APPLICATION FOR A RESEARCH & DEVELOPMENT PROJECT RELEVANT TO THE COMMUNITY PLANT VARIETY PROTECTION SYSTEM

Project: Harmonization of resistance test to diseases for DUS testing - 3 Final report

I Summary page:

Coordinator of the project: GEVES (F)
Other partners involved: Naktuinbouw (NL)
INIA (SP)
Central Institute for Supervising and Testing in Agriculture (CZ),
Bundessortenamt (D)
National Food Chain Safety Office (HU)
CREA (IT)
Science and Advice for Scottish Agriculture (UK)
French Technical Institute for Fruits and Vegetable (CTIFL)
Seed companies belonging to ESA (UE)

Name of the contact person: Sophie PERROT (GEVES)
Name of the scientific coordinator: Valérie GRIMAULT (GEVES)
Duration of the project: 2 years

Total estimated cost: 259199 €.

Project:

A new Harmores project is proposed on another set of host/race/pathogen combinations. The priorities have been selected in collaboration with partners of the project. These diseases were chosen according to the following criteria:

- they were compulsory and/or concerning an intermediate resistance (IR), or race specific response
- they were commonly used as a grouping character for DUS testing
- the protocols were known to be difficult and to give slightly different results depending on the test conditions
- they were of a high interest for the largest number of countries involved.

The objective of this new project is to harmonize the resistance tests in terms of reference material (isolates and varieties), test conditions and notation scales, and to propose new harmonized and robust protocols to CPVO. A focus for Harmores3 project is done on intermediate resistance, which makes it more difficult than the previous projects, but for which harmonized protocols and reproducible results is of great concerns.

The project aims at harmonizing, at the European level, resistance tests to seven vegetable diseases:

- *Meloidogyne incognita*/ tomato: IR, compulsory
- *Fusarium oxysporum* f. sp. *lycopersici* Race 0 (ex 1) and Race 1 (ex 2)/tomato for notation scale
- *Erysiphe pisi*/pea: field/greenhouse tests, different species, could become compulsory
- Powdery mildew/melon *(Podosphaera xanthii*): will be based on one race as model, and potentially modified with respect to the published results (Lebeda et al., 2010, 2016) and the CASDAR project for race definition, expected to be difficult, an additional period will perhaps be necessary to obtain a robust protocol.
- *Fusarium oxysporum* f. sp. *melonis* race 1.2/melon: IR
- *Fusarium oxysporum* f. sp. *melonis* race 2/melon: compulsory

List of partners

Institute responsible for the project:

GEVES (France)
Marie Noelle Racat (*Administrative Coordinator)*: GEVES: Rue Georges Morel, BP 90024, 49071 Beaucouzé, France. Phone : 33 (0)2 41 22 86 45, e-mail : marie-noelle.racat@geves.fr
Valerie Grimault (*Scientific Coordinator)*: GEVES-SNES, Rue Georges Morel, BP 90024, 49071 Beaucouzé Cedex, France. Phone : 33 (0)2 41 22 58 50, e-mail : valerie.grimault@geves.fr
Sophie Perrot: GEVES-SNES, Rue Georges Morel, BP 90024, 49071 Beaucouzé Cedex, France. Phone : 33 (0)2 41 22 58 58, e-mail : sophie.perrot@geves.fr

Other partners involved:

The Netherlands (NL):
Diederik Smilde: Naktuinbouw, Postbus 40, Sotaweg 22, 2370 AA Roelofarendsveen, Netherlands
Phone : 31 71 332 62 13, e-mail : d.smilde@naktuinbouw.nl
Richard Ludlage: Naktuinbouw, Postbus 40, Sotaweg 22, 2370 AA Roelofarensveen, Netherlands
Phone : 31 71 332 61 28, e-mail : r.ludlage@ naktuinbouw.nl

Spain (SP):
David Calvache: Centro de Ensayos de Valencia, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (I.N.I.A), Calle Joaquín Ballester, 39, 46009 Valencia, Spain.
Phone : 34 963079604, e-mail: oevvval@hotmail.es
Cristina Moyano Cárdaba: Laboratorio de Sanidad, Edificio de Semillas, Dirección Técnica de Evaluación de Variedades y Laboratorios, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (I.N.I.A), Ctra. La Coruña Km. 7.5, 28040 Madrid, Spain. Phone: 34 913474186, e-mail: cardaba@inia.es

Czech Republic (CZ):
Lenka Lefnerova: Central Institute for Supervising and Testing in Agriculture, National Plant Variety Office, Hroznová 2, 656 06 Brno, Czech Republic.
Phone: +420 543 548 227, 543 548 111, e-mail: lenka.lefnerova@ukzuz.cz

Hungary (HU):
Ferenc Kovács: National Food Chain Safety Office (NÉBIH), Hungary 1024 Budapest, Keleti K. u. 24.
Phone: 0036 1 336 9161, e-mail: kovacsf@nebih.gov.hu
Ágnes Halász: National Food Chain Safety Office (NÉBIH), Hungary 1118 Budapest, Budaörsi út 141-145.
Phone: 0036 1 3091000 /1801, e-mail: halasza@nebih.gov.hu

United Kingdom (UK):
Tom Christie: SASA (Science and Advice for Scottish Agriculture), Roddinglaw Road, Edinburgh, EH12 9FJ, UK. Phone: +44-(0)131-244 8961, e-mail: Tom.Christie@sasa.gsi.gov.uk
Marian Mc Ewan: SASA (Science and Advice for Scottish Agriculture), Roddinglaw Road, Edinburgh, EH12 9FJ, UK. Phone: +44-(0)131-244 8879, e-mail: Marian.McEwan@sasa.gsi.gov.uk

Italy (IT)
Romana Bravi : CREA - Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria – Centro per la Sperimentazione e Certificazione delle Sementi - SS 18 – km 77,700; 84091 – Battipaglia (SA) – Italy
Tel +39 0828309484 - Fax +39 0828302382, e-mail: romana.bravi@crea.gov.it
Loredana Sigillo: CREA - Consiglio per la Ricerca in agricoltura e l'analisi dell'economia agrarian, Laboratorio di Analisi Fitopatologiche, SS 18 – km 77,700; 84091 – Battipaglia (SA) – Italy Tel +39 0828309484
Fax +39 0828302382, e-mail: Loredana Sigillo [loredana.sigillo@entecra.it]

CTIFL :France (FR)
Marie Torres : CTIFL, Centre de Balandran, 751 chemin de Balandran, 30127 Bellegarde
Phone : 0033466011054, e-mail : torres@ctifl.fr
François Villeneuve : CTIFL, Centre de Lanxade, 28 route des Nebouts, 24130 Prigonrieux
Phone : 0033553580005, e-mail : villeneuve@ctifl.fr

ESA:

European Seed Association, 23, Rue du Luxembourg, 1000 Brussels, Belgium
Phone: +32 2 743 28 60
Fax: +32 2 743 28 69
Email: secretariat@euroseeds.eu
19 partners from ESA representing 9 companies (BASF-Nunhems, Enza zaden, Gautier, HM Clause, Monsanto-Bayer, Ramiro Arnedo, Rijk Zwaan, Sakata, Vilmorin-Mikado)

II Detailed description of the project:

1. Relevance for the system

Genetic resistance to diseases is one of the major focuses for breeding programs of vegetables and many resistances to bacteria, fungi, viruses and nematodes have been introduced in commercial varieties. Resistance tests are used as a character for DUS testing and also as a grouping character and published in UPOV guidelines and CPVO protocols. Some resistance tests are compulsory, while others are not, but a lot of countries in the European Union cannot afford to apply them. It appears that countries use different protocols while the published protocol is not used, by habit or because protocol conditions are not robust enough to be used in different laboratories conditions. So, in different countries, different isolates, resistance and susceptible controls, test conditions are used. This can make comparison of varieties between countries difficult, in particular for intermediate resistances. A need was therefore identified to harmonize protocols, in order to obtain robust and reliable tests, and to be able to compare varieties as required by the registration system. Having reliable resistance tests for grouping characters will reduce the costs of experiments and time spent in comparisons of varieties.
From 2004 to 2014, national examination offices in France, Spain, United Kingdom, Czech Republic, Hungary and Netherlands harmonized 14 protocols on tomato, pepper, pea, lettuce and bean. Updated harmonized protocols have been proposed to CPVO for publication in their website in the technical protocols section. For these protocols, common reference isolates, resistance and susceptible controls, differentials and test conditions have been validated. Protocols have also been updated compared to bibliography: taxonomy, new races…For the resistance tests involved in these projects, we should have better coherence of results between countries and between declarations of breeders and official tests, better definition and exchange of reference material (isolates, controls and differentials) and in the medium term improve collection management.

We now propose a new project for harmonization of resistance tests for DUS testing in the EU. We will work on important tomato, melon and pea pathogens, with a focus on intermediate resistance. This project proposal for one year (part 1) will be followed by a second project proposal of 2 years (part 2).

2. Quality of the project

Project description
Host/pathogen combination chosen:

- *Meloidogyne incognita*/ tomato: IR, compulsory.
- *Fusarium oxysporum* f. sp. *lycopersici* Race 0 (ex 1) and Race 1 (ex 2)/tomato for notation scale.
- *Erysiphe pisi*/pea: field/greenhouse tests, different species, could become compulsory
- Powdery mildew/melon (*Podosphaera xanthii*): will be based on one race as model, and potentially modified with respect to the published results (Lebeda et al., 2010, 2016) and the CASDAR project for race definition; expected to be difficult, an additional period will perhaps be necessary to obtain a robust protocol.
- *Fusarium oxysporum* f. sp. *melonis* race 1.2/melon: IR.
- *Fusarium oxysporum* f. sp. *melonis* race 2/melon: compulsory.

Priority host pathogen combinations have been chosen in collaboration with partners of the project. They were chosen because they were compulsory, concerned an intermediate resistance, number of entries was high, and protocols were commonly used in countries for DUS or grouping character, or protocols were known to be difficult and to give slightly different results depending on conditions used. We have also made an effort to choose host/pathogen combinations which are interesting for different countries involved in DUS tests.

During the setup of the project a better knowledge of protocols used was obtained by consultation of the partners:

- updated bibliography on host/pathogen combination and particularly genetic of resistance: resistance or intermediate resistance, influence of genetic background
- isolates used
  - virulence
  - culture
  - stability in culture
- resistant and susceptible controls used
- test conditions

This knowledge allowed to define for each pathogen the issues rose by protocols and include them in the project.

For this third project, as pathogens chosen represent different issues, it was not possible to define a general description of the project by phases common to each pathogen. So, the phases of the project are described by pathogen. For each pathogen, the following actions have been planned in collaboration with partners in web meetings. For each task and action, MTA will be sent with internal number of the lab for isolate and/or varieties, taking into account that material will need to be available at the end of the project to be used as reference material in the future protocols.

Task 1: Tomato:

- **Action 1: *Fusarium oxysporum* f. sp. *lycopersici* Race 0 (ex 1) and Race 1 (ex 2) (Fol)**: The goal of this action is to define a notation scale for this pathogen for which isolates, controls and protocols were harmonized in the Harmores1 project. A better notation scale is needed due to new varieties having a slight different level of resistance than controls.
  - **Action 1.1**: A workshop will be organized to compare notation scale of each partner and the way to interpret, to come to a common one. All partners will perform the test in their lab on a panel to be defined during the kick off committee, including controls of CPVO with isolates described in CPVO protocol. It will allow them to observe varieties in their lab conditions. Then, all partners will meet in one lab to make notation and interpretation all together. If partners do not have the isolates or controls, they will be sent on request by GEVES.
- **Action 2: *Meloidogyne***. The goal of this action is to harmonize the whole protocol using a panel and focus on IR
  - **Action 2.2 (Year 1 project part 2)**:
    - Protocols will be compared with a focus on the inoculation method
    - Notation scale and interpretation will be compared
    - A workshop will be organized after results of CTs have been received and analyzed. It will focus on the same panel as CT to allow participants to compare the plants in their condition and in the one of the workshops.
  - **Action 2.3 (Year 2 project part 2)**: The protocol and notation scale chosen in the previous years will be validated by a CT.

Task 2: Melon:

- **Action 1: *Fusarium oxysporum* f. sp. *melonis* race 1.2**. The goal of this action is to harmonize the whole protocol using a panel and focus on IR
    - **Action 1.2 (Year 1 project part 2)**:
        - A CT will be organized to compare methods of inoculation (dipping roots and transplanting or dipping tray; greenhouse or growth chamber, notation date) on isolates, controls and panel defined in 1st year.
        - A workshop will be organized after results of CTs have been received and analyzed. It will focus on the same panel as CT to allow participants to compare the plants in their condition and in the one of the workshops. It will include different dates of notation to compare evolution of symptoms and way to interpret them.
    - **Action 1.3 (Year 2 project part 2)**:
        - The protocol and notation scale chosen in the previous years will be validated by a CT.
- **Action 2: *Fusarium oxysporum* f. sp. *melonis* race 2**: For this compulsory characteristic, the goal is to harmonize the whole protocol. The second year of the project part 2, validation of the chosen protocol will be extended to *Fusarium oxysporum* f. sp. *melonis* race 0 and 1. Tests will be performed in the fall.
    - **Action 2.2 (Year 1 project part 2)**:
        - A CT will be organized to compare methods of inoculation (dipping roots and transplanting or dipping tray; concentration of inoculum, greenhouse or growth chamber, notation date) on isolates, controls and the panel defined in 1st year.
        - A workshop will be organized after results of CTs have been received and analyzed. It will focus on the same panel as CT to allow participants to compare the plants in their condition and in the one of the workshops. It will include different dates of notation to compare evolution of symptoms, notation scales and way to interpret them.
    - **Action 2.3 (Year 2 project part 2)**:
        - The protocol and notation scale chosen in the previous years will be validated by a CT. The CT will also be performed with Fom: 0 and 1 isolates.
- **Action 3: Powdery mildew (*Podosphaera xanthii*):** This action will be based on methodology published by Lebeda et al. (2016), Lebeda and Sedláková (2010) and results of the CASDAR project for race definition. One race will be chosen as model. *Podosphaera xanthii* is chosen as priority even if 2 species occur in Europe, because most melons are cultivated in Mediterranean areas where *Podosphaera xanthii* is predominant. The goal of this action is to harmonize the whole protocol using a panel and focus on varieties having an intermediate resistance. As this action is expected to be a difficult one, an additional period to the 3-year project will perhaps be necessary to obtain a robust protocol.

    - **Action 3.2(Year 1 project part 2):**
        - CTs will be organized on protocol(s) defined in year 1 on selected material
        - A workshop will be organized to validate notation scale and interpretation
        - **Action 3.3 (Year 2 project part 2)**: CT will be organized to validate reference material, protocol and notation scale and interpretation.

Task 3: Pea

Action 1: *Erysiphe*. For this pathogen, field and greenhouse tests are currently used by partners. Literature showed that different species: *E. pisi*, *E. trifolii*, *E. beaumleri*, have been identified as causing powdery mildew of pea and these species show a different virulence pattern on the different resistance genes of pea (Attanayake R.N. et al, 2010; Fondevilla S. et al, 2006; Fondevilla S. et al, 2010; Fondevilla S. and Rubiales D. , 2012; Fondevilla S. et al, 2013; Onderj M. et al, 2005). It could explain why in the past years, partners observed conflicting results between breeders or examination offices results and between different sowing dates. This would not be in favor of field tests in future protocols. The project will focus on *Erysiphe pisi*. A robust protocol is needed as this characteristic could become compulsory in the near future.

- **Action 1.2 (Year 1 project part 2)**:
  - The test of year 1 will focus on *Erysiphe pisi* in field and, in greenhouse with isolate(s) selected during project part 1 to allow to select reference material (strain and controls) and compare protocols.
- **Action 1.3 (Year 2 project part 2)**:
  - A CT will be organized on isolate (s) and panel chosen. It will allow to validate protocol and reference material defined during year 1 project part 2 and show if they are reproducible in different labs.

Indicative timetable

Task	Action	Pathogen	Description	Part 2 year 2
1: Tomato	1.1	Fol:0, 1	CT validation	x
	2.3	*Meloidogyne incognita*	CT validation	x
2: Melon	1.3	Fom: 1.2	CT validation	x
	2.3	Fom: 0, 1, 2	CT validation	x
	3.3	*Podosphaera xanthii*	CT validation	x
3: Pea	1.3	*E. pisi*	CT validation	x

Significant risks and alternative actions if milestones are not reached

It is considered unlikely that the majority of the milestones will not be reached, except for Powdery mildew of melon and pea which are difficult tasks. Partners highly experienced in pathology tests for DUS testing on vegetables will participate to first CTs, and they are aware of the requirements of the project. Regular meetings of the partners will be organised to ensure good progress is maintained. For powdery mildew of pea and melon, an additional period to the 3-year project will perhaps be necessary to obtain a robust protocol

Time spent by each partner (month)

Tasks	FR Org	FR CTIFL	NL	SP	CZ	DE	HU	UK	IT	PL
CT	3.33	0.83	1.67	1.67	0.8	0.67	1,2	1,2	1.67	0

Meetings including workshops	1.33	0.067	0.67	0.67	0,067	0,067	0,067	0,067	0.67	0,067
Reports	1.33	0.2			0,067	0,067	0,067	0,067		
Coordination	12.67				0,0					

Deliverables at the end of project part 1 and 2:

- updated bibliography on host/pathogens chosen
- available reference isolates with maintainers laboratories
- available reference resistant, intermediate resistant and susceptible controls
- culture conditions defined for pathogens
- test conditions defined
- proposed protocols to CPVO

Exploitation and Dissemination Plans

The practical deliverables from the project will be made available to all the partners, to the CPVO and ultimately to any other EU MS or UPOV members that may be interested. The results will be presented at the appropriate UPOV meetings and if suitable, will be published in the scientific press or in congresses (Eucarpia…).

III Report on part 2 year 2 activities:

Table 1: Details of part 1 and part 2

	Year	Date	Who?	Actions
Before project	2014	June	GEVES	Sending of the draft questionnaire for priorities and criteria of choice for host/pathogen
		June to December	All partners	Comments about priorities and criteria of choice
	2015	January	GEVES	Sending of the table summarizing the table of priorities
		April 22nd	All partners	priorization
		July 9th	GEVES	Sending of the questionnaire on test based on protocols and for definition of laboratories participating
		July to September	All partners	Comments about the draft questionnaire of comparison of protocol
		September 11th, 21st and 28th	All partners	WebEx meeting on pea, melon and tomato to define the different phases of Harmores 3 (CTs, WS and validation)
		September 28th	GEVES	Sending of mintutes of Web meeting, the table summarizing the participation of the partners for each host/pathogen couple, the table summarizing the comparison of protocols, the estimated budget to complete and the questionnaire of reference material proposed by partners
		September to mid of October	All partners	Comments about the estimated budget and the questionnaire of reference material proposed by partners
		October 15th	GEVES	Sending of Harmores 3 project to CPVO
	2016	June	CPVO	Acceptation of Harmores 3 project divided into two parts
Part 1	2016	June 24th	All partners	*Fusarium*/melon kick-off meeting by WebEx: analysis of the questionnaire to define controls, isolates and protocols and preparation of first comparative tests (CTs) and Workshops (WS) (exchanges of seeds and hosts, calendar of setting up of tests)
		June to October	All partners	Sending of isolates and seeds to GEVES for *Fusarium*/melon
		July to September	GEVES	Redaction of test plans and notation sheet for the CT *Fusarium* race 1.2/melon
		July to october	GEVES	Redaction of test plans and notation sheet for the CT *Fusarium* race 2/melon
		September 15th	All partners	Px/melon, tomato and pea kick-off meeting in France: analysis of the questionnaire to define controls, isolates and protocols and preparation of first comparative tests (CTs) and Workshops (WS) (exchanges of seeds and hosts, calendar of setting up of tests)
		September to October	All partners	Sending of isolates and seeds to GEVES for Fol/tomato

	September to beginning of November	GEVES	Redaction of test plans and notation sheet for the CT Mi/tomato
	September to December	All partners	Sending of isolates and seeds to GEVES for Mi/tomato and *E. pisi*/pea
		GEVES	Redaction of test plans and notation sheet for the CT Px/melon
	September to January	All partners	Sending of isolates and seeds to GEVES for Px/melon
	September 20th	GEVES	Sending of test plan and notation sheet for the CT *Fusarium* race 1.2/melon
	Beginning of October	GEVES	Preparation of materials for CTs *Fusarium* race 1.2/melon
	October 10th	GEVES	Redaction of test plans and notation sheet for the CT Fol/tomato
	October 18th	GEVES	Sending of materials for CTs *Fusarium* race 1.2/melon to partners Sending of test plan and notation sheet for the CT *Fusarium* race 2/melon
	October 20th	GEVES	Sending of test plan for the CT Fol/tomato
	End of October	GEVES	Preparation of materials for CTs *Fusarium* race 2/melon
	October 31st	GEVES	Sending of materials for CTs *Fusarium* race 2/melon to partners
	Beginning of November	GEVES	Preparation of materials for CT Mi and Fol/tomato
	November 9th	GEVES	Sending of materials and notation sheet for CT Fol/tomato
	November 10th	GEVES	Sending of materials for CT Mi/tomato
	November 14th	GEVES	Sending of test plan and notation sheet for the CT Mi/tomato
	December 5th	GEVES	Sending of additional varieties for CT Mi/tomato
2017	Beginning January	GEVES	Preparation of materials for CTs Px /melon
	January 10th	GEVES	Sending of test plan and notation sheet for the CT Px/melon
	January 11th	GEVES	Sending of materials for CTs Px /melon
	March	GEVES	Redaction of test plans, notation sheet and preparation of materials for the CT *E. pisi*/pea
	March 23rd	GEVES	Sending of test plan and notation sheet for the CT *E. pisi*/pea
	March-April	GEVES	Sending of materials for CT *E. pisi*/pea
	February - April	All partners	Sending of results for Mi/tomato
	March – April 7th	All partners	Sending of results for Fol/tomato
	March – April 26th	All partners	Sending of results for Px/melon
	March – May 12th	All partners	Sending of results for *Fusarium* race 1.2/melon
	March – May 16th	All partners	Sending of results for *Fusarium* race 2/melon
	April	GEVES	Analyse of results
	May 5th	GEVES	Sending of raw data
	May 17-18th	All partners	WS Px/melon in Angers
	May 18-19th	All partners	Annual meeting part 1 in Angers
	May	GEVES	Sending of annual meeting part 1 minutes

		July 6th	GEVES	Sending of part 1 report to partners
		September	GEVES	Sending of part 1 report to CPVO
Part 2 melon/Px	2017	November to December	GEVES	Redaction of test plans and notation sheet for the CT Px/melon
		December	GEVES	Preparation of materials for CTs Px /melon
	2018	January 24th	GEVES	Sending of test plan and notation sheet for the CT Px/melon
		January 22th	GEVES	Sending of materials for CTs Px /melon
		End of April to mid of May	All partners	Sending of results for Px/melon
Part 2 melon/Fom	2017	August to November	All partners	Sending of seeds to GEVES for *Fusarium*/melon
		June 2017 to December		Redaction of test plans and notation sheet for the CT *Fusarium* race 1.2/melon Redaction of test plans and notation sheet for the CT *Fusarium* race 2/melon
		November 15th		WS Fom: 1.2/melon at Murcia
		December	GEVES	Preparation of materials for CTs *Fusarium* race 1.2 and *Fusarium* race 2/melon
	2018	January 11th	GEVES	Sending of presentation, minutes and results of the melon Fom: 1.2 WS Sending of test plan and notation sheet for the CT *Fusarium* race 1.2/melon Sending of test plan and notation sheet for the CT *Fusarium* race 2/melon
		January 15th		Sending of materials for CTs *Fusarium* race 1.2/melon to partners Sending of materials for CTs *Fusarium* race 2/melon to partners
		April – June 1st	All partners	Sending of results for *Fusarium* race 1.2/melon
		March – June 4th	All partners	Sending of results for *Fusarium* race 2/melon
Part 2 tomato/Mi	2017	June	GEVES	Redaction of test plans and notation sheet for the CT Mi/tomato
		June 13th	GEVES	Sending of test plan and notation sheet for the CT Mi/tomato
		June to July	All partners	Sending of seeds to GEVES for Mi/tomato
		July	GEVES	Preparation of materials for CT Mi
		August	GEVES	Sending of materials for CT Mi/tomato
		October to February 2018	All partners	Sending of results for Mi/tomato
Part 2 tomato/Fol	2017	June 22nd	All partners	Sending of seeds to GEVES for WS Fol/tomato
		September	GEVES	Preparation of materials for WS Fol/tomato
		October	GEVES	Sending of materials for WS Fol/tomato
		November 15th	All partners	WS Fol/tomato at La Costière
		May 24th	GEVES	Sending of WS Fol/tomato minutes
Part 2 pea/*E. pisi*		September to October	Partners	Sending of CT results *E. pisi* in field part 1
		October 10th		WS *E. pisi* on field results by Skype

2018	January	GEVES	Redaction of test plans, notation sheet and preparation of materials for the CT *E. pisi*/pea in field and controlled conditions
	February 1st	GEVES	Sending of materials for CT *E. pisi*/pea
	February 2nd	GEVES	Sending of test plan and notation sheet for the CT *E. pisi*/pea in controlled conditions
	February	GEVES	Sending of material for WS *E. pisi*
	April 12th to May 14th		Sending of results for CT *E. pisi*/pea in controlled conditions
	May 15th	All partners	WS *E. pisi*/melon in Angers
	April to May	GEVES	Analyse of results
	May 18th	GEVES	Sending of raw data
	June 5th	All partners	WS Fom: 2/melon at Roelofarendsveen
	June 6th	All partners	WS Mi/tomato at Roelofarendsveen
	June 7-8th	All partners	Annual meeting part 2 at Roelofarendsveen
	August to September	All partners	Sending of results for CT in field *E. pisi*/pea

I. General information

The Harmores 3 project was accepted by CPVO the 15th June 2016. The draft of the consortium agreement was sent by GEVES the 28th February, the last return from partners was received beginning of May 2018. Following the withdrawal of UPOL to Harmores 3 project at the end of the part 1, the addendum to Grant agreements for part 1 part 2 was signed the 04th January 2018 and the addendum to Financing agreement was signed the 8th March 2018.

Following the withdrawal of UPOL from the Harmores 3 project, there has been no change to the CPVO funding of part 2, however the distribution of the budget may be adapted if additional tasks are undertaken by any partners.

The part 2 year 2 was the phase of validation that is why a larger number of participants was involved in the comparative tests.

II. PATHOSTAT-Veg

PATHOSTAT-Veg is an IT application developed by GEVES in the framework of a French Ministry project. The aim of the application is to propose selected statistical tests selected and adapted to the different bio-tests used for the registration and the protection of vegetable varieties. These statistical tests have to be used in complement of observation in bio-tests to help in the interpretation of varieties. This application will be soon available on the GEVES website.
PATHOSTAT was applied on one example of Harmores 3 project: tomato/*Fusarium oxysporum* f. sp. *lycopersici* from an Excel result table of partners data. It allows to validate of the minimum number of plants observed, to compare distributions between replicates (block and varieties) and to study of the resistance/sensitivity evaluation of varieties.

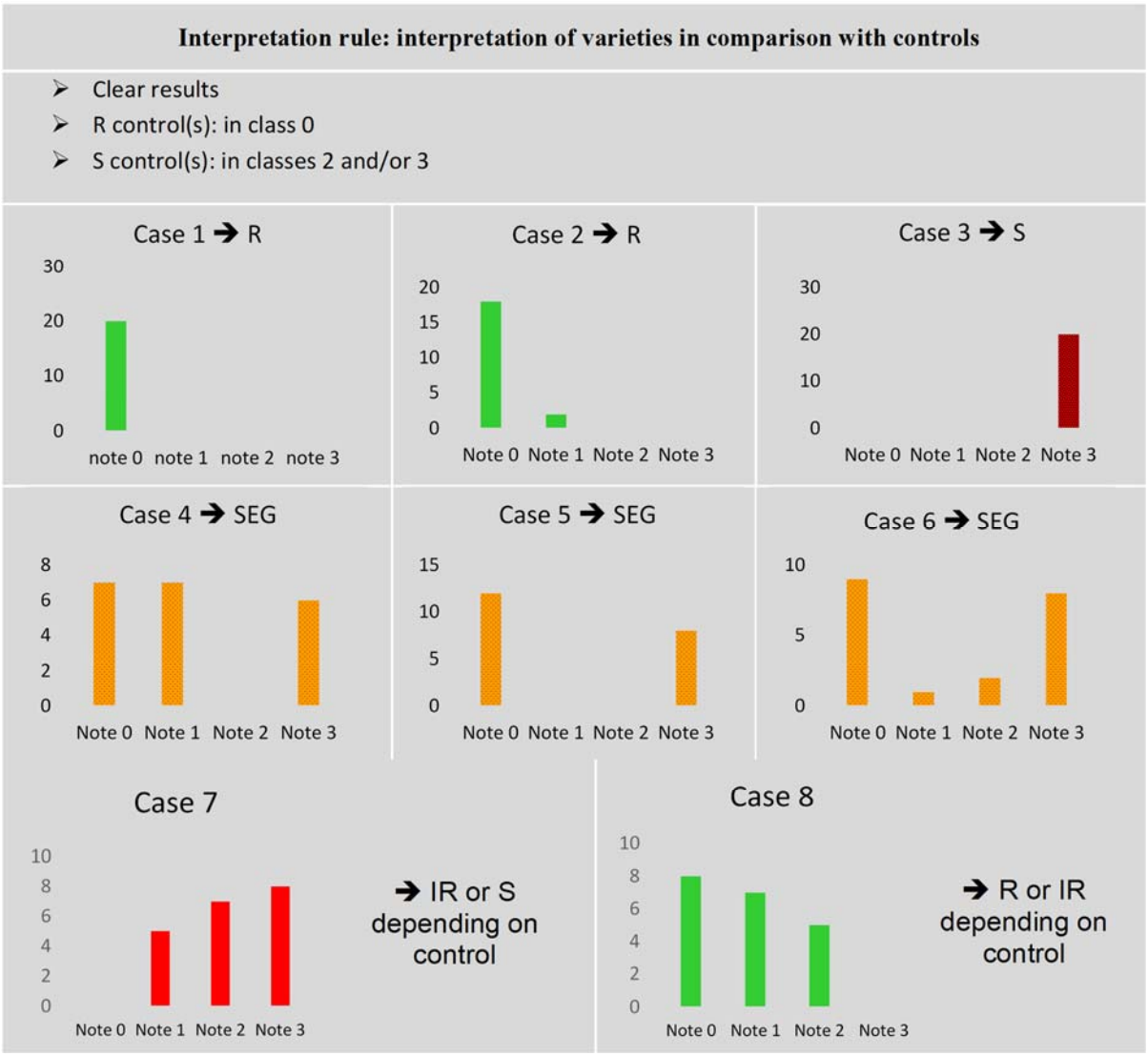
III. Tomato

A. Tomato/ *Fusarium oxysporum* f. sp. *lycopersici* race 0 and race 1

During the meeting of part 2 year 1, the steering committee decided to carry out a second comparative test to validate the new common notation scale (Table 2) and the interpretation rule (Figure 1).

Table 2: common notation scale defined in Harmores 3 project Part 2 year 1

Class 0	Class 1	Class 2	Class 3
Healthy compared to the non-inoculated control.	Healthy compared to the non-inoculated control with brown vessel above the cotyledon (observed when plants are cut in case of variety with different levels of symptoms)	Higher than 50% of growth reduction and/or yellowing and/or wilting on cotyledons and/or leaves.	Nearly dead: strong reduction with plants look dwarf (there can be necrosis but not always) or dead



R: resistant; S: susceptible; IR: intermediate resistant

Figure 1: common decision rule for tomato/Fusarium oxysporum f. sp. lycopersici

It was also proposed for voluntary partners to test the use of markers (protocol proposed to UPOV) and test on bigger plants for additional information in the aim to validate results of biotest. Markers on whole panel and test on big plants on varieties with a certain level of resistance plus one susceptible control were optional.

1. Materials and methods

a) Comparative test

14 laboratories were involved in the validation comparative test.
Each lab selected, among the three strains validated per race (Harmores 1), the most robust strain for each race based on its own test conditions (Tables 2 and 3).

Table 3: strains tested in Fol: 0 CT (one isolate chosen by lab)

Fusarium oxysporum f. sp. lycopersici race 0	PRI20698
	Orange 71
	Fol 071

Table 4: strains tested in Fol: 1 CT (one isolate chosen by lab)

Fusarium oxysporum f. sp. lycopersici race 1	4152 (more aggressive)
	PRI 10195 N
	RAF 70 (less aggressive)

The validation CT was performed on a panel made up of CPVO control and varieties tested in previous tests (Tables 5 and 6). Based on results on last workshop, it was proposed to include uncoded intermediate resistance cherry type controls (in addition to other uncoded controls from CPVO protocol):

- Fol: 0: variety G
- Fol: 1: variety H

New varieties with cherry type and/or an intermediate resistance but sufficiently resistant in field (tomato lines only, not rootstock) were also request from partners to be added for both races in the panel. But no proposition was received for the part 2 year 2.

Table 5: panel of varieties compared in tomato/Fol: 0 CT

Pathogen	Varieties	Expected	Cultigroup	
Fusarium oxysporum f. sp. *lycopersici* race 0	Marmande verte	Susceptible		Uncoded
	Marporum x Marmande verte	Resistant		
	Marporum	Resistant		
	Motelle	Resistant		
	Cherry type control 1/G	Intermediate Resistant	Small fruits	
	Vispo	Intermediate Resistant	Cherry	Coded
	Moneymaker	Susceptible	Classic round fruits	
	A	Intermediate Resistant	Cherry	
	B	Intermediate Resistant	Cherry	
	C	Intermediate Resistant	Cherry	
	D	Not uniform	Pear	
	E	Not uniform	Goose	
	G	Intermediate Resistant	Small fruits	

Table 6: panel of varieties compared in tomato/Fol: 1 CT

Pathogen	Varieties	Expected	Cultigroup	
Fusarium oxysporum f. sp. *lycopersici* race 1	Marmande verte	Susceptible		Uncoded
	Marporum	Susceptible		
	Motelle x Marmande verte	Resistant		
	Cherry type control 2/H	Resistant		
	Galaxy	Intermediate resistant	Classic	Coded
	Fol Harmo	Intermediate resistant		
	Moneymaker	Susceptible	Classic round fruits	
	H	Resistant	Cherry	
	I	Resistant	Cherry	
	J	Resistant	Cherry	
	K	Intermediate resistant	Small fruits	
	L	Intermediate resistant	Classic	

Tests were performed following CPVO protocol harmonized in Harmores 1 project (Figure 2). Notation were done with the common notation scale and comportment of varieties was defined by each partner depending on the common decision rule.

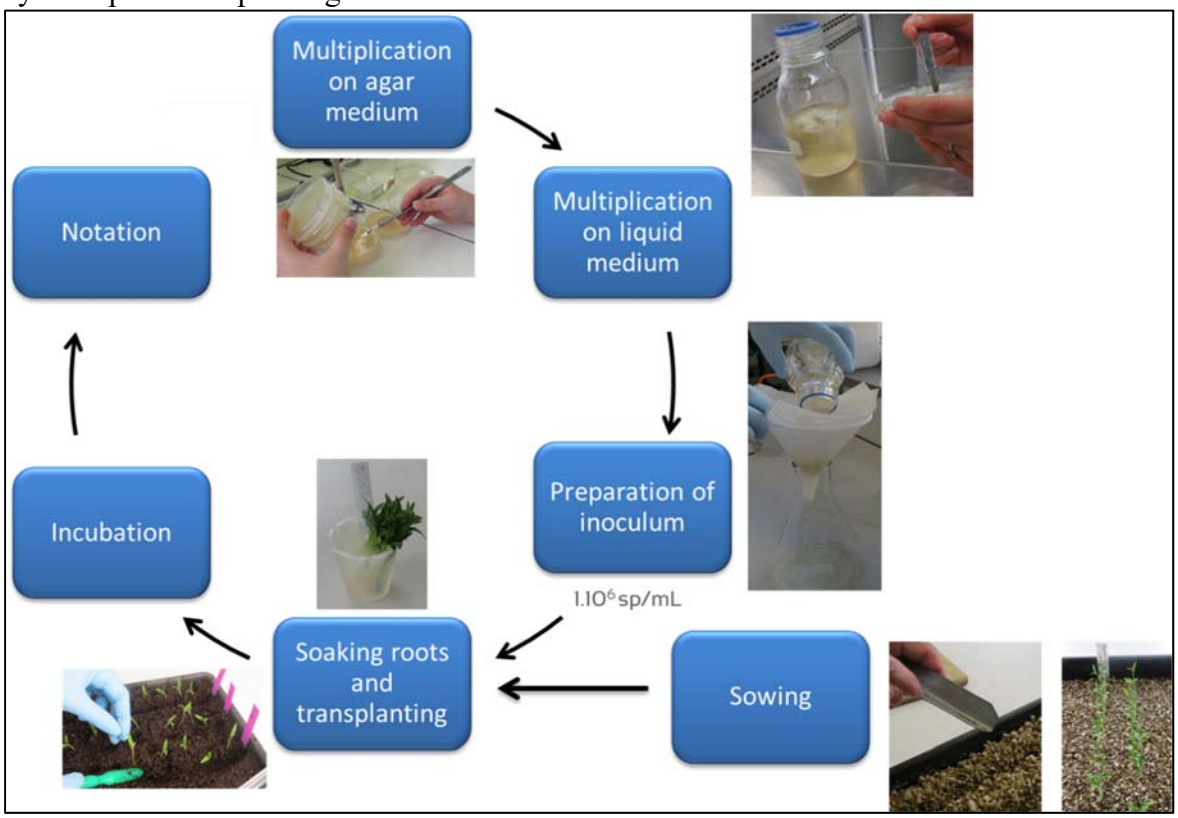


Figure 2: illustration of Tomato/*Fusarium oxysporum* f. sp. *lycopersici* protocol harmonized in Harmores 1

It was decided to perform test on 3 repetitions of 10 plants to allow statistical analysis of comparison with controls. Interpretation of results will be based on the repartition of plants per class and disease index and following the common decision rule.

b) Test on big plants

Test on big plants was proposed as optional and was carried out by 3 partners (CREA, GEVES and Naktuinbouw).
It was decided by the steering committee during the last meeting that the test will performed only on varieties on panel with no clear-cut comportment (that is to say for Fol 0: the varieties G and E and for Fol 1: the varieties H, I and J) and controls. The protocol used is detailed in annex 1.
Notation will be based on symptoms of brown vessels under cotyledons and yellowing and wilting on leaves.

c) Marker tests

Test on markers was proposed as optional and was carried out by 3 partners (CREA, GEVES and Naktuinbouw). It was planned on all varieties tested on comparative test on at least 20 plants.
The protocol is presented in annex 2.

2. Fol: 0 results

a) Fol : 0 results of CT

The results of interpretation of varieties by partners are presented in Table 7.

Table 7: results of tomato/Fol: 0 CT with lab's interpretation

Variety	Expected	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 8	Lab 9	Lab 10	Lab 11	Lab 13	Lab 14	Lab 17
D	Not uniform	R	R	IR	HG	R	IR	R	R	HG	IR	R	S
E	Not uniform	R	R	HG	HG	IR	R	IR	HG	R	R	R	S
Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S	S
Moneymaker	S	S	S	S	S	S	S	S	S	S	S	S	S
Cherry type control 1	IR	R	R	R	R	R	IR	R	R	R	R	R	R
Vispo	IR	R	R	IR	R	R	R	R	R	R	R	R	IR
A	IR	R	R	R	R	R	R	R	R	R	R	R	IR
B	IR	R	R	R	R	R	R	R	R	R	R	R	HG
C	IR	R	R	R	R	R	IR	R	R	R	R	R	R
G	IR	HG	R	R	HG	R?	IR	IR	S	HG	HG	HG	IR
Marporum x Marmande verte	R	R	R	R	R	HG	R	R	R	R	HG	R	R	R
Marporum	R	R	R	R	R	R	R	R	R	R	HG	R	R	R
Motelle	R	R	R	R	R	R	R	R	R	R	R	R	R	R

R: resistant; IR: intermediate resistant; S: susceptible; HG: heterogeneous; in blue: uncoded controls

The susceptible control (Marmande verte) and resistant controls (Marporum x Marmande verte, Marporum and Motelle) were conformed as expected in all labs excepted in one lab where Marporum x Marmande verte and Marporum were observed as heterogeneous.
The added control (Cherry type control 1/G) observed with an intermediate resistant level in previous tests was globally judged as resistant.
The susceptibility level of Moneymaker, observed in previous tests, was confirmed by all labs.
The varities Vispo, A, B, C, expected as intermediate resistant were globally judged as resistant. The variety G (coded) was judged with the different interpretations (S, IR, R or HG) depending on labs.
Both varieties D and E, expected as not uniform, showed again an important difference of interpretation depending on labs, even with the comparison to the Cherry type control 1.

The same raw data were analysed with the PATHOSTAT-Veg application. Results are presented in Table 8. The application proposed 2 levels of interpretation susceptible or resistant (using resistant controls as reference level of resistance) and highlight not uniform varieties.

Table 8: results of tomato/Fol: 0 CT with PATHOSTAT-Veg interpretation

Variety	Expected	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 8	Lab 9	Lab 10	Lab 11	Lab 13	Lab 14	Lab 17
D	Not uniform	R	R	R	R	R	R	R	R	R	HG/S	R	HG/S
E	Not uniform	R	R	R	R	HG/S	R	R	HG/S	R	R	R	S
Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S	S
Moneymaker	S	S	S	S	S	S	S	S	S	S	S	S	S
Cherry type control 1 = G	IR	R	R	R	R	R	R	R	R	R	R	R	R
Vispo	IR	R	R	R	R	R	R	R	R	R	R	R	R
A	IR	R	R	R	R	R	R	R	R	R	R	R	HG/S
B	IR	R	R	R	R	R	R	R	R	R	R	R	HG/S
C	IR	R	R	R	R	R	R	R	R	R	R	R	R
G	IR	R	R	R	R	R	R	R	S	R	HG/S	HG	HG/S
Marporum x Marmande verte	R	R	R	R	R	R	R	R	R	R	R	R	R
Marporum	R	R	R	R	R	R	R	R	R	R	R	R	R
Motelle	R	R	R	R	R	R	R	R	R	R	R	R	R

R: resistant; IR: intermediate resistant; S: susceptible; HG: heterogeneous; in blue: uncoded controls

More varieties were statistically judged not different than controls with the statistical application. The varieties Cherry type control 1/G**,** Vispo, A, B and C were judged as resistant, excepted in lab 17 where A and B were judged as the limit HG/S (not statistically susceptible compared to the susceptible control). The variety G, D and E were also judged with different levels (R, S or HG/S) depending on tests.

The varieties E and G were also tested on big plants to confirm their resistant level at a latest stage.

b) Fol: 0 results for test on big plants

Results on test at a latest stage are presented table 9.

Table 9: results of tomato/Fol: 0 CT with test on big plants

Variety	Expected	Lab 3	Lab 5	Lab 6
E	Not uniform	IR	R	R
Marmande verte	S	S	S	S
G	IR	IR	R	R
Marporum x Marmande verte	R	R	R	R
Marporum	R	R	R	R
Motelle	R	R	R	R

R: resistant; IR: intermediate resistant; S: susceptible; HG: heterogeneous; in blue: uncoded controls

A good correlation was observed between labs. Controls showed expected results. Varieties E and G were judged as IR or R compared to controls in the 3 labs. Even if E and G were interpreted either resistant or intermediate resistant depending on labs, analysis of raw data confirmed that same symptoms were observed by the 3 labs on these varieties. Depending on conditions of the test, either no symptoms on E and G, either slight ones were observed compared to resistant control but less than the susceptible one. The differences of judgement are due to lab's interpretation and not to differences between level of resistance.

c) Fol: 0 results for test with markers

Tests were carried out by 3 labs with marker for *I2* gene which confers resistance to Fol: 0 and Fol: 1.
Not all varieties and 20 plants were tested by all participants in contrast on what was planned (table 10). 2 varieties A and C were tested genotypically heterogeneous (homozygous and heterozygous *I2*) but with a homogeneous phenotype predicted (resistant), confirmed by biotest. The question is how to judge the homogeneity of these varieties? This issue was discussed by steering committee with the results obtained for Fol: 1.

Table 10: results of tomato/Fol: 0 CT with markers test

	Lab 3					Lab 5 (not 20 plants as defined)					Lab 6 (not 20 plants as defined)				
Variety	**Biotest**		**Homo I2**	**Hete I2**	**Homo S**	**Biotest**		**Homo I2**	**Hete I2**	**Homo S**	**Biotest**		**Homo I2**	**Hete I2**	**Homo S**
D	27-1-2-0	R			20										
E	25-4-1-0	R		1	20						15-0-5-0	IR			4
Marmande verte	1-0-14-15	S			28	1-0-6-13	S			7	0-0-20-0	S			4
Moneymaker	2-0-4-16	S			13										
Cherry type control 1 = G	27-1-2-0	R			18	18-0-2-0	R								
Vispo	24-0-0-0	R		18	1										
A	30-0-0-0	R	2	18											
B	29-1-0-0	R		20											
C	30-0-0-0	R	9	11											
G	25-4-1-0	R			18					5					
Marporum x Marmande verte	28-1-1-0	R			30	18-0-2-0	HG			5					
Marporum	28-0-1-0	R			30	20-0-0-0	R			10	16-3-3-0	R			4
Motelle	30-0-0-0	R	17	1		20-0-0-0	R								

R: resistant; S: susceptible; HG: heterogeneous; Homo: homozygous; Hete: heterozygous; in blue: uncoded controls; unexpected results

3. Fol: 1 results

a) Fol: 1 results of CT

The results of interpretation of varieties by partners are presented in Table 11.

Table 11: results of tomato/Fol: 1 CT with lab's interpretation

		Fol 1 - Standard test												
Variety	**Expected**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**	**Lab 8**	**Lab 9**	**Lab 10**	**Lab 11**	**Lab 13**	**Lab 14**	**Lab 17**	**Lab 18**
Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Marporum	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Moneymaker	S	S	S	HG	R	S	S		S	S	HG	S	S	S
Galaxy	IR	R	R	IR	HG	IR	IR	R	HG	HG	R	R	R	R
Fol Harmo	IR	R	HG	IR	S	IR?	IR	R		HG	R	R	R	IR
K	IR	R?	HG	R	S	S	HG	HG	IR	S	HG	HG	IR	IR
L	IR	R	HG	R	R	R	IR	R	R	R	R	R	R	R
Motelle x Marmande verte	R	R	R	R	HG	IR	R	R	R	R	R	R	R	IR
Cherry type control 2/H	R	R	R	R	HG	R	R	R	R	R	R	R	R	R
H	R	R	IR	IR	R	IR	R	IR	R	R	R	R	R	R
I	R	R	IR	R	HG	R	HG	R	R	R	R	R	R	R
J	R	R	HG	IR	S	IR	R	R	R	R	R	R	IR	R

R: resistant; IR: intermediate resistant; S: susceptible; HG: heterogeneous; in blue: uncoded controls

The susceptible controls (Marmande verte and Marporum) and resistant control (Motelle x Marmande verte) were conformed as expected in all labs excepted for the resistant hybrid which was observed as heterogeneous or intermediate resistant in three labs.
The added control (Cherry type control 2/H) observed with an intermediate resistant level in previous tests was globally observed as resistant.
The susceptibility level of Moneymaker, observed in previous tests, was confirmed by most of labs. But it was judged as heterogeneous or resistant by 3 labs.
The varieties Galaxy, Fol Harmo, K and L, expected as intermediate resistant and the varieties H, I and J, expected resistant were judged differently depending on labs.

The same raw data were analysed with the PATHOSTAT-Veg application. Results are presented in Table 12. The application proposed 2 levels of interpretation susceptible or resistant (using resistant controls as reference level of resistance) and highlight not uniform varieties.

Table 12: results of tomato/Fol: 1 CT with PATHOSTAT-Veg interpretation

		Fol 1 - Standard test												
Variety	**Expected**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 5**	**Lab 6**	**Lab 8**	**Lab 9**	**Lab 10**	**Lab 11**	**Lab 13**	**Lab 14**	**Lab 17**	**Lab 18**
Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Marporum	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Moneymaker	S	S	S	S	R	S	S		HG/S	S	HG/S	S	S	S
Galaxy	IR	R	R	HG/S	R	R	R	R	R	HG/S	R	R	R	R
Fol Harmo	IR	R	R	R	S	HG/S	R	R	HG/S	R	R	R	R	R
K	IR	HG/S	HG/S	R	HG/S	HG/S	HG/S	S	HG/S	S	HG/S	HG	HG/S	R
L	IR	R	HG/S	R	R	R	R	R	R	R	R	R	R	R
Motelle x Marmande verte	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Cherry type control 2	R	R	R	R	R	R	R	R	R	R	R	R	R	R
H	R	R	R	HG/S	R	R	R	R	R	R	R	R	R	R
I	R	R	R	R	R	R	HG/S	R	R	R	R	R	R	R
J	R	R	HG/S	HG/S	HG/S	HG/S	R	R	R	R	R	R	HG/S	R

R: resistant; IR: intermediate resistant; S: susceptible; HG: heterogeneous; in blue: uncoded controls

Varieties were statistically judged not different than controls in more tests with the statistical application. But in still many tests, the varieties were judged as the limit HG/S (not statistically susceptible compared to the susceptible control).

The varieties H, I and J were also tested on big plants to confirm their resistant level at a latest stage.

a) Fol: 1 results for test on big plants

Results for Fol: 1 on test at a latest stage are presented table 13.

Table 13: results of tomato/Fol: 1 CT with test on big plants

		Fol 1 - Test on big plants		
Variety	**Expected comportment**	**Lab 3**	**Lab 5**	**Lab 6**
Marmande verte	S	S	S	S
Marporum	S	S	HG	S
Motelle x Marmande verte	R	R	R	R
H	R	IR	R	R
I	R	IR	R	R
J	R	IR	R	R

R: resistant; IR: intermediate resistant; S: susceptible; HG: heterogeneous; in blue: uncoded controls

A good correlation was observed between labs. Controls showed expected results. Varieties H, I and J were judged as IR or R compared to controls in the 3 labs. In the same way as for Fol: 0, even if H, I and J were interpreted either resistant or intermediate resistant depending on labs, analysis of raw data confirmed that same symptoms were observed by the 3 labs on these varieties. The differences of judgement are due to lab's interpretation and not to differences between level of resistance.

b) Fol: 1 results for test with markers

Tests were carried out by the same 3 labs as Fol: 0 with marker for *I2* gene which confers resistance to Fol: 0 and Fol: 1. Not all varieties and 20 plants were tested by all participants in contrast on what was planned (table 14). Lab 3 informed that only 4 plants were tested because for the mit is enough

Table 14: results of tomato/Fol: 1 CT with markers test

Variety	Lab 3								Lab 5 (not 20 plants as defined)								Lab 6 (not 20 plants as defined)							
	Biotest					Homo I2	Hete I2	Homo S	Biotest					Homo I2	Hete I2	Homo S	Biotest					Homo I2	Hete I2	Homo S
	Class 0	Class 1	Class 2	Class 3	Judged				Class 0	Class 1	Class 2	Class 3	Judged				Class 0	Class 1	Class 2	Class 3	Judged			
Marmande verte	0	0	0	30	S			30									0	0	30	0	S			4
Marporum	0	0	1	29	S			30									0	1	16	14	S			4
Moneymaker	0	0	0	30	S			15									0	0	26	2	S			4
Galaxy	11	0	0	0	R		9	1	17	0	3	0	HG		5	1	19	7	4	0	IR		4	
Fol Harmo	24	1	4	0	HG		19										11-	8	11	0	IR?		4	
K	2	0	0	12	HG	1		5									0	3	21	6	S			4
L	18	0	3	3	HG		12	8									30	0	0	0	R		4	
Motelle x Marmande verte	26	1	2	0	R		28		18	0	2	0	HG		9		14	15	1	0	IR		4	
Cherry type control 2 = H	26-	0	2	0	R	20			15	1	4	0	HG	1	8	1	21	6	2	0	R	4		
H	24	2	4	0	IR	13	7										13	11	6	0	IR	4		
I	19	5	5	1	IR		20		17	2	1	0	HG			4	22	7	1	0	R		4	
J	9	2	10	0	HG	12	6		4	0	8	8	S			10	12	6	7	0	IR	4		

R: resistant; S: susceptible; HG: heterogeneous; Homo: homozygous; Hete: heterozygous; in blue: uncoded controls; unexpected results

No concordances between labs were observed:

- Genetic heterogeneity observed only in some labs: 1 or 2 labs out of 3 for varieties Galaxy, K, Cherry type control 2 and H.
- The *I2* marker not detected in all labs: for varieties I and J, marker detected in only in 2 labs out of 3.

Some unexpected results were obtained:

- *I2* marker detected but susceptible plants observed in biotest: 4 varieties (Fol Harmo, H, I and J) tested with *I2* but with susceptible plants in test (classes 2 and 3).
- Varieties genotypically heterogeneous (homozygous and heterozygous *I2*) but with a homogeneous phenotype predicted (resistant): 2 varieties H and J.

In addition to the problem on non-concordance between labs, these results raise the question as to whether how to judge the level of resistance of the varieties with presence of susceptible plants but presence of *I2* marker (resistant or intermediate resistant)? And how to judge the homogeneity of the varieties genotypically heterogeneous but with a homogeneous phenotype predicted**?** In this case, markers could predict the phenotype but could not be used to judge uniformity.
One of possible explanation of these differences between phenotype and genotype is the penetrance reaction: a modification of expression of *I2* gene when presence of *I* gene. Perhaps this reaction will not be visible at a latest stage of inoculum (hypothesis due to the fact that on big plants, varieties are judged as resistant).

It was also observed a problem of presence of non-specific bands on gels, including one band with high risk of confusion with 940 bp band (*I2*) (figure 3).

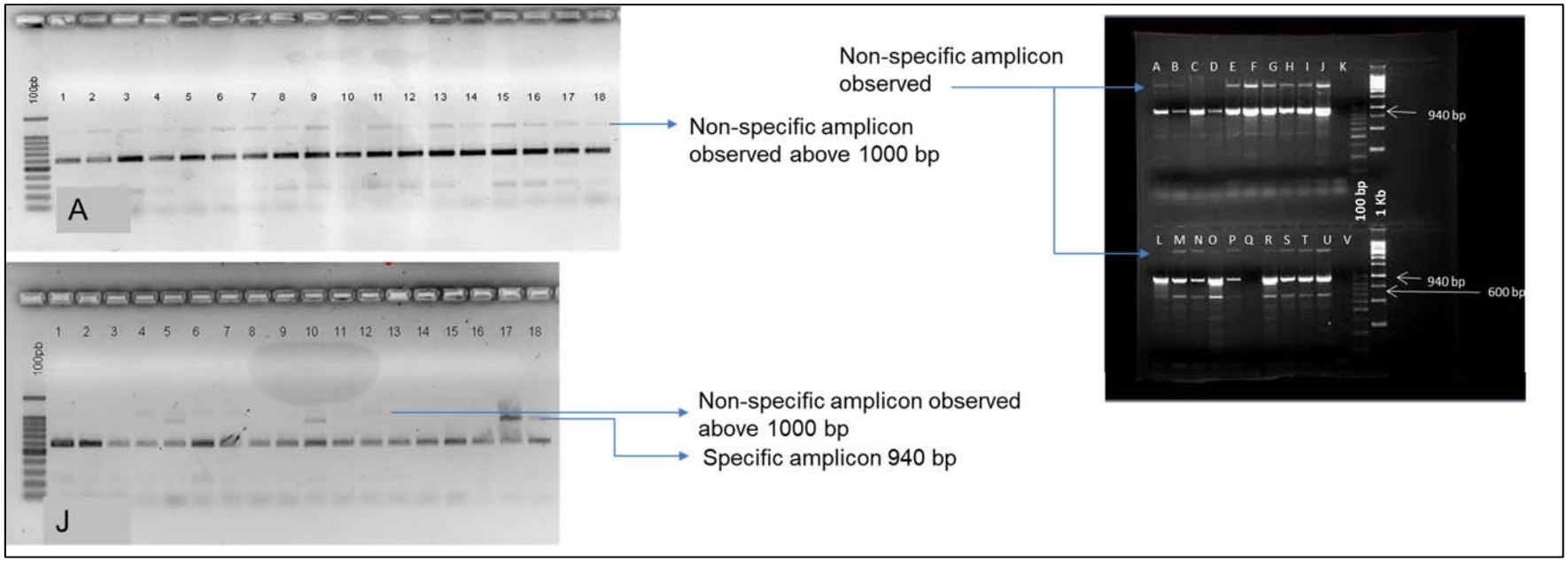


Figure 3: non-specific band on *I2* marker gels.

There is not enough data (not enough labs to have the expected number of plants) and too much inconsistencies between labs for a validation of *I2* markers. It is a necessity to repeat test with more plants and more labs to conclude.

4. Tomato/ *Fusarium oxysporum* f. sp. *lycopersici* conclusion

a) Protocol of biotest

Based on the results obtained in last year of Hamores 3 project, the steering committee decided to select for the updated protocol (annex 3):

- Isolates validated in Harmores 1 project (Tables 3 and 4)

- Susceptible and resistant controls validated in Harmores 1 project (Table 15)

Table 15: tomato/Fol controls validated in Harmores 1 project

	Fusarium oxysporum f. sp. *lycopersici* race 0	*Fusarium oxysporum* f. sp. *lycopersici* race 1
Susceptible controls	Marmande or Marmande verte or Resal	Marmande verte or Roma or Cherry Belle or Marporum
Resistant control	Motelle or Mohawk or Gourmet and Marporum or Larissa (resistant only to race 0) and Marporum x Marmande verte	Tradiro or Odisea or Motelle or Mohawk or Ranco And Motelle x Marmande verte (optional)

It was decided to add a resistant control as control for medium level in the ones used for big plant tests (judged as resistant on big plants):

- For Fol: 0, E or G depending on availability
- For Fol: 1, H, I or J depending on availability

- The common notation scale (Table 16). The fact to cut plant to observe brown vessels is kept as a tool to confirm susceptible or resistant behaviour on varieties with plants on different classes. It is also informative to confirm controls if plants in different classes.

Table 16: common notation scale defined in Harmores 3 project Part 2 year 2 for *Fusarium oxysporum* f. sp. *lycopersici*

Class 0	Class 1	Class 2	Class 3
Healthy compared to the non-inoculated control.	Healthy compared to the non-inoculated control with brown vessel above the cotyledon (observed when plants are cut in case of variety with different levels of symptoms)	Higher than 50% of growth reduction and/or yellowing and/or wilting on cotyledons and/or leaves.	Nearly dead: strong reduction with plants look dwarf (there can be necrosis but not always) or dead
			
If all plants in class 0 or if all plants in classes 2 and 3, it is not necessary to cut the plants.			
In case of variety or control with different levels of symptoms, cut the plants to check presence or not of strong brown vessel above cotyledons. In case of no brown vessels or below cotyledons, the plant is note 0. In case of brown vessels above cotyledons, the plant is note 1.			

- The common decision rule:

Partners studied repartition of plants per class (on results for Fol: 0 and Fol: 1) for varieties judged differently depending on labs. This discussion allowed to define rules for validation of test on controls and for interpretation of varieties depending on common notation scale:

- Validation of test on controls
  - Susceptible: most plants in 2 and 3, at most 2 plants can be observed at classes 0 and 1
  - Resistant: most plants in 0 and 1, at most 2 plants can be observed at classes 2 and 3

- Interpretation of varieties for validated test:
  - If not different from both resistant level controls, the variety is judged as resistant.
  - If lower level from lower resistant level control, the variety is judged as susceptible.
  - If no clear results, it is advice to use statistical test or to retest.

- An interpretation of data in terms of UPOV characteristics

3 possible scenarios were compared:

1) Addition on an intermediate resistant control to allow 3 levels of interpretation: susceptible, intermediate resistant and resistant. Partners were agreed to eliminate this hypothesis which was not enough robust.
2) Addition of a threshold of a lower level of resistance to define a spectrum of different level of resistance between the lower and the higher level (as done before for Pea/*Ascochyta* or Pea/*Fusarium*).
3) Only susceptible and resistant level with current controls. Partners were agreed to eliminate this hypothesis which was not representative of what is observed in test.

Finally, the steering committee decided to select the second one scenario and to propose two resistance levels: susceptible and resistant (Figure 4) with a threshold of a lower level of resistance. The resistant level corresponds to a spectrum of different levels of resistance and not to a highly level of resistance (illustrated by PATHOSTAT results).

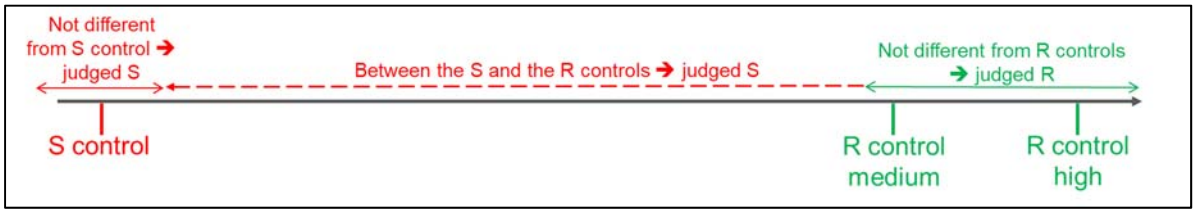


Figure 4: tomato/Fol interpretation

The interpretation of data in terms of UPOV characteristic states will be susceptible [1] or resistant [9].

b) Validation of marker test

For markers, it was decided a follow up meet with partners to set up a test plan for validation, presently there is not enough labs results and too much differences between labs. Before proposal of an alternative method, we have to obtain consistent results. The steering committee proposed a 2-step approach:

- A pretest so that each potential participant can implement the PCR protocol in their lab, and to confirm the capacity of the lab to apply the protocol in a future comparative test. For example, by sending around DNA extracts to be tested.
- The organization of an interlaboratory comparative test to fully validate the complete protocol. Including sowing seeds of varieties previously characterized in the Harmores 3 project, the DNA extraction step and the PCR test.

These tests are planned in autumn 2019. These extra tests will be outside CPVO project (without finding).

Question of the use of marker for *I* gene, which confers resistance to Fol: 0, was also discussed because it was validated on CPVO project on a panel of varieties. But experience of partners over the past few years showed that marker for *I* gene are not enough relevant. *I* gene is not well link. It is not yet ready to be proposed as alternative to biotest for evaluation of resistance of tomato to Fol: 0.

B. Tomato/ *Meloidogyne*

At the end of the part 2 year 1 of Harmores 3 project, a harmonized protocol was defined with the selection of the inoculation method (plants sown in soil contaminated with infested root) (figure 5), the number of plants to observe (at least 30 plants) and a common notation scale based on the previous workshop (table 17).

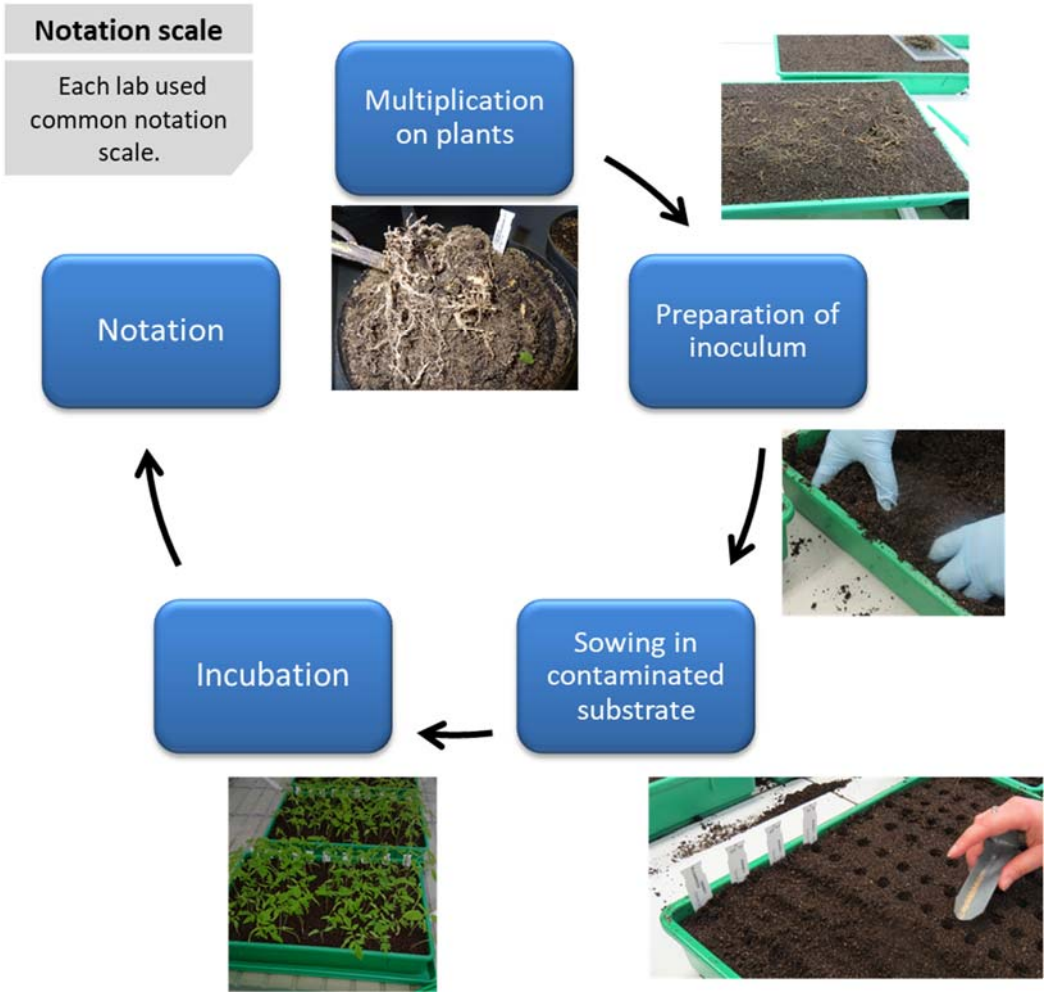
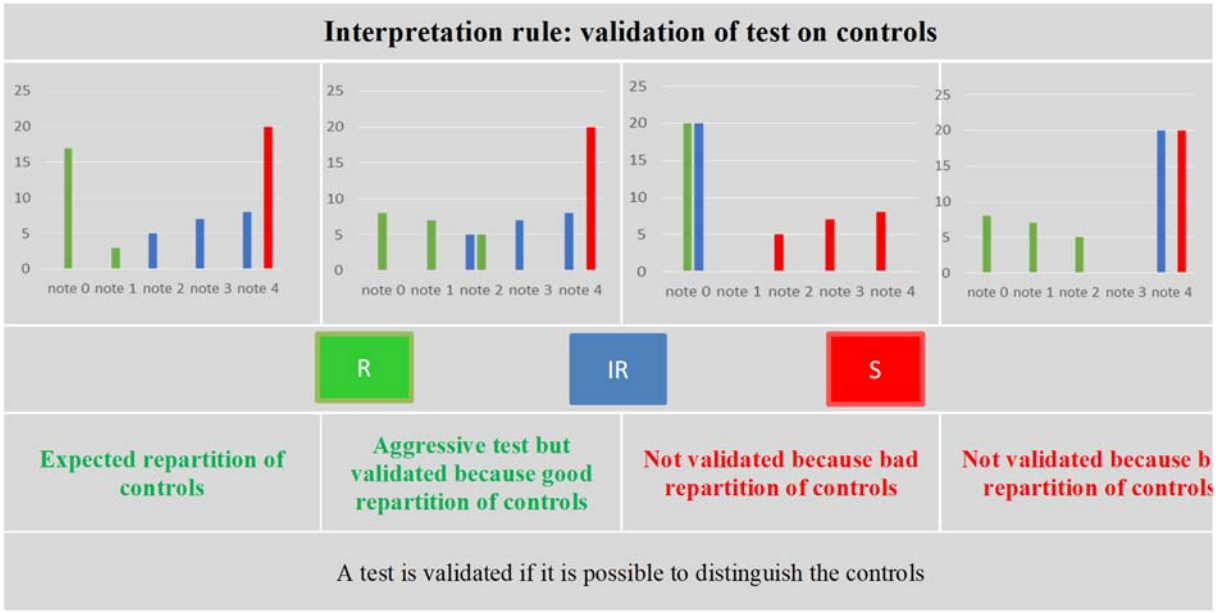


Figure 5: Protocol for plants sown in soil contaminated with infested root

Table 17: common notation scale defined in Harmores 3 project Part 2 year 1 for tomato/*Meloidogyne incognita*

Note 0: healthy plant, no galls	Note 1: few and little galls which are difficult to find (for example less than 5)	Note 2: few galls, easy to observe but on few roots, still a lot of roots without galls	Note 3: many individual galls on most but not all roots	Note 4: many galls on all roots sometimes in chains
				

A common decision rule was also described with the expected results on controls for validation of tests and the interpretation of varieties in comparison with controls (figure 6).



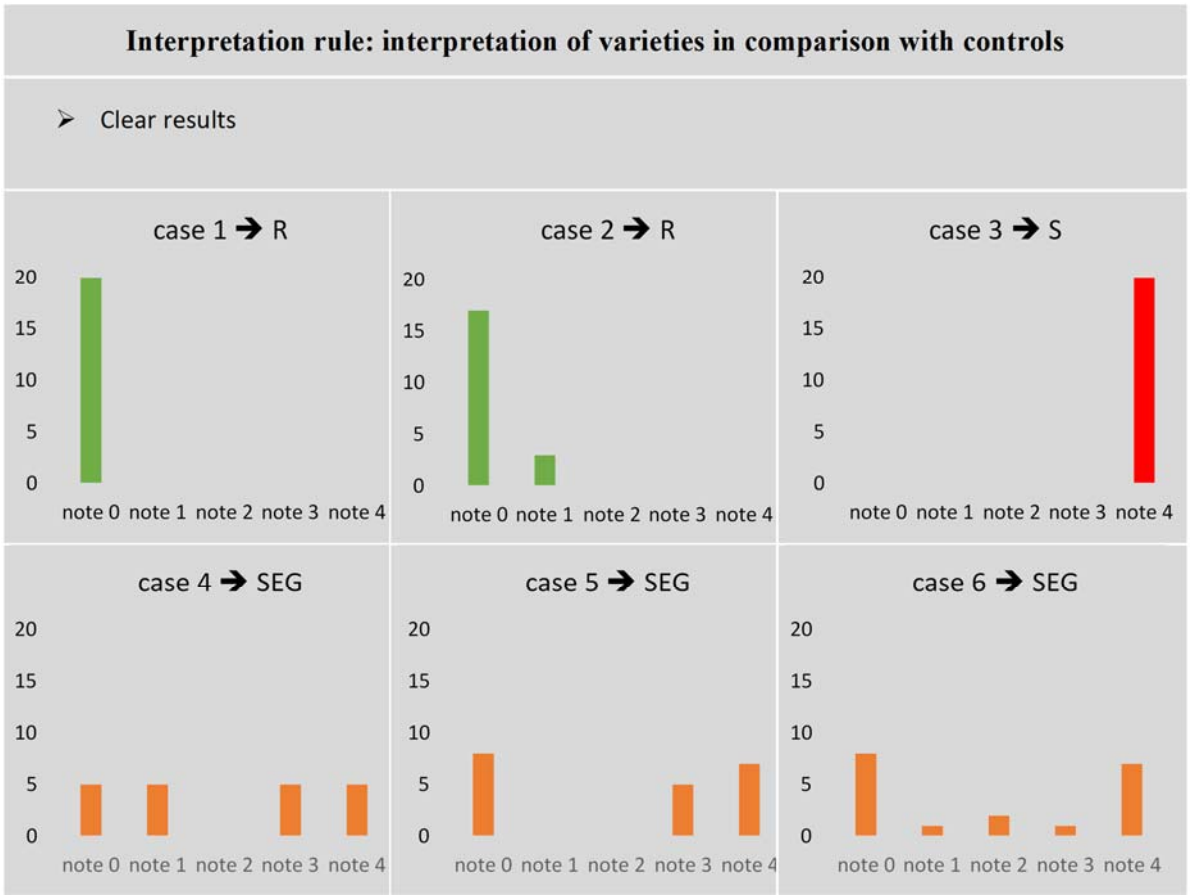


Figure 6: interpretation rule for tomato/*Meloidogyne incognita*

The objective of the last action is to select reference material and to validate the common interpretation rule.

1. Materials and methods

13 laboratories were involved in the validation comparative test.
The panel of height varieties of tomato (including CPVO controls) tested in part 2 year 1 was completed by 5 other varieties (Table 18):

- 4 varieties with different genetics and expected intermediate resistant or resistant
- 1 which is a F2 in segregation to confirm that the protocol allows to distinguish segregation from intermediate resistance.

Controls were uncoded to validate the test and to judge varieties comportment depending on the common decision rule (Figure 6).
Test was performed on thirty plants (plus 5 non-inoculated) with at least 3 repetitions per variety. Each lab used its own population of nematodes.

Table 18: panel of varieties compared in tomato/*Meloidogyne* CT part 2 year 2

Varieties	Expected comportment	
Casaque rouge	Susceptible	Uncoded
Anahu x Casaque rouge	Resistant	
Campeon	Intermediate resistant	
Tyonic	Intermediate resistant	
Casaque rouge	Susceptible	Coded
Bonny Best	Susceptible	
Altess	Intermediate resistant	
Campeon	Intermediate resistant	
Madyta	Intermediate resistant	
Mi-IR-RZ2018	Intermediate resistant	
Trujillo F1	Intermediate resistant	
Aguamiel F1	Intermediate resistant	
Tyonic	Resistant with Ty	
Anahu x Casaque rouge	Resistant	
Anahu	High resistant	
Mi-HR-RZ2018	Resistant	
F2	Segregation	

The selected inoculation method (the most commonly used method by the labs: plants sown in infested soil) was compared at two ratios (the ratio previously tested in part 2 year 1 and the ratio divided by 2). Indeed, due to the difference of aggressivity of test depending of lab's population and conditions, it was decided to test two different concentration of inoculum in all labs for the method "Plants sown in a mix soil with infested root": the concentration used in the previous CT and a halved concentration.
Furthermore, in option each laboratory had the opportunity to compare its own protocol to the selected protocol to allow to each partner to validate this additional method with its own conditions of test.
The repartition of tests per labs is described table 19.

Table 19: inoculation methods compared in tomato/*Meloidogyne* CT part 2 year 2

	Methods of inoculation						
	1: Plants sown in a mix soil with infested root Ratio Cf test plan	Plants sown in a mix soil with infested root Ratio of test plan divided by 2	Plants at 2 leaf stage contaminated with deposit of infested roots between lines	Plants at 2 leaf stage contaminated by deposit infested roots and transplanting	3 weeks stage plants contaminated with eggs (3000 eggs/pl)	Deposit juvenils at 14 days leaf stage (1st leaf emerging)	Plants sown in a mix soil with infested with specific number of nodules
HM Clause	X	X	X				
Naktuinbouw	X	X					
Bayer	X	X				X	
EZ	X	X					
Nebih	X	X					
Vilmorin	X	X					
BASF	X	X			X		X
GEVES	X	X	X				
INIA	X	X		X		X	
RZ	X	X					
CPPSI	X	X			X		
Sakata	X	X					
Gautier	X	X					

X: method used in routine by the lab, X: new method for the lab, no experience with this protocol

2. Results

a) Results of the common inoculation method (plants sown in a mix of soil with contaminated roots)

The results of lab's interpretation are presented in table 20.

Table 20: results of CT Harmores 3 part 2 for selected inoculation method for tomato/*Meloidogyne* CT part 2 year 2

		Plants sown in a mix soil with infested root Ratio 1											Plants sown in a mix soil with infested root Ratio 2											
Variety	**Expected**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 6**	**Lab 8**	**Lab 9**	**Lab 10**	**Lab 12**	**Lab 13**	**Lab 14**	**Lab 18**	**Lab 2**	**Lab 3**	**Lab 4**	**Lab 6**	**Lab 8**	**Lab 9**	**Lab 10**	**Lab 11**	**Lab 12**	**Lab 13**	**Lab 14**	**Lab 18**
F2	seg	R	HG	HG	R	IR	HG	HG	HG	HG	R	HG	IR	HG	HG	R	IR	HG	HG	HG	HG	HG	HG	HG
Casaque rouge (uncoded)	S	S	S	S	S	S	S	S	S	S		S	S	S	S	S	S	S	S	HG	S	S	S	
Casaque rouge (coded)	S	IR	S	S	S	S	S	S	S	HG		S	S	S	S	S	S	S	S	S	S	HG		S
Bonny Best	S	S	S	S	S	S	S	S	S	S		S	S	S	S	S	S	S	S	HG	S	S		S
Campeon (uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR	R	IR	HG	IR	IR	HG	IR
Campeon (coded)	IR	IR	IR	IR	IR	IR	IR	HG	IR	HG	IR	IR	IR	IR	IR	IR	IR	R	HG	S	IR	HG	HG	IR
Tyonic (uncoded)	IR	IR	IR	IR	S	IR	IR	IR	IR	IR			IR	IR	IR	IR	IR	S	IR	HG	IR	IR	S	
Tyonic (coded)	IR	S	IR	IR	IR	IR	HG	IR	IR	R	S	S	S	IR	IR	IR	IR	S	IR	HG	IR	R	S	S
Altess	IR	S	S	IR	S	IR	IR	S	IR	HG	S	S	IR	IR	IR	S	IR	IR	HG	S	IR	HG	S	S
Madyta	IR	S	IR	IR	R	IR	HG	S	IR	HG		S	S	IR	IR	IR	IR	S	S	HG	IR	HG	S	S
Mi-IR-RZ2018	IR	IR	IR	IR	IR	IR	HG	IR	IR	R	S	S	IR	IR	IR	IR	IR	S	IR	HG	IR	R	S	S
Trujillo F1	IR	S	IR	IR		S	HG	IR	IR	R		S	S	IR	IR	IR	IR	S	IR	HG	IR	R	S	S
Aguamiel F1	IR	S	IR	IR	IR	IR	HG	S	IR	IR	S	S	S	IR	IR	S	IR	R	IR	HG	IR	IR	S	S
Anahu x Casaque rouge (uncoded)	R	R	R	R	R	R	R	R	R	R	S		R	R	R	R	R	R	R	R	IR	R	IR	
Anahu x Casaque rouge (coded)	R	R	R	R	R	R	R	R	R	R	HG	R	R	R	R	R	R	R	R	R	R	R	HG	HG
Anahu	HR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	HG	R	R	R	R?
Mi-HR-RZ2018	HR	R	R	R	R	R	R	R	R	R	R	IR	R	R	R		R	R	R	R	IR	R	R	IR

R: resistant, IR: intermediate resistant, S: susceptible, HG/SEG: heterogeneous/segregation

Based on the decision rule for validation of controls, 5 tests were not validated (lab 11 ratio 2, lab 14 ratio 1 and 2, lab 18 ratio 1 and 2). These results were not included in the following analysis. For the other labs, controls were validated.

The variety F2 (in segregation) was well judged as heterogeneous in 14 tests out of 20. And it was judged as resistant or intermediate resistant in 6 tests out of 20. For these cases the repartition of plants per class was analysis by partners to confirm or not lab's interpretation (Figure 7).

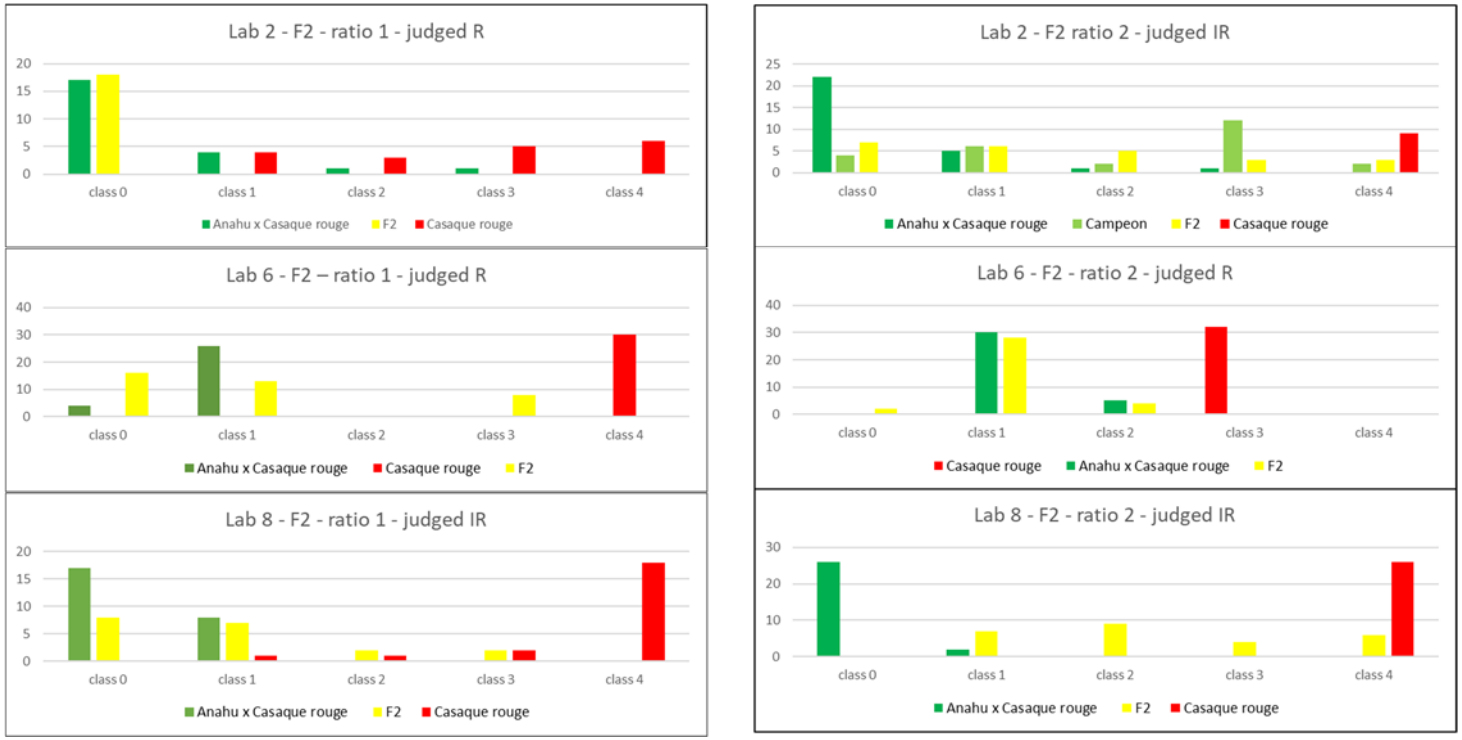


Figure 7: tomato/*Meloidogyne*, repartition of plants per class for F2 variety when not judged heterogeneous by labs

For lab 2 ratio 1, the test is not validated on the susceptible control Casaque rouge. For lab 2 ratio 2, the variety F2 would be also judged as intermediate resistant by other partners.
For lab 6 ratio 1, the variety F2 would be judged as heterogeneous by partners. And for ratio 2, the test was not validated on controls which are judged too closed.
For lab 8, both ratios were validated on controls, and variety F2 is judged as intermediate resistant.

In conclusion, the F2 variety was finally judged heterogeneous in 15 tests out of 18. It confirms that with the protocol allows to distinguish segregation from intermediate resistance.

Coded controls were included in the panel. They were not always judged as expected, but globally a good concordance was observed.
For the other varieties included in the panel, some differences were observed for varieties expected as intermediate resistant. Altess and Madyta confirmed the observation done in part 2 year 1 where they were already judged as susceptible in several labs. For the varieties Mi-IR-RZ2018, Trujillo F1 and Aguamiel F1 the repartition of plants per class of few tests was analyzed by partners (figure 8).

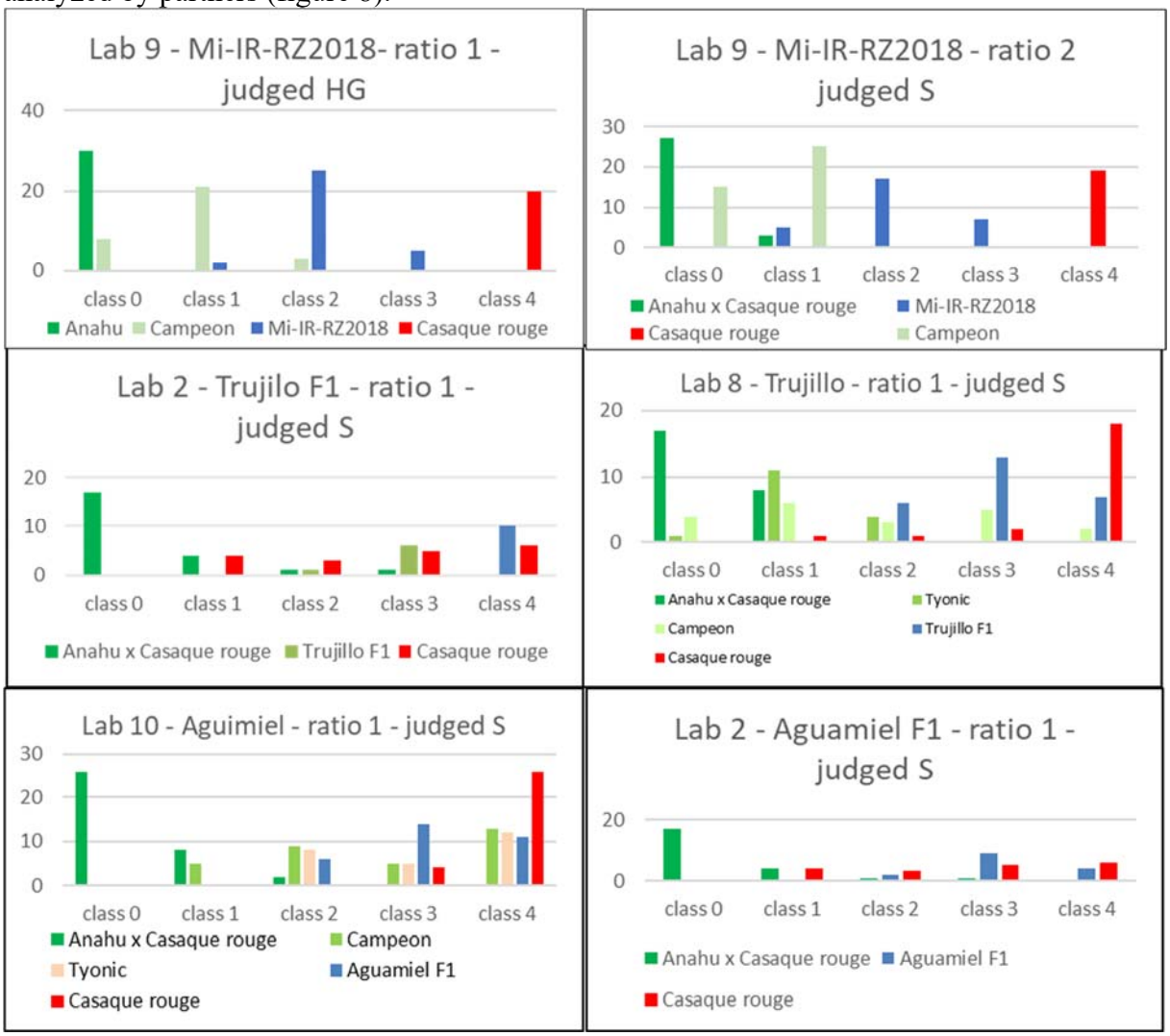


Figure 8: tomato/*Meloidogyne*, repartition of plants per class for intermediate resistant varieties when judged heterogeneous or susceptible by labs

The temperature of test was described as an explanation of the different levels of intermediate resistance observed. Indeed, in case of *Ty* resistance gene, *Ty* could be instable with a higher temperature. That why partners concluded that it is important to have a control with *Ty* resistance gene like the variety Tyonic.

The CPVO resistant control Anahu and the variety Mi-HR-RZ2018, expected as high resistant, were conformed in all tests.

During the comparative tests, it was observed that some tests can be very aggressive (sometimes dead plants with no galls because attacked very fast). It will be decided to add in the protocol that in case of aggressive test, it is advice to put seeds in a layer of non-contaminated soil or decrease the quantity of inoculum. It is also important to be careful to have an equal quantity of inoculum on root for inoculum (with not too much galls per roots).

b) Results of other inoculation methods

Finally, only 3 out of the 5 other methods were tested (Table 21). The inoculation methods by deposit of juveniles at 14 days leaf stage (1st leaf emerging) and plants sown in a mix soil with infested with specific number of nodules were not compared to the selected method.

Table 21: results of CT Harmores 3 part 2 for the other inoculation methods for tomato/*Meloidogyne* CT part 2 year 2

		Plants at 2 leaf stage contaminated with deposit of infested roots			3 weeks stage plants contaminated with eggs (3000 eggs/pl)
		between lines	and transplanting		
Variety	**Expected**	**Lab 3**	**Lab 2**	**Lab 17**	**Lab 14**
F2	seg	HG		R	HG
Casaque rouge (uncoded)	S	S	S	S	S
Casaque rouge (coded)	S	S		S	S
Bonny Best	S	S		S	S
Campeon (uncoded)	IR	IR	IR	IR	R
Campeon (coded)	IR	IR		R	R
Tyonic (uncoded)	IR	IR	S	IR	IR
Tyonic (coded)	IR	IR		IR	IR
Altess	IR	IR		IR	HG
Madyta	IR	IR		HG	IR
Mi-IR-RZ2018	IR	IR		IR	IR
Trujillo F1	IR	IR		S	HG
Aguamiel F1	IR	IR		S	HG
Anahu x Casaque rouge (uncoded)	R	R	R	R	R
Anahu x Casaque rouge (coded)	R	R		R	R
Anahu	HR	R		R	R
Mi-HR-RZ2018	HR	R		R	R

R: resistant, IR: intermediate resistant, S: susceptible, HG/SEG: heterogeneous/segregation

(1) Results of inoculation at 2 leaf stage with deposit of infested roots between lines

The inoculation at 2 leaf stage by deposit of infested roots stage between lines was tested only in one lab. Even if the interpretation of controls and varieties was conformed to expected, regarding the repartition of plants per class, the test was not validated on the susceptible control Casaque rouge with plants in the five classes as the both intermediate resistant controls (Campeon and Tyonic) (figure 9).

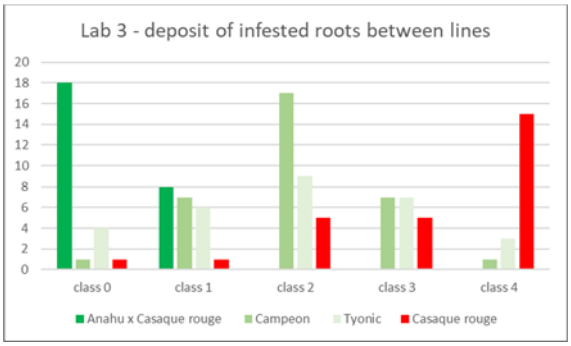


Figure 9: repartition of plants per class for control varieties

(2) Results of inoculation at 2 leaf stage with transplantation in contaminated soil

The inoculation by deposit of infested roots in soil and transplanting of plants at 2 leaf stage was not validated in lab 2 due to the susceptible comportment of the intermediate resistant control Tyonic with all plants in class 4 (figure 10). This inoculation method seems to be very aggressive in this lab with also 14 plants death for the susceptible control Casaque rouge.

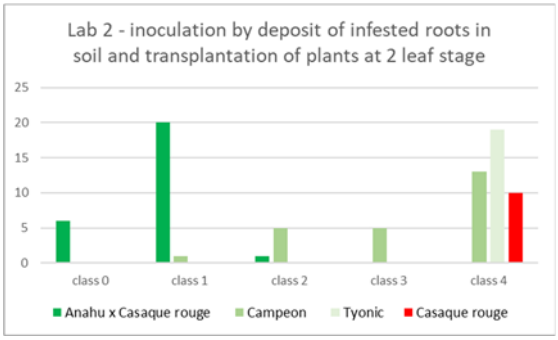


Figure 10: repartition of plants per class for control varieties

In lab 17, the results were conformed on the interpretation of controls and their repartition of plant per class. But the interpretations of varieties F2, Madyta, Campeon (coded), Trujillo F1 and Aguamiel F1 were non conformed with expected.

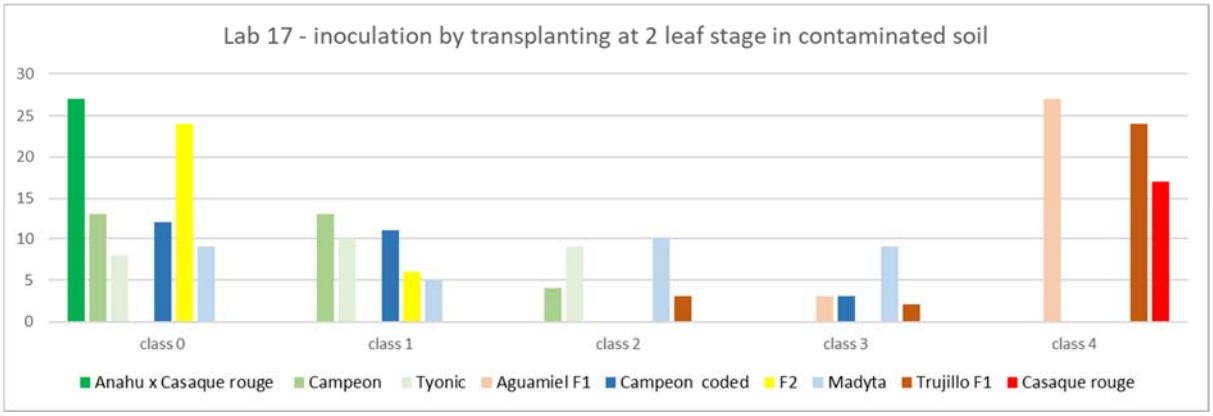


Figure 11: repartition of plants per notes for controls and varieties with unexpected interpretation

(3) Results of inoculation with deposit of eggs at 1-week stage

The controls are validated for this inoculation methods. Some differences are observed between the interpretation proposed by the lab and the repartition of plants per class. The varieties Aguamiel F1, Altess and Trujillo F1 were judged as heterogeneous by lab 14 but depending on the common decision rule these varieties should be interpreted intermediate resistant with a lower level than the threshold of Campean and Tyonic, and so judged as susceptible.
The variety F2 was well confirmed as heterogeneous with plants in classes 0 and 1 from one side and in classes 3 and 4 from another side.

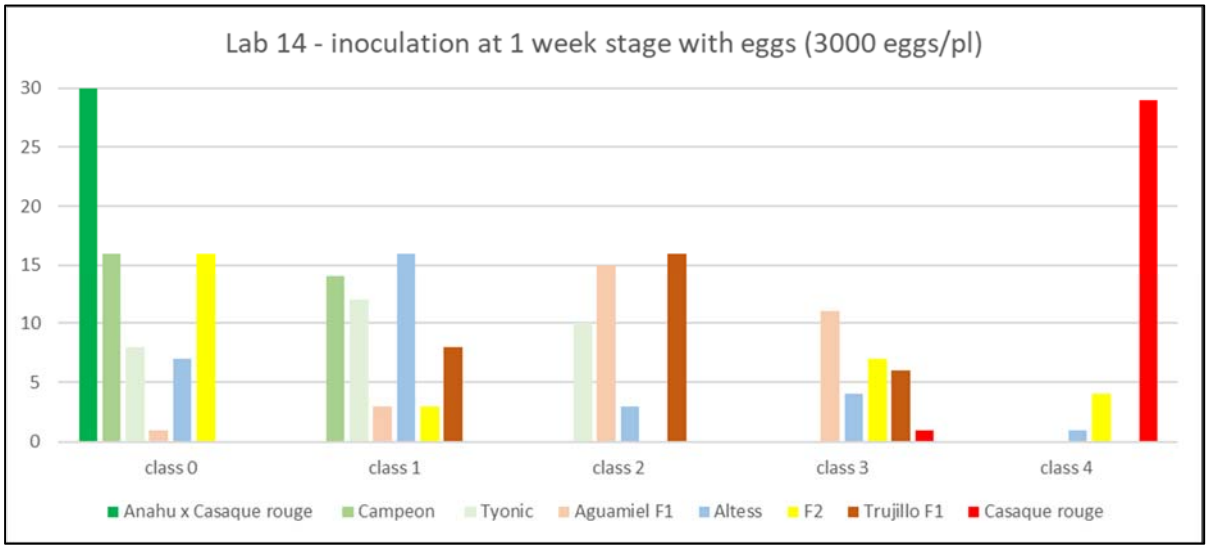


Figure 12: repartition of plants per notes for controls and varieties with unexpected interpretation

In conclusion, the inoculation methods at 2 leaf stage with deposit of infested roots between lines or with transplanting in contaminated soil were not validated in the labs who tested it. The inoculation method with deposit of eggs at 1-week stage was validated in the lab 14.

3. Tomato/ *Meloidogyne incognita* conclusion

Based on the results obtained in last year of Hamores 3 project, the steering committee decided to select for the updated protocol (annex 4):

- Susceptible control: Casaque rouge
- Intermediate resistant controls: Campeon (heterozygote Mi*1-2*) and Tyonic (*Ty*)
- Resistant control: Anahu x Casaque rouge. The hybrid was chosen more than Anahu because it is a hybrid variety and most of varieties in DUS tests are also hybrids. And it will allow to estimate the aggressivity of test.

The controls Casaque rouge, Campeon and Anahu x Casaque rouge are already included in MATREF network and available. The variety Tyonic (commercial variety) will be included in MATREF following the Harmores 3 project to be multiply and available in case of request.

Test will be performed on 30 plants in 3 repetitions of 10 plants in different trays (plus at least 10 non-inoculated plants to observe if the lack of germination is due to nematode or not). The steering committees decided that it was not necessary to have repetitions for non-inoculated plants.

The selected inoculation method with plants sown in soil contaminated by infested roots was validated. The steering committees validated the quantity of inoculum indicated in the test plan as ratio 1: Contaminated roots with galls are cut to approximately 2 to 3 cm and the small sections are mixed with the substrate. For example, the ratio of infested roots is between 30g to

60g, depending of aggressiveness of test and lab's conditions, for 100 plants to inoculate (in tray of 45*30 containing approximately 5.5 kg of substrate). It was precised that inoculum quantity is an indicative and each lab will have to adapt the quantity of inoculum to its own population of nematodes and environmental conditions.
For other methods, only the inoculation with deposit of eggs at 1-week stage was validated in one lab (lab 14).

The test conditions are between 20-26°C with the precision that the temperature has to be adapted depending on the aggressivity of test to obtain expected comportment of controls but should not be above 26°C.

The notation scale defined in part 2 year 1 was improved for class 4 (table 22).

Table 22: common notation scale validated for tomato/*Meloidogyne*

Class 0: healthy plant, no galls	Class 1: few and little galls which are difficult to find (for example less than 5)	Class 2: few galls, easy to observe but on few roots, still a lot of roots without galls	Class 3: many individual galls on most but not all roots	Class 4: many galls on all roots, sometimes in chains, can lead to dead plants and /or may suppress emergence
				

The common decision rule was review by partners depending on repartition of plants per class observed during the comparative test.

- Susceptible control: most plants at classes 3 and 4, at most 2 plants can be observed at class 2
- Resistant control: most plants at classes 0 and 1, at most 2 plants can be observed at class 2
- Intermediate resistant controls: clearly different from other controls with majority of plants around class 2

These rules for validation of test on controls are illustrated by the diagrams below (figures 13 and 14). For each control, the rule of 1 off-type allowed is also applied.

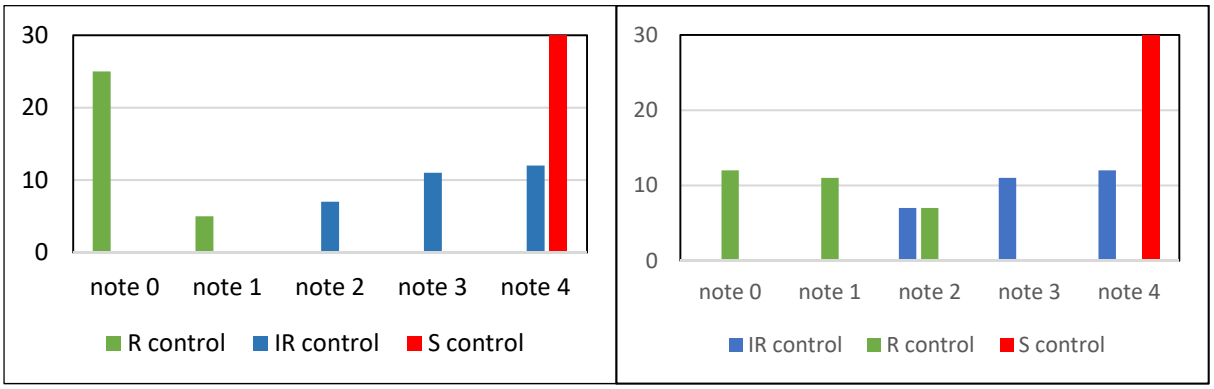


Figure 13: example of repartition of plants per class in case of validate test for tomato/*Meloidogyne incognita* (the example 2 is for aggressive test)

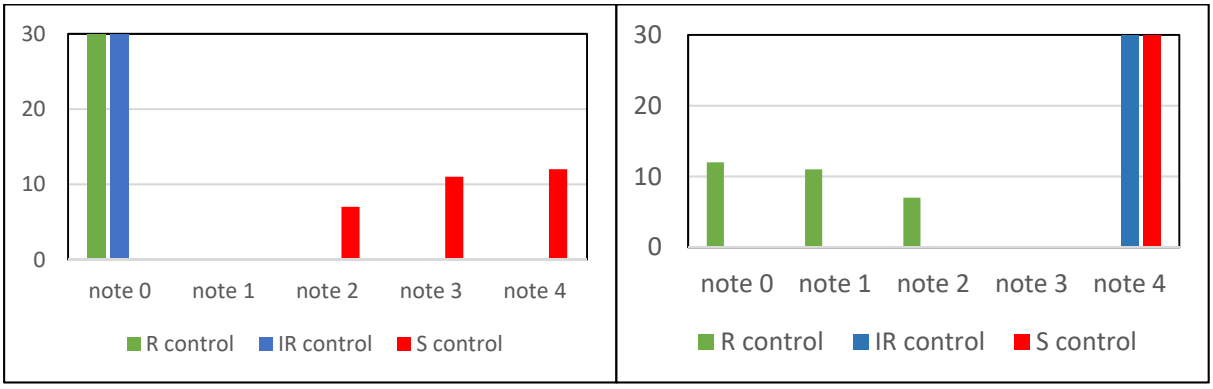


Figure 14: example of repartition of plants per class in case of not validate test for tomato/*Meloidogyne incognita* (the example 1 is for not enough aggressive test and the example 2 is for a too aggressive test)

The interpretation of varieties has to be done in comparison with controls (for validated tests):

- Variety very similar to resistant control is judged as resistant
- Variety very similar to susceptible control is judged as susceptible
- Variety very similar to intermediate resistant control is judged as intermediate resistant
- If significantly different from resistant and intermediate resistant control (notations are between resistant and intermediate resistant controls), the variety is judged as intermediate resistant
- If significantly different from intermediate resistant and susceptible control (notations are between intermediate resistant and susceptible controls), the variety is judged as susceptible
- If results not clear, statistical analysis is advised

This decision rule is illustrated by the figure 15:

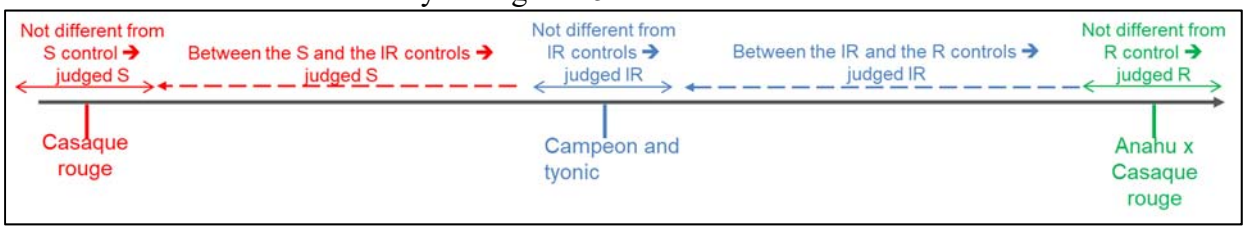


Figure 15: decision rule for tomato/*Meloidogyne incognita*

The steering committees discussed about the interpretation of data in terms of UPOV characteristic states. The question was if 3 states are defined (susceptible, intermediate resistant and resistant), it is important to be sure that the intermediate resistant level will be very stable between different tests. Is the test enough robust? From partners experience, most of varieties are between intermediate resistant and resistant levels. For some partners the risk is the no possibility to distinguish intermediate resistant and resistant between different tests.
Some compagnies will claim resistant or susceptible for the same variety depending on market. But the steering committee reminded that the needs of marketing are different from those of DUS tests and pathology for which the varieties have to be compare to the controls. Companies can decide to propose their varieties susceptible, intermediate resistant or resistant in the commercial catalogue depending on pressure of disease in field.

For DUS tests, grouping of varieties is done base on the hypothesis of a reliant declaration of applicant. If the claim is not correct, the candidate variety will not be growth in the trial with the right group of varieties of common knowledge. In France, grouping is done with resistant varieties from one side and intermediate resistant and susceptible varieties in another side. The

reason of this is to reduce the potential risk of additional test (due to error of claim). It was defined that there is less risk with grouping intermediate resistant with susceptible varieties to have an additional test. In other examination offices, grouping is done with resistant varieties and intermediate resistant from one side and susceptible varieties in another side It is important to have the same strategy between examination offices.
One option is to have 3 states, in this case, there are most comparisons and higher cost:

- susceptible varieties would be compared to intermediate resistant and susceptible varieties
- resistant varieties would be compared to intermediate resistant and resistant varieties
- intermediate resistant would be compared to susceptible, intermediate resistant and resistant varieties

But in this case, you will also have less varieties in each group, so it would be not less efficient.
Another option is to keep the 3 levels of interpretation but only 2 states.
The conclusion was that expert analysis and statistical analysis are robust. So, it is possible to have 3 robust groups using harmonized protocol and statistics (to avoid that the interpretation would be pathologist dependent and not always reproducible between examination offices).

IV. Melon

A. Melon/*Fusarium oxysporum* f. sp. *melonis* race 1.2 (Fom: 1.2)

At the end of the part 2 year 1 of Harmores 3 project, a harmonized protocol was defined with:

- The number of plants to observe (at least 30 plants with 3 repetitions of 10 plants in different trays) plus 5 non-inoculated plants

- Two inoculation methods (absorption and injection in soil) (figures 16 and 17).

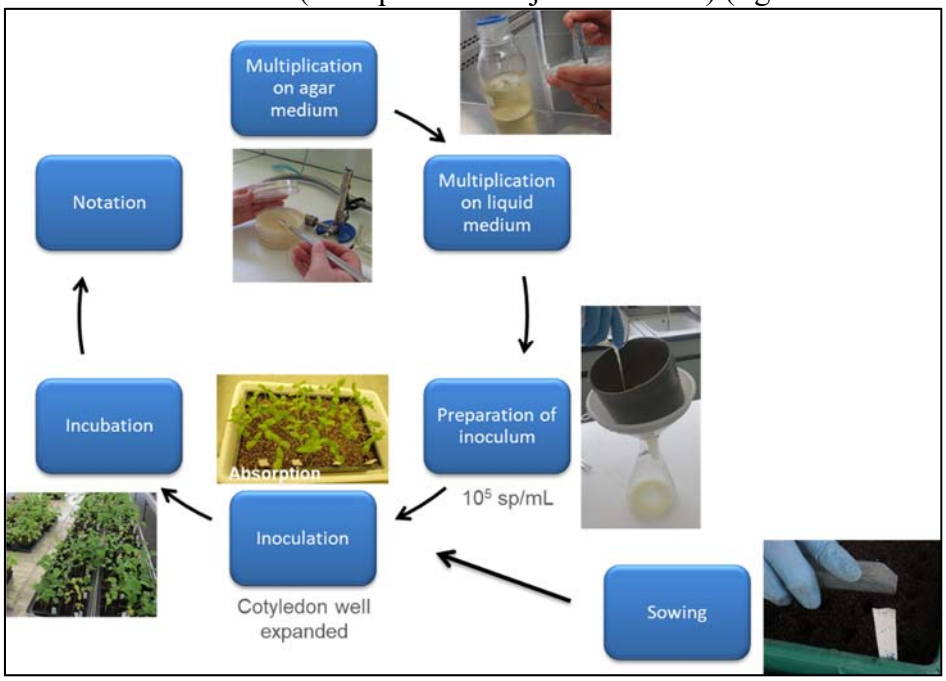


Figure 16: inoculation method by absorption for melon/*Fusarium oxysporum* f. sp. *melonis* race 1.2

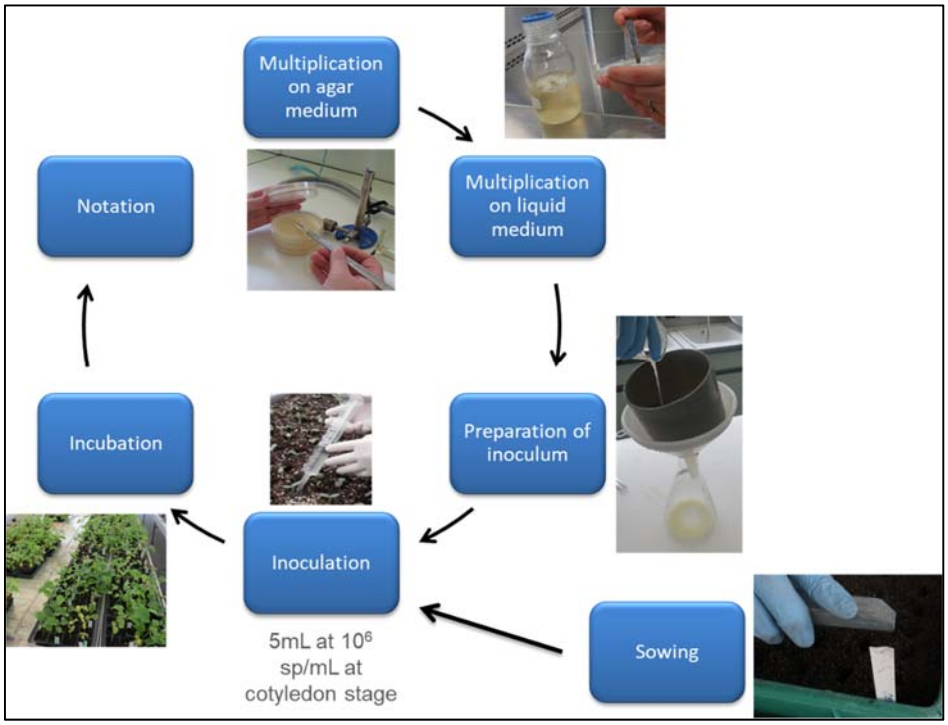


Figure 17: inoculation method by injection for melon/*Fusarium oxysporum* f. sp. *melonis* race 1.2

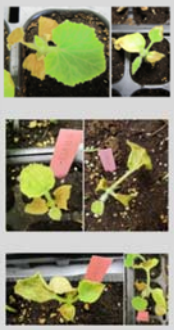
- Controls:
  - Susceptible controls: Charentais-Fom 1 and Virgos
  - Intermediaite resistant controls : Piboule and Lunasol
  - Resistant controls: Isabelle and Dinero

- The reference strains: MATREF/04-07-01-04 and MIAE 732

- The date of observation based on appearance of symptoms on the susceptible control:
  - 1st notation: symptoms on susceptible controls at class 3 [generally 10-21 dpi]
  - 2nd notation: symptoms well expressed on the susceptible controls [all plants at 4 or 5 class]

- A common notation scale (table 23)

Table 23: common notation scale for melon/*Fusarium oxysporum* f. sp. *melonis* race 1.2

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
					

- A common decision rule with two intermediate resistant controls used as threshold between susceptibility and intermediate resistance:

Figure 18: common decision rule for melon/*Fusarium oxysporum* f. sp. *melonis* race 1.2

The objective of the comparative test of part 2 year 2 was to validate the selected reference material and the protocol.

1. Materials and methods

14 laboratories were involved in the validation comparative test.
Both strains selected previously (MATREF/04-07-01-04 coded C and MIAE 732 coded B) were compared by each lab.
No new varieties were proposed for the comparative test which was done on the panel made up of controls previously used. These controls were uncoded and coded (table 24). The problem of availability of Piboule did not allow to test it coded and uncoded. It was decided to test it uncoded to be able to interpret varieties in comparison to Piboule.

Table 24: controls selected for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test

Varieties	Expected comportment	Supplied by	
Virgos	Susceptible	MATREF	Uncoded
Charentais Fom 1	Susceptible	Enza Zaden	
Piboule (INRA line)	Low Intermediate Resistant	Monsanto	
Lunasol	Low/High Intermediate Resistant	MATREF	
Isabelle	Intermediate Resistant	MATREF	
Dinero	Resistant	Enza Zaden	
Virgos	Susceptible	MATREF	Coded
Charentais Fom 1	Susceptible	Enza Zaden	
Lunasol	Low/High Intermediate Resistant	MATREF	
Isabelle	Intermediate Resistant	MATREF	
Dinero	Resistant	Enza Zaden	

The 12 seeds lots were tested by each lab on 30 seeds more 5 non-inoculated with at least 3 repetitions per variety (3 repetitions of 10 plants to allow statistical analysis in different trays) on both isolates with selected protocols (table 25):

Table 25: Repartition of methods per partners for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test

Methods	Absorption		Injection in soil	
Isolates	**Strain B**	**Strain C**	**Strain B**	**Strain C**
INIA	X	X	X	X
GEVES	X	X	X	X
NEBIH	X	X	X	X
CREA	X	X	X	X
Naktuinbouw	X	X	X	X
Ramiro Arnedo	X	X	X	X
Sakata	X	X	X	X
HM Clause	X	X	X	X
Monsanto	X	X	X	X
Rijk Zwaan	X	X	X	X
Enza Zaden	X	X	X	X
BCSVS	X	X	X	X
CTIFL	X	X	X	X
Gautier	X	X	X	X
CPPSI	X	X	X	X

The interpretation of coded varieties was based on the repartition of plants per notes (table 23) and also on disease index with the threshold between S and IR defined with the varieties Piboule and Lunasol (figure 18).

2. Results

The results of lab's interpretation of varieties are presented in tables 26 and 27.

Table 26: results for absoption method for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test

		Absorption																						
		Isolate B												Isolate C										
Variety	Expected	lab 2	lab 3	lab 4	lab 5	lab 6	lab 10	lab 11	lab 12	lab 13	lab 14	lab 15	lab 18	lab 2	lab 3	lab 4	lab 5	lab 6	lab 10	lab 11	lab 12	lab 13	lab 14	lab 15	lab 18
Virgos (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HG	S	S	S	S	S	S
Virgos (coded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HG	S	S	S	S	IR	S
Charentais Fom 1 (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HG	HG	S	S	S	S	S
Charentais Fom 1 (coded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HG	S	S	S		IR	S
Piboule (Uncoded)	IR	IR	IR	R	S	R	IR	IR	IR	IR	IR	IR	R	IR	IR	R	S	IR	R	R	IR	S	IR	S	R
Lunasol (Uncoded)	IR	IR	IR	IR	S	R	IR	IR	IR	S	IR	S	IR	IR	IR	IR	S	IR	IR	IR	IR	S	R	S	IR
Lunasol (coded)	IR	IR	IR	IR	S	R	HG	IR	IR	S	IR	IR	IR	IR	IR	IR	S	IR	R	R	IR	S	IR	R	IR
Isabelle (Uncoded)	IR	IR	IR	R	S	R	R	IR	R	IR	IR	R	R	IR	IR	R	S	IR	HG	R	IR	IR	IR	S	R
Isabelle (coded)	IR	IR	IR	R	S	IR	IR	R	R	S	R	IR	R	IR	IR	R	S	IR	R	R	IR	IR	IR	R	R
Dinero (Uncoded)	IR	R	IR	R	S	IR	R	R	R	IR	R	R	R	R	R	R	S	R	R	R	R	IR	R	R	R
Dinero (coded)	IR	R	IR	R	S	IR	R	R	R	IR	R	R	R	R	R	R	S	R	R	R	R	IR	R	R	R

R: resistant, IR: intermediate resistant, S: susceptible, HG: heterogenous/segregation

The tests were not validated on control in lab 5 with both strains. These results were not included in the following analysis.
Most of the partners have not respected the common decision rule with two levels of interpretation: susceptible and intermediate resistant. Uncoded and coded susceptible controls were globally judged as susceptible in all labs. Uncoded and coded intermediate resistant controls of higher level (Isabelle and Dinero) were globally judged as resistant or intermediate resistant in all tests. Uncoded and coded intermediate resistant controls of lower level (Piboule and Lunasol) were globally judged as resistant or intermediate resistant but were judged as susceptible in 8 tests.

Table 27: results for injection method for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test

		Injection in soil																				
		Isolate B											Isolate C									
Variety	Expected	lab 2	lab 3	lab 4	lab 5	lab 6	lab 8	lab 10	lab 11	lab 12	lab 13	lab 14	lab 2	lab 3	lab 4	lab 5	lab 8	lab 10	lab 11	lab 12	lab 13	lab 14
Virgos (Uncoded)	S	S	S	S	S	S	S	HG	S	S	S	S	S	S	S	S	S	HG	S	S	S	S
Virgos (coded)	S	S	S	HG	S	S	S	HG	S	S	S	S	S	S	S	HG	S	HG	S	S	S	S
Charentais Fom 1 (Uncoded)	S	S	S	S	S	S	S	HG	S	S	S	S	S	S	S	HG	S	R	S	S	S	S
Charentais Fom 1 (coded)	S	S	S	HG	S	IR	S	R	S	S	S	S	S	S	S	HG	S	R	S	S	S	
Piboule (Uncoded)	IR	IR	IR	IR	HG	R	IR	R	IR	IR	IR	IR	IR	IR	R	HG	IR	R	IR	IR	IR	IR
Lunasol (Uncoded)	IR	IR	S	IR	HG	R	IR	R	IR	IR	IR	IR	IR	IR	IR	HG	IR	R	R	R	IR	IR
Lunasol (coded)	IR	IR	S	R	HG	R	IR	R	IR	IR	S	IR	IR	IR	IR	HG	R	R	IR	R	IR	IR
Isabelle (Uncoded)	IR	IR	IR	IR	HG	R	IR	R	R	R	IR	R	IR	IR	R	HG	R	R	R	R	IR	R
Isabelle (coded)	IR	IR	IR	R	S	R	IR	R	R	R	IR	R	IR	IR	R	HG	IR	R	R	R	IR	R
Dinero (Uncoded)	IR	R	R	R	HG	R	R	R	R	R	IR	R	R	R	R	HG	R	R	R	R	IR	R
Dinero (coded)	IR	R	R	R	S	R	R	R	R	R	IR	R	R	R	R	HG	R	R	R	R	IR	R

R: resistant, IR: intermediate resistant, S: susceptible, HG: heterogenous/segregation

The tests were not validated on control in labs 5 and 10 with both strains. These results were not included in the following analysis.
Most of the partners have not respected the common decision rule with two levels of interpretation: susceptible and intermediate resistant. Uncoded and coded susceptible controls were globally judged as susceptible in all labs. Uncoded and coded intermediate resistant controls of higher level (Isabelle and Dinero) were judged as resistant or intermediate resistant in all tests. Uncoded intermediate resistant controls of lower level (Piboule and Lunasol) were globally judged as resistant or intermediate resistant but were judged as susceptible in 3 tests.

It was decided to reinterpret tests following the common decision rule. Results are presented in tables 28 and 29 for Lunasol used as threshold and in tables 30 and 31 for Piboule used as threshold.

Table 28: results for absoption method for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test with common decision rule based on Lunasol as threshold

		Absorption										
		Isolate B										
Variety	Expected	lab 2	lab 3	lab 4	lab 5	lab 6	lab 10	lab 11	lab 12	lab 14	lab 15	lab 18
Virgos (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S
Virgos (coded)	S	S	S	S	S	S	S	S	S	S	IR	S
Charentais Fom 1 (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S
Charentais Fom 1 (coded)	S	S	S	S	S	S	S	S	S	S	IR	S
Piboule (Uncoded)	IR	S	IR	IR	S	IR	IR	IR	IR	IR	IR	IR
Lunasol (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Lunasol (coded)	IR	S	IR	IR	IR	S	IR	S	IR	IR	IR	IR
Isabelle (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Isabelle (coded)	IR	S	IR	IR	IR	S	IR	IR	IR	IR	IR	IR
Dinero (Uncoded)	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR
Dinero (coded)	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR

		Absorption										
		Isolate C										
Variety	Expected	lab 2	lab 3	lab 4	lab 5	lab 6	lab 10	lab 11	lab 12	lab 14	lab 15	lab 18
Virgos (Uncoded)	S	S	S	S	S	S	HG	S	S	S	S	S
Virgos (coded)	S	S	S	S	S	S	S	S	S	S	IR	S
Charentais Fom 1 (Uncoded)	S	S	S	S	S	S	S	HG	S	S	S	S
Charentais Fom 1 (coded)	S	S	S	S	S	S	S	S	S		IR	S
Piboule (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	S	S	S	IR
Lunasol (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Lunasol (coded)	IR	IR	IR	IR	S	IR	IR	IR	S	S	IR	IR
Isabelle (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR
Isabelle (coded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR
Dinero (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Dinero (coded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR

R: resistant, IR: intermediate resistant, S: susceptible, HG: heterogenous/segregation

Lunasol was observed with a higher level of intermediate resistance than Piboule in few labs (2, 6 with isolate B and in labs 12, 14 and 15) what is explain that Piboule was judged as susceptible in these labs. In these labs some of other intermediate resistant varieties were also judged as susceptible.

Table 29: results for injection method for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test with common decision rule based on Lunasol as threshold

		Injection in soil																
		Isolate B									Isolate C							
Variety	Expected	lab 2	lab 3	lab 4	lab 6	lab 8	lab 11	lab 12	lab 13	lab 14	lab 2	lab 3	lab 4	lab 8	lab 11	lab 12	lab 13	lab 14
Virgos (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Virgos (coded)	S	S	S	HG	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Charentais Fom 1 (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Charentais Fom 1 (coded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
Piboule (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	S	S	IR	IR
Lunasol (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Lunasol (coded)	IR	IR	IR	IR	IR	IR	IR	S	S	IR	IR	IR	IR	IR	IR	S	IR	IR
Isabelle (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	S	IR	IR
Isabelle (coded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR	IR
Dinero (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Dinero (coded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR

R: resistant, IR: intermediate resistant, S: susceptible, HG: heterogenous/segregation

The same observation of a higher level of intermediate resistance than Piboule was also done for injection method but not in the same labs.

No clear difference of pathogenicity was observed between isolates B and C with both inoculation methods.

Table 30: results for absoption method for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test with common decision rule based on Piboule as threshold

		Absorption																					
		Isolate B											Isolate C										
Variety	Expected	lab 2	lab 3	lab 4	lab 6	lab 10	lab 11	lab 12	lab 13	lab 14	lab 15	lab 18	lab 2	lab 3	lab 4	lab 5	lab 6	Lab 10	lab 11	lab 12	lab 14	lab 15	lab 18
Virgos (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HG	S	S	S	S	S
Virgos (coded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	IR	S
Charentais Fom 1 (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	HG	S	S	S	S
Charentais Fom 1 (coded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S		IR	S
Piboule (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Lunasol (Uncoded)	IR	IR	S	S	IR	IR	IR	S	S	S	S	S	S	S	S	S	S	IR	S	IR	IR	IR	S
Lunasol (coded)	IR	S	IR	S	S	IR	S	S	S	S	IR	IR	S	IR	S	S	IR	IR	IR	IR	S	IR	S
Isabelle (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR	S	IR	IR	IR	IR	IR	S	IR
Isabelle (coded)	IR	S	IR	IR	S	IR	IR	IR	S	IR	IR	IR	S	IR	IR	IR	IR	IR	IR	IR	S	IR	IR
Dinero (Uncoded)	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Dinero (coded)	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR

R: resistant, IR: intermediate resistant, S: susceptible, HG: heterogenous/segregation

Piboule was observed with a higher level of intermediate resistance than Lunasol in some labs (3, 4, 12, 13, 14, 15 and 18 with isolate B and in labs 2, 3, 4, 5, 6, 11 and 18 with isolate C) what is explain that Lunasol was judged as susceptible in these labs. In these labs other intermediate resistant varieties were also judged as susceptible.

Table 31: results for injection method for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test with common decision rule based on Piboule as threshold

		Injection in soil																
		Isolate B									Isolate C							
Variety	Expected	lab 2	lab 3	lab 4	lab 6	lab 8	lab 11	lab 12	lab 13	lab 14	lab 2	lab 3	lab 4	lab 8	lab 11	lab 12	lab 13	lab 14
Virgos (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Virgos (coded)	S	S	S	HG	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Charentais Fom 1 (Uncoded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
Charentais Fom 1 (coded)	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
Piboule (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Lunasol (Uncoded)	IR	IR	IR	IR	IR	S	S	S	IR	S	IR	IR	S	IR	IR	IR	S	S
Lunasol (coded)	IR	IR	IR	IR	IR	IR	IR	S	S	S	IR	IR	S	IR	IR	IR	IR	S
Isabelle (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR	IR
Isabelle (coded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	S	IR	IR	IR	IR	IR	IR	IR
Dinero (Uncoded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR
Dinero (coded)	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR	IR

R: resistant, IR: intermediate resistant, S: susceptible, HG: heterogenous/segregation

The same observation of a higher level of intermediate resistance than Piboule was also done for injection method but in the less labs.

Globally, a good concordance was observed between labs independently of inoculation method or isolate tested. No clear difference of pathogenicity was observed between isolates C and B. The variety Piboule seems to be more restrictive than Lunasol as lower limit of intermediate resistance (more varieties judged as susceptible with Piboule as threshold than with Lunasol). Depending on the choice of Piboule or Lunasol as intermediate resistant low-level control, the interpretation of a variety will not be the same. Steering committee discussed if it is preferable to have only one. In this case, the choice between Lunasol and Piboule would be based on the availability on the long term of these varieties. Finally, it was decided to keep both intermediate resistant controls low-level Piboule and Lunasol and to try to have long term available seeds or during availability of seeds to validate a new intermediate resistant variety with the same level.
No differences were observed for Isabelle and Dinero (intermediate resistant controls of higher level) or between Virgos and Charentais Fom-1 (susceptible controls).

The disease index of controls (Virgos susceptible, Piboule intermediate resistant low level and Isabelle intermediate resistant high level) in each test was analysis to define the rules of validation of controls in addition of the repartition of plants per class (table 32).

Table 32: disease index for *Fusarium oxysporum* f. sp. *melonis* race 1.2 comparative test

	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 8	Lab 10	Lab 11	Lab 12	Lab 13	Lab 14	Lab 15	Lab 18
Abs B													
Virgos	94%	96%	88%	97%	81%		97%	98%	99%	100%	78%	88%	94%
Piboule	9%	32%	1%	99%	1%		38%	33%	35%	89%	33%	65%	9%
Isabelle	0%	17%	0%	83%	4%		18%	20%	14%	86%	29%	36%	0%
Abs C													
Virgos	89%	60%	64%	83%	41%		72%	67%	31%	100%	88%	100%	86%
Piboule	52%	9%	3%	71%	16%		29%	6%	19%	100%	28%	91%	0%
Isabelle	71%	22%	0%	75%	12%		23%	2%	11%	86%	34%	81%	6%
Inj B													
Virgos	100%	100%	89%	94%	60%	69%	55%	93%	100%	100%	87%		
Piboule	71%	86%	8%	79%	0%	2%	8%	22%	26%	79%	27%		
Isabelle	54%	59%	11%	46%	0%	2%	4%	5%	4%	48%	13%		
Inj C													
Virgos	100%	82%	43%	89%	9%	43%	53%	56%	43%	100%	96%		
Piboule	28%	38%	0%	49%	3%	1%	5%	19%	16%	76%	30%		
Isabelle	64%	29%	0%	43%	0%	0%	6%	7%	6%	29%	14%		

Abs: absorption method of inoculation; inj: injection method of inoculation

Partners established that controls have to be clearly different between each other to validate the test. For example, in lab 5 with absorption and strain B, controls were too close with disease index of 97%, 99% and 83%, this test was not validated. In the same way the classification of controls by resistance level have to be respected. For example, in lab 2 with absorption and strain C, Isabelle (intermediate resistant high level) has a higher disease index 71% than Piboule (intermediate resistant low level) 52%, in this case the test was not validated.
The steering committee has defined a range of disease index for each control: above 80 % for the susceptible one, generally below 40% for the intermediate resistant one.

On the same way, the repartition of plant per class was analyse by partners to described what is expected on controls for the validation of the test. The conclusion is presented below in point 3.

3. Melon/Fom: 1.2 conclusion

Based on the results obtained in last year of Hamores 3 project, the steering committee decided to select for the updated protocol (annex 5):

- Susceptible control: Virgos
- Intermediate resistant low-level controls: Piboule and Lunasol
- Intermediate resistant high-level control: Isabelle.

Virgos and Isabelle were validated because partners have used them from a long time and have had more experience with these varieties. Lunasol and Piboule were selected as intermediate resistant low-level controls. Piboule will be requested to INRA to be included in MATREF following the Harmores 3 project to be multiply and available in case of request.
The other validated controls (Lunasol, Virgos and Isabelle) are already included in MATREF network and available.

For *Fusarium oxysporum* f. sp. *melonis* race 1.2, even if not clearly difference of pathogenicity was observed between isolates, steering committee decided to select only one. The reason was that there is a risk to select two isolates, even if they have apparently the same comportment. Because in case of new genetic (not observed in comparative tests), it would be possible to observed that there would be differences between tests performed by the two isolates and discrepancies between results. The isolate MATREF/04-07-01-04 was kept because it was already used as reference isolate from many years by several partners for registration and there is more experience with this strain. The second reason was that tis isolate was less aggressive and is better to see different levels of resistance. The same reasoning was applied for *Fusarium oxysporum* f. sp. *melonis* race 2.

Test will be performed on 30 plants in 3 repetitions of 10 plants in different trays (plus at least 5 non-inoculated plants to be able to judge growth reduction).

Both selected inoculation methods (absorption and injection) were validated. It was observed that labs are more reproducible with the method they are used to apply. Each lab has to choose its inoculation method, between both selected, depending on results on controls, with one or the other validate method, in its own condition of test.

As the virulence of the test is depending of lab conditions, that is why the date of notation is based on appearance of symptoms on the susceptible control. The first observation will be done when the symptoms on the susceptible controls are at least at class 3 [generally 10-21 dpi] and a second notation can be necessary few days later to re-evaluate some unclear varieties.

The common notation scale defined in part 2 year 1 (table 23) was validated. The validation of test on controls was defined depending on repartition of plants per class and on the disease index:

- Susceptible control: plants at classes 3 and 4, and in some cases few plants at class 2. Very high disease index above 80%
- Intermediate resistant controls: 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 (Isabelle is expected with lower disease index than Piboule and Lunasol)

The steering committees discussed about the interpretation of data in terms of UPOV characteristic states. 2 options were studied:

- 2 states: susceptible/intermediate resistant, with the intermediate resistant low-level controls (Piboule and Lunasol) as threshold between susceptibility and intermediate resistance.
- 3 states: susceptible/intermediate resistant/resistant. The question was which note to give to each state: [1]/[2]/[3] or [1]/[7]/[9]. In the first case, it would be nor possible to distinguish varieties at note [2] from varieties at notes [1] or [3]. In the second case, it would be possible to distinguish varieties at note [7] from varieties at notes [1] or [9].

It was decided that it is safer to have only two states: susceptible [1] and intermediate resistant [9]. This decision rule is illustrated by the figure 19:

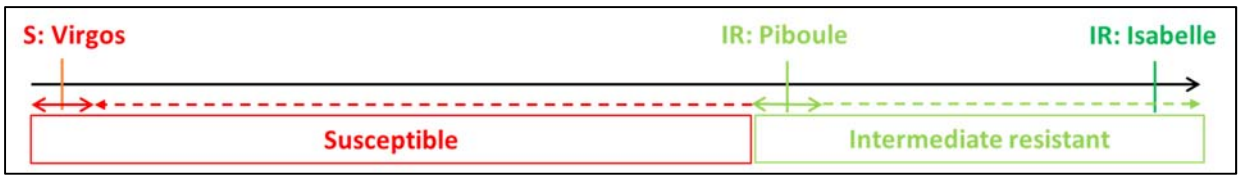


Figure 19: decision rule for *Fusarium oxysporum* f. sp. *melonis* race 1.2

The interpretation of varieties has to be done in comparison with controls (for validated tests):

- Variety very similar to one of the intermediate resistant low-level controls, or with a higher level of resistance, is judged as intermediate resistant.
- Variety very similar to susceptible control is judged as susceptible.
- If significantly different from intermediate resistant low-level controls and susceptible control (notations are between intermediate resistant and susceptible controls), the variety is judged as susceptible.
- If results not clear, statistical analysis is advised.

B. Melon/Fusarium oxysporum f. sp. melonis race 2

During the part 2 year 1 of Harmores 3 project, different steps of a harmonized protocol were defined:

- The number of plants to observe (at least 30 plants with 3 repetitions of 10 plants in different trays) plus 5 non-inoculated plants to be able to judge growth reduction.

- Two inoculation methods (injection and soaking roots) (figures 20 and 21).

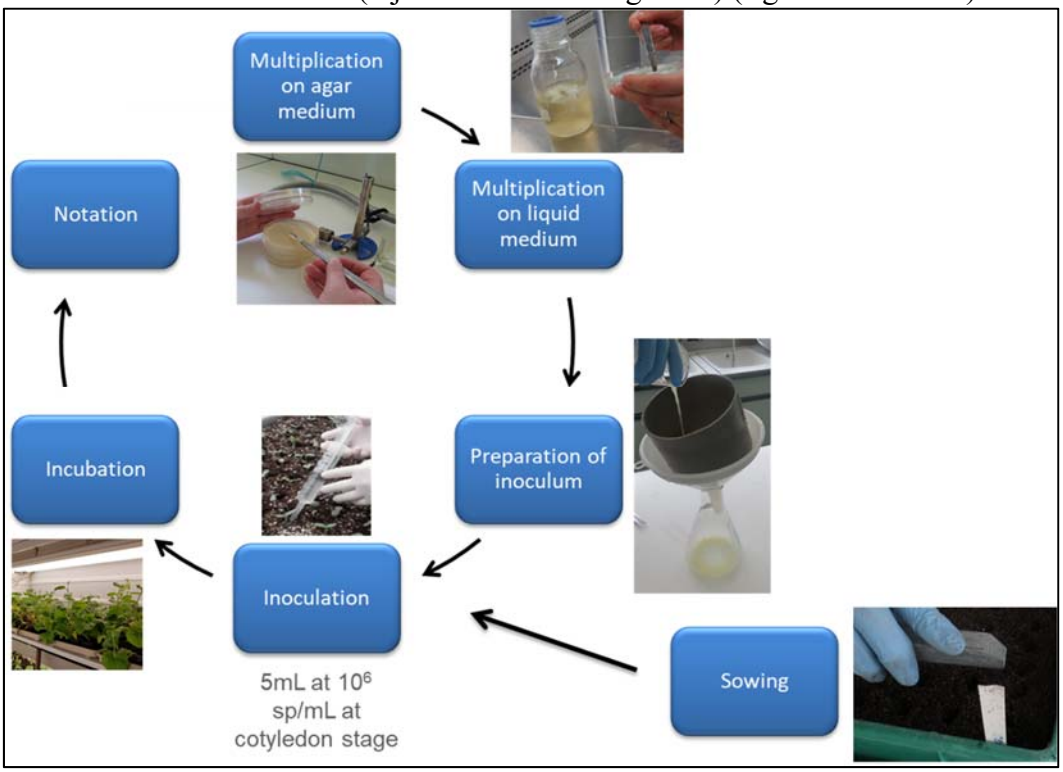


Figure 20: inoculation method by injection for melon/*Fusarium oxysporum* f. sp. *melonis* race 2

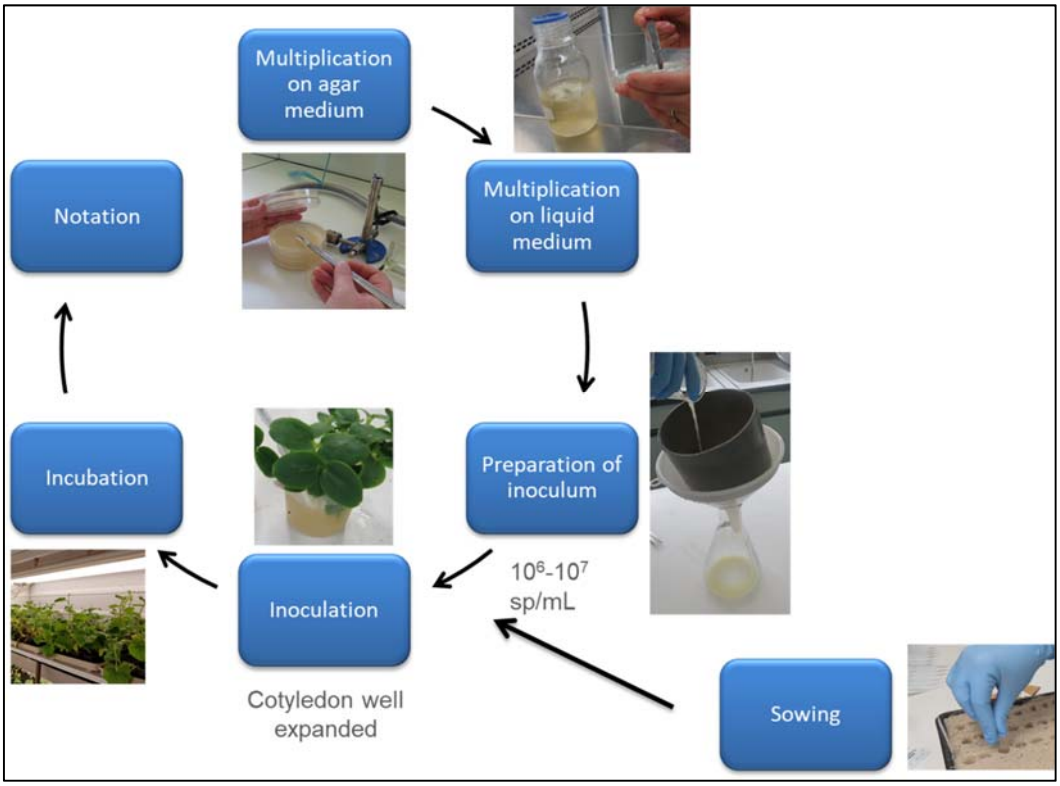


Figure 21: inoculation method by soaking roots and transplanting for melon/*Fusarium oxysporum* f. sp. *melonis* race 2

- 3 varieties were selected as candidate controls:
  - Solemio and Marianna as susceptible. Solemio expected with a high susceptibility and Marianna selected in part 2 year 1 to be used as susceptible control for an indication of the level of the aggressiveness of the test.
  - Charentais Fom-1 as resistant

- The date of notation, with two observations at 10-14 days post-inoculation and 21 days post-inoculation:
  - First notation around 10 to 14 dpi when all plants of the susceptible control beginning to express symptoms
  - Second notation when symptoms are well expressed on the susceptible control (notes 2 and 3 with a strong proportion at 3).
  -
- A common notation scale (table 33), with the specification of other symptoms: vein clearing difficult to judge, for which it is advised to make a later notation to observe the evolution of this symptom over the time (figure 22).

Table 33: common notation scale for melon/*Fusarium oxysporum* f. sp. *melonis* race 2

Non-inoculated	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 observe some yellowing, different from the symptoms of *Fusarium*	Light symptoms of yellowing/wilting	typical symptoms : yellowing, wilting and necrosis, stunting (growth stopped)	Death of plant (Dead)
				

Figure 22: symptoms of vein clearing not due to *Fusarium oxysporum* f. sp. *melonis* race 2

No common decision rule has been defined; the steering committee considered that it was too early based on the results of part 2 year 1. The common decision rule was planned to be defined after the validation comparative test in part 2 year 2.

1. Materials and methods

15 laboratories were involved in the validation comparative test on *Fusarium oxysporum* f. sp. *melonis* race 2.
At the end of part 2 year 1, it was planned, that during the second year of the project part 2, the validation of the chosen protocol for *Fusarium oxysporum* f. sp. *melonis* race 2 will be extended to *Fusarium oxysporum* f. sp. *melonis* races 0 and 1. This validation was defined as a comparative test performed with Fom: 0 and 1 isolates following the protocol and with the reference material selected in part 2 year 1. This comparative test was done on controls at the same time than the Fom: 2 comparative test. This additional test was done by 6 volunteer laboratories with the MATERF isolate or the lab's isolates.

The strains of *Fusarium oxysporum* f. sp. *melonis* compared in comparative tests were presented table 34.

Table 34: isolates compared for melon/*Fusarium oxysporum* f. sp. *melonis* races 0, 1 and 2

Race	Code	Isolat	Part 2 year 1
Fom: 0	N	MAT/REF/04-07-01-03-02	
Fom: 1	P	MAT/REF/04-07-01-01	
Fom: 2	M = R	F185	Less agressive
Fom: 2	K = L	MAT/REF/04-07-01-02	More agressive

For races 0 and 1, the panel was made up of uncoded differentials and on one variety (Harmo-19E), provided by one partner and expected susceptible for race 0 and with non-known comportment for race 1. For race 2, the panel was made up of varieties tested previously with 3 other varieties: Harmo-19D (expected as intermediate resistant), Harmo-19C (variety Ducral proposed by one partner as resistant) and Harmo-19E (table 35).

Table 35: panel of varieties tested for melon/*Fusarium oxysporum* f. sp. *melonis* races 0, 1 and 2

Variety	Expected Fom: 0	Expected Fom: 1	Expected Fom: 2
Charentais T	Susceptible	Susceptible	
Solemio			Susceptible
Marianna			Susceptible
Charentais Fom-2	Resistant	Resistant	Susceptible
Harmo2018-1			Intermediate resistant
SEL 5			Intermediate resistant
Harmo-19D			Intermediate resistant
MR-1			Resistant
Vedrantais	Resistant	Susceptible	Resistant
Kiros			Resistant
Charantais Fom-1			Resistant
Harmo-19C Ducral			Resistant
Harmo-19E	Susceptible	non-known	Resistant

Candidate control varieties Marianna, Solemio (susceptible) and Charentais Fom-1 (resistant) were uncoded is the comparative test to establish the varieties interpretation in comparison with these candidate controls.

Tests were done on 30 seeds per variety (more 5 non-inoculated) on selected isolates with only one inoculation method to choose per partner based on results of previous test (Injection in soil or Soaking root and transplanting). Soaking method was advised but if infection method gave expected results in the lab, this could be chosen.

2. Results

a) *Fusarium oxysporum* f. sp. *melonis* races 0 and 1

Results for *Fusarium oxysporum* f. sp. *melonis* races 0 and 1 are presented in table 36.

Table 36: results of comparative test for melon/*Fusarium oxysporum* f. sp. *melonis* race 0

		Isolate N - Fom: 0											
		Soaking root and transplanting						Injection in soil					
Variety	Expected	lab 2	lab 3	lab 4	lab 5	lab 6	lab 8	lab 2	lab 3	lab 4	lab 5	lab 6	lab 8
Charentais T	S	S	S	S	S	S	S	S	S	S	S	S	S
Védrantais	R	R	R	R	R	R	R	R	R	R	R	R	R
Charentais Fom-2	R	R	R	R	R	R	R	R	R	R	R	R	R
Harmo-19E	S	R						R					

		Isolate P - Fom: 1											
		Soaking root and transplanting						Injection in soil					
Variety	Expected	lab 2	lab 3	lab 4	lab 5	lab 6	lab 8	lab 2	lab 3	lab 4	lab 5	lab 6	lab 8
Charentais T	S	S	S	S	S	S	S	S	S	S	S	S	S
Védrantais	S	S	S	S	S	S	S	S	S	S	S	S	S
Charentais Fom-2	R	R	R	R	R	R	R	R	R	R	R	R	R
Harmo-19E		S	S					S	S				

R: resistant, S: susceptible, * Lab's isolates

Both strains were validated on controls. A good concordance was observed between labs. These results confirmed that the common notation scale defined for Fom: 2 is also adapted for the races Fom: 0 and 1. The variety Harmo-19E (expected susceptible for race 0) was observed resistant for both races.

b) *Fusarium oxysporum* f. sp. *melonis* race 2

For *Fusarium oxysporum* f. sp. *melonis* race 2, the large majority of labs decided to use the inoculation method by soaking roots in a suspension of spores and transplanting.

(1) *Fom: 2 strain* MATREF 04-07-01-02

The results for the strain L (= K / MATREF 04-07-01-02) are presented table 37.

Table 37: results of comparative test for melon/*Fusarium oxysporum* f. sp. *melonis* race 2 strain L

		Soaking root and transplanting										Injection
Variety	Expected	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
Solemio	S	S	S	S	S	S	S	S	S	S	S	IR
Marianna *	S	S	HG	S	S	S	S	S	S	S	S	S
Marianna	S	S	HG	HG	S	S	S	S	S	S	IR	S
Charentais Fom-2	S	S	HG	HG	S	S	S	S	S	S	S	S
Harmo2018-1	IR	S	HG	S	S	S	HG	S	S	S	IR	S
Harmo-19D	IR	IR	HG	S	S	S	S	S	HG	S	IR	IR
Charentais Fom-1 *	R	R	R	R	R	R	HG	R	R	R	R	R
Charentais Fom-1	R	R	R	R	R	R	HG	R	R	R	R	IR
Kiros	R	R	R	R	R	R	R	R	R	R	R	R
MR-1	R	R	R	R	R	R	R	R	R	R	R	IR
Vedrantais	R	R	R	R	R	R	R	R	R	R	R	R
Harmo-19C	R	HG	R	HG	S	IR=S?	HG	S	R	S	R	IR
Harmo-19E	R	HG								HG		

S: susceptible; IR: intermediate resistant; R: resistant; HG: heterogeneous; *: uncoded controls

Globally a good concordance was observed between labs. The inoculation method by injection was observed less aggressive in the only one lab using it. The controls Solemio, Marianna and Charentais Fom-1 were validated in most of labs. But some heterogeneous results were observed. Partners analysed the repartition of plants per class for some examples of heterogeneous results to conclude if it was a heterogeneous comportment or a continuum of repartition of plants on the four classes.

For some examples, the susceptible control Marianna was not validated with presence of plants at notes 0 and 1 (figure 23). In this case, the test is not validated.

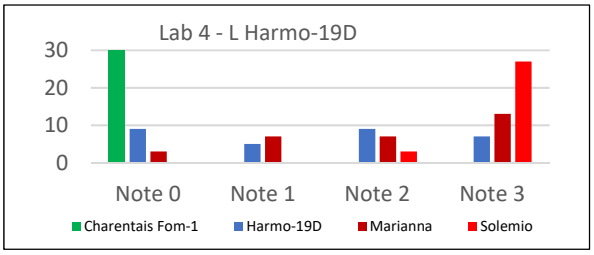


Figure 23: example of repartition of plants per class for melon/Fom: 2 with Marianna control not validated

For other examples for which the controls were validated, the repartition of plants per class was not heterogeneous but with a continuum. In this case, the test has to be repeat with higher concentration to confirm the homogeneity of the variety (figure 24).

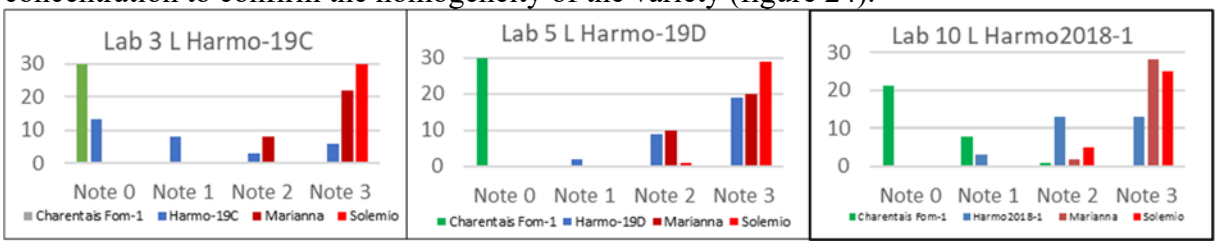


Figure 24: examples of repartition of plants per class for Fom: 2 strain L for varieties judged as heterogeneous by labs

(2) *Fom: 2 strain* F185

The results for the strain R (= M / F185) are presented table 38.

Table 38: results of comparative test for melon/*Fusarium oxysporum* f. sp. *melonis* race 2 strain R

		Soaking root and transplanting										Injection	
Variety	**Expected**	**lab 3**	**lab 4**	**lab 5**	**lab 6**	**lab 8**	**lab 10**	**lab 11**	**lab 12**	**lab 14**	**lab 18**	**lab 2**	**lab 16**
Solemio *	S	S	S	S	S	S	S	S	S	S	S	S	S
Solemio	S	S	S	S	S	S	S	S	S	S	IR	S	IR
Marianna *	S	S	S	S	S	S	S	S	S	S	S	S	S
Marianna	S	S	S	S	S	S	S	S	S	S	S	S	IR
Charantais Fom-2	S	S	S	S	S	S	S	S	S	S	S	S	IR
Harmo2018-1	IR	S	S	S	S	S	S	S	S	S	IR	S	IR
Harmo-19D	IR	S	S	HG	S	S	S	S	IR	S	IR	IR	IR
Charentais Fom-1 *	R	R	R	R	R	R	HG	R	R	IR	R	R	R
Charentais Fom-1	R	R	R	R	R	R	R	R	R	HG	R	R	R
Kiros	R	R	R	R	R	R	HG	R	R	S	R	R	R
MR-1	R	IR	IR	R	R	R	R	R	R	IR	R	R	IR
Vedrantais	R	R	IR	R	R	R	R	R	R	IR	R	R	R
Harmo-19C	R	HG	S	S	S	S	S	S	HG	S	R	R	IR

S: susceptible; IR: intermediate resistant; R: resistant; HG: heterogeneous; *: uncoded controls

Globally a good concordance was observed between labs. This isolate was observed more aggressive than the isolate MATREF 04-07-01-02 (contrary to what was observed in part 2 year 1). As for the other strain, the inoculation method by injection was observed less aggressive in the only two labs using it. The controls Solemio, Marianna and Charentais Fom-1 were validated in most of labs. But few heterogeneous results were observed. Partners analysed the repartition of plants per class for some examples of heterogeneous results to conclude if it was a heterogeneous comportment or a continuum of repartition of plants on the four classes (figure 25).

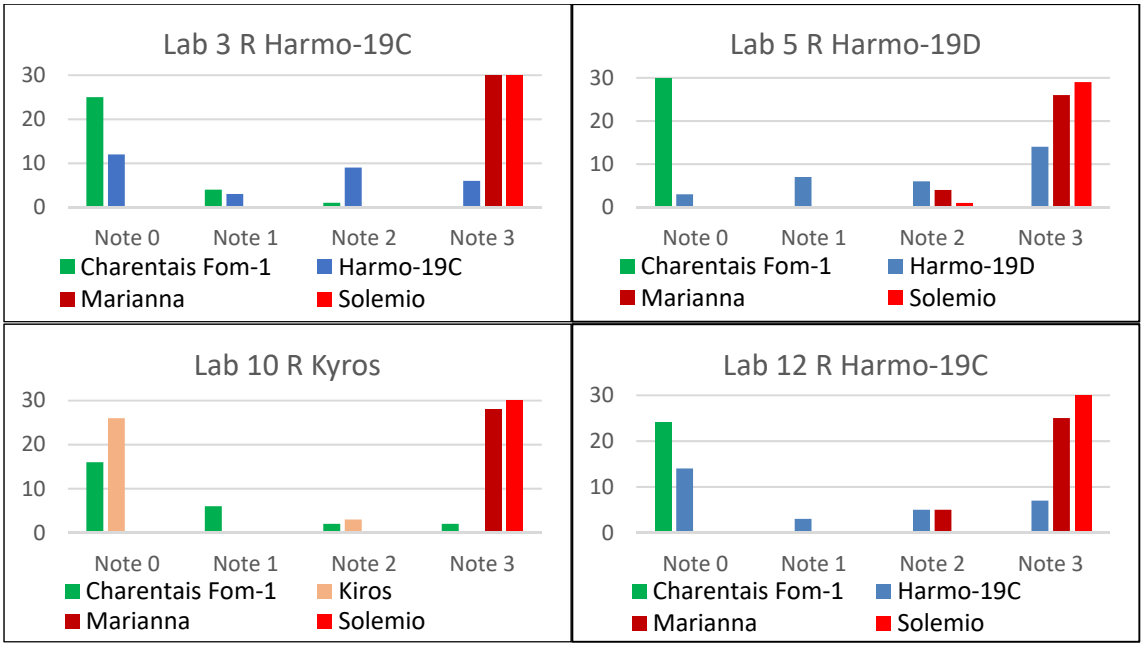


Figure 25: examples of repartition of plants per class for Fom: 2 strain R for varieties judged as heterogeneous by labs

Tests were validated on controls in the four labs. The varieties Harmo-19C and Harmore-19D were observed with a repartition of plants per note corresponding to a continuum. In this case, the test has to be repeat with higher concentration to confirm the homogeneity of the variety. The variety Kiros was observed as heterogeneous with 3 plants at note 2 while the other plants were at note 0.

3. *Fusarium oxysporum* f. sp. *melonis* race 2 conclusions

Based on the results obtained during the last year of Hamores 3 project, the steering committee decided to select for the updated protocol (annex 6):

- Susceptible control: Marianna.
- Resistant control: Charentais Fom-1

Solemio was not selected as susceptible control because it is a commercial hybrid with the question of the availability of seeds over the time and because Solemio is very susceptible, so it gives no information about the level of aggressivity of test. Marianna was selected as susceptible control because it was observed less susceptible than Solemio and it can show if the test is not aggressive enough. The availability of Marianna will be confirmed with the provider to be included in MATREF.

Both strains F185 and MATREF 04/007/01/02 were validated in the comparative test. But, for the same reason as *Fusarium oxysporum* f. sp. *melonis* race 1.2, only one strain will be selected and indicated in the CPVO protocol to avoid, in case of new genetic (not observed in comparative tests), to observed differences between tests performed by the two isolates and

discrepancies between results. The strain F185 was chosen because it presented less problems of interpretation.

Both inoculation methods injection and soaking roots were selected in part 2 year 1. Only one lab has been in favour keeping the injection inoculation method since it was more used of this one. In the comparative tests, this method was tested only by one or two labs with non-conform results in some cases. The advice has been that the inoculation method by soaking roots is more robust and more reproducible, that is why this method was selected by the steering committee.

The date of notation defined previously has been adapted depending on observation of results of comparative tests. As the virulence of the test is depending of lab conditions, the date of notation is based on appearance of symptoms on the susceptible control. Both dates of notation were retained but the second one is optional:

- 1st notation: symptoms on susceptible control at classes 2 and 3 with strong proportion at three.
- A 2nd notation can be necessary to re-evaluate some unclear varieties.

The common notation (table 33) was validated. The validation of test on controls was defined depending on repartition of plants per class and on the disease index:

- Susceptible control: plants at classes 2 and 3.
- Resistant control: plants at classes 0 and 1, sometimes very few plants at classes 2 or 3.

The steering committees discussed about the interpretation of data in terms of UPOV characteristic states. It was decided to have 2 states: susceptible [1] and resistant [9]. The question was how to place the threshold between susceptibility and resistance. The interpretation of varieties has to be done in comparison with controls (for validated tests):

- Susceptible: comparable to the susceptible control
- Resistant: comparable to the resistant control
- Between: if the variety is statistically judged different from susceptible and resistant controls, the result is inconclusive. The test will be repeated with a higher concentration to confirm the homogeneity of the variety. In case of confirmation of the first result, the variety will be judged as heterogeneous.

This decision rule is illustrated by the figure 26:

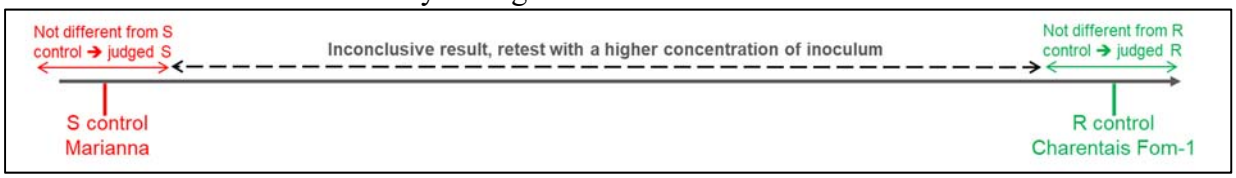


Figure 26: decision rule for *Fusarium oxysporum* f. sp. *melonis* race 1.2

In case of unclear varieties, the variety has to be retested or to be tested in another lab.

C. Melon/*Podosphaera xanthii*

During the part 2 year 1 of Harmores 3 project, different steps of a harmonized protocol were defined:

- The stage of plants at inoculation (figure 27):

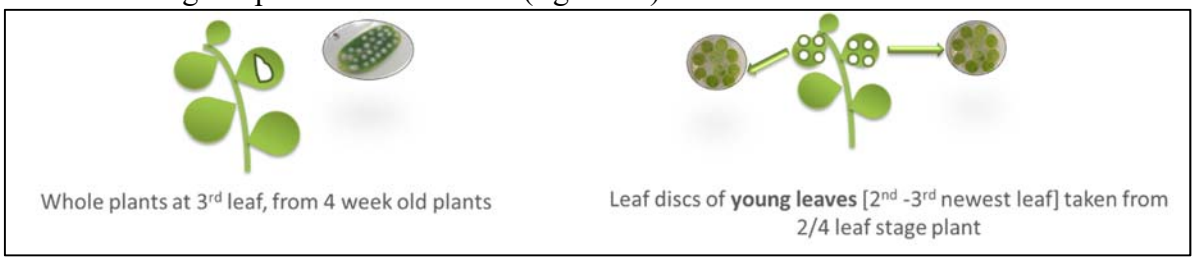


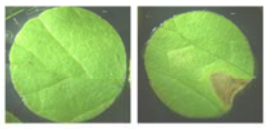
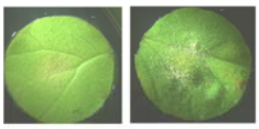
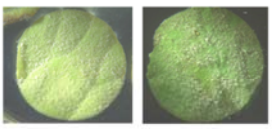
Figure 27: stage of plants at inoculation for both inoculation methods whole plants (left) and leaf discs (right)

- The number of plants: at least 20 plants per variety plus controls, 5 plants for other differentials. For leaf discs method: 1 disc = 1 plant = 1 data point.

- The date of notation: when symptoms are well expressed on the susceptible control (around 10 to 17 days post-inoculation).

- The common notation sale (table 39):

Table 39: common notation scale for melon/*Podosphaera xanthii*

	Resistant 1: No development of the fungus (no mycelium or dead mycelium) or no sporulation	**Intermediate resistant** 5: weak sporulation	**Susceptible** 9: strong sporulation
On leaf discs			
On whole plants			

- The common interpretation rule based on the repartition of plants per class (table 40):

Table 40: common notation scale for melon/*Podosphaera xanthii*

Interpretation rule: validation of test on controls											
Validated if: ➢ **Resistant control at class 1** ➢ **Susceptible control at class 9**											
Examples:											
	Class 1	**Class 5**	**Class 9**		**Class 1**	**Class 5**	**Class 9**		**Class 1**	**Class 5**	**Class 9**
Rep 1	0	4	3	Rep 1	5	4	0	Rep 1	1	1	5
Rep 2	0	6	1	Rep 2	7	2	0	Rep 2	1	5	1
Rep 3	0	2	4					Rep 3	0	2	2
S				**IR**				**At the limit S or HG, retest to check**			

The objective of the comparative test of part 2 year 2 was to validate the four selected inoculation methods (table 41).

Table 41: selected inoculation method compared for melon/*Podosphaera xanthii*

Leaf discs 2-4 leaf stage on agar			Whole plants at 2-4 leaf stage
Direct contact	Settling tower	Spraying	Local deposit

1. Materials and methods

12 laboratories were involved in the validation comparative test.

Two strains of *Podosphaera xanthii* race 3.5 were compared by each lab. The first strain was the MATREF isolate (MAT/REF/04-07-03-05) and the second one was the isolate Harmo which has been characterized as more aggressive than Px: 3.5 MATREF during a previous project (DIVERSOID) with symptoms on additional differentials. Even if this strain is characterized as a race 3.5 on the current differentials, its comportment is different from reference Px: 3.5 race.

Both strains pf Px: 3.5 were tested by labs following the selected inoculation methods depending on labs facilities and used (table 43) and using the common notation scale and interpretation rule.

Table 42: repartition of tests for *Podosphaera xanthii* comparative test

Partner	Leaf discs 2-4 leaf stage			Whole plants
	Direct contact	Settling tower	Spraying	Local deposit
	Agar	Agar	Agar	
Bayer				X
Naktuinbouw	X	X	X	
Monsanto		X		X
HM Clause			X	
EZ	X		X	
GEVES		X		X
Gautier		X		X
INIA				X
Sakata		X		
CPSSI				X
Ramiro				X
RZ		X		

In part 2 year 1, candidate controls were defined: Vedrantais (susceptible) and Arum (resistant). No intermediate resistant control was selected. It was decided to define it at the end of the comparative test of validation in last year. Three new varieties expected as intermediate resistant were proposed for the comparative test (Harmo 19A, Harmo 19B and Harmo 19D). Therefore, a mix lot (with seeds of susceptible and resistant control) was included in the panel to confirm that the protocol allows to distinguish intermediate resistance and segregation (table 42).

Table 43: Varieties selected for *Podosphaera xanthii* comparative test

Varieties	Expected comportment for Px: 3-5 (*Podosphaera xanthii)*		Number of plants to test by isolate
Arum	R	Control Uncoded	20 (+ 5 non-inoculated)
Vedrantais	S	Differentials Uncoded (used as Control)	20 (+ 5 non-inoculated)
PMR45	S	Differentials Uncoded	5
PMR5	S		
Edisto 47	S		
ARHBJ	R		
Harmo-19A	IR	Coded	20 (+ 5 non-inoculated)
Harmo-19B	IR		
Harmo-19D	IR		
Hispano	IR		
Arum	R		
Forterra	IR		
Gustabel	S		
Mix	SEG		

2. Results

(1) Strain Px : 3.5 MATREF

Results obtained with the reference strain Px: 3.5 MATREF were presented table 44.

Table 44: results of comparative tests for *Podosphaera xanthii* race 3.5 strain MATREF

		Leaf discs 2-4 leaf stage							Whole plants			
		Settling tower					Spraying					
Variety	**Expected**	**lab 3**	**lab 8**	**lab 11**	**lab 12**	**lab 18**	**lab 6**	**lab 10**	**lab 3**	**lab 11**	**lab 14**	**lab 18**
Mix	seg	HG	HG	HG	HG	HG	S	HG	HG	HG	HG	HG
Vedrantais *	S	S	S	S	S	S	S	S	S	S	S	S
PMR45	S	S	S	S	S	S	S	S	S	S	S	S
PMR5	S	S	S	IR	IR	S	R	IR	IR	R	S	S
Edisto 47	S	S	S	S	S	S	S	S	S	S	S	S
Gustabel	S	HG	S	S	S	RI	R	IR	S	IR	S	S
Hispano	IR	HG	IR	R	R	R	R	R	R	R	R	R
Forterra	IR	S	S	S	S	RI	S	S	S	IR	S	S
Harmo-19A	IR	IR	IR	IR	IR	R	R	R	IR	R	R	R
Harmo-19B	IR	S	S	S	S	S	R	IR	IR	R	IR	S
Harmo-19D	IR	S	IR	IR	HG	R	R	IR	IR	R	IR	R
ARHBJ	R	IR	IR	IR	S	R	R	IR	R	R	R	R
Arum *	R	R	R	R	R	R	R	R	R	R	R	R
Arum	R	R	R	R	R	R	R	R	R	R	R	R

S: susceptible; IR: intermediate resistant; R: resistant; HG: heterogeneous; *: uncoded controls

Globally, a good concordance was observed between labs. The most of differences are between interpretation resistant or intermediate resistant. Candidate controls Vedrantais and Arum were validated in all tests. Gustabel (expected susceptible) was judged as resistant or heterogeneous in some tests. Forterra (expected intermediate resistant) was judged as susceptible in some tests. These results confirmed those obtained in part 2 year 1. The variety harmo-19B (expected intermediate resistant) was judged as susceptible in some tests.

The strain was validated as a race 3.5 in 5 tests out of 11. In the 6 other tests, the race was not validated on the differential PMR5. In these cases, the strain was observed as a race Px: 5.
The mix lot was judged, as expected, heterogeneous in 10 tests out of 11. This result confirmed that it is possible to distinguish heterogeneous varieties from intermediate resistant varieties.
No clear differences were observed between inoculation methods with this strain in the interpretation, but the method on whole plants seemed to be less aggressive than the leaf discs method.

(2) Strain Px : 3.5 Harmo

Results obtained with the reference strain Px: 3.5 MATREF were presented table 45.

Table 45: results of comparative tests for *Podosphaera xanthii* race 3.5 strain Harmo

		Leaf discs 2-4 leaf stage							**Whole plants**			
		Direct contact	**Settling tower**					**Spraying**				
Variety	**Expected**	**lab 6**	**lab 3**	**lab 8**	**lab 11**	**lab 12**	**lab 18**	**lab 6**	**lab 3**	**lab 11**	**lab 14**	**lab 18**
Mix	seg	HG	HG	HG	IR	S	HG	HG	HG	HG	HG	HG
Vedrantais *	S	S	S	S	S	S	S	S	S	S	S	S
PMR45	S	S	S	S	S	S	S	S	S	S	S	S
PMR5	S	S	S	S	S	S	S	S	S	S	S	S
Edisto 47	S	S	S	S	S	S	S	S	S	S	S	S
Gustabel	S	R	S	S	S	S	S	IR	S	S	S	S
Hispano	IR	R	HG	IR	IR	HG	IR	R	IR	R	IR	IR
Forterra	IR	IR	S	S	S	S	S	IR	S	S	S	S
Harmo-19A	IR	R	S	S	S	S	IR	IR	S	IR	IR	S
Harmo-19B	IR	R	S	S	S	S	S	IR	S	IR	IR	S
Harmo-19D	IR	R	S	S	S	S	IR	IR	S	R	IR	IR
ARHBJ	R	R	S	S	IR	IR	IR	R	IR	R	IR	IR
Arum *	R	R	S	IR	R	IR	IR	R	IR	R	R	IR
Arum	R	R	IR	S	IR	HG	IR	R	IR	R	R	IR

S: susceptible; IR: intermediate resistant; R: resistant; HG: heterogeneous; *: uncoded controls

Candidate controls Vedrantais and Arum were validated in all tests (excepted in 1 lab). The mix lot was judged heterogeneous in 9 tests out of 11.
The strain was validated as a race 3.5 but was judged as more aggressive than Px: 3.5 MATREF with susceptibility of some varieties expected as intermediate resistant or resistant (Gustabel, Forterra, Harmo-19A, Harmo-19B and Harmo-19D).
The settling tower inoculation method was observed as more aggressive than the other methods with this strain. Results were less reproducible than Px: 3.5 MATREF compared to expected results for the inoculation method with leaf discs.

Steering committee discussed about the validation of the strain Px: 3.5 MATREF as a race 3.5 because its lack of aggressivity on the differential PMR5 in some labs. One proposal was to define the strain Px: 3.5 Harmo as reference strain. But due the aggressive comportment of this strain on additional differentials (identified in Diversoid project), this strain would be characterized as another race than race 3.5 in the future. Partners decided to research for a more stable Px: 3.5MATREF among the isolates sampled and characterized in the DRT ISF project which is coming after Harmores 3 project.

(3) Intermediate resistant control candidates

One of the aims of the comparative test of validation was to define an intermediate resistant control. Two varieties were identified as candidate: Hispano and ARHBJ. The repartition of plants per class were studied by partners for both strains for each variety (figures 28 and 29).

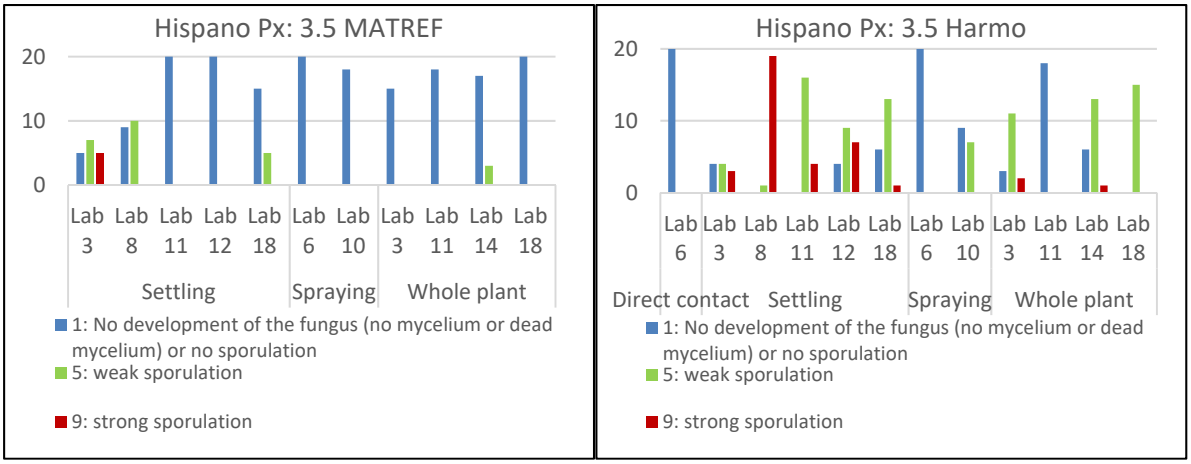


Figure 28: repartition of plants per class for Hispano for melon/Px comparative test

The variety Hispano was observed too much resistant to be validated as an intermediate resistant control.

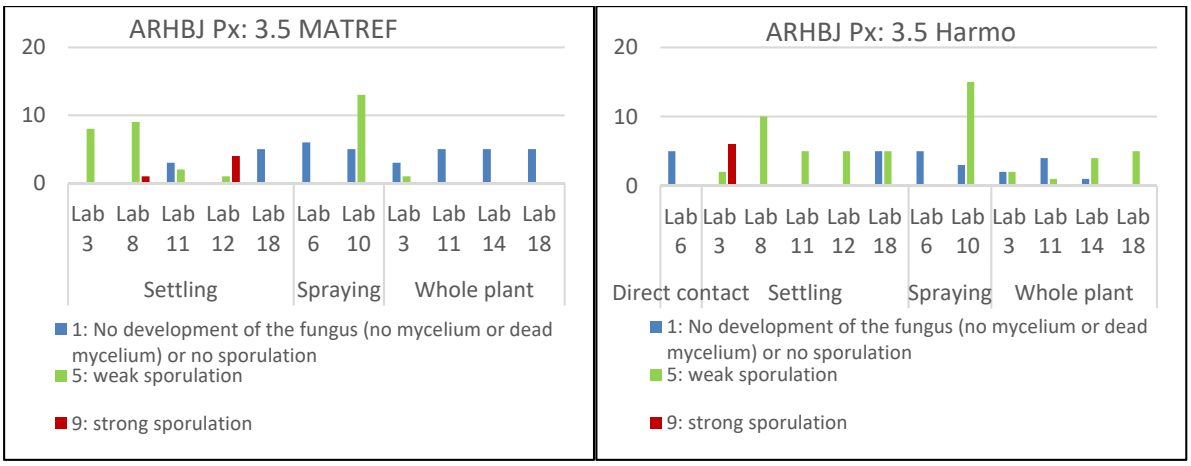


Figure 29: repartition of plants per class for ARHBJ for melon/Px comparative test

The variety ARHBJ was not validated as an intermediate resistant control. The steering committee proposed to review the repartition of plants per class and the disease index a next Skype meeting planned in June.

(4) Inoculation methods

The inoculation method on leaf discs with direct contact was tested only in one test with the strain Px Harmo. There is not enough data to be validated and this method not used in routine by several labs. So, this method was not selected.
The inoculation method on leaf discs with spraying was tested only in three tests and was the less aggressive. There is not enough data to be validated and this method not used in routine by several labs. So, this method was not selected.

The inoculation method on leaf discs by settling tower was the most used method and the most aggressive. This method shown some variation and difficult interpretation. Sometimes the resistant control Arum was observed with different results between coded and uncoded samples.

The inoculation method on whole plants was observed as the more reproducible method and was selected by the steering committee.

(5) Notation scale and interpretation rule

During the comparative test, significant differences of levels of sporulation were brought to light in the class 5 (figure 30).

Figure 30: different levels of symptoms observed in the class 5 for melon/Px comparative test

It was proposed a four classes notation scale (1-3-5-9) allowing more flexibility to be able to distinguish regular isolates from aggressive isolates and high resistant from resistance. This new common notation scale would be tried in the ISF DRT ring test. Partners discussed about the importance to communicate with the market about what are the resistant and the intermediate resistant levels.

A date of notation was validated based on expected symptoms on the controls when the sporulation is well expressed on the susceptible control.
The common decision rule for validation of controls was defined:

- Resistant:
  - 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
  - Not plants at classes 5 or 9

- Intermediate resistant:
  - Between the resistant and the susceptible control
  - Generally, plants at classes 3 and 5
- Susceptible:
  - 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
  - Not plants at class 1

A common decision rule for interpretation of varieties was proposed (figure 32):

- Quantitative analysis based on the disease index and the repartition of plants per class compared to the controls.
- The varieties between the IR and the R control has to be judged as IR (not enough R).
- The varieties between the S and the IR control has to be judged as S (not enough IR).

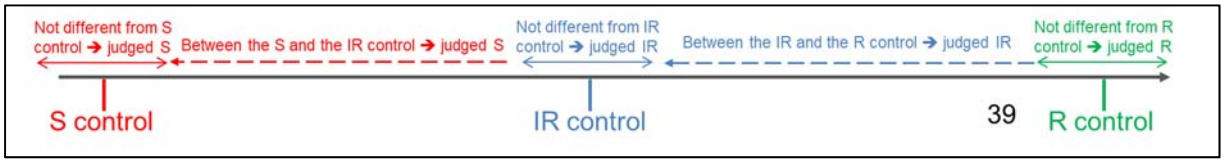


Figure 31: common decision rule for interpretation for melon/Px

(6) Meeting of 14-15 May conclusions

The steering committee decided that it was too early to validate the conclusions of Harmores 3 project for melon/Px and proposed another meeting in June to analyse more in detail results on melon/Px with the repartition of plants per class and reinterpretation with the common rule of results on whole plants for Px: 3.5 MATREF.
It was decided to use Pathostat statistic tool with the different scenario of intermediate resistant control candidates. It was required to each partner to send pictures of the next classes 3 and 5 to illustrate the new notation scale.

3. Meeting of 3rd June 2019

The objectives of this meeting were to work on the interpretation rule based on repartition of notes and to define then if the protocol was ready to be written.

The first step was for each lab to analyse results obtained in the comparative test of validation with the whole plant inoculation method and with the strain Px: 3.5 MATREF according the repartition of plants per class, the disease index and the dendrogram generated by Pathostat application (figure 32). Groups of varieties with different levels of resistance were identified.

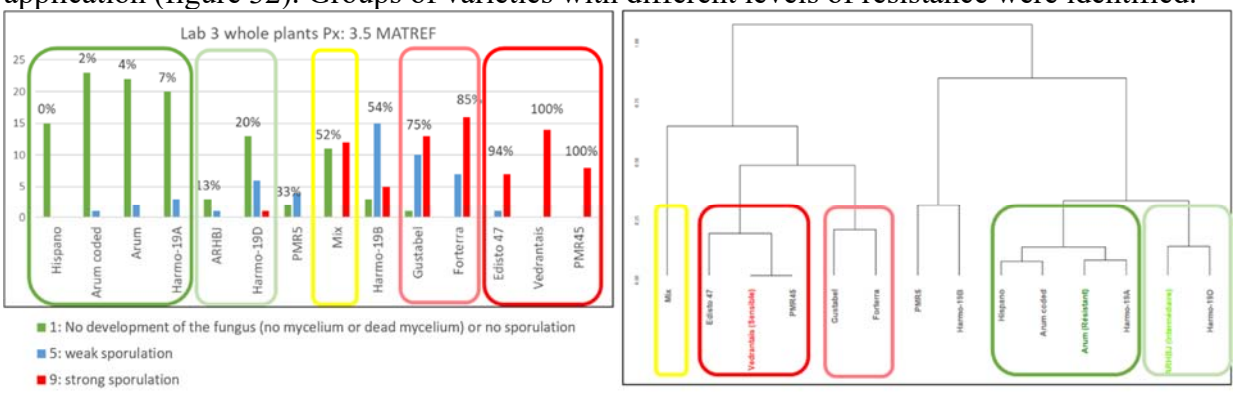


Figure 32: example of detailed results obtained for melon/Px with the whole plant inoculation and Px: 3.5 MATREF (green: resistant, red: susceptible, yellow heterogeneous)

According to this analyse, three varieties were identified as intermediate resistant candidate controls: ARHBJ, Harmo-19D and Harmo-19B.

For each lab, the varieties comportment was reinterpreted using PATHOSTAT with respectively ARHBJ, Harmo-19D and Harmo-19B as intermediate resistant control (figure 33).

Figure 33: example of Pathostat analysis for lab 3 with the variety ARHBJ used as IR control (green: resistant, red: susceptible, yellow heterogeneous)

The application classified varieties in four groups: resistant (statistically not different from Arum), intermediate resistant (statistically not different from the intermediate resistant control candidate), susceptible (statistically not different from Védrantais), heterogeneous (statistically different from controls).

The conclusions were summarized in the (table 46).

Table 46: results of comparative tests for *Podosphaera xanthii* race 3.5 strain MATREF on whole plants reinterpreted by Pathostat statistical application.

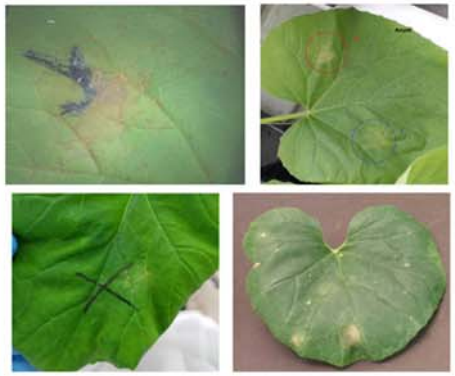
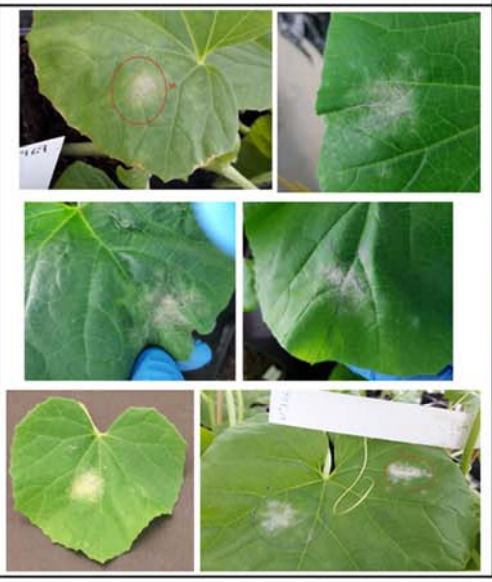
		IR = ARHBJ					IR = Harmo-19D					IR = Harmo-19B				
Variety	**Expected**	**Lab 2**	**lab 3**	**lab 11**	**lab 14**	**lab 18**	**Lab 2**	**lab 3**	**lab 11**	**lab 14**	**lab 18**	**Lab 2**	**lab 3**	**lab 11**	**lab 14**	**lab 18**
Mix*	seg	IR	HG	HG	HG	HG	IR	HG	HG	HG	HG	IR	HG	HG	HG	HG
Vedrantais	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S
PMR45	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	IR(S)
PMR5	S	S	IR	R	S	S	S	IR	R	S	S	S	IR	R	S	IR(S)
Edisto 47	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	IR(S)
Gustabel	S	S	S	S	S	S	IR	S	S	S	S	S	S	S	S	IR(S)
Forterra	IR	S	S	S	S	S	IR	S	S	S	S	S	S	S	S	IR(S)
Harmo-19B	IR	IR	S	R	S	S	IR	S	S	S	S	IR	IR	IR	IR	IR(S)
Hispano	IR	R	R	R	R	R	R	R	R	IR	R	R	R	R	IR	R
Harmo-19A	IR	IR	R	R	R	R	IR	R	R	IR	R	IR	R	R	IR	R
Harmo-19D	IR	S	IR	R	S	R	IR	IR	IR	IR	IR (R)	S	IR	R	IR	R
ARHBJ	R	R	IR	IR (R)	IR	IR (R)	R	R	R	R	R	R	R	R	R	R
Arum (Uncoded)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Arum (coded)	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

(R): observed resistant with all plants at class 1; (S): observed susceptible with all plants at class 9

Globally, no clear differences were observed depending on the intermediate resistant control chosen.
ARHBJ is identified as a potential new differential in the DRT ISF test, in this case this variety could not be used as intermediate resistant control.
Harmo-19D (variety Durango) could be a good intermediate resistant control. It was confirmed by partners that there is a difference of level of resistance between the resistant control (Arum) and Durango in tests in field. It would be a good control of the level of the aggressivity of the test.
Harmo-19B (variety Arango) was observed as more restrictive intermediate resistant control than Harmo-19 D with more varieties judged as susceptible with this threshold.
The steering committee decided to keep harmo-19B (Arango) and Harmo-19D (Durango) as candidates for validation in DRT ISF test. They will be tested on more plants in different labs on some isolates using the new common notation scale.

Based on pictures send by partners on the different level of sporulation observed for the previous class 5, an updated notation scale was defined with an additional note (to validate during ISF ring test under preparation). This notation scale was established only for test on whole plants (table 47):

Table 47: updated common notation scale for *Podosphaera xanthii*

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
			

In addition, an example of contamination by environment on the susceptible control was described. In this case, the test would not be validated (figure 34).

Figure 34: example of environment contamination

4. Melon/*Podosphaera xanthii* conclusions

A draft of protocol was defined which has to be validated during the DRT ISF ring test for the validation of the intermediate resistant control and the selection of reference isolates (annex 7) with:

- The inoculation method by local deposit on whole plant was validated. It was advised to place the inoculum on a mark on the leaf to avoid confusion with environment contamination.

- The number of plants: at least 20 plants per variety plus controls, 5 plants for other differentials. For leaf discs method: 1 disc = 1 plant = 1 data point.

- The date of notation: when symptoms are well expressed on the susceptible control (around 10 to 17 days post-inoculation).

- The controls selected:
  - Resistant: Arum
  - Intermediate resistant: Harmo-19D (Durango) and/or Harmo-19B (Arango) as candidates
  - Susceptible: Védrantais
  - It was decided to add differentials (at least 5 plants) in each test to validate the race and to compare the level of sporulation.

- The notation scale in four classes (table 47).

- The calculation of the disease index which give a synthetic picture of the test:

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

Nx: Number of plants at class X

- The common decision rule to interpret varieties (figure 35):
  - Interpretation of varieties depending on controls
  - Quantitative analysis based on the disease index and the repartition of plants per class compared to the controls.
  - The varieties between the intermediate resistant and the resistant control has to be judged as intermediate resistant (not enough resistant).
  - The varieties between the susceptible and the intermediate resistant control has to be judged as susceptible (not enough intermediate resistant).

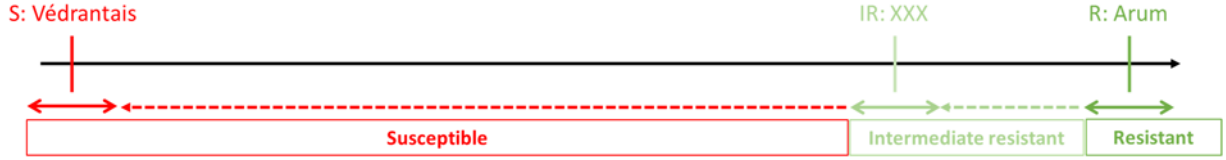


Figure 35: common notation scale for melon/*Podosphaera xanthii*

In case of no clear results, the test has to be repeated to confirm the comportment of the variety.

V. Pea

A. Pea/*Erysiphe pisi*

At the end of the part 1 year 1 of Harmores 3 project, a reference collection of *Erysiphe pisi* isolates was set up (table 48).

Table 48: collection of isolates for *Erysiphe pisi*

	Field test			**Controlled conditions**		
	Isolate	Sequencing	Maintenance	Isolate	Sequencing	Maintenance
Lab 3	**2431 (Field 2017)**	*E. pisi*	Yes	**2430 (Greenhouse)**	*E. pisi*	Yes
Lab 6	Field 2017	*E. pisi*	/	/	/	/
Lab 7	**Field 2017**	*E. pisi*	Yes (in lab 3)	Field 2017	*E. pisi*	/
Lab 9	/	/	/	Lm16	*E. pisi*	Isolate used in CT lost
Lab 9	/	/	/	**Greenhouse 2017**	*E. pisi*	New isolate not used in CT

All isolates used in test (controlled conditions and field) have been sequenced as *E. pisi* by Naktuinbouw (based on specific EryF/EryR primers).

In the part 2 year 1, two inoculation methods on controlled condition were validated: inoculation by spraying (figure 36) and inoculation by sprinkling (figure 37).

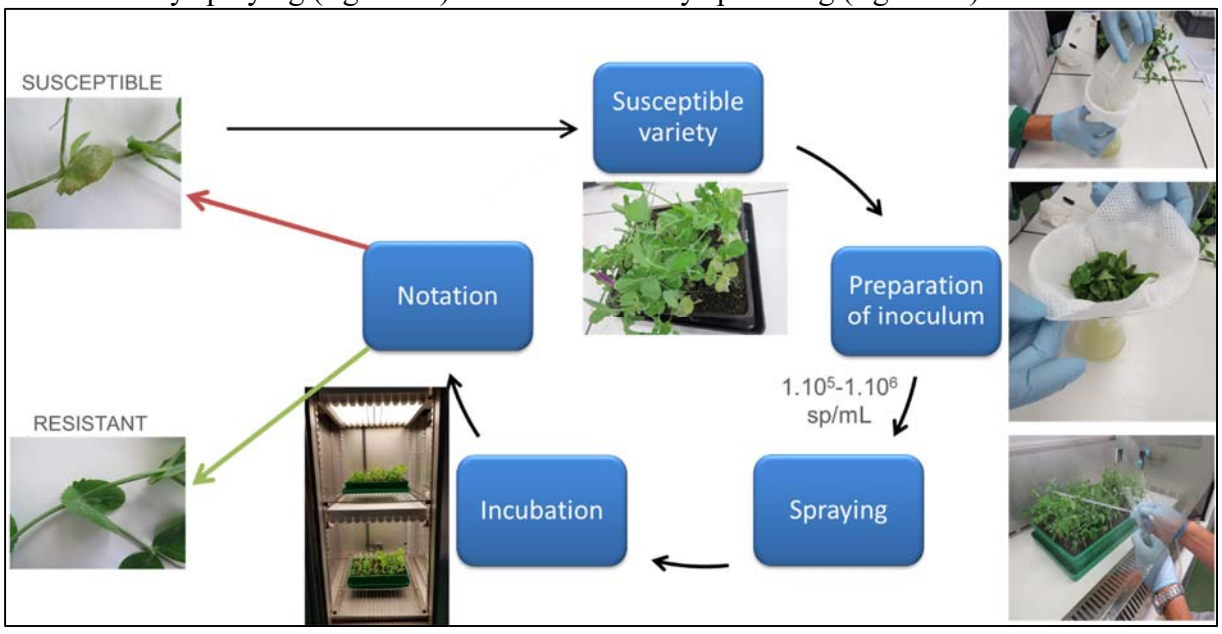


Figure 36: protocol of inoculation by spraying for pea/*Erysiphe pisi*

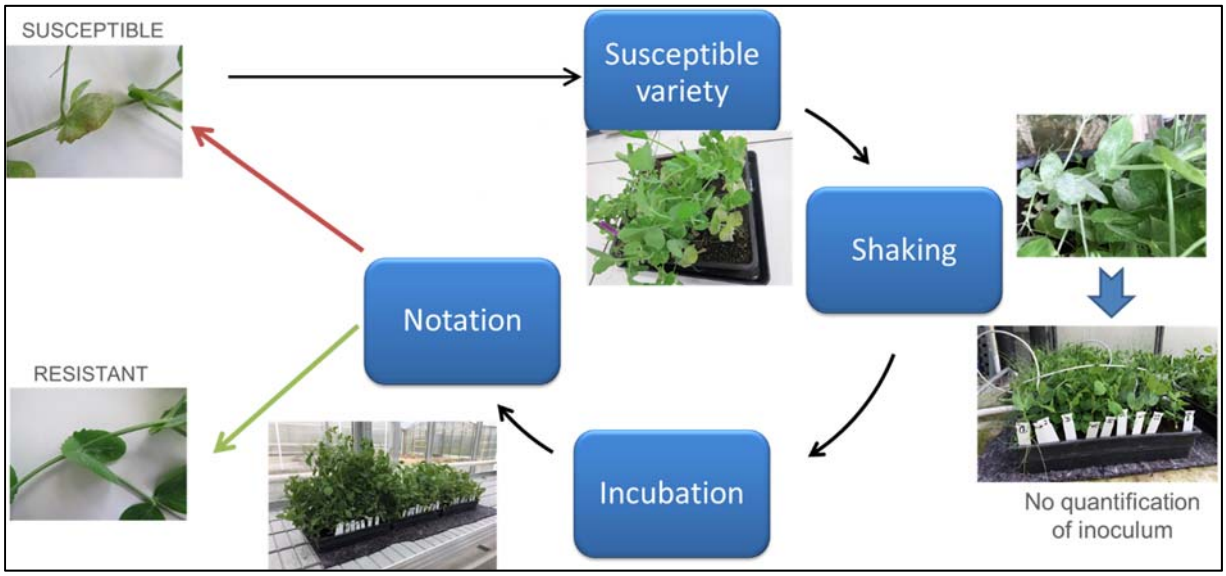


Figure 37: protocol of inoculation by sprinkling for pea/*Erysiphe pisi*

A common notation scale, which can be used for both conditions (controlled and field tests), was defined (table 49).

Table 49: common notation scale for *Erysiphe pisi*

Susceptible: sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant)				
Resistant: No sporulation or few mycelia pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms				
Symptoms which should not be confused with *E. pisi*: senescence of older leaves, yellowing, discoloration of leaves and insect damages	 senescing	 yellowing	 discoloration	 insect damage

The objectives of the part 2 year 2 were to compare the results of the second test performed in field with the test performed in controlled conditions.

1. Comparative tests (field and controlled conditions)

a) Materials and methods

4 laboratories were involved in the validation comparative test.

The panel was made up of the varieties tested in the previous year of the project (table 50).

Table 50: panel for *Erysiphe pisi* comparative test

Varieties	Crop type	Growth control	Expected
Aladin	Agricultural	Later growth	S
Cabree	Vegetable	Early growth	S
Ottoman	Agricultural		S
Ema	Vegetable		R
Sugar Bon	Vegetable	Early growth	R
Alezan	Agricultural		R
Vivaldi	Vegetable		R (CPVO control)
JI2302		Later growth	R

Four varieties were identified as growth control. Two notations were decided in field to observe evolution of symptoms. The dates of notation had to be defined depending on symptoms on early and later growth controls.
For test in controlled conditions, the date of notation was around 14 days post-inoculation depending of symptoms on susceptible control.

The repartition of tests between labs is presented table 51.

Table 51: repartition of comparative tests for *Erysiphe pisi*

Partner	Field	Controlled condition	
		Climatic chamber	Greenhouse
GEVES	X	X + X (isolate from Lab 7 field)	
Naktuinbouw	X		X
SASA	X		X
Vilmorin	X		X

Each lab used its own *Erysiphe pisi* isolate. In the previous test, a difference of aggressivity was observed with the isolate from Lab 7. This isolate was tested in comparison of GEVES isolate in controlled conditions.

b) Results

Results obtained in controlled conditions are presented table 52.

Table 52: results of comparative tests for *Erysiphe pisi* in controlled conditions

Variety	Expected	Lab 3 (isolate 430)	Lab 3 (Lab 7 isolate)	Lab 6 (isolate 2430)	Lab 9
Cabree	Susceptible	S	S	S	S
Ottoman	Susceptible	S	S	S	S
Aladin	Susceptible	S	S	S	S
JI2302	Resistant	R	R	R	R
Ema	Resistant	R	R	R	R
Vivaldi	Resistant	R	R	R	R
Alezan	Resistant	R	R	R	R
Sugar Bon	Resistant	R	R	R	R

S: susceptible, R: resistant

Results were consistent in all labs for controlled conditions and conformed to expected results.

Results obtained in field condition are presented table 53.

Table 53: results of comparative tests for *Erysiphe pisi* in field

Variety	Expected	Lab 3	Lab 6	Lab 7	Lab 9
Cabree	Susceptible	*	(0)	*	R
Ottoman	Susceptible	*	S (7)	S	R
Aladin	Susceptible	*	R (4)	S	R
JI2302	Resistant	*	R (1)	S	R
Ema	Resistant	*	R (15)	R	R
Vivaldi	Resistant	*	R (3)	R	R
Alezan	Resistant	*	R (2)	S	R
Sugar Bon	Resistant	*	R (2)	R	R

S: susceptible, R: resistant, (x): number of plants observed, *: all plants senesced before notation

The steering committee compared results obtained in the two comparative tests performed in the Harmores 3 project (tables 54 and 55).

Table 54: results of the two comparative tests for *Erysiphe pisi* in controlled conditions

		CT 1 – part 1			CT 2 – part 2			
Variety	Expected	Lab 3	Lab 7	Lab 9	Lab 3 (isolate 2430)	Lab 3 (Lab 7 isolate)	Lab 6 (isolate 2430)	Lab 9
Cabree	S	S	S	S	S	S	S	S
Ottoman	S	S	R/S	S	S	S	S	S
Aladin	S	S	S	S	S	S	S	S
JI2302	R	R	R	R	R	R	R	R
Ema	R	R	R	R	R	R	R	R
Vivaldi	R	R	S	R	R	R	R	R
Alezan	R	R	R	R	R	R	R	R
Sugar Bon	R	R	R	R	R	R	R	R

S: susceptible, R: resistant,

For tests in controlled condition, it was observed consistent results on the 2 CT with a clear cut between susceptible and resistant comportment and a high level of accuracy and reproducibility.
The advantages of tests in controlled conditions are:

- That is a quick test which needs less space compared to field test.
- The PCR validation of isolate is necessary after the sample of the isolate but not for subsequent tests.
- The same isolate can be used year after year.

The disadvantage is that the isolate needs to be maintain by the lab.
The conclusion was that test in controlled conditions is validated.

Table 55: results of the two comparative tests for *Erysiphe pisi* in field

		CT 1 – part 1				CT 2 – part 2			
Variety	Expected	Lab 3 (test 1)	Lab 3 (test 2)	Lab 6	Lab 7	Lab 3 (test 1)	Lab 6	Lab 7	Lab 9
Cabree	S	R	/	S	R?	*	(0)	*	R
Ottoman	S	R	S	S	S	*	S (7)	S	R
Aladin	S	R	S	S	R	*	R (4)	S	R
JI2302	R	R	R	S	S	*	R (1)	S	R
Ema	R	R	IR	R	R*/S	*	R (15)	R	R
Vivaldi	R	R	/	R	S	*	R (3)	R	R
Alezan	R	R	IR	IR	S	*	R (2)	S	R
Sugar Bon	R	R	R	IR	S	*	R (2)	R	R

S: susceptible, R: resistant, IR: intermediate resistant, (x): number of plants observed, *: all plants senesced before notation

For tests in field, it was observed non-consistent results obtained from either of the two comparative tests.
The advantage of tests in field in the observation of different levels of resistance.
The disadvantages are:

- That the validation of test is reliant on weather/season/isolates,
- It is a long test which requires a lot of space compared to controlled conditions,
- The need of a validation by PCR of isolate at the end of each test (if isolate is not *E. pisi*, test cannot be validated)
- Four controls are required (early and later growth)
- No guarantee that isolate is the same each year

For these reasons, the field condition is not validated.

A training test in controlled conditions was planned during the last year of the project (instead of the comparative test initially planned) to allow to each partner to practice using the method of multiplication of isolate and conditions of test.

2. Training test

a) Materials and methods

4 laboratories were involved in the training test.

The panel was made up of the varieties tested in the previous year of the project (table 56). The varieties were uncoded.

Table 56: panel for *Erysiphe pisi* training test

Varieties	Crop type	Expected
Aladin	Agricultural	S
Cabree	Vegetable	S
Ottoman	Agricultural	S
Ema	Vegetable	R
Sugar Bon	Vegetable	R
Alezan	Agricultural	R
Vivaldi	Vegetable	R (CPVO control)
JI2302 (Stratagem)	Vegetable	R

At least 20 plants were tested per variety. All labs used the *Erysiphe pisi* isolate 2430 (sent by GEVES).
Plants stage ta inoculation was 3-4 leaf stage. And each lab chosen one of the two selected inoculation methods depending on its used and facilities.

b) Results

Results of the training test are presented in table 57:

Table 57: results for *Erysiphe pisi* training test

		Controlled conditions			
Variety	**Expected**	**Lab 2**	**Lab 3**	**Lab 6**	**Lab 7**
Cabree	Susceptible	S	S	S	S
Ottoman	Susceptible	S	S	S	S
Aladin	Susceptible	S	S	S	S
JL2302	Resistant	R	R	R	R
Ema	Resistant	R	R	R	R
Vivaldi	Resistant	R	R	R	R
Alezan	Resistant	R	R	R	R
Sugar Bon	Resistant	R	R	R	R

S: susceptible, R: resistant

Tests were validated in all labs.

4. Pea/*Erysiphe pisi* conclusions

Based on the results obtained during the Hamores 3 project, the steering committee decided to select for the updated protocol (annex 7):

- Controls:
  - Susceptible controls: Cabree (Vegetable) and Aladin or Ottoman (agricultural)
  - Resistant control: Ema, Sugar Bon or Vivaldi (Vegetable) and Alezan (agricultural)

One question was about the choice or not between vegetable or agricultural type. As the characteristic is compulsory only for vegetable, it was decided to keep both and to precise if the variety is a control for vegetable or agricultural type. Controls will be chosen depending on the type of the variety in study.

- Design of test:
  - The test has to be performed on at least 20 plants per variety.
  - It will be precise not non-inoculated control in the CPVO protocol due to the fact of no possibility to place them exactly on the same conditions

- Conditions of test:
  - Climatic chamber or greenhouse
  - 20°C (+5°C) but it is advised not to go below 18°C, in some conditions it has been observed that increasing the day temperature up to 27°C was allowed to obtain a good sporulation on the susceptible control
  - Luminosity: at least 12h
  - Watering on the substrate (no spraying)

- Method of inoculation:
  - By spraying
  - By sprinkling

- Isolate: isolate 2430 selected

The steering committee discussed about the necessity to select or not an isolate. If no isolate is selected, it is possible for labs to sample an isolate from field, and it is not necessary in this case to maintain the isolate (it is not possible to freeze spores, isolate has to be maintain on plants), but the isolate has to be validated by sequencing. But for all other pathogens, one reference isolate was defined to ensure the reproducibility of results between labs. In conclusion, the isolate 2430 was selected and GEVES was identified as maintainer.

- Date of notation: between 14-21 dpi, when sporulation is well expressed on the susceptible control.

- Common notation scale (table 49).

VI. Harmores 3 conclusion

For tomato/ *Fusarium oxysporum* f. sp. *lycopersici* races 0 and 1, tomato/*Meloidogyne incognita*, melon/*Fusarium oxysporum* f. sp. *melonis* races 0, 1, 2 and 1.2 and for pea/*Erysiphe pisi*, workshops and comparative tests has allowed to define harmonized protocols with:

- Reference strains with maintainers laboratories,
- Culture conditions defined for pathogens
- Available reference resistant, intermediate resistant and susceptible controls,
- Stages and methods of inoculation,
- Conditions of tests,
- Notation scales,
- Decision rules.

Following the validation comparative tests performed on the last year of the project for these couple host/pathogen, the steering committee will propose to CPVO updated robust protocols validated in different laboratories.

For melon/*Podosphaera xanthii*, a draft of protocol will be proposed to CPVO including the new notation scale and the intermediate resistant candidate controls Durango and Arango which will be validated during the DRT ISF ring test. The protocol will be also updated after this project with reference isolates validated for each race and new additional differentials defined to distinguish the new races present in field.

For tomato/ *Fusarium oxysporum* f. sp. *lycopersici* race 1, the alternative protocol with marker test for the resistance gene *I2* was also evaluated. But, at this time, more consistent results are needed to prove the reproducibility and the repeatability of this method before the validation.
An extra-test outside the CPVO project will be organized. Firstly, a pretest so that each potential participant can implement the PCR protocol in their lab, and to confirm the capacity of the lab to apply the protocol in a future comparative test. For example, by sending around DNA extracts to be tested. Secondly, the organization of an interlaboratory comparative test to fully validate the complete protocol. Including sowing seeds of varieties previously characterized in the Harmores 3 project, the DNA extraction step and the PCR test.

The results of the Harmores 3 project were presented to UPOV in May 2019 in preparation of a future updating of UPOV protocol including Harmores 3 project results.

ANNEXES

ANNEX 1: TOMATO/*FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* MARKER TEST PROTOCOL

Execution of test:

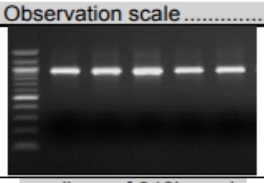
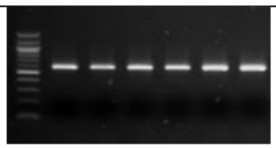
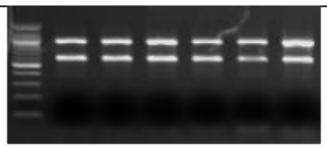
The marker test is optional, and it was decided by the steering committee during the last meeting that the test will perform on all varieties on panel.

(ii) DNA marker test

Resistance to both race 0 (ex 1) and race 1 (ex 2) is often based on resistance gene I2. The presence of the resistant and/or susceptible allele of gene I2 can be detected by the co-dominant marker as described in this method.

1. Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
2. Functional gene	I2
3. Primers	
3.1 Susceptible allele	Z1063-i2-F 5'-GTT TGA CAG CTT GGT TTT GT-3' Z1063-i2-R 5'-CTC AAA CTC ACC ATC ATT GA-3'
3.2 Resistant allele	TFusF1 5'-CTG AAA CTC TCC GTA TTT C-3' TFusRR1 5'-CGA AGA GTG ATT GGA GAT-3'
4. Format of the test	
4.1 Number of plants per genotype	at least 20 plants
4.2 Control varieties	homozygous susceptible allele present: (*Solanum lycopersicum*) Moneymaker homozygous resistant allele present: (*Solanum lycopersicum*) Tradiro
5. Preparation	
5.1 Preparation DNA	harvest per individual plant a part of a young leaf. Isolate total DNA with a standard DNA isolation protocol (CTAB/SDS based). Re-suspend in 100 µl $T_{10}E_{0.1}$. Dilute total DNA to 1/10 (H_2O) to obtain a DNA concentration between 1-10 ng/µl.
5.1 Preparation PCR	use 3 µl of each diluted DNA sample into individuals PCR reactions. Prepare the PCR master mix, 20µl reaction volume: • 3 µl of 10x diluted DNA • 2,5 µl of 10x reaction buffer • 2 mM $MgCl_2$ • 0.1 µM of resistance primers each • 0.2 µM of susceptible primers each • 200 µM of each of the four dNTPs • 1 unit of Taq DNA polymerase
6. PCR conditions	1. initial denaturation step at 94°C for 3 minutes 2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes 3. final extension step of 72°C for 10 minutes
7. Observations	
7.1 Method	visual

7.2 Observation scale

		
amplicon of 940bp only homozygous susceptible allele present	amplicon of 600bp only homozygous resistant allele present	amplicons of 940bp and 600bp susceptible and resistant allele present: heterozygous resistant

7.3 Validation of test	control varieties should give the expected band(s).
8. Interpretation of test results	
24.1 Race 0 (ex 1)	
present	[9] homozygous or heterozygous resistant in DNA marker test in case homozygous susceptible allele present a bio-assay on race 0 (ex 1) should be performed. in case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene I2 without I).
24.2 Race 1 (ex 2)	
absent	[1] homozygous susceptible in DNA merker test
present	[9] homozygous or heterozygous resistant in DNA merker test in case the DNA marker test result does not confirm the declaration in the TQ, a bio-assay should be performed to observe whether the resistance is absent or present for the variety (on another mechanism, e.g. gene I3).

ANNEX 2: TOMATO/*FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* TEST ON BIG PLANTS PROTOCOL

Execution of test

The test on big plants is optional and it was decided by the steering committee during the last meeting that the test will perform only on varieties on panel with no clear-cut comportment and controls.

Table 4: panel selected for *Fusarium oxysporum* f. sp. *lycopersici* race 0 and race 1 for test on big plants

Pathogen	Varieties	Expected comportment	Cultigroup	
Fusarium oxysporum f. sp. *lycopersici* race 0	Marmande verte	Susceptible		Uncoded
	Marporum x Marmande verte	Resistant		
	Marporum	Resistant		
	Motelle	Resistant		
	Cherry type control 1	Intermediate Resistant	Small fruits	
	E	Not uniform	Goose	Coded
	G	Intermediate Resistant	Small fruits	

Pathogen	Varieties	Expected comportment	Cultigroup	
Fusarium oxysporum f. sp. *lycopersici* race 1	Marmande verte	Susceptible		Uncoded
	Marporum	Susceptible		
	Motelle x Marmande verte	Resistant		
	Cherry type control 2	Resistant		
	H	Resistant	Cherry	Coded
	I	Resistant	Cherry	
	J	Resistant	Cherry	

Growth stage of plants
Plants are grown in greenhouse or growth chamber about 21 days (around 3-4 leaf stage)

Temperature:
Test performed in climatic chambers or greenhouse at 24-28°C. In case of aggressive test, Temperature can be decreased to 20-24°C.

Inoculum:
Fusarium oxysporum f. sp. *lycopersici* is grown on PDB or S of Messiaen media or in aerated liquid cultures for 7 to 10 days. Spores are harvested with a scraper and adjusted to 10^6 sp/ml for strains grown on media. In case of aggressive test, inoculum concentration can be decreased.

Method of inoculation:
Deposit about 135 mL of inoculum suspension per plants

Duration of test:
At least 28 days from sowing to notation. Depending to the susceptible control.

Number of plants tested:
At least 20 plants.

Notation:
At least 21 days after inoculation.

Notation Scale: (observation)
Number of yellow/wilting leaves
Number of leaves with brown vessels

ANNEX 3: TOMATO/*FUSARIUM OXYSPORUM* F. SP. *LYCOPERSICI* PROTOCOL

Ad. 45.1 + 45.2 + 45.3: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol) – Race 0EU/1US, Race 1EU/2US and Race 2EU/3US

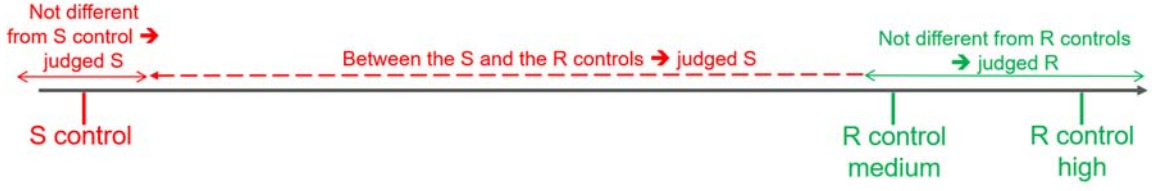
1. Pathogen	*Fusarium oxysporum* f. sp. *lycopersici*
3. Host species	*Solanum lycopersicum* L.
4. Source of inoculum	GEVES[1] (FR), INIA[2] (ES) or Naktuinbouw[3] (NL),
5. Isolate	Race 0EU/1US (e.g. strains Orange 71 or PRI 20698 or Fol 071 validated in Harmores project), race 1EU/2US (e.g. strains 4152 or PRI40698 or RAF 70 validated in Harmores project) and race 2EU/3US Individual strains may vary in pathogenicity
6. Establishment isolate identity	use differential varieties, see ISF website : https://www.worldseed.org/wp-content/uploads/2019/09/Tomato-Fusarium-wilt_July2019_Final.pdf
7. Establishment pathogenicity	on susceptible tomato varieties
8. Multiplication inoculum	
8.1 Multiplication medium	Potato Dextrose Agar or Medium "S" of Messiaen or Czapek-Dox
8.4 Inoculation medium	water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
8.6 Harvest of inoculum	filter through double muslin cloth
8.7 Check of harvested inoculum	see 10.2
8.8 Shelf-life/viability inoculum	4-8 h, keep cool to prevent spore germination
9. Format of the test	
9.1 Number of plants per genotype	at least 20 plants plus at least 5 non-inoculated plants
9.2 Number of replicates	plants have to be divided into at least 2 replicates
9.3.1 Control varieties for the test with race 0EU/1US	
Susceptible	Marmande, Marmande verte, Resal
Resistant	Marporum, Larissa, "Marporum x Marmande verte", Motelle, Gourmet, Mohawk and Riesling as additional resistant control for medium level
9.3.2 Control varieties for the test with race 1EU/2US	
Susceptible	Marmande verte, Cherry Belle, Roma, Marporum, Ranco
Resistant	Tradiro, Odisea or "Motelle x Marmande verte" and Agostino as additional resistant control for medium level
9.3.3 Control varieties for the test with race 2EU/3US	
Susceptible	Marmande verte, Motelle, Marporum
Resistant	Alliance, Florida, Ivanhoé, Tributes, Murdoch, "Marmande verte x Florida"
9.5 Test facility	glasshouse or climate room
9.6 Temperature	24-28°C (severe test, with mild isolate), 20-24°C (mild test, with severe isolate)

[1] GEVES: matref@geves.fr
[2] INIA: cardaba@inia.sp
[3] Naktuinbouw: resistentie@naktuinbouw.nl

9.7 Light ..... 12 hours per day or longer
9.8 Season..... all seasons
10. Inoculation
10.1 Preparation inoculum ..... 3-5 days in aerated liquid cultures like PDB, Czapek Dox or S of Messiaen or scraping of plates of 10 days cultures on agar medium.
10.2 Quantification inoculum ..... spore count, adjust to 10^6 spores per ml, in case of very aggressive isolate inoculum concentration can be decreased
10.3 Plant stage at inoculation..... 10-18 d, cotyledon to first leaf
10.4 Inoculation method ..... plants at the inoculation stage are harvested carefully, roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option, and transplanted in trays
10.5 End of test ..... 14-21 days after inoculation
11. Observations
11.1 Method ..... visual
11.2 Observation scale .....

Class 0	Class 1	Class 2	Class 3
Healthy compared to the non-inoculated control.	Healthy compared to the non-inoculated control with brown vessel above the cotyledon (observed when plants are cut in case of variety with different levels of symptoms)	Higher than 50% of growth reduction and/or yellowing and/or wilting on cotyledons and/or leaves.	Nearly dead: strong reduction with plants look dwarf (there can be necrosis but not always) or dead
			
If all plants in class 0 or if all plants in classes 2 and 3, it is not necessary to cut the plants.			
In case of variety or control with different levels of symptoms, cut the plants to check presence or not of strong brown vessel above cotyledons. In case of no brown vessels or below cotyledons, the plant is note 0. In case of brown vessels above cotyledons, the plant is note 1.			

11.3 Validation of test ..... validation on controls. Expected comportment of controls:
Susceptible: most plants in 2 and 3, at most 2 plants out of 20 can be observed at classes 0 and 1
Resistant: most plants in 0 and 1, at most 2 plants out of 20 can be observed at classes 2 and 3
12. Interpretation of data in terms of UPOV characteristic states
If not different from both resistant level controls, the variety is judged resistant
If lower level than the medium resistant level control, the variety is judged susceptible.
If no clear results, statistics must be used.



13. Critical control points

ANNEX 4: TOMATO/*MELOIDOGYNE INCOGNITA* PROTOCOL

Ad 43: Resistance to *Meloidogyne incognita* (Mi)

1. Pathogen	*Meloidogyne incognita*
3. Host species	Tomato - *Solanum lycopersicum*
4. Source of inoculum	GEVES[4] (F) or INIA (SP)[5] or Naktuinbouw (NL[6])
5. Isolate	non-resistance breaking
6. Establishment isolate identity	use rootstock or tomato standards
7. Establishment pathogenicity	use susceptible rootstock or tomato standard
8. Multiplication inoculum	
8.1 Multiplication medium	living plant
8.2 Multiplication variety	preferably resistant to powdery mildew
8.3 Plant stage at inoculation	2 leaf stage
8.5 Inoculation method	deposit of piece of contaminated roots in soil (around 5-10g per plant, to adapt depending of the population aggressivity)
8.6 Harvest of inoculum	6 at 10 weeks after inoculation, root systems are cut with scissors into pieces of about 1 cm length
8.7 Check of harvested inoculum ..	visual check for presence of root knots and ripe egg masses
8.8 Shelf life/viability inoculum	1 day
9. Format of the test	
9.1 Number of plants per genotype	30 plants, plus at least 10 non-inoculated plants to observe if a possible lack of germination is due to nematode or not
9.3 Control varieties	Susceptible: Casaque Rouge Intermediate resistant: Campeon and Tyonic Resistant: Anahu x Casaque Rouge
9.4 Test design	3 repetitions of 10 plants in different trays by variety to allow statistical analysis
9.5 Test facility	greenhouse or climate room
9.6 Temperature	20-26°C, the temperature must be adapted depending on the aggressivity of the test to obtain expected comportment of controls but should not be above 26°C
9.7 Light	at least 12 h per day
10. Inoculation	
10.1 Preparation inoculum	small pieces of diseased roots mixed with soil,
10.2 Quantification inoculum	the ratio is depending of aggressiveness of test and lab's conditions (e.g. between 30g to 60g of infested roots, for 100 plants in a tray of 45*30 cm containing approximately 5.5 kg of substrate,), galls must have an equal repartition on the soil.
10.3 Plant stage at inoculation	seed
10.4 Inoculation method	plants sown in soil contaminated with infested root homogeneously mixed with soil
10.7 End of test	28 to 45 days after inoculation depending on test conditions (temperature, season)
11. Observations	

[4] GEVES; matref@geves.fr
[5] INIA; cardaba@inia.sp
[6] Naktuinbouw; resistentie@naktuinbouw.nl

11.1 Method ..... root inspection
11.2 Observation scale .....

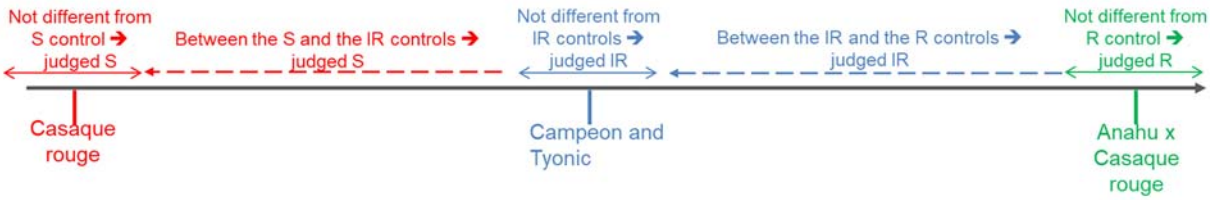
Note 0: healthy plant, no galls	Note 1: few and little galls which are difficult to find (for example less than 5)	Note 2: few galls, easy to observe but on few roots, still a lot of roots without galls	Note 3: many individual galls on most but not all roots	Note 4: many galls on all roots, sometimes in chains, can lead to dead plants and /or may suppress emergence
				

11.3 Validation of test ..... Validation on controls. Expected comportment of controls:
Susceptible: most plants at classes 3 and 4, at most 2 plants can be observed at class 2.
Resistant: most plants at classes 0 and 1, at most 2 plants can be observed at class 2.
Intermediate resistant: clearly different from other controls with majority of plants around class 2.

11.4 Off-types..... resistant varieties may have a few plants with a few galls

12. Interpretation of data in terms of UPOV characteristic states

Variety very similar to resistant control is judged as resistant.
Variety very similar to susceptible control is judged as susceptible.
Variety very similar to intermediate resistant control is judged as intermediate resistant.
If significantly different from resistant and intermediate resistant control (notations are between resistant and intermediate resistant intermediate resistant controls), the variety is judged as intermediate resistant.
If significantly different from intermediate resistant and susceptible control (notations are between intermediate resistant and susceptible controls), the variety is judged as susceptible.
If results are not clear, statistical analysis is advised.



13. Critical control points:
Avoid rotting of roots; high temperature causes breakdown of resistance.
In case of aggressive test, put seeds in a layer of non-contaminated soil or decrease the quantity of inoculum.

ANNEX 5: MELON/*FUSARIUM OXYSPORUM* F. SP. *MELONIS* RACE 1.2 PROTOCOL

Ad 68.4: Resistance to *Fusarium oxysporum* f. sp. *melonis* race 1.2 (Fom: 1.2)

1. Pathogen	*Fusarium oxysporum* f. sp. *melonis* race 1.2
2. Quarantine status	No
3. Host species	Melon - *Cucumis melo* L.
4. Source of inoculum	GEVES (FR)[7]
5. Isolate	Fom: 1.2, e.g. MATREF/04-07-01-04 validated in Harmores 3 project
6. Establishment isolate identity	test on differentials. The most recent table is available through ISF at https://www.worldseed.org/wp-content/uploads/2015/10/Melon-Fusarium_wilt_2012.pdf
7. Establishment pathogenicity	test on susceptible varieties
8. Multiplication inoculum	
8.1 Multiplication medium	on agar medium (e.g. Sabouraud, PDA) 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	-
9. Format of the test	
9.1 Number of plants per genotype	30 plants per variety plus 5 non-inoculated controls, 3 repetitions of 10 plants to allow statistical analysis (in different trays)
9.3 Control varieties	Susceptible: Virgos Intermediate resistant: Piboule and Lunasol and Isabelle (Isabelle is expected with lower disease index than Piboule and Lunasol)
9.4 Test design	3 repetitions of 10 plants to allow statistical analysis (in different trays) and non-inoculated control in another tray.
9.5 Test facility	Climatic chamber or greenhouse
9.6 Temperature	18-24°C
9.7 Light	at least 12 hours
10. Inoculation	
10.1 Preparation inoculum	Scrap 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 obscurity 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	1.10^5-1.10^6 sp/mL, depending on inoculation method (see 10.4) and lab conditions
10.3 Plant stage at inoculation	cotyledon expanded, first leaf emerging
10.4 Inoculation method	two methods can be used for inoculation. Absorption:

[7] matref@geves.fr

Absorption of a suspension of spores, e.g. 700mL of a suspension at 1.10^5 sp/mL for 50 plants in a tray 30 cm*30 cm.

Injection:

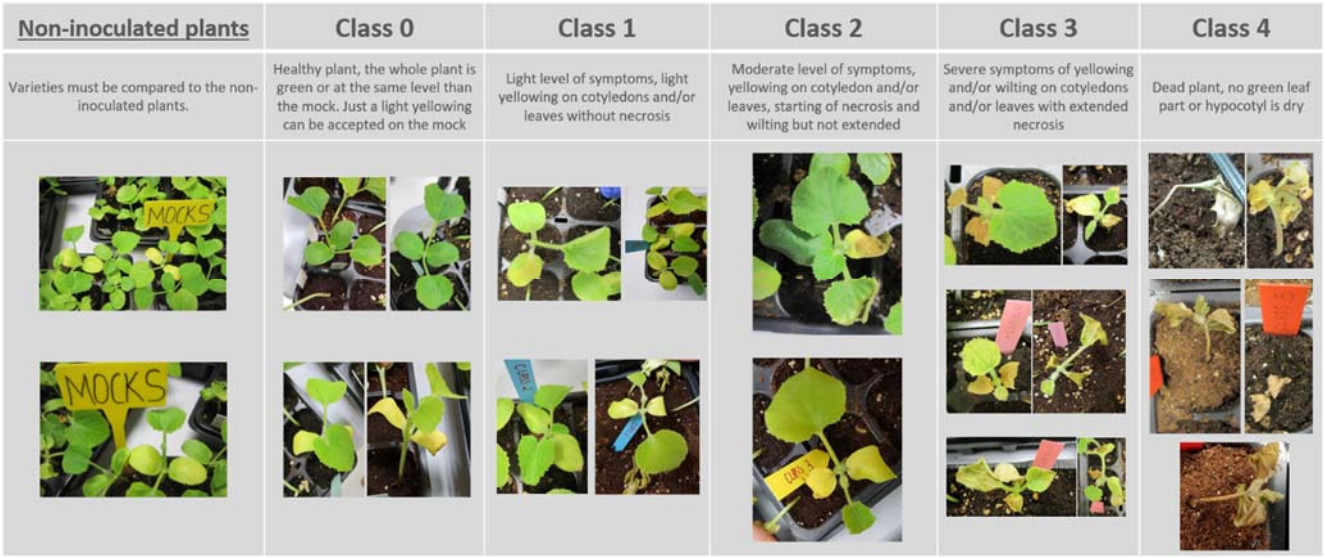
Injection of a suspension of spores at the base of the plant, e.g. 5mL at 10^6 sp/mL per plant.

10.7 End of test ..... 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

11.2 Observation scale .....



11.3 Validation of test ..... Validation on controls. Controls expected comportment:

Intermediate Resistant:

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

Susceptible:

Plants at classes 3 and 4, and in some cases few plants at class 2. Very high disease index above 80%.

11.4 Off-types.....

12. Interpretation of data in terms of UPOV characteristic states

Interpretation of varieties depending on controls (figure 1)

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

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.

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

If no clear results, statistics must be used.

$$DI = \frac{(N0 * 0) + (N1 * 1) + (N2 * 2) + (N3 * 3) + (N4 * 4)}{(N0 + N1 + N2 + \mathrm{N3} + \mathrm{N4})} * 100$$

Nx : number of plants at class x

Figure 38: disease index

13. Critical control points:

ANNEX 6: MELON/*FUSARIUM OXYSPORUM* F. SP. *MELONIS* RACES 0, 1, 2 PROTOCOL

Ad 68.1 – 68.3: Resistance to *Fusarium oxysporum* f. sp. *melonis*, races 0, 1 and 2 (Fom: 0, 1, 2)

1. Pathogen	*Fusarium oxysporum* f. sp. *melonis* races 0, 1 and 2
2. Quarantine status	no
3. Host species	Melon - *Cucumis melo* L
4. Source of inoculum	GEVES (FR)[8]
5. Isolate	Fom: 0 (e.g. MAT/REF/04-07-01-03-02 validated in Harmores 3 project), Fom: 1 (e.g. MAT/REF/04-07-01-01 validated in Harmores 3 project), Fom: 2 (e.g. F185 validated in Harmores 3 project)
6. Establishment isolate identity	test on differentials. The most recent table is available through ISF at https://www.worldseed.org/wp-content/uploads/2015/10/Melon-Fusarium_wilt_2012.pdf
7. Establishment pathogenicity	test on susceptible varieties
8. Multiplication inoculum	
8.1 Multiplication medium	on agar medium (e.g. Malt agar, PDA) 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.....	4-8 h, keep cool to prevent spore germination
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
9.3.1 Control varieties for the test with race 0	Susceptible: Charantais T Resistant: Védrantais, Charentais Fom-2
9.3.2 Control varieties for the test with race 1	Susceptible: Charantais T, Védrantais Resistant: Charentais Fom-2
9.3.3 Control varieties for the test with race 2	Susceptible: Marianna Resistant: Charentais Fom-1
9.4 Test design	3 repetitions of 10 plants to allow statistical analysis (in different trays) and at least 5 non-inoculated plants per genotype
9.5 Test facility	Climatic chamber or greenhouse
9.6 Temperature	18-24°C
9.7 Light	at least 12 hours
9.9 Special measures	recommend having really 18°C at night, and not above 25°C during the day. Under summer greenhouse conditions (high temperatures, high light intensity, long days), intermediate resistance (IR) can perform as high resistance (HR).
10. Inoculation	

[8] matref@geves.fr

10.1 Preparation inoculum ..... Scrap cultures with water on 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 Czapek-Dox culture medium for 5-7 days to room temperature.

10.2 Quantification inoculum..... 4.10^5 to 1.10^6 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, and transplanted in trays

10.7 End of test ..... 1st notation: symptoms on susceptible control at classes 2 and 3 with a strong proportion at 3. A second notation can be necessary to reevaluate some unclear varieties

11. Observations

11.1 Method ..... visual observation

11.2 Observation scale .....

Mock	Note 0	Note 1	Note 2	Note 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 observe some yellowing, different from the symptoms of *Fusarium*	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.

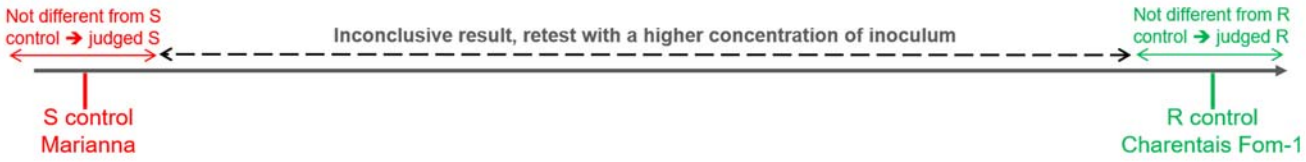
11.3 Validation of test ..... Validation on controls. Controls expected comportment:
<u>Resistant:</u>
Plants at classes 0 and 1, sometimes very few plants at classes 2 or 3

Susceptible:
Plants at classes 2 and 3

11.4 Off-types.....

12. Interpretation of data in terms of UPOV characteristic states

Interpretation of varieties depending on controls.
Susceptible: not different to the susceptible control.
Resistant: not different to the resistant control. In case of variety with a comportment between the susceptible and the resistant control, repeat the test with a higher concentration, in case of confirmation of the result, the variety will be judged heterogeneous.
In case of unclear varieties retest, or test in another lab.



13. Critical control points: ..... 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.
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 off spores collected on a Millipore filter with vacuum force.

ANNEX 7: MELON/*PODOSPHAERA XANTHII* RACES 1, 2, 5, 3.5 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)[9]
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 58: 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. Dry the cotyledons with sterile paper towel, then place them in Petri dishes with the following medium:

Sucrose	10g
Mannitol	20g

[9] matref@geves.fr

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

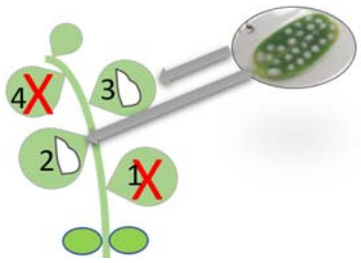
9.7 Light .....at least 12 hours

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.

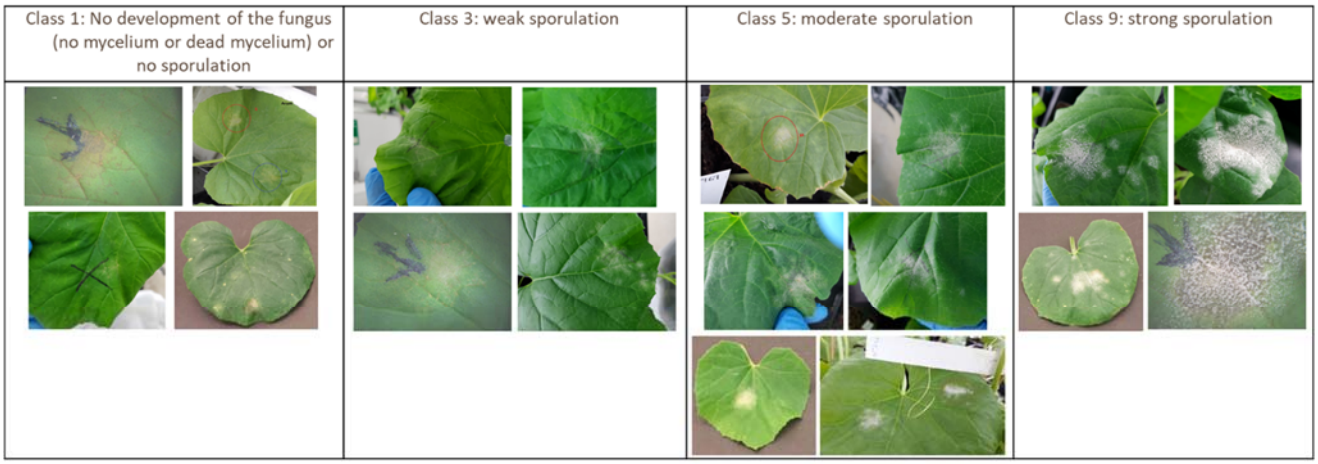


10.4 Inoculation method .....Take spores from a cotyledon already covered with conidia and deposit them on a leaf. Different isolates can be tested on the same plant (or the same leaf) if the local deposit is well separated from each other and if a mark indicates the place of the deposit.

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*:

Resistant:

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

Intermediate Resistant:

Between the resistant and the susceptible control

Generally, plants at classes 3 and 5

Susceptible:

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

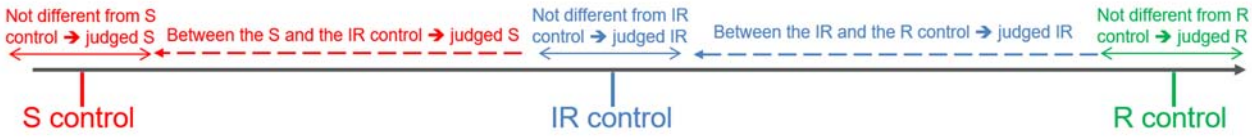
Interpretation of varieties depending on controls

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 39: 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 9: PEA/*ERYSIPHE PISI* PROTOCOL

Ad 57: Resistance to *Erysiphe pisi* (Ep)

1. Pathogen	Powdery mildew – *Erysiphe pisi*
2. Quarantine status	No
3. Host species	Pea – *Pisum sativum* L
4. Source of inoculum	GEVES[10] (FR)
5. Isolate	*Erysiphe pisi e.g.* isolate 2430 validated in Harmores 3 project
6. Establishment isolate identity	validation by use specific EryF/EryR primers to validate the species of *Erysiphe* (use ITS primers from Attanayake et al, 2010[11].)
7. Establishment pathogenicity	use susceptible variety (e.g. Aladin, Cabree or Ottoman)
8. Multiplication inoculum	
8.1 Multiplication medium	living plant
8.2 Multiplication variety.....	see 7
8.3 Plant stage at inoculation.....	see 10.3
8.5 Inoculation method.....	see 10.4
8.6 Harvest of inoculum.....	For spraying by washing off with demineralized water For sprinkling by detaching leaves of a susceptible host plant
8.7 Check of harvested inoculum...	visual check for presence of sporulation
8.8 Shelf life/viability inoculum.....	1-2 hours
9. Format of the test	
9.1 Number of plants per genotype	20 plants
9.3 Control varieties	Susceptible: For vegetable crops: Cabree For agricultural crops: Aladin, Ottoman Resistant: For vegetable crop: Ema, Sugar Bon, Vivaldi, Stratagem (JI2302) For agricultural crop: Alezan
9.4 Test design	No non-inoculated control due as it is impossible to place them exactly in the same conditions (risk of contamination).
9.5 Test facility	greenhouse or climate room
9.6 Temperature	20°C (+5°C) but it is advised not to go below 18°C. In some conditions it has been observed that increasing the day temperature up to 27°C was allowed to obtain a good sporulation on the susceptible control.
9.7 Light	at least 12 h per day
10. Inoculation	
10.1 Preparation inoculum	By spraying: Washing off from leaves by vigorous shaking in a closed container containing water. Sieve the suspension through muslin cloth. By sprinkling:

[10] GEVES; matref@geves.fr

[11] Attanayake et al, 2010. *Erysiphe trifolii*– a newly recognized powdery mildew pathogen of pea, Plant Pathology, Volume 59, Issue 4, p712-720

Selection of leaves with strong sporulation.

10.2 Quantification inoculum..... By spraying:
Counting spores; spores density should be 1.10^5 to 1.10^6 spores/mL
By sprinkling:
Approximately 1 well-sporulating plant to inoculate 10 plants.

10.3 Plant stage at inoculation ..... 3-4 leaf stage

10.4 Inoculation method ..... By spraying:
Spraying of the suspension of spores on leaves
By sprinkling:
Shaking of sporulated leaves above the plants to be contaminated.

10.7 End of test ..... Between 14-21 dpi, when sporulation is well expressed on the susceptible control

11. Observations

11.1 Method ..... visual

11.2 Observation scale .....

Susceptible: sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant)				
Resistant: No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms				
Symptoms which should not be confused with *E. pisi*: senescence of older leaves, yellowing, discoloration of leaves and insect damages	 senescing	 yellowing	 discoloration	 insect damage

11.3 Validation of test ..... Analysis of results should be calibrated with results of R and S controls.

11.4 Off-types.....

12. Interpretation of data in terms of UPOV characteristic states

Absent (susceptible) [1] sporulation on leaves. Symptoms can be observed on stem and tendril (not always on the whole plant)

Present (resistant) [9] No sporulation or few mycelial pustules only on the lower leaves in case of high disease pressure, no evolution of the symptoms

13. Critical control points:

Watering on the substrate (no spraying) to avoid washing the spores off the surface of the leaves.

It is not possible to freeze spores. Need to maintain on plants