5	100.0	16.7	36.1	100.0	57.6	66.7	100.0	100.0	0.0	0.0	5.6	0.0	97.2	76.2	90.5	97.2	78.8	38.9
Arago	97.2	37.5	66.7	86.1	63.9	8.3	93.3	0.0	0.0	66.7	0.0	0.0	77.8	100.0	0.0	2.8	0.0	25.0	0.0	19.4	58.3	91.7	100.0	77.8	88.9	100.0	75.0	100.0	86.1	100.0	66.7	100.0	22.2	56.7	100.0	100.0	30.6	69.4	100.0	77.8	0.0	100.0	100.0	0.0	0.0	3.0	0.0	94.4	77.8	83.3	100.0	94.4	19.4

It's the table legend of this table and it used for the following of the report:

Table legend
Susceptible
Resistant
IR
Heterogeneous
Non concluded

At this stage, we observed discrepancies between labs regarding interpretations of similar results (equal results = different interpretations). It was necessary to harmonize the interpretation rules before discussing.

That's why the following interpretations rules were defined for analyzing results of partners based on the disease index:

Interpretation	S	IR	R
Disease index	>60 (+-2)	Between 15 and 60 (+-2)	<15 (+- 2)

Some results from some labs were withdrawn based of susceptible control no validated (hatched data)

.

The results of interpretation were compiled in the tables below.

Table 3: result before the discussion

	Mon 18-1	Mon 19-6	RZ_ID873	SRY 18.0109..	RZ_ID4578	SAK-SPA-18-0	Venturia	Envero	RZ_ID1330	SRY -18-105..	RZ_ID470	CM18043	Uber race	Px:5 Matref	Px: 3-5 Matre	Mon 19-04	Mel 2381-18-	Isolat ARUM	Px:2 Matref	Px:3 Matref	RZ_ID1296	Px:1 Matref	SalGH2018	SRY 18-0105-1
Variety	**Proposed Strain X**	**Proposed Strain T**	**Proposed Strain Q**	**Proposed Strain N**	**Proposed Strain S**	**Proposed Strain B**	**Proposed Strain M**	**Proposed Strain G**	**Proposed Strain W**	**Proposed Strain K**	**Proposed Strain V**	**Proposed Strain A**	**Proposed Strain R**	**Proposed Strain Y**	**Proposed Strain J**	**Proposed Strain E**	**Proposed Strain C**	**Proposed Strain O**	**Proposed Strain I**	**Proposed Strain P**	**Proposed Strain F**	**Proposed Strain U**	**Proposed Strain H**	**Proposed Strain D**
Vedrantais	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Ames 31282	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HG/S	S	S	S	S	S	HG/S
PMR45	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R/RI
WMR29	S	S	RI/S	R/S	S	S	S	S	S	S	S	S	S	S	S	S	S	R/S	R	R	R	R	R	R
PMR5	S	S	S	S	S	S	S	S	S	S	RI	S	S	R	R	S	RI	S	R	RI	R	R	R	R
Edisto47	S	S	RI	R/RI	S	S	S	S	S	S	S	S	S	S	S	RI	HG/S	R/S	R	R	R	R	R	R
Arago	S	S	S	S	S	S	S	S	S	S	S	S/RI	S/RI	RI	R/RI	RI	R/S	RI/S	R/RI	RI	R	R	R	R
PI482420	S	RI	S	S	R	S	S	S	S/RI	S/R/RI	S	RI/R	R/RI	R	R/RI	RI	S	S	R	RI	S	R	R/RI	R
RIL 1 = RIL N°14	S	S	S	S	RI	RI	S/IR	RI	S	S/R/RI	S	RI/R	R/RI	S	R/RI/S	RI	S	RI/ R	R/RI	RI	S	R/RI	R/RI	R/RI
Durango	S	S	S	S	S	S	RI/S	RI	S	RI/S	S	RI/R	RI	RI	R/RI	RI	S	RI/R	R/RI	RI	R	R	R	R
RIL 4 =RIL N°44	S	S	S	S	S	RI	RI	RI	R	RI	RI	R	R/RI	RI	R/RI	R	S/RI	S/R	R	RI	R	R	R	R
PI124112	S	S	R	R	RI	S/RI	S	RI	R	RI/S/RI	S	RI	S/RI	R	R	R	RI	R	R	R	R	R	R	R
Arum	RI	RI/R	R	R	RI	S	S	S	R	RI/R/RI	S	R	R	R	R	R	RI	S/RI	R	R	R	R	R	R
SVI105	RI	RI	R	R/S	R	RI/R	RI	R	R	RI/RI/R	RI	R	R	R	R	R	R	RI/R	R	R	R	R	R	R
PI313970	RI	RI	R	R/RI	RI	RI	RI	R	R	RI	RI	R	RI	RI	R	R	R	R	R	R	R	R	R/RI	R

Table 4: Results after discuss during meeting 03/09/2020 and 12/10/2020.

Isolate		Selected	rejected	Confirmation needed	Comments
RZ_ID470	**Isolate V**		x		Only one lab result validated, weak on PMR 5 (expected Px:3.5)
Venturia	**Isolate M**	x			Very agressive , consistant between RIL 1 and Durango
Mon 19-6	**Isolate T**			x	Most results quite consistent except Arum Confirmation needed
RZ_ID4578	**Isolate S**			x	Only 2 labs results and not consistent- Confirmation needed
Mon 18-1	**Isolate X**			x	Only 2 labs results and not consistent- Confirmation needed
RZ_ID873	**Isolate Q**	x			
SRY 18.0109.2	**Isolate N**		x		inconsistent result, inversion probably
RZ_ID1330	**Isolate W**	x			
Envero	**Isolate G**	x			
Uber race	**Isolate R**	x			
Mel 2381-18-27	**Isolate C**			x	no information on pattern, added after characterization = inconsistent result, confirmation needed
CM18043	**Isolate A**	x			
Px5 - 98Sm65	**Isolate Y**	x			
Px3-5 - 04Sm2	**Isolate J**	x			Matref Px3.5, No Px :3.5 more Px :5. (pb on PMR5)
Mon 19-04	**isolate E**			x	No expected result on Edisto 47 (expected Px:3.5). Confirmation needed
Race 2 US	**Isolate 2 US**		x		not available
Px2- S87.7	**Isolate I**	x			
Px3- 00Sm39	**Isolate P**	x			Matref Px3 low on PMR5
RZ_ID1296	**Isolate F**			x	Confirmation needed
Px1 -Sm3	**Isolate U**			x	Confirmation needed
SalGH2018	**Isolate H**	x			
SRY 18-0105-1	**Isolate D**	x			Expected as Px: 3.5 type but was observed as Px:1.
SAK-SPA-18-1	**Isolate B**		x		Very aggressive isolate, difficult to interpret
SRY18-0043	**Isolate K**		x		inconsistent result, difficult to interpret
Isolat 22- Arum, basf	**Isolate O**		x		Could be a mix of races, this isolate is not pure
SAK (Race 1 US)	**Isolate L**		x		no result (1 no validated result)

Variety	Selected	rejected	confirmation needed	Comments
Vedrantais	x			Close results it's decided to keep only Vedrantais
Ames 31282		x		
PMR45	x			
WMR29			x	WMR29 not always stable
Edisto47			x	
PMR5	x			It's kept even if problem of necrosis (important genetic)
Arago			x	
RIL 1 = RIL N°14			x	
Durango			x	
PI482420			x	
PI124112		x		
RIL 4 =RIL N°44			x	
Arum	x			
SVI105	x			No difference observed in this survey, keep both for now
PI313970		x		

➔ 6 isolates were excluded at this step as well as 3 differentials candidates. For the others, a complementary test is necessary.

At the end of the first year of ring test, some results were not clear and needed to be completed by test in different labs. Only lacking information had been tested in 2021.

Pattern table before the complementary test of 2021:

	isolate D	Isolate H	isolate U	isolate F	isolate P	isolate I	Isolate E	Isolate J	Isolate Y	Isolate A	Isolate C	Isolate R	Isolate G	Isolate W	Isolate Q	Isolate X	Isolate S	Isolate T	Isolate M
Vedrantais	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PMR45	IR/R	R	R	R	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
WMR29	R	R	R	R	R	R	S	S	S	S	S	S	S	S	IR/S	S	S	S	S
Edisto47	R	R	R	R	R	R	IR	S	S	S	S	S	S	S	IR	S	S	S	S
PMR5	R	R	R	R	IR	R	S	IR	R	S	IR	S	IR/S	IR/S	S	IR/S	IR/S	S	S
Arago	R	R	R	R	IR	IR/R	IR	IR/R	IR	IR/S	R/S	IR	S	S	S	S	S	S	S
RIL 1 = RIL N°14	IR/R	IR/R	IR	S	IR	IR/R	IR	R/IR/S	S	IR/R	S	IR/R	IR	S	S	S	IR/S	S	IR/S
Durango	R	R	R	R	IR	IR/R	IR	IR/R	IR	IR/R	S	IR	IR	S	S	S	S	S	IR/S
PI482420	R	IR/R	R	IR/S	IR/R	R	IR	IR/R	R	IR/R	IR/S	IR/R	S	IR/S	S	IR/S	R	IR	S
RIL 4 =RIL N°44	R	R	R	IR/R	IR	R	R	IR/R	IR	R	IR/S	IR/R	IR	R	S	IR/S	S	S	IR
Arum	R	R	R	R	R	R	R	R	R	R	IR/R	R	IR/S	R	R	IR/R	IR/R	IR/R	S
SVI105	R	R	R	R	R	R	R	R	R	R	R	R	IR/R	R	R	IR/S	IR/R	IR	IR

	S
	S/IR
	IR/R
	ir
	R

The results were compiled with previous results (2020-2021) to compare. We also used some graph dotplot of repartition of note for each differential and each isolate (annex 2). The discussion during meeting of 19/10/2021, permit to reduce the number of isolates and confirmed the differentials set.

Isolate		Selected	rejected	Comments
Venturia	**Isolate M**	x		
Mon 19-6	**Isolate T**	x		
RZ_ID4578	**Isolate S**		x	Close to MON 18-01
Mon 18-1	**Isolate X**	x		
RZ_ID873	**Isolate Q**		x	Close to MON 18-01
RZ_ID1330	**Isolate W**	x		
Envero	**Isolate G**		x	Close to Venturia
Uber race	**Isolate R**	x		
Mel 2381-18-27	**Isolate C**	x		
CM18043	**Isolate A**		x	Close to Uber race
Px5 - 98Sm65	**Isolate Y**	x		
Px3-5 - 04Sm2	**Isolate J**		x	Not validated on PMR 5 - substituted by race uber
Mon 19-04	**isolate E**		x	Close to Px :5
Px2- S87.7	**Isolate I**	x		
Px3- 00Sm39	**Isolate P**	x		
RZ_ID1296	**Isolate F**	x		
Px1 -Sm3	**Isolate U**	x		
SalGH2018	**Isolate H**		x	Close to Px :1
SRY 18-0105-1	**Isolate D**		x	Close to Px :1

Based on the latest results from Px:3.5 MATREF, confirmed not validated, the steering members decided to replace the 04Sm2 isolate with the UBER race as new official Px:3.5 MATREF.

During the discussion, it was confirmed the discard of WMR 29.

This second phase of test permitted to obtain this completed table based on the disease index and the repartition per class results. These patterns will to be validated by a largest number of laboratory in the next step.

➔**Definition of a set of differentials and pattern of interest**

New coding	Px:1	Px:2	Px:3	Px:5	Px:3-5	Px:6	Px:7	Px:8	Px:9	Px:10	Px:11
	Px:1 Matref	Px:2 Matref	Px:3 Matref	Px:5 Matref	Uber race	RZ_ID1296	Venturia	RZ_ID1330	Mon 19-6	Mel 2381-18-27	Mon 18-1
Coding test	isolate U	isolate I	Isolate P	Isolate Y	Isolate R	isolate F	Isolate M	Isolate W	Isolate T	Isolate C	Isolate X
origins	Matref	Matref	Matref	Matref	Yolo county	Qingdao (China)	Italian isolate 2018	Johannesburg (South Africa)	Spain (La Mancha)	Guatemala	Spain ElEjido
Vedrantais	S	S	S	S	S	S	S	S	S	S	S
PMR45	R	S	S	S	S	R	S	S	S	S	S
Edisto47	R	R	R	S	S	R	S	S	S	S	S
PMR5	R	R	S	R	S	R	S	S	S	IR	S
Arago	R	R	IR	IR	IR	R	S	S	S	S	S
RIL 1 = RIL N°14	R	R	IR	S	R	S	IR	S	S	S	S
Durango	R	R	IR	IR	IR	R	IR	S	S	S	S
PI482420	R	R	IR	R	R	S	S	S	IR	S	S
RIL 4 =RIL N°44	R	R	IR	IR	R	IR	IR	R	S	S	S
Arum	R	R	R	R	R	R	S	IR	IR	IR	IR
SVI105	R	R	R	R	R	R	IR	R	R	R	IR

4. Ring test of validation with the restricted set of isolate and differentials.

During the web meeting (25/01/2022), The selected pattern results of the isolates were presented by GEVES, and definition of controls were discussed.
The next step consisted of a validation of the results obtained in step 3.
All the tests were divided between the different labs available trying to share isolates. Each participant tested 4-6 candidate isolates (according to the capacity of each lab). Each isolate was tested by 4-5 participants.

To the 11 differentials host to be tested, two varieties: Escrito and Pendragon were added to confirm results of controls on Px:1 and 2 (seeds of theses varieties only were sent to the laboratories testing Px: 1 and 2). Uncoded seeds of the differential set were provided to each participant. 20 seeds per differential were sent. The same plants were used to test the different candidate isolates.

In the web meeting of October 2022, the results of this validation step were presented by GEVES. The discussion about these results and comparison with the previous results permit to obtain pattern validated on a part of those isolates.

It was reported by several participants that the variety PI 482420 present some necrosis at notations. It was decided to exclude this variety from the panel.

<u>Pattern validated:</u>

Isolate	Px:1 Matref	Px:2 Matref	Uber race	RZ_ID1296	RZ_ID1330
Coding test	isolate U	isolate I	Isolate R	isolate F	Isolate W
Vedrantais	S	S	S	S	S
PMR45	R	S	S	R	S
Edisto47	R	R	S	R	S
PMR5	R	R	S	R	S
Arago	R	R	IR	R	S
RIL 1 = RIL N°14	R	R	R	S	S
Durango	R	R	IR	R	S
RIL 4 =RIL N°44	R	R	R	R	R
Arum	R	R	R	R	IR
SVI105	R	R	R	R	R

Pattern to be confirm:

For the second part of the isolates, the results are less clear. It was decided to repeat certain results to reinforce these data.

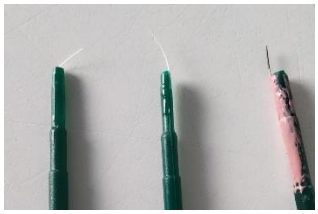
Isolate	Px:3 Matref	Px:3 Matref	Px:5 Matref	Px:5 Matref	Venturia	Venturia	Mon 19-6	Mon 19-6	Mel	Mel	Mon 18-1	Mon 18-1
Coding test	Isolate P	Isolate P	Isolate Y	Isolate Y	Isolate M	Isolate M	Isolate T	Isolate T	Isolate C	Isolate C	Isolate X	Isolate X
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
Vedrantais	S	S	S	?	S	S	S	S	S	S	S	?
PMR45	S	S	S	?	S	S	S	S	S	S	S	?
Edisto47	R	R	S	?	S	S	S	S	S	S	S	?
PMR5	S	S	R	?	S	S	S	S	IR	IR	S	?
Arago	IR	IR/S	IR	?	S	S	S	S	S	IR/S	S	?
RIL 1 = RIL N°14	IR	R	S	?	IR	IR	S	S	S	S	S	?
Durango	IR	IR/S	IR	?	IR	IR/S	S	S	S	S	S	?
~~PI482420~~	~~IR/RèIR~~	~~IR/S~~	~~R~~	~~?~~	~~S~~	~~S~~	~~IR~~	~~? NECROTIC~~	~~S~~	~~S~~	~~S~~	~~?~~
RIL 4 =RIL N°44	IR	R	IR	?	IR	IR	S	S	S	IR/S	S	?
Arum	R	R	R	?	S	S	IR	IR/R	IR	IR	IR	?
SVI105	R	R	R	?	IR	IR	IR/R➔R	R	R	R	IR	?
Conclusion	find another one		repeat		repeat on Durango		repeat on Arum		repeat		repeat	

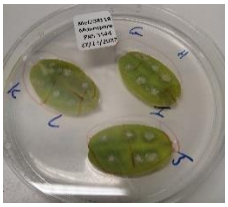
The partners decided to add a step of purification to increase the stability of isolates.

5. Purification of isolates and confirmation ring test

Between the end of 2022 and beginning 2023, a strain purification was carried out by GEVES using the single spore technique. This technique involves isolating a spore using a cat's

whiskers under binocular. The GEVES realized around 4-5 single spores by isolate to be purified.





Each single spore candidate was validated on the differentials panel. Only one candidate was selected to be tested by partners in the last step (except one with two versions kept).

Results of validation step of single spore:

	Px:3		Px:5		Mon 19-6		Mon 18-1		Mel 2381-18-27		Venturia		Venturia	
	Single spore P5		Single spore Y3		Single spore T4		Single spore X1		Single spore C1		Single spore M1		Single spore M3	
	IM	Interpretation	IM	Interpretation	IM	Interpretation	IM	Interpretation	IM	Interpretation	IM	Interpretation	IM	Interpretation
Védrantais	96.67	S	100	S	96.67	S	100.00	S	70	S	96.67	S	100	S
PMR45	100.00	S	96.67	S	100.00	S	100.00	S	80	S	86.67	S	100	S
Edisto 47	3.33	R	90.00	S	96.67	S	96.67	S	73.33	S	80.00	S	93.33	S
PMR 5	93.33	S	10.00	R	85.19	S	83.33	S	36.67	IR	83.33	S	90.00	S
Arago	93.33	S	30.00	IR		S	93.33	S	80	S	70	S	66.67	S
Ril 1 = RIL n°14	54.17	IR	100.00	S		S	96.30	S	90	S	55.56	IR	74.07	S
Durango	74.07	S	45.83	IR	90.00	S	83.33	S	79.17	S	50.00	IR	55.56	IR
RIL 4 = RIL n°44	50.00	IR	90.00	S	91.67	S	70.00	S	26.67	IR	38.89	IR	44.44	IR
Arum	0.00	R	3.33	R	0.00	R	10	R	20	IR	90	S	93.33	S
SVI 105	0.00	R	0	R	0.00	R	0.00	R	0	R	0	R	0	R

The final stage was aimed at confirming the behaviour of the purified isolates (single spore). The seven participants tested seven purified isolates on a set of ten differentials candidates with the protocol on whole plant (only one lab on leaf discs).

All the result were collected by GEVES between June and August and the raw data were sent to each partner before the meeting. The results were discussed one by one during the meeting of 26/09/2023. After discussion and examination of isolates and candidate differential varieties at the same time, the following table is obtained:

	Px:3 Matref	Px:5 Matref	Venturia	Venturia	Mon 19-6	Mel 2381-18-27	Mon 18-1
Coding test	Isolate P	Isolate Y	Isolate M1	Isolate M3	Isolate T	Isolate C	Isolate X
	2023	2023	2023	2023	2023	2023	2023
Vedrantais	S	S	S	S	S	S	S
PMR45	S	S	S	S	S	S	S
Edisto47	R	S	S	S	S	S	S
PMR5	S	R	S	S	S	IR/S	S
Arago	S	IR	IR/S	S	S	S	S
RIL 1 = RIL N°14	IR/R	S	IR/R	S/IR/R	S	S	IR
Durango	IR	IR	IR/R	S/IR/R	S	S	IR
RIL 4 =RIL N°44	IR	IR	IR/R	IR	S	IR	IR
Arum	R	R	S	S	R	IR	IR
SVI105	R	R	IR/R	IR	R	R	IR
Conclusion	ok (RIL1)	ok	not selected	ok (ril 1, dur)	ok	OK (pmr5 nt stable)	OK

The varieties RIL1 and Durango have been excluded due to their unstable results. The isolates kept were the historic Isolates Px:1 to Px:3.5 (MATREF Network). Two isolates were added: Px: 6 Mon 19-6 as more aggressive one and Px: 7 Venturia M (M3 single spore version) as one representative of the isolates commonly found in field.

Summary of discussion in the meeting of 26 September 2023
On isolates:

Isolate		Selected	rejected	Comments	Origin
Px1 -Sm3	**Isolate U**	x		Reference isolate	MATREF
Px2- S87.7	**Isolate I**	x		Reference isolate	MATREF
Px3- 00Sm39	**Isolate P**	x		Reference isolate ok with pattern but not stable on RIL 1 Put on note for PMR5: test is not validated if not strong sporulation on PMR5.	MATREF
Px5 - 98Sm65	**Isolate Y**	x		Reference isolate Durango and RIL 4 between IR and R ==> conclusion IR for both differentials.	MATREF
Px3-5 - Uber race	**Isolate J**	x		Reference isolate	USA Yolo county
Venturia	**Isolate M (M1 and M3)**	x		M3 selected, more aggressive on arago; representative of field	(Venturina) Italy
Mon 19-6	**Isolate T**	x		All four are more or less the same (a Px:3.5 box). Only few differences on RIL1 and RIL4,	Spain (La Mancha)
Mon 18-1	**Isolate X**		x		Spain El Ejido

				due only to difference of test aggressivity. Mon 19-6 the more aggressive one is kept.	
RZ_ID1330	**Isolate W**		x		Johannesburg (South Africa)
Mel 2381-18-27	**Isolate C**		x		Guatemala
RZ_ID1296	**Isolate F**		x	not kept because too close that Px: 1.	Qingdao (China

On differentials:

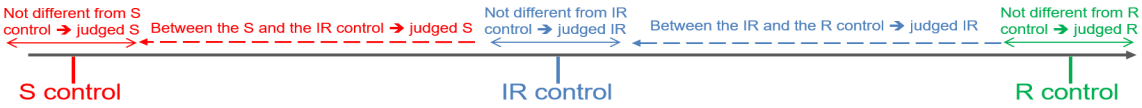
Variety	Selected	rejected	Comments	availability
Vedrantais	x		Reference differentials	MATREF
PMR45	x		Reference differentials	MATREF
Edisto47	x		Reference differentials	MATREF
PMR5	x		Reference differentials	MATREF
Arago	x			MATREF
RIL 1 = RIL N°14		x	Instable results	Produced by RZ
Durango		x	Instable results	MATREF
RIL 4 =RIL N°44	x			Produced by RZ
Arum	x		More interesting for the future evolution of race	MATREF Produced by BASF, easy supplied of seeds
SVI105	x			Produced by RZ

Following the conclusions of the discussion in the meeting of 26 September 2023, the following new coding system was proposed:

New coding	**Px:1**	**Px:2**	**Px:3**	**Px:5**	**Px:3-5**	**Px:6**	**Px:7**
Isolate	Px:1 Matref	Px:2 Matref	Px:3 Matref	Px:5 Matref	Uber race	Mon 19-6	Venturia
Coding test	isolate U	isolate I	Isolate P	Isolate Y	Isolate R	Isolate T	Isolate M3
year	2022	2022	2023	2023	2022	2023	2023
Vedrantais	**S**	S	S	S	S	S	S
PMR45	**R**	**S**	S	S	S	S	S
Edisto47	R	**R**	**R**	**S**	**S**	S	S
PMR5	R	R	**S**	R	**S**	**S**	S
Arago	R	R	**S**	**IR**	**IR**	S	S
RIL 4 =RIL N°44	R	R	**IR**	IR	**R**	**S**	IR
Arum	R	R	R	**R**	**R**	**R**	**S**
SVI105	R	R	R	R	R	R	**IR**

Varieties marked in bold in the table below were selected as controls for each level of interpretation for each isolate.

During the project, different versions of the interpretation rules were considered. Finally, 3 interpretations note were retained (as in actual CPVO protocol), as the latest results based on more different laboratories are clearer and more robust.



The controls of each level were defined in table below:

New coding	Px:1	Px:2	Px:3	Px:5	Px:3-5	Px:6	Px:7
Code	Px:1 Matref	Px:2 Matref	Px:3 Matref	Px:5 Matref	Uber race	Mon 19-6	Venturia
Isolate Coding test	isolate U	isolate I	Isolate P	Isolate Y	Isolate R	Isolate T	Isolate M3
origins	MATREF	MATREF	MATREF	MATREF	USA Yolo county	Spain (la Mancha)	Italian isolate 2018
1- Susceptible Control	Vedrantais	~~Vedrantais~~ PMR 45	~~Vedrantais~~ PMR 5 Arago	~~Vedrantais~~ Edisto 47	~~Vedrantais~~ PMR 5 Edisto 47	~~Vedrantais~~ RIL 4 PMR 5	Arum
IR	Not necesary	Not necesary	~~Arago~~ ~~Durango~~ RIL 4	Arago ~~Durango~~	Arago ~~Durango~~	/	SVI105
9- Resistant Control	~~Arum~~ PMR 45	~~Arum~~ Edisto 47	~~Arum~~ Edisto 47	Arum PMR 5	Arum RIL 4	Arum	/
Choice of race for DUS	Compulsory					Propose not compulsory	Propose not compulsory

Actual controls New controls

The race use for the DUS description were discuss and it's decided the two new races were propose only not compulsory.

For conclude, a new pattern of the different isolates could be defined on new differentials set, and new controls for each isolate were also defined. This new set of differentials and these new isolates provide a better representation of the diversity of isolates observed today. The isolates and seeds of differentials will be available from MATREF.

7. Publication of the results

The report of this project will be published on the website of ISF.The new coding system and differentials sets for *Podosphaera xanthii* will be published on the same site int the differential hosts section.

The description of the controls will also be added tothe table HRT of Euroseeds.

The conclusion of the project will be presented to the VEM CPVO and TWV UPOV to propose to add the two new races Px: 6 and Px: 7.
The controls will be proposed to modified at the next revision of the protocol .

Meetings of the project:

Initial meeting 04/04/2019: Collection of isolate

Web meeting 01/10/2019: initial characterization

Web meeting 03/09/2020 and 12/10/2020: Characterisation

Web meeting 19/10/2021: Complementary test

Web meeting 25/01/2022: Validation test

Web meeting 06/10/2022: Purification and confirmation test

Meeting in Paris 26/09/2023: Conclusion

Annex

Annex 1: CPVO protocol

Ad 69.1 to 69.4: Resistance to *Sphaerotheca fuliginea (Podosphaera xanthii)*, races 1, 2, 5, 3.5 (Px: 1, 2, 5, 3.5)

Ad 70: Resistance to *Erysiphe cichoracearum* (*Golovinomyces cichoracearum*)

1. Pathogen ..... Powdery mildew *Podosphaera xanthii (Spaherotheca fuliginea), Erysiphe cichoracearum (Golovinomyces cichoracearum)*. Only *Podosphaera xanthii* was validated in Harmores 3 project.
2. Quarantine status ..... no
3. Host species ..... Melon - *Cucumis melo* L.
4. Source of inoculum ..... GEVES (FR)[1]
5. Isolate ..... Px: 1, Px: 2, Px: 5, Px : 3.5, Gc : 1 (MATREF/04-07-02-01)
6. Establishment isolate identity ... test on differentials

Table 1: races of Podosphaera xanthii and Golovinomyces cichoracearum, J. McCreight and M. Pitrat

	Podosphaera xanthii (Spaherotheca fuliginea)						*Golovinomyces cichoracearum (Erysiphe cichoracearum)*	
	Race 0	Race 1	Race 2	Race 4	Race 5	Race 3.5	Race 0	Race 1
Iran H	S	S	S	S	S	S	S	S
Védrantais	R	S	S	S	S	S	R	S
PMR45	R	R	S	S	S	S	R	S
WMR29	R	R	R	S	S	S	R	S
Edisto 47	R	R	R	R	S	S	R	R
MR-1, PI124112	R	R	R	R	R	R	R	R
PMR5	R	R	R	S	R	S	R	R
Nantais Oblong	R	S	S	S	S	S	R	R

7. Establishment pathogenicity ..... test on susceptible varieties
8. Multiplication inoculum

8.1 Multiplication medium ..... melon plantlets

8.2 Multiplication variety ..... susceptible variety, for example Védrantais. For higher isolates like 3.5 or 5, a variety with broken resistance may be preferable to keep the isolate pure.

8.3 Plant stage at inoculation ..... cotyledon

8.5 Inoculation method ..... 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.

[1] matref@geves.fr

Dry the cotyledons with sterile paper towel, then place them in Petri dishes with the following medium:

Sucrose	10g
Mannitol	20g
Agar	5g
Distilled water	1 liter

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.

8.6 Harvest of inoculum..... Sporulation on cotyledons

8.7 Check of harvested inoculum ..

8.8 Shelf life/viability inoculum..... maximum 1 to 1.5 months after the inoculation.

9. Format of the test

9.1 Number of plants per genotype at least 20 plants per variety and controls, 5 plants for other differentials

9.3 Control varieties .....For *Podosphaera xanthii:*
Susceptible: Védrantais
Intermediate resistant: Durango and/or Arango
Resistant: Arum
For *Golovinomyces cichoracearum:*
Susceptible: to choose in the table of differentials
Resistant: to choose in the table of differentials

9.4 Test design .....Include differentials to validate the race (at least 5 plants per differentials) and compare the level of sporulation.

9.5 Test facility .....Climatic chamber or greenhouse

9.6 Temperature .....20-24°C

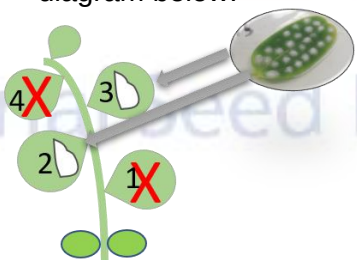
9.7 Light .....at least 12 hours

10. Inoculation

10.1 Preparation inoculum.....-

10.2 Quantification inoculum .....-

10.3 Plant stage at inoculation .....Whole plants at 3-4 true leaf fully expanded stage. Inoculation on the leaves 2 and 3 indicated on the diagram below.



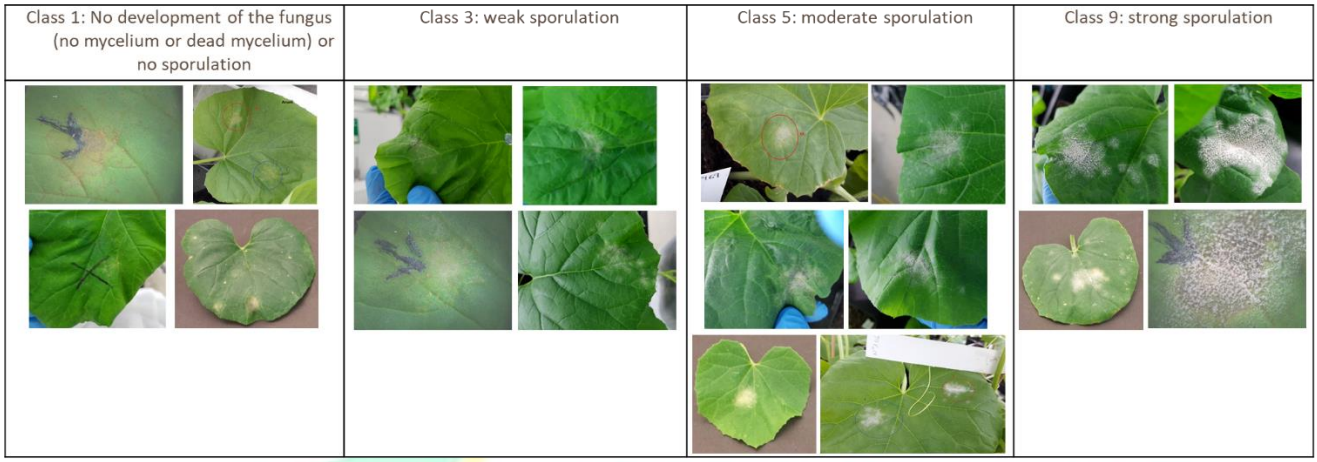
10.4 Inoculation method .....Take spores from a cotyledon already covered with conidia and deposit them on a leaf. Different isolates can be tested on the same plant (or the same leaf) if the local deposit is well separated from each other and if a mark indicates the place of the deposit.

10.7 End of test ..... 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.....



Example of contamination by environment on the susceptible control, test not validated

11.3 Validation of test..... Validation on controls.

Controls expected comportment for *Podosphaera xanthii*:

<u>Resistant:</u>

Plants at class 1

Most of the plants at class 1 and few plants at class 3 (very low disease index)

Plants at class 3 but in this case the susceptible control should be all at class 9

No plants at classes 5 or 9

<u>Intermediate Resistant:</u>

Between the resistant and the susceptible control

Generally, plants at classes 3 and 5

<u>Susceptible:</u>

Plants at class 9

Most of the plants at class 9 and few plants at class 5 (high disease index)

Few plants at class 3 but in this case the resistant control should be all at class 1 and the intermediate resistant control at classes 3 and 1

No plants at class 1

11.4 Off-types.....

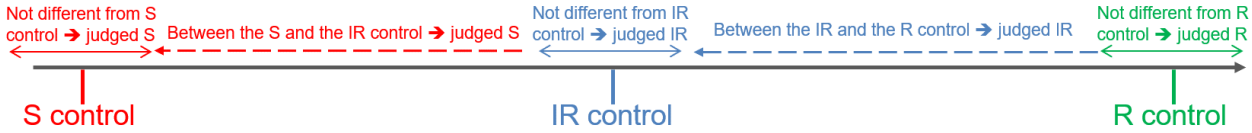
12. Interpretation of data in terms of UPOV characteristic states

Interpretation of varieties depending on controls

Quantitative analysis based on the disease index and the repartition of plants per class compared to the controls.

For *Podosphaera xanthii:*

The varieties between the intermediate resistant and the resistant control have to be judged as intermediate resistant (not enough resistant).
The varieties between the susceptible and the intermediate resistant control have to be judged as susceptible (not enough intermediate resistant).



$$DI = \frac{(N1*0)+(N3*1)+(N5*2)+(N9*3)}{(N1+N3+N5+N9)*3} *100$$

Nx: Number of plants at class X

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

Annex 2: Graph use for analyses