

**Project 2003-2006 for the Community Plant Variety Office (CPVO)
Research and Development Section**

**Harmonization of resistance test to diseases of vegetables crops in the European Union
Final report**

(December 15, 2004 – December 15, 2006)

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Table of contents

I. Introduction.....	5
II. Background on the first ring test in 2005.....	7
III. Results of the ring tests in 2006 and comparisons with 2005.....	11
A. Tomato Ring test	11
1. FOL race 0.....	11
2. FOL race 1.....	15
3. ToMV pathotype 0	19
4. <i>Verticillium dahliae</i> race 0	23
B. French bean ring test.....	27
1. <i>Colletotrichum lindemuthianum</i>	27
2. BCMV or BCMNV	31
3. <i>Pseudomonas savastanoi</i> pv. <i>phaseolicola</i>	35
IV. Conclusion	39
V. Perspectives and discussion about resistance tests in EU	39
A. Protocols	39
1. Potential revisions of protocols are proposed to CPVO.....	39
2. Discussion about CPVO protocols	39
B. Reference collections.....	41
1. Providing of R and S control varieties	41
2. Strains.....	43
C. Harmonization of new resistance tests	45
VI. Partners personal comments and opinions.....	46
Appendix	47
Appendix 1: report of the 3rd meeting in Spain, the 19-20th October 2006.....	49
Appendix 2: data of the second ring tests in 2006	127
Appendix 3: updated protocols	145

Table 1: Detailed schedule of the program.

	Year	Date	Who ?	Actions
Phase 1	2004	End of march	Naktuinbouw OEVV	Comments about the draft questionnaire written by GEVES
		Beginning of april	GEVES - Brion	Sending of the final questionnaire to Naktuinbouw and OEVV
		Mid-may	SNES Naktuinbouw OEVV	Sending of the completed questionnaires to GEVES Brion (valerie.cadot@geves.fr)
		Mid-may to mid-june	GEVES - SNES	Analysis of the questionnaire
		June 21 st	GEVES - SNES Naktuinbouw OEVV	1 st meeting in France: results of the questionnaire and preparation of ring tests (exchanges of seeds and hosts, calendar of setting up of tests)
		Dec 15th	GEVES - SNES	1st intermediate report
Phase 2	2004 - 2005	Autumn 2004 and spring 2005	GEVES - SNES Naktuinbouw OEVV	Ring test for the bean and tomato trials
		2005	GEVES - SNES Naktuinbouw OEVV	2 nd meeting in the Netherlands : Results of ring tests
		Dec 15th	GEVES - SNES	2nd intermediate report
Phase 3	2006	January to august	GEVES - SNES Naktuinbouw OEVV	New trials again if problems of reliability
		October	GEVES - SNES Naktuinbouw OEVV	3 rd meeting in Spain : final synthesis
		Dec 15th	GEVES - SNES	Final report

Table 2: Tomato disease resistance tests in 2005: percentage of non-conformity of strains and laboratory protocols.

The best results of non-conformity for strains and laboratories are given in grey in table 2

% non conformity	Strains			Laboratories		
	ES	FR	NL	ES	FR	NL
FOL race 0	27.8	22.2	25	41.7	16.7	16.7
FOL race 1	27.8	0	25	30.6	19.4	2.8
ToMV	24.2	18.2	27.3	30.3	6.0	33.3
Verticillium dahliae	3.7	0	7.4	3.7	0	7.4

I. Introduction

In the present program, three national variety examination offices in France (FR), Spain (ES) and the Netherlands (NL) proposed to evaluate and harmonize a set of disease resistance tests on vegetable crops. Nine host pathogen combinations and two species, tomato and French bean, have been chosen as models:

Tomato :

- *Fusarium oxysporum* f. sp *lycopersici* (Fol) races 0* and 1*
- ToMV race 0*
- *Verticillium dahliae**

French Bean:

- *Colletotricum lindemuthianum* (races delta, gamma, lambda*, race 6)
- *Bean Common Mosaic Virus (BCMV)**
- *Pseudomonas savastanoi* pv *phaseolicola*

*: Compulsory by CPVO

Taking place between december 2003 and december 2006, the program was divided in 3 phases:

- Phase 1: survey of the pathogens and protocols used within the three countries and preparation of the ring test (2004)
- Phase 2: first ring test carried out by each partner in order to characterize and compare the aggressiveness and the virulence of each strain versus standard varieties of each country (2004-2005)
- Phase 3: second ring test ; analysis and comparison of the results, in relation with the CPVO protocols, in order to define reliable standardised procedures (2005-2006).

The detailed schedule of the program is presented in Table 1.

This report presents the results of ring tests obtained after the realization of the 3 phases, which enabled to propose reference standards for cultivars, strains and protocols.

The results presented here were shared and discussed between partners during the third annual meeting of the program which was held in Madrid (Spain), on October 19-20th, 2006 (see Appendix 1: report of the 3rd meeting in Power point format and detailed results of the second ring test in Appendix 2).

For the majority of disease resistance tests, updated protocols (reference controls, reference strains and conditions of tests) are proposed to CPVO (see Appendix 3), and for a few tests, it is still necessary to have one year more tests before concluding about protocols.

This final report indicates also the perspectives about disease resistance tests: establishment of reference collection maintenance and proposals to harmonize new disease resistance tests.

At the end of this report, the partners expressed freely their comments and opinion about the work done at the end of this program.

Table 3. Tomato in 2005: Varieties, strains and protocols with high conformity

A. Varieties

Test	Variety	Reaction
Fol: 0	Marmande verte	S
	Resal	S
	Gourmet	R
	Mohawk	R
	Motelle	R
	Marporum x Marmande verte	RH
	Motelle x Marmande verte	RH
Fol:1	Marmande verte	S
	Cherry Belle	S
	Roma	S (strain F)
	Marporum	S
	Ranco	S
	Walter	R (strain F)
	Odisea	R (strain F)
	Motelle	R (strain F)
	Mohawk	R (strain F)
	Tradiro	R (strain F)
	Motelle x Monalbo	?
	Motelle x Marmande verte	?
ToMV:0	Monalbo	S
	Santa	S (strain F)
	Marmande	S (strain F)
	Dorina	R
	Momor	R
	Gourmet	R
	Mopérou	R
	Monalbo x Momor	RH
Verticillium	Marmande verte	S
	Flix	S
	Clarion	S (strain F and N)
	Clairvil	R
	Marmande VR	R
	Elias	R
	Marmande VR x Marmande verte	RH

B. Strains

Test	French strain	Dutch strain	Spanish strain
Fol:0	OK	OK	more aggressive
Fol:1	OK		
ToMV:0	OK		
Verticillium	OK	OK	

C. Protocols

Test	French protocol	Dutch protocol	Spanish protocol
Fol:0	OK	OK	more aggressive
Fol:1	OK (strain F and S)	OK	OK (strain F and S)
ToMV:0	OK		
Verticillium	OK	OK	OK

II. Background on the first ring test in 2005

Tomato (Tables 2 and 3) :

***Fusarium oxysporum* f. sp *lycopersici* race 0 (Fol 0):**

Globally, there was a good concordance between French and Dutch results, with a weak rate of non-conformity (16.7%). For both countries, there were only 2 varieties, Marmande (Spanish origin) and Ranco (Dutch origin) which were not susceptible. Spain had a higher rate of non-conformity (41.7). The Spanish lab may have inoculated too heavily by submerging the roots for a longer period of time than the French and Dutch labs. This could explain the discrepancy in the results, especially when susceptible cultivars were found resistant.

***Fusarium oxysporum* f. sp *lycopersici* race 1 (Fol 1):**

With the French strain, all the laboratories had 0% of non-conformity and a very good concordance was obtained between laboratories.

Necessity to harmonize notations for Fol 0 and 1:

- Scale of notation: in 2005, SNES and Naktuinbouw gave directly the percentage of diseased plants whereas INIA considered that the number of susceptible plants can be calculated by taking account the following classes:
number of diseased plants=number of dead plants + number of necrotic vessels plants + number of slight necrosis in vessels. So, it was decided to harmonize the scale of notation for the second ring test in 2006.
- Methods of notations: as 2 types of notations were used in 2005, each laboratory agreed to test both methods for Fol 0 and Fol 1 in the second ring test in 2006.

***Verticillium dahliae*:**

There was globally a good correlation between laboratories; the test was reproducible except for a few varieties.

Strain NL did not belong to *Verticillium dahliae* but to *Verticillium albo-atrum* but should give the same results as Ve varieties are resistant to both *Verticillium dahliae* and *Verticillium albo-atrum*.

ToMV race 0:

With 6% of non-conformity, the French strain and protocol showed a better reliability than the strains and protocols of the other countries (SP: 30.3% of non-conformity; NL: 33.3% of non-conformity). This difference was mainly due to the interpretation of the symptoms. INRA Montfavet considered that resistant cultivars have no symptoms or necrosis. Necrosis can occur in resistant heterozygotes when the test is very aggressive.

Table 4: Bean disease resistance tests in 2005: Percentages of non-conformity for strains and protocols

A. Percentage of non-conformity for protocols

Test	French protocol	Dutch protocol	Spanish protocol
Colletotrichum	7	29*	14**
BCMV	0	11	14***
Pseudomonas	?		

* unreadable by France; ** unreadable by Netherlands; ***F strain was unreadable

B. Percentage of non-conformity for strains

Test	French strain	Dutch strain	Spanish strains
Colletotrichum	17	6	13
BCMV	6	6	no strain available
Pseudomonas	?		

Table 5: Bean in 2005: Varieties, strains and protocols with high conformity

A. Varieties

Test	Variety	Reaction
Colletotrichum	Goldrush	S
	Michelet	S
	Masai	S
	Pastoral	R
	Booster	R
BCMV	Dufrix	S
	Flandria	S
	Aneto	S
	Bizet	R
	Booster	R
	Odessa	R
Pseudomonas	Michelet à longue cosse	S
	Masai	R

B. Strains

Test	French strain	Dutch strain	Spanish strain
Colletotrichum	delta & gamma (mixture)	lambda (stability problem)	race 6
BCMV	NL5 OK	NL3 OK	no strain available
Pseudomonas	4 isolates	PRI113	race 6

C. Protocols

Test	French protocol	Dutch protocol	Spanish protocol
Colletotrichum	OK	OK	more aggressive
BCMV	OK (strain F and S)	OK	OK (strain F and S)
Pseudomonas	OK		

Bean (Tables 4 and 5):

***Colletotrichum lindemuthianum* gene Are:**

It was difficult to conclude about test conformity because of some troubles with strains :

- race lambda was unreadable in France,
- race 6 was unreadable in the Netherlands,
- delta and gamma races were not used in mixing in Spain and in the Netherlands.

A problem of stability of lambda was also noticed.

However, each country had good results with their own strain and hosts. A good concordance was also found between the Netherlands and Spain with the Dutch race, but also between Spain and France with the French strains (delta and gamma).

BCMV:

A good correlation was found between all labs for the Dutch strain and between France and the Netherlands for the French strain. Nevertheless, the French strain (NL5) was unreadable by Spain.

A problem of identification of strains was raised for NL3 and NL5: do they belong to BCMNV or BCMV ? This question was answered by a literature study: NL3 and NL5 strains are described as BCMNV.

Psp :

It was difficult to conclude about conformity for two reasons:

- firstly, each country used various strains which can belong to different races;
- secondly, the scale of notation was not harmonized.

The Spanish strain (race 6) was the less aggressive for the 3 countries:

In France, the French strains were the most discriminant ; the Spanish race was not aggressive and the Dutch strain gave light symptoms.

However, whatever races and scales used, Masai was found resistant and Michelet à longue cosse susceptible for the 3 countries.

Preparation of the second ring test:

For the second ring test, it was decided to test again all the strains and cultivars of the first ring test, in order to have more experiments before concluding about conformity. Nevertheless, some cultivars were not tested again, either because they were clearly not in conformity during the first ring test or because seeds were not available, particularly for some CPVO controls.

Table 6: For tomato: planned protocols test for the second ring test in 2006

A. Varieties

Test	Origin	Variety	Expected Reaction
Fol: 0	FR	Marmande verte	S
	NL	Resal	S
	NL	Ranco	S
	ES	Marmande	S
	FR	Motelle	R
	FR	Marporum	R
	NL	Gourmet	R
	NL	Mohawk	R
	ES	Larissa	R
	FR	Marporum x Marmande verte	RH
Fol:1	FR	Marmande verte (F)	S
	FR	Marporum	S
	NL	Cherry Belle	S
	ES	Roma	S
	NL	Ranco	S
	FR	Motelle	R
	NL	Mohawk	R
	NL	Tradiro	R
	ES	Odisea	R
ToMV:0	FR	Monalbo	S
	NL	Santa	S
	ES	Marmande	S
	FR	Momor	R
	NL	Gourmet	R
	ES	Dorina	R
	CPVO	Mop��rou	R
	FR	Monalbo x Momor	RH
Verticillium	FR	Marmande verte	S
	NL	Clarion	S
	ES	Flix	S
	FR	Marmande VR	R
	NL	Tradiro	R
	ES	Elias	R
	CPVO	Clairvil	R
	FR	Monalbo x Marmande verte	RH

B. Strains

Test	French strain	Dutch strain	Spanish strain
Fol:0	Orange 71	PRI20698	Fol 071 (INRA Avignon)
Fol:1	Fol1 4152	PRI 10195N	RAF 70 (INRA Avignon)
ToMV:0	INRA Avignon	MA *	-
Verticillium	Vd Toreilles	Va	Vd:0 Almeria

*ToMV isolate MA later found to react as ToMV pathotype 1

C. Protocols

<u>Test</u>		
Fol	Inoculation	soaking in inoculum suspension for 15 min
	Radicels	cut (FR), not cut (NL, ES)
	Temperature	20-24��C (FR), 24��C (NL), 25-28��C (ES)
	Notation	21 dpi (NL), 25 dpi (FR, ES)
	Record	above cotyledon (FR), >2 cm (NL), >3 cm (ES and FR)
ToMV	Inoculation (rubbing)	cotyledon (FR), first leaf (NL), first and second leaf (ES)
	Temperature	24-26��C
	Notation	12-20 dpi
Verticillium	Record necrosis	mosaic or necrosis or no symptoms
	Inoculum	Czapek Dox or S Messiaen medium
	Inoculation	soaking roots of 10-18 days old plants for 4-10 min
	Density	10 ⁶ spores per ml
	Temperature	18-22��C
	Notation	25-30 dpi: cotyledons fully expanded to first leaf fully developed
	Record	growth retardation and brown vessel

III. Results of the ring tests in 2006 and comparisons with 2005

The activities of the phase III were organized in 6 steps by France and presented during the third meeting in Spain (V. Grimault, V. Cadot):

- 1. Schedule of the ring tests 2006 :** exchange of seeds and strains between partners ; harmonization of notation scale before the beginning of tests with common notation sheets taking into account all kinds of notation,
- 2. Ring tests carried out by FR, NL, ES in 2006 and results sent to FR.**
- 3. Analysis of results and synthesis by FR**
- 4. Presentation of results 2006 and comparison with 2005 (planned and performed tests, results) during the third meeting in Spain.**
- 5. Discussion with partners about the conformity (resistant and susceptible controls, strains, notation scale, conditions of tests),**
- 6. Proposals of modification of CPVO protocols.**

The detailed data of the second ring test are reported in Appendix 2.

A. Tomato Ring test

1. FOL race 0

1.1 Material and methods:

Varieties, strains and protocols used in the ring tests 2006 on tomato for Fol0 are described in the Table 6.

Following the results of 2005, it was decided that during the second ring test in 2006, each laboratory had to compare the 2 methods of notation for Fol0 and Fol1:

- 1) The French notation, used by SNES and by INRA Montfavet: The stem is cut to look for the progression of the fungus in the vessels: if red vessels are seen only below the cotyledons, the cultivar is considered resistant, and if the fungus passes above the cotyledons, the plant is considered as susceptible.
- 2) The Dutch and Spanish notation: only the length of discoloration is recorded. If there is a growth reduction (compared to non-inoculated controls) and if the length of the brown vessel is more than 2 or 3 cm, the plant is considered as susceptible.

Table 7: Results of the second ring test in 2006 on FOL 0 on tomato

			Country											
			FR (above cotyledons)			FR (> 3 cm)			NL (> 2 cm)			ES (> 3 cm)		
			isolate			isolate			isolate			isolate		
Expected result	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	ES	Marmande	S	S	S	S	S	S	S	S	S	S	S	S
S	CPVO, FR	Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S
S	NL	Resal	S	S	S	S	S	S?	S	S	S	S	S	S
S?	NL	Ranco	S (esc)	S	S (esc)	S (esc)	S	S?	R	R?	R	R	S (esc)	S (esc)
R	FR	Marporum x Marmande verte	S (esc)	S (esc)	S (esc)	R?	S (esc)	R?	R	R?	R	R?	S (esc)	S (esc)
R	SP	Larissa	R	R?	R	R	R?	R	R	R	R	R	R?	R?
R	CPVO, FR	Marporum	R?	R?	R?	R	R?	R?	R	R?	R	R	R?	S (esc)
R	CPVO, FR	Motelle	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Gourmet	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Mohawk	R	R	R	R	R	R	R	R	R	R	R	R

S: susceptible; S (esc): Susceptible with escapes; R: resistant; R?: resistant with diseased plants.

: result different from what expected

1.2 Results:

1.2.1 Ring test 2006 (Table 7)

Control varieties:

The best cultivars of this ring test were:

- **Susceptible (S) controls** : Marmande, Marmande verte, Resal.
Ranco was not kept as susceptible control because it did not have a uniform response in all the tests.
- **Resistant (R) controls**: Motelle, Gourmet, Mohawk, Marporum and Larissa
(these last two are susceptible to race 1).
- **Heterozygous R** : Marporum x Marmande verte
This control gave expected results but with some susceptible plants

Marporum and Larissa (both susceptible to race 1), described as resistant control to race 1, had often some susceptible plants in the test. It seems that genotypes resistant only to race 0 and not to race 1 have a weaker resistance to race 0 than genotypes resistant to races 0 and 1.

The heterozygous resistant control had some susceptible plants, which is expected as plants heterozygous for the resistance gene have a weaker resistance than homozygous ones and can show some susceptible plants.

Strains:

All the strains gave expected results, even if the aggressiveness was not the same, with the following range: isolate NL > isolate ES > isolate FR. When test conditions are very severe, the less aggressive strain (FR isolate) would be preferred.

Protocols:

All the protocols gave the expected results. The FR and ES tests seemed to be more drastic than the NL one.

Different conditions were found suitable in different laboratories, so a range of conditions may be valid in the common protocol:

- Inoculation: soaking of roots from 10 to 15 min in inoculum suspension did not affect the results as well as cutting or not the radicels.
- Concentration: in case of drastic test, we observed that inoculum concentration had to be decreased to obtain the expected results.
- Stage of inoculation did not affect the results.
- Temperature: in case of drastic test, we observed that temperature had to be decreased.
- Notation could be done from 21 to 25 days after inoculation, without affecting the results.

Notation scale:

The symptoms were very different according to the laboratories. The comparisons between the both notation methods (above cotyledons and > 3 cm), only performed by France, showed that the notation ">3cm" appeared less drastic than the notation method "above cotyledons". The length of red/brown vessels is not always a sufficient criterion allowing a decision between susceptible or resistant; it was sometimes difficult to record in case of very small susceptible plants where all the plants are smaller than 3 cm.

In France and Spain, a new level of notation was added for dead plants because the test was very drastic. This was not observed in the Netherlands where only in case of growth reduction, stems were cut to look for vessels.

Change of scale, same changes done on the protocol:

Table 8: Results of the second ring test in 2006 on Fol 1 on tomato

			Country											
			FR (above cotyledons)			FR (> 3 cm)			NL (> 2 cm)			ES(> 3 cm)		
			isolate			isolate			isolate			isolate		
Expected result	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	ES	Roma	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S(esc)	S	S	S
S	FR	Marporum	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
S	CPVO, FR	Marmande verte	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
S	NL	Cherry Belle	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
S	NL	Ranco	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
R	ES	Odisea	R	R	R	R	R	R	R	R	R	R	R	R
R	CPVO, FR	Motelle	R	R	R	R	R	R	R	R	R	R	R	R
R	FR	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
R	NL	Mohawk	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Tradiro	R	R	R	R	R	R	R	R	R	R	R	R

S: susceptible; S (esc): Susceptible with escapes; R: resistant; R?: resistant with diseased plants.

: result different from what expected

We agreed about a new notation scale with 4 qualitative classes:

- 0: no symptoms,
- 1: external healthy aspect of plant (without growth reduction) with brown vessels (sometimes extending above cotyledons, generally remaining below cotyledons),
- 2: growth reduction and brown vessels above cotyledons,
- 3: dead plant.

Generally resistance corresponds to classes 0 and 1, and susceptibility corresponds to classes 2 and 3, but analyses of results have to be calibrated with results of R and S controls.

1.2.2 Comparison with 2005:

Control varieties:

The best cultivars of this ring test were:

- R: Gourmet, Mohawk, Motelle
- S: Marmande verte, Resal.

Ranco and Marmande (SP) were not in conformity with expected results.

- Heterozygous R : Marporum x Marmande verte, Motelle x Marmande verte.

We noticed a difference of 20% of diseased plants between the 2 heterozygotes explained by the genotype of the resistant parents (Marporum resistant to race 0 only). So, we decided to keep only Marporum x Marmande verte for the second ring test.

Strains: Strain FR and NL gave expected results. Strain ES was judged too aggressive.

Protocols: FR and NL gave expected results (ES: problem of protocol with resistant cultivars found susceptible).

1.3 Proposals for modification of CPVO protocol: Fol 0 : see chapter 2.3.

2. FOL race 1

2.1 Material and methods

Varieties, strains and protocols used in the ring tests 2006 on tomato for Fol 1 are described in table 6.

2.2 Results

2.2.1 Ring test 2006 (Table 8)

Control varieties:

The best cultivars of this ring test were:

- R: Mohawk, Tradiro, Odisea, Motelle
- S: Marmande verte, Roma, Ranco, Cherry Belle, Marporum

Walter, the resistant control of CPVO, verified resistant in 2005, could not be tested in 2006, due to a lack of available seeds.

Strains: FR strain (the most aggressive) gave the most reproducible results.

Protocol:

- **French protocol gave expected results with FR strain.**
- **Spanish protocol gave also expected results with all the strains.**
- **In the Netherlands, the test was not enough aggressive as many plants escaped infection in susceptible controls.**

The Fol1 test was judged more reproducible than the Fol0 test as no susceptible plants were observed in resistant varieties.

Different conditions adapted to environmental conditions of the tests were defined:

- Inoculation by soaking roots from 10 to 15 min in inoculum suspension did not affect the results as well as cutting or not the radicels.
- Concentration: in case of drastic test, we observed that inoculum concentration had to be decreased to obtain the expected results.
- Temperature: in case of drastic test, we observed that temperature had to be decreased.

Notation scale:

The same problems as for Fol0 were observed. Based on these results, a new notation scale similar to Fol0 one was proposed.

2.2.2 Comparison with 2005 results:

With strain FR, all the laboratories had 0% of non-conformity and they had a very good concordance between them.

Control varieties: all the cultivars were in conformity with the strain FR.

Strains: strain FR gave expected results for the 3 countries.

Protocols:

- with the strain FR: all the labs were in conformity.
- the scale of notation was not harmonized in 2005.

2.3 Proposals for modification of CPVO protocol: FOL0 and 1 on tomato

Based on 2005 and 2006 ring tests, we defined new components for a proposal of updated protocol (see appendix 2):

Control standard varieties:

- New R:

We propose to use one variety resistant to race 1 like Motelle, Mohawk, Gourmet and one resistant only to race 0 like Marporum or Larissa as controls for Fol0:

- For Fol0: Motelle or Gourmet or Mohawk (R to race 0 and 1) and Marporum or Larissa (R to race 0, S to race 1)
- For Fol1: Tradiro or Odisea or Motelle or Mohawk or Ranco.

- New S:
 - For Fol0: Marmande, Marmande verte, Resal
 - For Fol1: Marmande verte, Roma, Cherry Belle, Marporum.
- Heterozygous R:
 - For Fol0: Marporum x Marmande verte
 - For Fol1: Motelle x Marmande verte (optional)

Reference strains:

- For Fol0: FR=Orange 71 or NL= PRI 20698 or ES=Fol 071(INRA of Avignon)
The range of aggressiveness of isolates could be adapted to aggressiveness of test, with the following classification of aggressiveness: PRI 20698 > Fol 071 > Orange 071.
- For Fol1: FR=Fol 1 4152 (the most aggressive).

Protocol:

- Number of plants: as it is indicated in CPVO, and as results are quite heterogeneous for heterozygous resistance, we propose at least 20 plants.
- Temperature: in CPVO protocol, it is indicated between 25 - 28°C; we propose the range 24-28°C, and in case of aggressive test, the temperature can be decreased to 20-24°C.
- Sowing: it is not important to indicate the conditions.
- Inoculation: in CPVO protocol, it is indicated “by cutting”; we propose to make “cutting” optional.
- Notation: in CPVO protocol, it is indicated 20-25 days after inoculation. We propose at least 21 days.
- Notation scale: harmonization of the scale with the proposal of 4 qualitative classes:
 - 0: no symptoms,
 - 1: external healthy plant (without growth reduction) with brown vessels (sometimes extending above cotyledons, generally remaining below cotyledons),
 - 2: growth reduction and brown vessels above cotyledons,
 - 3: dead plant.

Comments:

Generally resistance corresponds to classes 0 and 1, and susceptibility corresponds to classes 2 and 3, but analyses of results have to be calibrated with results of R and S controls.

Table 9: Results of the second ring test in 2006 on ToMV pathotype 0 on tomato

			Country					
			FR		NL		ES	
			isolate		isolate		isolate	
Expected result	Standard	Name	FR	NL	FR	NL	FR	NL
S	ES	Marmande	S	S	S	S	S	S
S	CPVO, FR	Monalbo	S	S	S	S	S	S (esc)
S	NL	Santa	S	S	S	S	S	S
R	CPVO, FR	Moperou	R	R	R	R	R	R
R	NL	Gourmet	R	R	R	R	R	R
R	FR	Momor	R	R	nt	nt	R	R
R	ES	Dorina	R*	R*	R	R*	R	R*
R	CPVO, FR	Monalbo X Momor	R*	R*	R*	R*	R*	?

*R**: symptoms of necrosis that show that variety is probably heterozygous for *R* gene

S: susceptible; S (esc): Susceptible with escapes; R: resistant; R?: resistant with diseased plants.

 : result different from what expected

3. ToMV pathotype 0

3.1 Material and methods

Varieties, strains and protocols used in the ring tests 2006 on tomato for ToMV pathotype 0 are described in table 6.

3.2 Results

3.2.1 Ring test 2006 (Table 9)

Control varieties:

The best standards of this ring test were:

- R: Momor, Monalbo x Momor, Moperou, Gourmet.

Dorina showed necrosis like Monalbo x Momor and is probably an heterozygous for the resistance gene. But as we don't know the genetic constitution of this variety, we did not select it as a standard.

- S: Marmande, Monalbo,

Santa showed very weak symptoms in ToMV pathotype 1 in a former NL test; for this reason, we did not select it as a standard.

Strains :

- The French ToMV strain, INRA Avignon, gave expected results. The FR strain is an Aucuba mosaic strain allowing an easy recording of mosaics.
- The Dutch ToMV strain, PRI MA, gave expected results on controls. This strain was tested on Mobaci, a differential variety with gene Tm1 only, used in French DUS testing. Mobaci was susceptible to this strain, showing that the NL strain is not a ToMV pathotype 0 but perhaps a ToMV pathotype 1. This should have no major consequences on DUS testing as modern varieties all contain the Tm 2-2 gene, but it does not correspond to guidelines which differentiate resistance to ToMV pathotype 0, ToMV pathotype 1 and ToMV pathotype 2. Moreover, this strain gave more faint mosaics than the French one. So this strain was not retained.

Protocols:

- all laboratories had expected results with the French strain (INRA Avignon)
- results were very homogeneous.

No differences of results were shown with the different protocols:

- inoculation can be done from 12 to 22 days after sowing with a reading from 12 to 20 days after inoculation.
- during spring season, the test can be run in greenhouse. Temperatures higher than 25-26°C may break the resistance. the FR strain could give an easier interpretation of results. The Aucuba mosaic strain induces clear, unmistakable mosaic symptoms.

Notation scale:

This year, we decided to record also the necrosis. It allowed us to observe that:

- susceptible cultivars showed 100% mosaic symptoms.
- homozygous resistant varieties had no symptoms.
- heterozygous resistant varieties had no symptom or showed necrosis on some plants. The phenotypic variation for resistance has no genetic basis and therefore ignored in DUS research. The early symptom of necrosis could be confused with mosaics, particularly with the NL PRI MA strain which gave faint green mosaics compared to the FR isolate which gave yellow mosaics. This could generate confusions in analysis of the results and could explain the non conformity of 2005.

So the recording of necrosis as a characteristic of resistance was proposed to be included in the new protocol.

3.2.2 Comparison with 2005:

Control varieties:

The best cultivars of this ring test were:

- R: Momor, Gourmet, Mopérou
- Monalbo x Momor (resistant with necrosis)
- S: Monalbo, Marmande

Strains: FR strain gave expected results.

Protocols:

- The French laboratory gave expected results.
- Dutch and Spanish laboratories had problems of interpretation of symptoms: In the Netherlands, this problem was aggravated by the fact that they did the test at a minimum day temperature of 24°C in the glasshouse while the other labs did it at 25°C. Higher temperatures will increase the amount of necrosis (Weber et al 1993, J. Virol: 6432-6438). In 2006, they have adapted their protocol and increased the glasshouse temperature to 25°C in the ToMV ring test .

➔ Progress in 2006: notation scale was harmonized and strains better identified. Homogeneous results were obtained between laboratories which enable to define a reference protocol.

3.3 Proposals for modification of CPVO protocol: ToMV on tomato

Based on 2005 and 2006 ring tests, we defined new components for a proposal of updated protocol (see appendix 3):

Control standard varieties:

- New S: Marmande or Monalbo
- New R: Momor or Gourmet (R to ToMV 0, 1, 2) and Moperou (R to ToMV 0 and 1) and Mobaci (R to ToMV 0 and 2 which could help to control isolate identity)
- Heterozygous R: Monalbo x Momor

We propose to add an heterozygous resistant control in this protocol like for French DUS tests, as many varieties are heterozygous for Tm2-2 and could give necrotic symptoms which do not correspond to symptoms observed only with susceptible and homozygous resistant controls.

In the set of controls, it would be also useful to include a differential for controlling the identity of the race, like Mobaci (S to ToMV 0, R to ToMV 1).

Reference strains:

ToMV 0 INRA Avignon (aucuba mosaic isolate)

Correct denomination of the tobamovirus strains: ToMV 0

It is advisable to stress that the only acronym recognized by the International Committee on Taxonomy of Viruses (ICTV) for Tomato Mosaic Virus is ToMV and not TMV. Since Ohno et al (1984) sequenced ToMV, this virus is recognized as a species that is distinct from TMV by ICTV. TMV is seldom found in tomato and poses any threat, while ToMV is an important pathogen. Before 1984, both viruses were commonly called TMV. Strains have been classified by Pelham (1972) into four pathotypes (0, 1, 2 and 1-2) on the basis of their differential reaction to specific resistance genes. Pathotype 1-2 is not relevant for DUS research.

Protocol:

- Number of plants: 15 to 30 are defined in CPVO protocol. As results were homogeneous, we propose at least 20 plants.
- Stage of inoculation: 12-14 days are defined in CPVO protocol; we propose cotyledons (first leaves emerging) to 2 expanded leaves.
- Temperature: 30-35°C are defined in CPVO protocol. As resistance can break at high temperatures, we propose 24-26°C.
- Light is important for symptom expression
- Notation: 10-12 days after inoculation are defined in CPVO protocol. As it is a too short time for symptom expression, we propose 12-21 days.
- Notation scale:
 - Resistance: no symptoms or necrosis on a variable proportion of plants heterozygous for resistance gene.
 - Susceptible: mosaics.
- We propose to cancel ToMV 1-2 as it does not exist
- We propose to delete asterisk for ToMV 1 and ToMV 2 in CPVO protocol because all the modern resistant cultivars have the Tm2² gene which can be checked by ToMV 0 strain..

Table 10: Results of the second ring test in 2006 on *Verticillium dahliae/albo-atrum* on tomato

			Country								
			FR			NL			ES		
			isolate			isolate			isolate		
Expected result	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	CPVO, FR	Marmande verte	S (esc)	S (esc)	S (esc)	S	S	S	S	S	S
S	NL	Clarion	S	S (esc)	S	S (esc)	S	S	S	S	S (esc)
S	ES	Flix	S	S (esc)	S (esc)	S	S	S	S	S	S (esc)
R	FR	Monalbo	R	R	R?	R	R	S	R	R?	S
R	FR	Monalbo x Marm verte	R	R	S (esc)	R	R	S	R	R?	R?
R	NL	Tradiro	R	R	R?	R	R	S	R	R?	R?
R	CPVO	Clairvil	nt	nt	nt	nt	nt	nt	nt	nt	nt
R	ES	Elias	R	R	R?	R	R	S	R	R	R?

S: susceptible; S (esc): Susceptible with escapes; R: resistant; R?: resistant with diseased plants.

: result different from what expected

4. *Verticillium dahliae* race 0

4.1 Material and methods

Varieties, strains and protocols used in the ring tests 2006 on tomato for *Verticillium dahliae* are described in the table 6.

The Netherlands have been using *Verticillium albo-atrum* race 0 instead of *V. dahliae* race 0, but non resistance breaking race 0 is defined across these two species.

4.2 Results

4.2.1 Ring test 2006 (Table 10)

Control varieties:

The best cultivars of this ring test were:

- S: Marmande verte, Flix.
- Clarion, less susceptible, could help for monitoring test aggressiveness.
- R: Monalbo, Tradiro, Elias.
- Heterozygous R: Monalbo x Marmande verte.

Heterozygous controls are interesting to include for helping interpretation of results, and they could be optional in case of aggressive test.

Strains

- FR and NL strains provided expected results but FR strain gave the most reproducible results. With the NL strain, many escapes were observed in France (strain less aggressive?). Even if *V albo atrum* strain was efficiently used for years in NL DUS testing, we do not recommend it because it is not a *V dahliae* strain.
- In contrast with 2005, ES strain did not provide expected results on resistant plants in the three laboratories (more disease). With the ES strain, the resistance was broken. A mutation on the Spanish Vd could explain the resistance breaking, with two hypothesis:
 - this mutation may have caused a loss of virulence. The ES strain may correspond to race 1 instead of race 0.
 - the resistance may also be broken by increasing general aggressiveness.

Protocols:

- **Notation scale:** the scale with symptoms/without symptoms gave expected results.
- **Test:** with FR strain, results were reproducible in all the laboratories.

4.2.2 Comparison with 2005

In 2005, a good correlation was globally found between laboratories. Except for a few varieties, the test was reproducible. The Dutch laboratory used *V. albo-atrum*.

Control varieties:

The best cultivars of this ring test were:

- R: Clairvil, Marmande VR, Elias.
Tradiro did not give expected results in Spain with NL strain.
The heterozygous Marmande VR x Marmande verte was as resistant as homozygous resistant controls, except with the strain NL in France.
- S: Marmande verte, Flix.
Clarion showed less diseased plants than Marmande verte and Flix.

Strains : In 2005, strains ES and FR gave the best results.

Protocols: all the protocols gave expected results but it is necessary to harmonize scale of notation.

➔ **Progress 2006: improvement of the notation scale and confirmation of susceptible and some resistant controls** (Elias and Tradiro).

4.3 Proposals for modification of CPVO protocol: *Verticillium dahliae* on tomato

Based on 2005 and 2006 ring tests, we defined new components for a proposal of updated protocol (see appendix 3):

Control standard varieties:

- New R: Monalbo or Elias,
- New S: Marmande Verte or Flix.

Clarion could be interesting to add because it is less susceptible and could help to control the aggressiveness of the test

- Heterozygous R: Monalbo x Marmande verte

The heterozygous control is interesting to include to help interpretation of results. It could be optional in case of aggressive test.

Reference strains:

- FR strain : *V. dahliae* Toreilles.

Protocol:

- Number of plants: 10 to 20 plants are defined in CPVO protocol; as results were quite homogeneous, we advice at least 20 plants.
- Sowing to inoculation: 15 to 20 days are defined in CPVO protocol; we propose 10-20 days (cotyledons to first leaf stage).

- **Notation scale:** a new description of symptoms for susceptible plants is proposed :
 - R: no symptoms
 - S: chlorosis in the lower leaves, growth reduced and brown vessels or growth not reduced and brown vessels. The analyses of results should be calibrated with results on resistant and susceptible controls.

-

General conclusion on tomato tests: See table 15.for reference strain and table 16 for reference controls.

Table 11: For bean, planned protocols for the second ring test in 2006

Bean	Controls		Strains	Protocols
	Susceptible	Resistant		
<i>Colletotricum lindemuthianum</i> (gene <i>Are</i>)	<ul style="list-style-type: none"> • Goldrush (ES), • Michelet à longue cosse, (FR) • Tuf (CPVO), • Masai (NL) 	<ul style="list-style-type: none"> • Pastoral (ES), • Booster (FR), • Talisman (NL) 	<ul style="list-style-type: none"> • FR : Delta and Gamma • NL: 418. Described as Lambda. • ES: race 6 (binary code) • Ref CPVO: Lambda 	<ul style="list-style-type: none"> • 2 methods of inoculation: <ul style="list-style-type: none"> - by soaking seeds - by spraying cotyledons • Plants: 30 • T°: 20°C in F et NL, 20-22°C in SP • Notation: 7 to 15 days after inoculation • Symptoms recorded: necrosis, dying plants
BCMV	<ul style="list-style-type: none"> • Dufrix (FR), • Flandria (NL), • Aneto (ES and NL) 	<ul style="list-style-type: none"> • Bizet (NL), • Booster (FR), • Odessa (CPVO) <p>Rapier (CPVO control) was cancelled after 2005 ring test</p>	<ul style="list-style-type: none"> • NI 3 from NL : BCMNV, PG-6 • NI5 from FR: BCMNV, PG-6 • Ref CPVO: BCMV, NI3 or PG-6 	<p>Inoculation at first leaf stage in all labs</p> <ul style="list-style-type: none"> • Plants: 30 • T°: 30°C followed by 25°C in F and only for NL5 in SP; 25°C in NL and SP; • Notation: 13 to 21 days after inoculation • Symptoms recorded: mosaics, local necrosis, top necrosis
<i>Pseudomonas savastanoi</i>	<ul style="list-style-type: none"> • Michelet à longue cosse (FR) • Astun (ES) • Slenderette (NL) 	<ul style="list-style-type: none"> • Masai (ES, FR, NL) 	<ul style="list-style-type: none"> • 7722 from FR (race 6) in 2006 instead of a mixing of strains in 2005 (whose race 6). • HRI 1449B from ES: race 7 in 2006 instead of race 6 in 2005 which were not virulent • PRI 113 from NL (race to be determined) • Ref CPVO: US race 1 (can be now race 1,5,7 or 9) or US race 2 (can be now race 2,6 or 8) 	<ul style="list-style-type: none"> • Inoculation: in field in F, a small test in Greenhouse in F, in growth chamber in ES, tests under progress in F and NL in greenhouse. At cotyledon + 1st leaf in F, at fist leaf in ES and NL • Inoculum: 108cfu/ml • Plants: 30 to 60 plants • T°: greenhouse: no summer trial possible • Notation: 2 to 3 weeks after inoculation • Symptoms recorded: water soaked lesion, halo

B. French bean ring test

1. *Colletotrichum lindemuthianum* race lambda (race 55), delta (race 23) and gamma (race 102) in mixing, race 6

There is a traditional designation of races (lambda, delta...) and a binary designation with numbers (according to reactions in the differential cultivars, Pastor Corrales, 1991)".

Race 6 (Ferreira and al, 1998) is the binary designation, thus it does not have a traditional designation like the other races used in this project, as lambda (race 55), gamma (race 102), and delta (race 23).

1.1 Material and methods:

Varieties, strains and protocols used in the ring tests 2006 on bean for *Colletotrichum lindemuthianum* are described in the table 11.

1.2 Results

1.2.1 Ring test 2006 (Table 12)

Control varieties:

The best cultivars of this ring test were:

- R: Booster: with a high level of resistance
- R: Pastoral, Talisman: with a weaker resistance
- S: Goldrush, Michelet, Tuf, Masai
-

Strains :

Different races have been used by the three laboratories but their common aim is to test the presence of gene "Are".

- Lambda (race 55), isolate number 418, gave expected results but this strain showed many problems:
 - a lower aggressiveness than other strains leading to the presence of many escapes.
 - a low sporulation in the Netherlands.
 - a lack of stability in its identification as lambda race (race 55) , and possibly a virulence spectrum which is not expected for race lambda (race 55).
- Delta (race 23) + Gamma (race 102) in mixing gave expected results (Delta (race 23) did not sporulate in ES). But as delta (race 23) and gamma (race 102) are used in mixing, the population of spores may be different for each race included in the mixing. So, even if the mixing was efficient for years in FR DUS testings, we do not recommend it.
- Race 6 gave also expected results. As it was more aggressive than delta (race 23) and gamma (race 102) in mixing, Spain found some susceptible plants on weak resistant cultivars, as Talisman and Pastoral.

Protocol:

- Tests were in conformity in France and in Spain and after a second test in the Netherlands, except with Lambda strain (race 55). The problem of a low disease pressure in the Netherlands test has been solved.
- Inoculation method: the both methods, by spraying cotyledons or by soaking seeds were tested only in FR and gave equivalent results.
- Light: the both conditions, 12L 12D (F) or 16L 8D (SP and NL) did not affect results.

Table 12: Results of the second ring test in 2006 on *Colletotrichum lindemuthianum* on bean

			Country											
			Spraying cotyledons			Inoculation by soaking seeds								
			F			F			NL			SP		
			isolate			isolate			isolate			isolate		
Expected result	Standard	Name	F	NL	SP	F	NL	SP	F	NL	SP	F	NL	SP
S	SP	Goldrush	S	S?	S	S	S	S	S (esc)	R	S	S	S	S
S	F	Michelet	S	S?	S	S	nt	S	S (esc)	R	S	S	S	S
S	CPVO	Tuf	S	S?	S	S	S (esc)	S	S (esc)	R	S (esc)	S	S (esc)	S
S	NL	Masai	S	R	S	S	R	S	S (esc)	R	S (esc)	S	S (esc)	S
R	SP	Pastoral	R	R	R	R	R	R	R	R	R	R	R	R?
R	F	Booster	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Talisman	R	R	R	R	R	R	R	R	R	R?	R	S?

S: susceptible; S (esc): Susceptible with escapes; R: resistant; R?: resistant with diseased plants.

: result different from what expected

Notation scale:

- The quantitative notation for soaking seeds was validated, but could be grouped as qualitative notation for S and R by grouping classes
 - for S: no symptoms and weak reaction with small superficial necrosis,
 - for R: deeply sunken necrotic flecks and dying plants.
- The method by spraying cotyledons presents the advantage to have a qualitative notation, with 2 classes:
 - for R: no symptoms,
 - for S: necrosis.

1.2.2 Comparison with 2005 results:**Control varieties:**

The best cultivars of this ring test were:

- R: Booster and Pastoral
Talisman did not give expected resistance in Spain with ES and NL strains.
- S: Goldrush, Masai, Michelet
Tuf did not give expected results with ES strain in France.

Strains : no conclusions were given in 2005 because

- strain ES was unreadable by the Netherlands and strain NL was unreadable by France
- Strain FR was not tested in mixing by Spain and by the Netherlands.

➔ Progress on isolates in 2006: fewer problems occurred on R and S controls, and problems with low inoculum pressure were identified.

1.3 Proposals for modification of CPVO protocol: *Colletotrichum lindemuthianum* on bean

Based on 2005 and 2006 ring tests, we defined new components for a proposal of updated protocol (see appendix 3):

Control standard varieties:

- New R: Booster;
Pastoral can be added as it has a weaker resistance and can give an indication on aggressiveness of the test.
- New S: Goldrush, Michelet, Masai

References:

- Balardin R.S., Jarosz A.M., Kelly J.D. 1997. Virulence and molecular diversity in *Colletotrichum lindemuthianum* from South, Central, and North America. *Phytopathology*, 87 n°12 : 1184-1191.
- Ferreira .J., Fueyo M.A., González A.J. and Giraldez R.. 1998. Pathogenic variability within *Colletotrichum lindemuthianum* in Northern Spain. *Annual Report of Dean Improvement Cooperative* 41: 163-164
- Pastor Corrales M.A., 1991. Estandarizacion de variedades diferenciales y de designacion de razas de *Colletotrichum lindemuthianum*. *Phytopathology* 81:694.

Reference strains :

- Race 6 (binary code) would be preferred to race lambda (race 55) as it is more aggressive and stable than lambda (race 55). Even if both races detect the Are gene, race 6 detects also a few more resistance genes. Thus, would varieties without Are genes need to be retested?

Protocol:

- Number of plants: at least 20 plants.
- Method of inoculation: we propose to keep the soaking seeds method and to add the method by “spraying cotyledons”. France, which tests the both methods, found that SNES method is more discriminatory for the expression of symptoms (no symptoms for resistant cultivars) and it is also faster (inoculation and duration of the test).
- Notations should be done when symptoms are well developed on susceptible controls (usually after 7 dpi to 14 dpi).
- It is important to mention that the humidity is an essential factor for symptoms development.
- Notation scale: a qualitative scale is proposed :

For soaking seeds:

R: classes 0: no symptoms and 1: superficial lesions

S: classes 2: deeply sunken lesions and 3: plant death

For spraying cotyledons:

R: no symptoms, some flecks of necrosis can occur in the stem and some necrosis in the cotyledons.

S: deep necrosis observed on plants

Proposal to modify the denomination of races:

We propose to use the new binary code for races denomination described in the ISF differentials, based on Balardin et al 1997 (Phytopathology, 87(12)) and to keep in brackets the old name given in the current CPVO protocols.

2. **BCMV (Bean Common Mosaic Virus) or BCMNV (Bean Common Mosaic Necrotic Virus)**

2.1 Material and methods

Varieties, strains and protocols used in the ring tests 2006 on bean for **BCMNV** are described in the table 11.

Virus species and strain identification:

Bean common mosaic (BCM) symptoms may be caused by two distinct virus species (BCMNV and BCMV) corresponding with serotype A (BCMNV) and B (BCMV). These two viruses have been classified into seven pathogenicity groups based on their virulence pattern on a differential set of 11 varieties. All strains of BCMNV may induce a temperature insensitive hypersensitive necrosis response, in opposition to BCMV which may induce temperature sensitive necrosis response, in case of strains belonging to pathogenicity groups IV and V. Higher temperatures (26-32°C) generally enhance necrosis and mosaic symptom expression compared with lower temperatures (20-25°C). The former CPVO described first week after inoculation at 30°C to enhance necrosis but then decreased to 25°C (better for mosaic).

Pathogenicity group VI comprises the BCMNV strains NL3 and NL5. NL3 and NL5 have the ability to induce necrosis on bean varieties with gene I. The extent of necrosis may vary from local vein necrosis to top necrosis or in extreme cases whole plant necrosis (commonly called blackroot).

Genetic background of resistance:

One dominant and several recessive resistance genes have been described:

- The dominant gene I is responsible for the necrotic response to specific virus strains and symptomless to other strains.
- Several recessive bc genes may cause resistance and in particular, an absence of symptoms: these are divided over three loci (bc-1, bc-2 and bc-3) and are dependant on the presence of still another recessive gene (bc-u). The loci bc-1 and bc-2 may be occupied by two distinct alleles of the resistance. These bc genes need to be combined with one or more other bc genes for being effective. The presence of bc genes or gene combinations may suppress the necrotic response of the I gene partially or completely. In that case the I gene is said to be "protected" by the action of the bc gene or genes.

Table 13: Results of the second ring test in 2006 on BCMV/BCMNV on bean

			Country					
			FR		NL		ES	
			isolate		isolate		isolate	
Expected result	Standard	Name	FR	NL	FR	NL	FR	NL
S	ES	Aneto (SP)	R?	S	S	S	S	S
S	NL	Aneto (NL)	S	S	--	--	?	?
S	FR	Dufrix	S	S	S	S	S	S
S	NL	Flandria	S	S	S	S	S	S
R	FR	Booster	R (top necrosis)	R	R	R	R	R
R	CPVO	Odessa	R (top necrosis)	R	R	R	R	R
R	NL	Bizet	R (no symptoms)	R	R	R	R	R

S: susceptible; S (esc): Susceptible with escapes; R: resistant; R?: resistant with diseased plants.

: result different from what expected

2.2 Results

2.2.1 Ring test 2006 (Table 13)

Control varieties:

The best cultivars of this ring test were:

- R:
 - (1) Booster, Odessa : with top necrosis or vein necrosis
 - (2) Bizet: no symptoms.
 Bizet never gives top necrosis, it could be a protected I variety.
 According to their genetic, resistant controls can have these two types of behaviour;
- S: Dufrix, Flandria

Aneto did not give expected results, with too weak symptoms, generally chlorosis and no mosaics.

Strains:

- NL5 was homogeneous in all labs, but needed a 30°C period in Spain to obtain top necrosis. A new result in France showed that top necrosis can occur at 25°C without a 30°C period on Booster.
- NL3 was not homogeneous for top necrosis in the different laboratories. A new result in France showed no top necrosis at 25°C without a 30°C period on Booster, but late vein necrosis.
- These two strains belong to PG -6 of BCMNV and not to BCMV.
- The fact that NL5 appears more uniform for top necrosis than NL 3 may be explained by genetics:
 With NL5, top necrosis occurs on cultivars with bc1 or bc1² genes whereas with NL3, top necrosis occurs only on cultivars with bc1 genes. With bc1² genes, NL3 provokes local and intervein necrosis which can also be found on susceptible cultivars.

Protocols:

- Expected and very homogeneous results were found in all labs (no escapes, no S plants in R controls).

Notation scale:

- Mosaics: only in S controls
- 1: no symptoms. 2: top necrosis, only in R controls
- Local necrosis: in R or S controls: could be due to interpretation of local necrosis
 - For R: vein necrosis and sometimes spots on leaves
 - For S: mosaic, sometimes intervein necrosis can appear with mosaics
- Proposed notation scale:
 - For R: 1: top necrosis and/or vein necrosis; 2: no symptoms
 - For S: mosaics, intervein necrosis can occur, leaf deformation

2.2.2 Comparison with 2005 results:

There was an excellent correlation between France and The Netherlands for the two strains tested except for Rapier for the Dutch laboratory (predicted susceptible by CPVO, and found resistant). After the meeting, Spain provided results: for the Dutch strain, there was also a good correlation between all labs except for Aneto for the Spanish laboratory; with the French strain,

the Spanish laboratory did not find any symptoms. Booster and Odessa were also found resistant with top necrosis, and Bizet without symptoms.

➔ **Comparable results were found in 2005 and 2006.**

2.3 Proposals for modification of CPVO protocol: BCMV/BCMNV on bean

Based on 2005 and 2006 ring tests, we defined new components for a proposal of updated protocol (see appendix 3):

Control standard varieties:

- R control are defined into two classes like in current CPVO protocol:
 - 1) with top necrosis
 - 2) without symptoms
- New R: Booster or Odessa (top necrosis) and Bizet (no top necrosis)
- New S: Dufrix, Flandria
We propose to cancel Rapier (2005) and we do not advice Aneto, which is not enough uniformly susceptible.

Reference strains :

- PG-6 represented by NL3 or NL5.
- We should notice that France uses NL5 and the Netherlands uses NL3 which is strictly speaking BCMNV according to the latest development in virology. But we will have to verify the identity of the 2 strains in 2007. These two isolates may differ markedly in their ability to induce necrosis, although both isolates break the same resistance genes.
- In CPVO protocol, we propose to change BCMV in BCMNV because BCMV was split in BCMV and BCMNV and that NL3 and NL5 belong to BCMNV (independent of temperature for top necrosis) and not to BCMV (dependent on high temperature for developing top necrosis).

Protocol:

- Number of plants: at least 20 plants are defined in CPVO protocol. As results were very homogeneous, we propose the same: at least 20 (general requirements of UPOV)
- Temperature: 25°C, with an optional period at 30°C
- Notation: 6 days after inoculation are defined in CPVO protocol. We propose to make the notation when mosaic symptoms are well developed on S control: usually after 13-21 days), with one or more notations.
- We confirm 3 notations like in current CPVO protocol:
 - 1: S : mosaics, intervein necrosis can occur, leaf deformation
 - 2: R with top necrosis, vein necrosis and/or small necrotic lesions in the leaf.
 - 3: R without symptoms
- We propose to change black root in top necrosis. The term 'blackroot', that is used in the CPVO protocol for the symptom described as top necrosis, is misleading because it suggests that only extremely severe necrosis falls into this category, whereas in reality the extent of necrosis may vary considerably.

3. *Pseudomonas savastanoi* pv. *phaseolicola*

3.1 Material and methods

Varieties, strains and protocols used in the ring tests 2006 on tomato for *Pseudomonas savastanoi* pv. *phaseolicola* are described in the table 11.

3.2 Results

3.2.1 Ring test 2006 (Table 14)

Control varieties:

The best cultivars of this ring test were:

- R: Masai
- S: Michelet à longue cosse,
Slenderette and Astun did not give uniform results in France and in the Netherlands.

Strains :

- Differentials were acquired in France to identify 7722 as race 6 (to be confirmed); the Netherlands also plans to identify PRI 113. Multiplications are under progress to be able to verify our strains.
- FR 7722 (race 6) and NL PRI 113 (race not yet identified) gave expected results.
- ES HRI 1449B (race 7 obtained from HRI) was judged less aggressive than the FR and NL strains.

Protocol:

- The test in field, performed by France, was in conformity in 2005 and 2006; but the reproducibility in different countries was not tested. In France, tests were performed on leaves and on pods because pods have a polygenic resistance and no genetic linkage exists between leaf and pod reaction.
- Tests in greenhouse, performed by the Netherlands and France, need confirmation.
- The test in growth chamber, performed by Spain, seems more reproducible than in greenhouse to control environmental parameters (light, humidity, temperature), but need also confirmation.

Notation scale: The new scale seems accurate but in France in greenhouse, on susceptible controls, no water soaked lesions were observed but only chlorotic halos and the size of the lesion was not a criteria. In NL the size of water soaked area was used to make the classes but could be confusing.

3.2.2 Comparison with 2005 results:

Control varieties:

The best cultivars of this ring test were:

- R: Masai
- S: Michelet à longue cosse.
Astun and Slenderette were not uniformly susceptible.

Table 14: Results of the second ring test in 2006 on *Pseudomonas savastanoi* pv. *phaseolicola* on bean

			Field			Greenhouse								
			Country											
			FR			FR			NL			ES		
			isolate			isolate			isolate			isolate		
Expected	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	ES	Astun	S(esc)	S(esc)	R	R	S	S	S	S	S(esc)	S	S	S
S	FR	Michelet	S	S	R	S	S	S	S	S	S	S(esc)	S	S
S	NL	Slenderette	S	S	R	S	S	S	S	S	S	S	S	S
R	FR	Masai (F)	R	R	R	R	R	R	R	R	R	R	R	R
R	ES	Masai (SP)	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Masai (NL)	R	R	R	R	R	R	R	R	R	R?	R?	R?
R	FR	Vaillant	R	R	R	R	R	R	R	R	R	nt	nt	nt

S: susceptible; S (esc): Susceptible with escapes; R: resistant; R?: resistant with diseased plants.

In green : results obtained after the 3rd meeting

: result different from what expected

Strains :

- ES strain (race 6) was not aggressive in field in France.
- As countries did not use the same races, it was not possible to retain one strain:
 - in Spain: race 6 (Sp, SITA 689, from Valladolid),
 - in France: mixing of 4 strains (with race 6),
 - in the Netherlands: NL strain, not identified.
- Bibliography indicates the predominance of the race 6 in EU. The question was which race to use: race 2 US (including new races 2, 6 and 8) predicted by CPVO?

Protocols:**In field:**

- In France, the test in field was in conformity on leaves and on pods but the inconvenient is that races were not controlled (mixing). Masai was resistant on leaves but also on pods and Michelet à longue cosse appeared as the most susceptible cultivar on pods. The French protocol gave better results with the French strain than with the others. Nevertheless, external races or strains may contaminate a test in the field. For that reason, in 2006, SNES decided to test both in the field and in greenhouse.

Protocol in greenhouse or in climatic chamber:

- In greenhouse or climatic chamber, the strain or race inoculated can be controlled. But according to the Dutch laboratory, the protocol is not always robust in greenhouse, as it was clearly demonstrated by the absence of symptoms for their second test. They believe that it was not due to a loss of virulence. It became apparent that inoculation in the glasshouse may fail due to high temperature. The failure of one experiment in the Netherlands was most likely due to some sunshine on the day of inoculation.
- In climatic chamber, Spain had good results both years and had race 6 characterized that was supplied by HRI.

➔ Progress were performed in 2006 on race identification, on understanding of race predominance, on notation scale, but we still need confirmation about reproducibility in greenhouse. In the field in France, the protocol gives a good reproducibility but it was not tested in other countries and strains cannot be controlled. As it was a too complicated model for a 2 years ring-test, it is necessary to carry on more experimentation for one year.

3.3 Proposals for modification of CPVO protocol: *Pseudomonas savastanoi* pv. *phaseolicola* on bean

Based on 2005 and 2006 ring tests, we defined new components for a proposal of updated protocol (see appendix 3):

Control standard varieties:

- New R: Masai and Vaillant
The origin of resistance for Vaillant is PI 50414 ; this resistance is effective to all known races. It seems a little bit less uniform in its reaction than Masai resistance.
- New S: Michelet à longue cosse
- Slenderette and Astun gave not an homogeneous response in 2005 and in 2006.

Reference strains :

- **Which race or strain to choose for the future?**

CPVO proposes two US races: race 1 and race 2 but this races had been split in several new races :

- the old race 1 includes the new races 5, 7 and 9.
- the old race 2 includes the new races 6 and 8.
- the old race 3 includes the new race 4.

These nine races were identified by Taylor et al (1996a). So the races defined in CPVO protocol have to be updated.

As the race 6 is the most frequent in Europe, it could be preferred as a new choice. Moreover, the race 6 is interesting because it breaks resistances of the 8 differential hosts defined by Taylor et al (1996a), and notably the resistance of *P. acutifolius* 1072 and ZAA12 (A43), but not the one of Vaillant.

- **Race specific resistance or not?** The group thinks that it is not important to have a race specific test but it is essential to have the same race tested to have an uniform grouping of varieties. It seems that we do not test for race specific genes, so the identity of the race would not be important. But for harmonization and to be sure that depending of the race used, a variety could not be classified in different groups, it would be better to propose only one race.

Protocol: to be set up

- Number of plants: at least 20 plants
- Temperature: in greenhouse, it is necessary to do the test in spring or autumn conditions to avoid high temperature.
- Humidity: high humidity is required.
- Notation: It is important to define DI (disease index), the analyses of results are on progress but it is too early to define something.

3.3 Follow up Psp on bean

- Confirm race identification:
 - isolates selected: 7722 (FR), new race 6 (ES, HRI 1299A supplied and characterized by Taylor), PRI 113 (NL)
- Test the 3 isolates on differentials defined by Taylor
- Select 1 to 3 race 6 isolates for ring test
- Ring test: Masai, Michelet, Slenderette, Red Mexican, UI3, Vaillant, 3-4 registered resistant varieties to define

References:

J.D. Taylor, D.M.: Teverson, D.J. Allen, and M.A. Pastor Corrales (1996a) Identification and origin of races of *Pseudomonas syringae* pv. *phaseolicola* from Africa and other bean growing areas. Plant Pathology 45: 469-478.

J.D. Taylor, D.M.: Teverson and J.H.C. Davis (1996b) Sources of resistance to *Pseudomonas syringae* pv. *phaseolicola* in *Phaseolus vulgaris*. Plant Pathology 45: 479-485.

IV. Conclusion

A synthesis about reference strains and control standards varieties are summarized in the tables 15 and 16 respectively.

- ♦ This project allowed to:
 - define isolates
 - define R and S controls
 - define notation scales and classes of resistance which would not be identified by markers
 - Propose updated robust protocols validated in different laboratories
- ♦ A lot of questions arised from this project:
 - Isolate identification
 - Varieties availability
 - Differentials identification and availability
- ♦ Many subjects remains to discover on other protocols not yet harmonized.

V. Perspectives and discussion about resistance tests in EU

A. Protocols

1. Potential revisions of protocols are proposed to CPVO: see Appendix 3
2. Discussion about CPVO protocols

Proposals of modifications for CPVO protocols: compulsory characteristic or not?

- We propose to keep compulsory based on our technical results (repeatable enough to use this character for DUS) the following diseases:
 - Fol 0
 - Fol 1
 - Ve
 - ToMV 0
 - Cl: with 1 more year to confirm the race 6. and notably their identification.
 - BCMNV: with 1 year more to confirm isolates to be used and notably their identification.
- We question about the possibility to add a new compulsory for Psp: 1 more year for harmonization (and more than one test a year) is necessary to give an advice
- We propose to cancel compulsory characteristic for ToMV 1 and ToMV 2 because all resistant cultivars have the Tm2² gene which can be checked with ToMV 0 strain.

We propose to cancel the characteristic ToMV 1-2 because this strain does not exist naturally.

Table 15: Strains validated after 2005 and 2006 ring tests

Species	Pathogen	Isolate	Comments
Bean	BCMN	<ul style="list-style-type: none"> NL5 NL3 	To be verified on differentials and for temperature
	Psp	<ul style="list-style-type: none"> 7722 (FR race 6) PRI 113 new race 6 (ES) 	To be verified on differentials, select an aggressive race 6 isolate
	CI	<ul style="list-style-type: none"> race 6 	
Tomato	Fol 0	<ul style="list-style-type: none"> Orange 71 PRI 20698 Fol 071 	Aggressiveness: PRI 20698> Fol 071 > Orange 071. Range of aggressiveness of isolates could be adapted to aggressiveness of test
	Fol 1	<ul style="list-style-type: none"> Fol 1 FR 	
	Vd and Va	<ul style="list-style-type: none"> Toreilles 	
	ToMV	<ul style="list-style-type: none"> I NRA Avignon 	

Table 16: R and S controls validated after 2005 and 2006 ring tests

Species	Pathogen	Resistant control	Heterozygous Resistant control	Susceptible control
Bean	BCMN	Top necrosis:Booster, Odessa No symptoms: Bizet		Dufrix Flandria
	Psp	Masai		Michelet à longue cosse
	CI	Booster Pastoral optional (less resistant)		Goldrush Michelet Masai
Tomato	Fol 0	Motelle Gourmet Mowhawk Marporum Larissa	Marporum x Marmande verte	Marmande Marmande verte Resal
	Fol 1	Mohawk Odisea Tradiro Motelle	Motelle x Marmande verte (optional)	Marmande verte Roma Cherry Belle Marporum Ranco
	Vd and Va	Monalbo Tradiro Elias		Marmande verte Flix
	ToMV	R to 0,1,2: Momor or Gourmet R to 0,1: Moperou R to 0 and 2 : Mobaci	Momor x Monalbo	Marmande Monalbo

General questions about CPVO protocols :

Strains/isolates :

- It would be necessary to use the correct terminology in protocols for the following terms: species, pathotypes, races, strains/isolates which can mean the same thing and need a reference number.
- It would be sometime necessary to allow more than one possible strain, according to the aggressiveness of the protocol, if this one is identified and gives the same results on controls.
- Identity control of isolates on differentials is necessary to verify race or pathotype identification. CPVO protocol could be improved by adding indications about the reference laboratories in charge of differentials and strains.

Control varieties:

- It would be sometime necessary to allow more than one possible variety depending of the protocols, if they give the same results
- It would be an improvement in CPVO protocol to add indications about the references of available control varieties

Protocols :

- A question arised about the obligation to apply the protocol or the possibility to propose a guideline, with more flexibility. The use of only one protocol without range indication where needed is not possible because a protocol is dependant of laboratory conditions and strain choice; thus we suggest to write protocols with some flexibility at different steps.
- We advice to add one or several contact laboratory.

We suggest to validate in priority the protocols which are robust in different laboratories conditions. Indeed, a protocol working well in one laboratory may not be adapted to another.

A "resistance testing" group at UPOV or CPVO: We propose the creation of a resistance testing group at UPOV or CPVO to discuss about the validation of protocols, with one pathologist and one person in charge of DUS tests by country.

B. Reference collections

In this programme, we encountered difficulties to obtain seeds of the reference varieties described in the CPVO protocols. R and S controls and strains availability needs to be improved to perform resistance tests in good conditions.

1. Providing of R and S control varieties

- Two examples have been previously mentionned as possible models:
 - **the French network (GEVES, INRA and breeders) in charge of maintaining the reference collections for controls and strains on vegetables.** In the French network, controls are free for members, and a fee is required for non members.

Table 17: Available Resistant and Susceptible controls validated after 2005 and 2006 ring tests

In red: available controls in FR network: not all R and S controls validated

Species	Pathogen	R control	Heteroz. control	Lower partial resistance	S control
Bean	BCMNV	Booster			Dufrix
	Psp	Masai			Michelet
	CI	Booster			Michelet or Masai
Tomato	Fol 0	Motelle + Marporum			Marmande or Marmande verte
	Fol 1	Mohawk and Motelle			Marmande verte Marporum
	Vd and Va	Monalbo			Marmande verte
	ToMV	Momor + Moperou + Mobaci	Momor x Monalbo		Marmande or Monalbo

- **IBEB (International Bremia Evaluation Board) GEVES, Naktuinbouw, and breeders in charge of maintaining the reference collections for Bremia of lettuce (hosts and races).**

In IBEb, controls are free for all and strains can be obtained for a handling fee.

Participants are obliged to share important results.

- For the seven diseases studied in tomato and bean in this program, only the French controls are available within the French network (Table 17). Seeds are available in small quantities for persons out of the network.
- The other R and S controls described in our proposed updated protocols are not easily available.

2. Strains

- We checked if all differentials are described and available. For the 7 diseases studied, we summarized this information in Table 18. All are described by ISF except for Verticillium, and only those common to the French R and S controls are available. For many other diseases, ISF did not describe differentials. As NL and FR laboratories are represented in the pathogen coding group of ISF which describes the differentials, we would have the possibility to propose to ISF new differentials description.
- Possibility to include in a test some extra controls critical for isolates identification
- Maintenance:
 - We propose a long term storage: in current CPVO protocols, maintenance of strains is described on medium and example of media to be used are given. This is more an indication for multiplication, as maintenance on media could favour mutations of loss of aggressiveness of strains. Long term storage could be done at -80°C or in liquid N2 to avoid degradation of the strains.
 - The reference strains should be maintained in 2 places (official laboratories) and available for the others. For example in NL, Naktuinbouw has a backup of companies strains, and in FR: companies have a backup of SNES strains used for DUS. In FR and NL: this strain backups are done with a free collaborative network and a fee is asked for isolates for non members of the network
- We propose to set up a list of reference isolates separated from protocols, which could be updated regularly ex: race x is well represented by isolate y and indicate maintainers, identity...
- We propose to control identity of strains at least 2 times each 5 years (share between the two maintainers laboratories)

To properly organize reference collections for differentials, R and S controls and strains, several topics still need to be discussed:

- Availability of controls and strains (examples: French network and IBEb)
- Seed health status of reference controls for the sending of seeds with appropriate phytosanitary passports or certificates
- Cost of production and maintenance of R and S controls, differentials and strains
- We suggest to discuss, with all parties, the possibility of developing a European system for the maintenance and the distribution of reference R and S controls that could be based on the French system.

Table 18: Differentials for strain identification

species	pathogen	differentials described?	same as some of the R and S controls
bean	BCMNV	yes I SF	no
	Psp	yes Taylor, 1996 and I SF	no
	CI	yes, Balardin et al 1997 and I SF	no
tomato	Fol 0	yes, I SF	most of them: Marmande verte, Marporum, Motelle
	Fol 1	yes, I SF	most of them: Marmande verte, Marporum, Motelle
	Vd and Va	No	yes for Vd race 0, we can use our R and S controls
	ToMV	yes, I SF	most of them: Monalbo, Mobaci, Moperou, Momor

C. Harmonization of new resistance tests

New host/pathogen couples are also proposed to harmonize the protocols used by the official laboratories of the European Union (Table 19).

Species	Pathogen	In CPVO?	Compulsory?	Comments	Interest to harmonize?		
					F	NL	SP
Bean	CI race Kappa	yes	no				
	Xap	yes	no				
Tomato	Meloidogyne	yes	yes	different notation scale possible, intermediate levels	3	1	2
	FORL	yes	no			1	
	Ff	yes	no	different races			
	Pi	yes	no				
	PI	yes	no				
	S	yes	no				
	Pst	yes	no				3
	Rs	yes	no				
	TYLCV	yes	no				
	TSWV	yes	no				2
	Lt	yes	no		2		
	OI	yes	no		2		
	BI	yes	yes not all races	strains and differentials OK, differences in notation dates, number of plants		3 for BI 16	3
Pea	LMV	yes	no		1	1	1
	Nr	yes	no		2		
	Fop	yes	yes for race 1		1	2	3
	Ep	yes	no	field, many races			
	Ap	yes	no		1		
	Psp	yes	no		2		
	PSbMV	yes	no				
	BYMV	yes	no		2		
	PEMV	yes	no		2		
	Fom	yes	yes for races 0,1,2		3	1 for race 2, 2 for race 0 and 1	1
Melon	Ag	yes	no				
	ZYMV	yes	no				
	PRSV	yes	no				
	MNSV	yes	no				2
	Sf (Px)	yes	no				2
	Ec (Gc)	yes	no				
	Tobamoviruses	yes	yes for ToMV 0		3	1 for ToMV 0, 2 for PMMV 1-2 and 1-2-3	1
	PVY	yes	no		1	2	1
	Pc	yes	no				
	Cca	yes	no			1	

VI. Partners personal comments and opinions

(On this page, each partner could express briefly his own point of view and personal comments on the project after two years of work.)

GEVES (France):

The ring tests were very successful and fruitful to point out the communalities and the differences between the partners' protocols, to raise problems of offering reference collections, well identified and available. For 5 pathogens, common strains, susceptible/resistant controls and protocols have been defined and harmonized. A follow-up of the program of one year may be necessary for one disease.

NAK-tuinbouw (The Netherlands):

The project has been useful the purpose of harmonizing protocols between our labs, and useful suggestions can be made to improve the guideline for disease resistance testing in the CPVO protocol for DUS research. Our discussions revealed that the linkages between the regulatory system and the scientific community are rather weak. The use of outdated terminology weakens the authority of these protocols. It is advisable that disease resistance protocols should be regularly and rigorously reviewed to reflect progress in the two fields of plant pathology and plant breeding. Another weakness is that many of the standard varieties are not easy to obtain"

OEVV (Spain):

The main aim of the project of harmonizing the CPVO protocols for resistance testing has been fulfilled very positively. During the duration of the project has been evident the necessity of this harmonization and it is remarkable the very fruitful outcome of the work done during the three years. Protocols of each lab have been tested by all labs and discussed. This has resulted in a protocol for each pathogen-plant species combination that compiles the necessary changes in order to achieve a protocol with updated information related to the pathogen, an easier and more reliable method of inoculation, evaluation and interpretation of results and a reference collection of standard varieties and isolates.

We can also conclude that it would be convenient to do harmonization of other CPVO protocols.

Appendix

Appendix 1: report of the 3rd meeting in Spain, the 19-20th October 2006

Appendix 2: data of the second ring tests in 2006

Appendix 3: updated protocols



Harmonization of resistance tests to diseases of vegetable crops in the European Union

Supported by CPVO

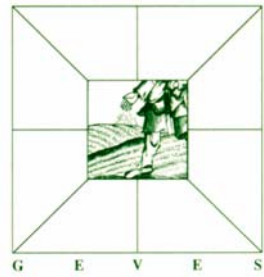
3rd annual meeting, Spain,
19-20 October 2006

Results of second ring test



Purpose

- ◆ Harmonization of resistance tests, define R and S controls, define strains, validate fiability (repetability, reproducibility) of resistance tests
- ◆ Host /pathogen chosen
 - Bean: BCMNV/BCMV, *Colletotrichum*, *Pseudomonas savastanoi* pv. *phaseolicola*
 - Tomato: *Fusarium oxysporum* fsp. *lycopersici* race 0 and 1, *Verticillium albo-atrum* or *dahliae*, ToMV



Scientific contractors

◆ France : GEVES

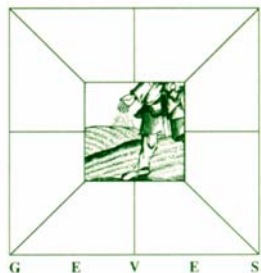
- François Boulineau
- Valérie Cadot
- Valérie Grimault

◆ Netherland : NAK-Twuinbouw

- Kees Van Ettekoven
- Diederik Smilde

◆ Spain : OEVV

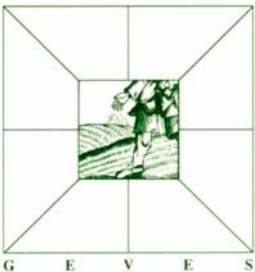
- Luis Salaices
- David Calvache
- Cristina Moyano
- Margarita Recuenco



Schedule

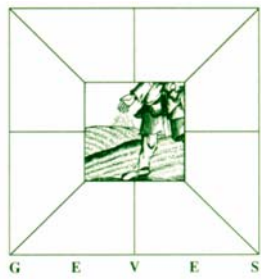
51

	Year	Date	Who ?	Actions
Phase 1	2004	End of march	Naktuinbouw OEVV	Comments about the draft questionnaire written by GEVES
		Beginning of april	GEVES - Brion	Sending of the final questionnaire to Naktuinbouw and OEVV
		Mid-may	SNES Naktuinbouw OEVV	Sending of the completed questionnaires to GEVES Brion (valerie.cadot@geves.fr)
		Mid-may to mid-june	GEVES - SNES	Analysis of the questionnaire
		June 21 st	GEVES - SNES Naktuinbouw OEVV	1 st meeting in France: results of the questionnaire and preparation of ring tests (exchanges of seeds and hosts, calendar of setting up of tests)
		Dec 15th	GEVES - SNES	1st intermediate report
Phase 2	2004 - 2005	Autumn 2004 and spring 2005	GEVES - SNES Naktuinbouw OEVV	Ring test for the bean and tomato trials
	2005	Sept 12/13th	GEVES - SNES Naktuinbouw OEVV	2 nd meeting in the Netherlands : Results of ring tests
		Dec 15th	GEVES - SNES	2nd intermediate report
Phase 3	2006	January to august	GEVES - SNES Naktuinbouw OEVV	New trials again if problems of reliability
		October 19/20 th	GEVES - SNES Naktuinbouw OEVV	3 rd meeting in Spain : final synthesis
		Dec 15th	GEVES - SNES	Final report



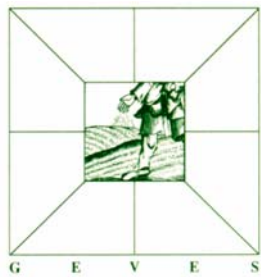
AGENDA

- ◆ Results of the 2nd ring test
- ◆ Reexamination of protocols
 - R and S controls
 - Strains
 - Protocols
- ◆ Preparation of the final report by 15 th December 2006
- ◆ Perspectives for resistance tests in EU
 - Protocols
 - Reference collections
 - Harmonization of new resistance tests
- ◆ Miscellaneous



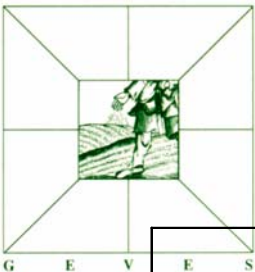
Background : 1st ring test in 2005⁵³ for french bean

Bean	Varieties		Strains	Protocols
	Susceptible	Resistant		
<i>Colletotricum lindemuthianum</i> race lambda, (delta + gamma in mixing ?)	Goldrush, Michelet, Masai,	Pastoral, Booster	- Lambda but problem of stability. - Delta + gamma? - Race 6	ES, FR, and NL ?
BCMV	Dufrix, Flandria, Aneto ?	Bizet, Booster, Odessa	FR and NL	FR and NL except for Rapier
<i>Pseudomonas savastanoi</i>	Michelet à longue cosse	Masai	FR, and NL? ES ?	?



Background : 1st ring test in 2005⁵⁴ for tomato

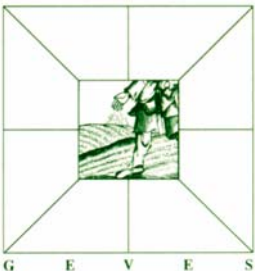
Tomato	Controls			Strains	Protocols
	Susceptible	Resistant	Heterozygote		
FOL 0	Marmande verte (FR), Resal,	Gourmet, Mohawk, Motelle	Marporum X Marmande verte, Motelle x Marmande verte	FR, NL and ES (more aggressive)	FR, NL
FOL 1	Cherry Belle (all strains) Strain FR: also Roma, Marmande verte (FR), Marporum, Ranco	Walter (all strains) Strain FR : also Odisea, Motelle, Mohawk, Tradiro,	MotelleX Monalbo ?, Motelle X Marmande verte ?	FR	NL (all strains) FR & ES with the French strain
TMV 0	<u>Monalbo (all strains)</u> , Strain FR : Santa, Marmande	Dorina, Momor (Tm-2 ²), Gourmet, Mopérrou (Tm-2)	Monalbo X Momor	FR	FR
Verticillium	Marmande verte, Flix, (Clarion : not good with strain SP)	Clairvil, Marmande VR, Elias, (Tradiro : not good with strain SP)	Marmande VR x Marmande verte	FR and ES	all



Planned ring test 2006 for tomato

55

Tomato	Controls			Strains	Protocols
	Susceptible	Resistant	Heterozygote		
FOL 0	Marmande verte (F), Resal, Ranco, Marmande (SP)	Gourmet, Mohawk, Motelle, Marporum, Larissa	Marporum X Marmande verte,	FR, NL and SP	All (5 or 10 mn of soaking, cut stem); <u>2 notations:</u> 1: above/below cotyledons 2: 2 cm of red vessels
FOL 1	Roma, Marmande verte (F), Marporum, Cherry Belle, Ranco	Odisea, Motelle, Mohawk, Tradiro, Walter,	MotelleX Monalbo ?, Motelle X Marmande verte ?	FR, NL and SP	All (5 or 10 mn of soaking, cut stem); <u>2 notations:</u> 1: above/below cotyledons 2: 2 cm of red vessels
TMV	Marmande, Monalbo, Santa	Dorina, Momor, Gourmet, Mopérou	Monalbo X Momor	FR, NL	All ; to clarify necrotic spots
Verticillium	Marmande verte, Flix, Clarion	Clairvil, Monalbo, Elias, Tradiro	Monalbo X Marmande verte	ES and FR and NL	All



Planned ring test 2006 for french bean

56

Bean	Controls		Strains	Protocols
	Susceptible	Resistant		
<i>Colletotricum lindemuthianum</i> gene <i>Are</i>	Goldrush, Michelet à longue cosse, Tuf, Masai	Pastoral, Booster, Talisman	Delta and Gamma in mixing, Lambda (NL checked by Spain), race 6	All: seeds and leaves inoculation.
BCMV	Dufrix, Flandria, Aneto (ES and NL)	Bizet, Booster, Odessa	FR and NL	FR and NL except for Rapier
<i>Pseudomonas savastanoi</i>	Michelet à longue cosse and differential hosts of race 2 and 6	Masai (ES, FR, NL) and differential hosts of race 2 and 6	Strains used or present per country: Race 2: FR and NL Race 6: ES	Harmonization of the race and of the scale of notation



Planned ring test 2006

- ◆ For all host/pathogen combination:
 - Harmonization of notation scale before the tests: common notation sheets taking into account all kinds of notation



Results ring test 2006

- ◆ Presentation of the test plan: varieties, conditions of the test, notation scale...
- ◆ Presentation of the results
- ◆ Discussion on the results
 - R and S controls, strains, notation scale, parameters of the test...
- ◆ Proposals of modifications of the CPVO protocol.
- ◆ In green: post meeting additions



Ring test 2006: BCMNV/BCMV on Bean⁵⁹

◆ Strains used:

- NL 5 from FR: BCMNV, PG-6
- NL 3 from NL: BCMNV, PG-6
- Ref CPVO: BCMV, NI 3 or PG-6
- ➔ OK except not BCMV

◆ Varieties:

- S: Aneto F and SP, Dufrix, Flandria
- R: Booster, Odessa, Bizet
- Varieties: Rapier (CPVO control) was cancelled after 2005 ring test

◆ Protocols:

- Inoculation at first leaf stage in all labs
- Plants: 30
- T°: 30°C followed by 25°C in F and only for NL5 in SP; 25°C in NL and SP;
- Notation: 13 to 21 days after inoculation
- Symptoms recorded: mosaics, local necrosis, top necrosis



Notation scale: BCMNV/BCMV on ⁶⁰Bean



Local necrosis

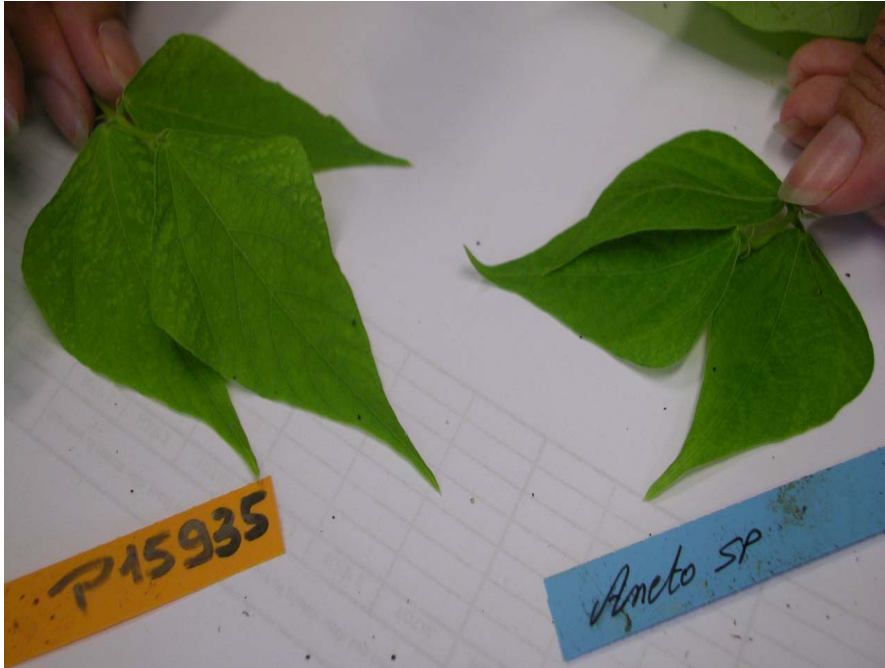
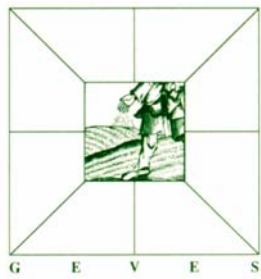


Mosaics



Top necrosis

BCMNIV symptoms



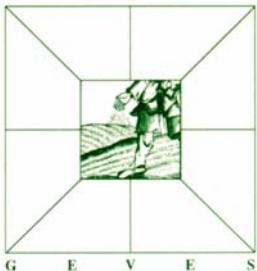
Weak symptoms for Aneto



Local necrosis, other symptoms



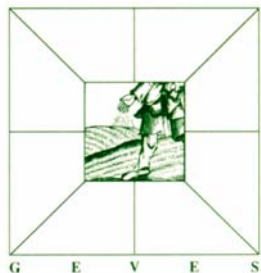
Curled leaf



Results BCMNV/BCMV on Bean

			Country					
			FR		NL		ES	
			isolate		isolate		isolate	
Expected result	Standard	Name						
			FR	NL	FR	NL	FR	NL
S	ES	Aneto (SP)	R?	S	S	S	S	S
S	NL	Aneto (NL)	S	S	--	--	?	?
S	FR	Dufrix	S	S	S	S	S	S
S	NL	Flandria	S	S	S	S	S	S
R	FR	Booster	R (top necrosis)	R	R	R	R	R
R	CPVO	Odessa	R (top necrosis)	R	R	R	R	R
R	NL	Bizet	R (no symptoms)	R	R	R	R	R

Results BCMNV/BCMV on Bean



◆ R and S controls

- R: Booster, Odessa, Bizet: expected results
- S: Dufrix, Flandria: expected results, Aneto not expected results (weak symptoms)

◆ Strains

- NL 5 homogenous in all labs, but need a 30°C period in ES to obtain top necrosis, **new result in F: top necrosis at 25°C without a 30°C period on Booster**
- NL 3 not homogenous for top necrosis in the different labs, **new result in FR: no top necrosis at 25°C without a 30°C period on Booster, but late vein necrosis**

◆ Notation scale:

- Mosaics: only in S controls
- Top necrosis or no symptoms: only in R controls
- Local necrosis: in R or S controls: could be due to interpretation of local necrosis
 - For R: vein necrosis and sometimes spots on leaves
 - For S: intervein necrosis and mosaics
- Proposed notation scale:
 - R: 1: top necrosis and/or vein necrosis; 2: no symptoms
 - S: mosaics, intervein necrosis can occur, leaf deformation



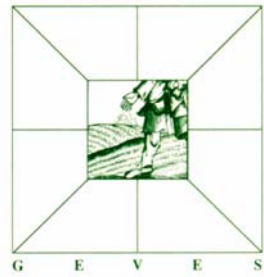
Results BCMNV/BCMV on Bean

◆ Test:

- Expected results in all labs
- very homogenous results: no escapes, no S plants in R controls

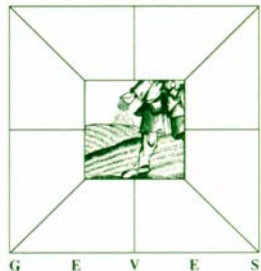
◆ Comparison with 2005:

- Test OK in NL and FR with NL 5 and NL 3 strains (not OK in ES). For ES : test after the meeting 2005 with NL 3: Aneto not OK, Booster and Odessa OK with top necrosis, Bizet without top necrosis. No symptoms in ES with NL 5.
- ➔ comparable results in 2005 and 2006, progress as expected results in 2006 in all labs



Proposals for modification of CPVO⁸⁵ protocol : BCMV / BCMNV on Bean

- ◆ R and S controls
 - Cancel Rapiere (2005) and do not advise Aneto
 - R control with top necrosis and another without like in current CPVO protocol
 - New R: Booster or Odessa (top necrosis) and Bizet (no symptoms)
 - New S: Dufrix, Flandria
 - Propose to change black root in top necrosis
 - 3 notations like in current CPVO protocol:
 - 1: S
 - 2: R with top or vein necrosis
 - 3: R without symptoms
- ◆ Change BCMV in BCMNV and explain in introduction that BCMV was splitted in BCMV and BCMNV
- ◆ Define strains
 - PG-6 represented by NL3 or NL5



Proposals for modification of CPVO⁹⁶ protocol : BCMV / BCMNV on Bean

◆ Protocol:

- Number of plants: at least 20 in CPVO, very homogeneous results, at least 20 (general requirements of UPOV)
- Temperature: 25°C, with an optional period at 30°C
- Notation: 6 days after inoculation in CPVO protocol, here 13 to 21 days, one or more notations?. Advise when mosaic symptoms are well developed on S control (usually after 13-21 days)
- Notation scale:
 - 1: S: mosaic, intervein necrosis, leaf deformation
 - 2: R with top or vein necrosis
 - 3: R without symptoms



Follow up BCMNV on bean

- ◆ Verify identity of the 2 strains in 2007

Ring test 2006: Pseudomonas on Bean



◆ Strains used:

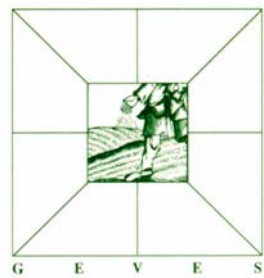
- 7722 from FR (race 6)
- HRI 1449B from ES (race 7) (changed after ring test 2005: race 6 not virulent)
- PRI 113 from NL (race to be determined)
- Ref CPVO: US race 1 (can be now race 1,5,7 or 9) or US race 2 (can be now race 2,6 or 8)

◆ Varieties:

- S: Astun, Michelet, Slenderette
- R: Masai from FR, NL, ES

◆ Protocols:

- Inoculation:
 - in FR: in field and a small test in greenhouse,
 - in ES: in growth chamber,
 - in NL: in greenhouse
 tests under progress in FR and NL in greenhouse. At cotyledon + 1st leaf in FR, at first leaf in ES and NL
- Inoculum: 10^8 cfu/ml
- Plants: 30 to 60 plants
- T°: greenhouse: no summer trial possible
- Notation: 2 to 3 weeks after inoculation
- Symptoms recorded: water soaked lesion, halo



Notation scale: Psp on Bean

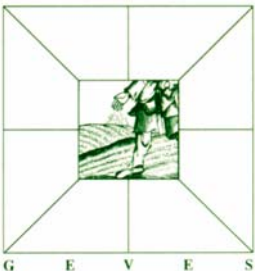
- 0: necrosis in the area of maximum inoculation either side of the leaf midrib (R)
- 1: some necrosis and water-soaking largely confined to the area of maximum inoculation(R)
- 2: Water-soaked lesions less than 1 mm distributed over the leaf undersurface (S)
- 3: Water-soaked lesions more than 1 mm distributed over the leaf undersurface (S)
- R: note 0 or 1 or DI <20%
- S: note 2 and 3 or disease index >20%



Chlorotic halo in the field



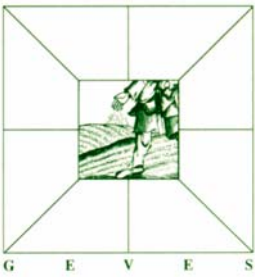
Note 3 in growth chamber



Results Psp on Bean

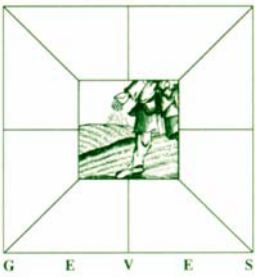
			Field			Greenhouse								
			Country											
			FR			FR			NL			ES		
			isolate			isolate			isolate			isolate		
Expected	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	ES	Astun	S(esc)	S(esc)	R	R	S	S	S	S	S(esc)	S	S	S
S	FR	Michelet	S	S	R	S	S	S	S	S	S	S(esc)	S	S
S	NL	Slenderette	S	S	R	S	S	S	S	S	S	S	S	S
R	FR	Masai (F)	R	R	R	R	R	R	R	R	R	R	R	R
R	ES	Masai (SP)	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Masai (NL)	R	R	R	R	R	R	R	R	R	R?	R?	R?
R	FR	Vaillant	R	R	R	R	R	R	R	R	R	nt	nt	nt

Masai is also a CPVO standard; in green new results in NL and FR after meeting



Results Psp on Bean

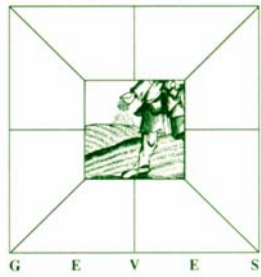
- ◆ R and S controls
 - R: Masai: expected results
 - S: Michelet: expected results ; Slenderette and Astun: not expected results
- ◆ Strains
 - Differentials acquired in FR to identify 7722 as race 6 (to be confirmed), same in NL to identify PRI 113. Multiplication under progress to be able to verify our strains
 - 7722, PRI 113: expected results
 - HRI 14 49B: ? **Less aggressive**
- ◆ Notation scale:
 - OK
- ◆ Test:
 - OK in field (2005 and 2006), reproducibility in different countries not tested
 - Need confirmation in greenhouse



Results Psp on Bean

◆ Comparison with 2005:

- Isolates: ?
 - ES strain (race 6): not aggressive in the field in FR
 - Not the same races used by countries: ES : race 6, F: mixing of 4 strains (with race 6), NL : strain not identified. Predominance of the race 6 in EU.
 - Which race to use? race 2 US (including new races 2, 6 and 8) predicted by CPVO?
- Cultivars:
 - R: Masai OK
 - S: Michelet OK. Astun and Slenderette : not OK
- Protocols:
 - In field: OK on leaves and pods in FR but races not controlled (mixing).
 - In greenhouse : not always robust in NL (no symptoms in september 2005)
- ➔ progress in 2006 on race identification, on understanding of race importance or not, on notation scale, but still need confirmation in greenhouse, repeatable in the field in FR but reproducibility in other countries not tested. A too complicated model for a 2 years ring-test



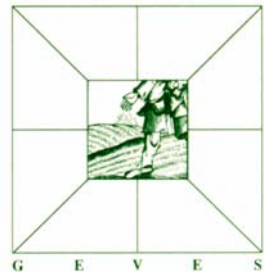
Proposals for modification of CPVO⁷³ protocol :Psp on Bean

◆ R and S controls

- New R: Masai ; origin of resistance: PI 50 414: Vaillant
- New S: Michelet
- Define and find a race 6 isolate enough aggressive
- Not race 1 or 2, speak about new races, confirmation of race to be made. A race 6 would be preferred as more frequent in Europe. Not a race specific test but one same race to have uniform grouping of varieties.

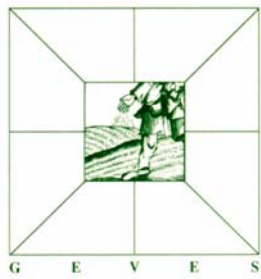
◆ Protocol: to be set up

- Number of plants: at least 20
- Temperature: spring or autumn conditions in greenhouse, avoid high temperature.
- Humidity: high
- Notation: importance to define Disease Index, analysis of results but too early to define something



Follow up Psp on bean

- ◆ Confirm race identification:
 - isolates selected: 7722, new race 6 SP, PRI 113
 - test the 3 isolates on differentials defined by Taylor
- ◆ Select 1 to 3 race 6 isolate for ring test
- ◆ Ring test: Masai, Michelet, Slenderette, Red Mexican, UI 3, Vaillant, 3-4 registred resistant varieties to be defined



Ring test 2006: Colletotrichum on Bean⁷⁵

◆ Strains used:

- FR : Delta and Gamma
- NL: 418. Described as Lambda.
- ES: race 6 (binary code)
- Ref CPVO: Lambda

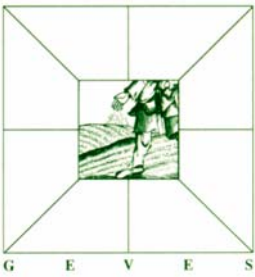
◆ Varieties:

- S: Goldrush, Michelet, Tuf, Masai
- R: Pastoral, Booster, Talisman

◆ Protocols:

- Inoculation: by soaking seeds or spraying cotyledons
- Plants: 30
- T°: 20°C in F et NL, 20-22°C in SP
- Notation: 7 to 15 days after inoculation
- Symptoms recorded: necrosis, dying plants

Notation scale: Colletotrichum on Bean⁷⁶



0: no symptoms

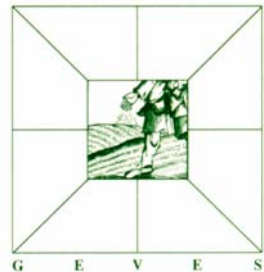
1: weak reaction with small superficial necrosis (dots or stripes)

2: deeply sunken necrotic flecks on hypocotyl or stem or strong reaction with necrosis larger than 3 mm sunk deeply into the tissue

3: dying plants

R: 0 and 1, DI > 50%; S: 2 and 3, DI < 50%





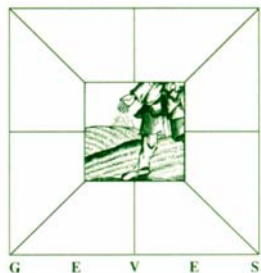
Colletotrichum symptoms



Weak symptoms for NL strain

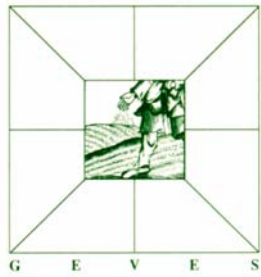


NL, F and SP strains



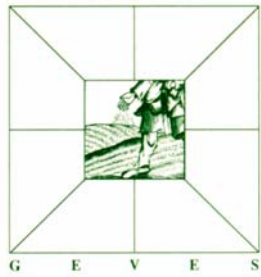
Results Colletotrichum on Bean

			Country											
			Spraying cotyledons			Inoculation by soaking seeds								
			F			F			NL			SP		
			isolate			isolate			isolate			isolate		
Expected result	Standard	Name	F	NL	SP	F	NL	SP	F	NL	SP	F	NL	SP
S	SP	Goldrush	S	S?	S	S	S	S	S (esc)	R	S	S	S	S
S	F	Michelet	S	S?	S	S	nt	S	S (esc)	R	S	S	S	S
S	CPVO	Tuf	S	S?	S	S	S (esc)	S	S (esc)	R	S (esc)	S	S (esc)	S
S	NL	Masai	S	R	S	S	R	S	S (esc)	R	S (esc)	S	S (esc)	S
R	SP	Pastoral	R	R	R	R	R	R	R	R	R	R	R	R?
R	F	Booster	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Talisman	R	R	R	R	R	R	R	R	R	R?	R	S?



Results Colletotrichum on Bean

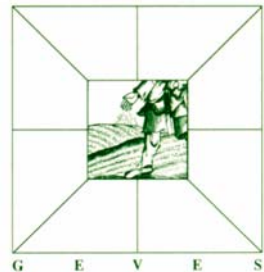
- ◆ R and S controls
 - R: Booster: expected results
 - R: Pastoral, Talisman: weaker resistance
 - S: Goldrush, Michelet, Tuf, Masai: expected results
- ◆ Strains
 - Delta + Gamma and race 6 expected results (Delta did not sporulate in SP), race 6 more aggressive
 - Lambda (?) 418: in line with expected results but so low aggressiveness that many escapes occur.
- ◆ Notation scale:
 - OK, quantitative notation for soaking seeds, interest of a qualitative notation for spraying cotyledons with 2 classes :
 - S: no symptoms and weak reaction with small superficial necrosis,
 - R: deeply sunken necrotic flecks and dying plants
- ◆ Test:
 - OK in FR, ES and after 2nd test in NL except with Lambda strain. Low disease pressure in NL solved.
 - Inoculation method: spraying cotyledons or soaking seeds, gave equivalent results
 - Light: 12L 12D (FR) or 16L 8D (ES and NL) did not affect results



Results Colletotrichum on Bean

◆ Comparison with 2005:

- Isolates: no conclusions because
 - strain ES unreadable by NL and strain NL unreadable by FR
 - Strain FR not tested in mixing by ES and NL
- R and S controls:
 - R: Booster and Pastoral: OK. Talisman : not R in ES with ES and NL strains
 - S: Goldrush, Masai, Michelet OK. Tuf: not OK with ES strain in FR
- ➔ progress on isolates in 2006, less problems on R and S controls, problems of low inoculum pressure identified.



Proposals for modification of CPVO⁸¹ protocol : Colletotrichum on Bean

◆ R and S controls

- New R: Booster; Pastoral can be added as it has a weaker resistance and can give an indication on aggressiveness of the test
- New S: Goldrush, Michelet, Masai

◆ Define strains

- Use numeric code. Race 6 would be preferred as it is more aggressive and stable than Lambda. Both races detect the Are gene. Race 6 detects a few more resistance genes. **Would varieties without Are need to be retested?**

◆ Protocol:

- Number of plants: at least 20
- Method of inoculation: soaking seeds. Spraying cotyledons is proposed to be added as an alternative method (ES does not agree)
- Notation: when symptoms are well developed on S control (usually after 7DPI to 14DPI)
- Importance of humidity for symptoms development
- Notation scale: qualitative
 - S: 0: no symptoms and 1: weak reaction with small superficial necrosis
 - R: 2: deeply sunken necrotic flecks and 3: dying plants

Ring test 2006: Fusarium on tomato



◆ Strains used:

- Fol 0:
 - FR: Orange 71
 - NL: PRI 20698
 - ES: Fol 071
- Fol 1:
 - FR: Fol 1 F
 - NL: PRI 10195 N
 - ES: RAF 70
- Ref CPVO: race 0, race 1

◆ Varieties:

- S to race 0: Marmande, Marmande verte, Resal, Ranco
- R to race 0: Larissa, Marporum, Motelle, Marporum x Marmande verte, Gourmet, Mohawk
- S to race 1: Roma, Marporum, Marmande verte, Cherry belle, Ranco
- R to race 1: Odisea, Motelle, Mohawk, Tradiro

◆ Protocols:

- Inoculation: soaking in inoculum suspension, cut (FR) or not (ES, NL) radicels
- Plants: 30
- T°: 24°C (NL), 20-24°C (FR), 25-28°C (ES)
- Notation: 21 (NL) to 25 (FR and ES) DPI
- Symptoms recorded: above cotyledons (F), > 2cm (NL), > 3cm (FR and ES)

Notation scale: Fusarium on tomato



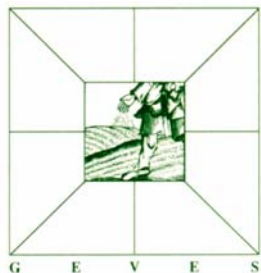
Resistant (left) and susceptible (right) plants



Dead plants (susceptible)

Fol 0 symptoms





Results Fol 0 on tomato

			Country											
			FR (above cotyledons)			FR (> 3 cm)			NL (> 2 cm)			ES (> 3 cm)		
			isolate			isolate			isolate			isolate		
Expected result	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	ES	Marmande	S	S	S	S	S	S	S	S	S	S	S	S
S	CPVO, FR	Marmande verte	S	S	S	S	S	S	S	S	S	S	S	S
S	NL	Resal	S	S	S	S	S	S?	S	S	S	S	S	S
S?	NL	Ranco	S (esc)	S	S (esc)	S (esc)	S	S?	R	R?	R	R	S (esc)	S (esc)
R	FR	Marporum x Marmande verte	S (esc)	S (esc)	S (esc)	R?	S (esc)	R?	R	R?	R	R?	S (esc)	S (esc)
R	SP	Larissa	R	R?	R	R	R?	R	R	R	R	R	R?	R?
R	CPVO, FR	Marporum	R?	R?	R?	R	R?	R?	R	R?	R	R	R?	S (esc)
R	CPVO, FR	Motelle	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Gourmet	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Mohawk	R	R	R	R	R	R	R	R	R	R	R	R



Results Fol 0 on tomato

◆ R and S controls

- R: Motelle, Gourmet, Mohawk + Marporum, Larissa (S race 1): expected results
- S: Marmande, Marmande verte, Resal: expected results, Ranco not expected results (last info from DS: Ranco R to race 0 and not to race 1)
- Heterozygous R : Marporum x Marmande verte: expected results but some S plants
- Cancel Ranco

◆ Strains

- All OK, [Range of aggressiveness](#): NL>ES>FR

◆ Notation scale:

- Not homogeneous
- add a third class for intermediate plants: no growth stopped but above cotyledons
- Would be 4 qualitative classes: 0: no symptoms, 1: healthy plant with brown vessels (above cotyledons), 2: growth reduction and brown vessels above cotyledons, 3: dead plant. Generally R: 0 and 1, S: 2 and 3 but analysis of results calibrated with results of R and S controls



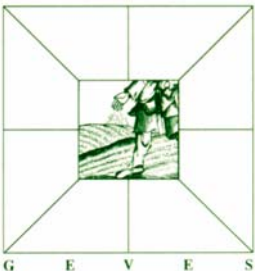
Results Fol 0 on tomato

◆ Test:

- Reproducible in all laboratories, with all strains for R and S, problems of notations for Heterozygous R or varieties S to race 1: proposal to improve notation scale
- Sowing on vermiculite or soil
- Cut or not radicles
- Inoculum concentration 10^6 sp/ml can be decreased if aggressive test
- T° : 24-28°C, in case of aggressive test T° can be decreased to 20°C

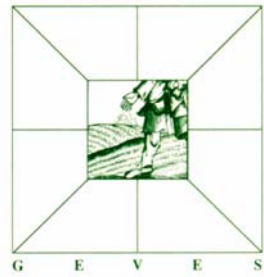
◆ Comparaison 2005

- Isolates: Strain FR and NL OK. Strain ES too aggressive.
- Cultivars:
 - R: Gourmet, Mohawk, Motelle OK.
 - S: Marmande verte, Resal OK. Ranco and Marmande (SP) not OK
 - Marporum x Marmande verte, Motelle x Marmande verte ?
- Protocols: FR and NL OK. (ES : pb of resistant cultivars found susceptible). Method of notation to harmonize ?
- ➔ progress in 2006: R and S confirmed, 3 strains OK, improve notation scale for heterozygous R



Results Fol 1 on tomato

			Country											
			FR (above cotyledons)			FR (> 3 cm)			NL (> 2 cm)			ES(> 3 cm)		
			isolate			isolate			isolate			isolate		
Expected result	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	ES	Roma	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S(esc)	S	S	S
S	FR	Marporum	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
S	CPVO, FR	Marmande verte	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
S	NL	Cherry Belle	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
S	NL	Ranco	S	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S(esc)	S	S	S
R	ES	Odisea	R	R	R	R	R	R	R	R	R	R	R	R
R	CPVO, FR	Motelle	R	R	R	R	R	R	R	R	R	R	R	R
R	FR	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
R	NL	Mohawk	R	R	R	R	R	R	R	R	R	R	R	R
R	NL	Tradiro	R	R	R	R	R	R	R	R	R	R	R	R



Results Fol 1 on tomato

◆ R and S controls

- R: Mohawk, Tradiro, Odisea, Motelle: expected results
- S: Marmande verte, Roma, Ranco, Cherry belle, Marporum: expected results

◆ Strains

- More reproducible results with FR strain (more aggressive)

◆ Notation scale:

- Same as Fol 0



Results Fol 1 on tomato

◆ Test:

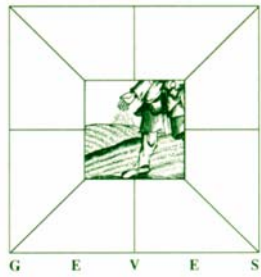
- More reproducible than Fol 0, OK in FR with FR strain and ES
- Sowing on vermiculite or soil
- Cut or not radicels
- Inoculum concentration 10^6 sp/ml can be decreased if aggressive test
- T° : 24-28°C, in case of aggressive test T° can be decreased to 20°C



Results Fol 1 on tomato

◆ Comparison with 2005:

- Isolates: strain FR OK for the 3 countries.
- Cultivars: all the cultivars with the strain FR.
With the 3 strains :
 - R: Walter
 - S: Cherry Belle
- Protocols: all the labs OK with the strain FR. If not, lab NL OK. Not the same scale of notation in ES in 2005.
- ➔ progress 2006: R and S confirmed, confirm FR strain, proposal to improve notation scale



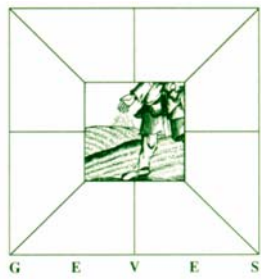
Proposals for modification of CPVO⁹² protocol : Fusarium on tomato

◆ R and S controls

- New R:
 - For Fol 0: Motelle or Gourmet or Mohawk (R to race 0 and 1) and Marporum or Larissa (R to race 0, S to race 1)
 - For Fol1: Tradiro or Odisea or Motelle or Mohawk
- New S:
 - For Fol 0: Marmande, Marmande verte, Resal
 - For Fol 1: Marmande verte, Roma, Ranco, Cherry belle, Marporum. Walter the S control of CPVO was not tested due to no availability of seeds: not confirmed in ring test
- Heterozygous R: Marporum x Marmande verte for Fol 0, Motelle x Marmande verte for Fol 1 (optional)

◆ Define strains

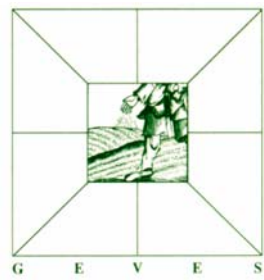
- **Fol 0:** Orange 71 or Fol 0 NL or Fol 071
- **Fol 1:** Fol 1 FR (more aggressive) or Fol 1 NL or RAF 70 (less aggressive)



Proposals for modification of CPVO⁹³ protocol : Fusarium on tomato

◆ Protocol:

- Number of plants: not indicated in CPVO, quite heterogeneous results for F1, at least 20
- Temperature: 28-25°C in CPVO, 24-28°C . In case of aggressive test, T° can be decreased to 20-24°C
- Sowing: not important to indicate the conditions
- Inoculation: cutting in CPVO, propose cutting in option
- Notation: 20-25 days after inoculation in CPVO protocol. Propose at least 21 days.
- Notation scale: 4 qualitative classes:
 - 0: no symptoms,
 - 1: healthy plant with brown vessels (above cotyledons),
 - 2: growth reduction and brown vessels above cotyledons,
 - 3: dead plant.
 - Generally R: 0 and 1, S: 2 and 3 but analysis of results calibrated with results of R and S controls



Ring test 2006: *Verticillium* on tomato

94

◆ Strains used:

- FR and ES : *V. dahliae* (Toreilles and Almeria)
- NL: *V. albo atrum* (PRI)
- Ref CPVO: *V. dahliae*
- ➔OK because R to Vd and Va

◆ Varieties:

- S: Marmande verte, Clarion, Flix
- R: Monalbo, Monalbo x Marmande verte, Tradiro, Elias

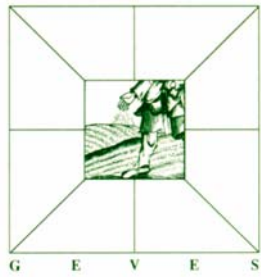
◆ Protocols:

- Inoculation: 10 to 18 days after sowing, by cutting radicels and soaking in inoculum suspension
- Plants: 30
- T°: 18°C to 22°C
- Notation: 25 to 30 DPI
- Symptoms recorded: number of diseased plants: growth retarded, brown vessels

Notation scale: Verticillium on tomato



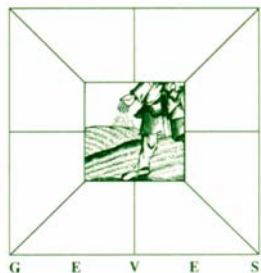
Verticillium symptoms



Aggressive test mistake in F

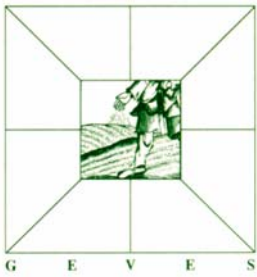


Normal symptoms in F



Results Verticillium on tomato

			Country								
			FR			NL			ES		
			isolate			isolate			isolate		
Expected result	Standard	Name	FR	NL	ES	FR	NL	ES	FR	NL	ES
S	CPVO, FR	Marmande verte	S (esc)	S (esc)	S (esc)	S	S	S	S	S	S
S	NL	Clarion	S	S (esc)	S	S (esc)	S	S	S	S	S (esc)
S	ES	Flix	S	S (esc)	S (esc)	S	S	S	S	S	S (esc)
R	FR	Monalbo	R	R	R?	R	R	S	R	R?	S
R	FR	Monalbo x Marm verte	R	R	S (esc)	R	R	S	R	R?	R?
R	NL	Tradiro	R	R	R?	R	R	S	R	R?	R?
R	CPVO	Clairvil	nt	nt	nt	nt	nt	nt	nt	nt	nt
R	ES	Elias	R	R	R?	R	R	S	R	R	R?



Results Verticillium on tomato

◆ R and S controls

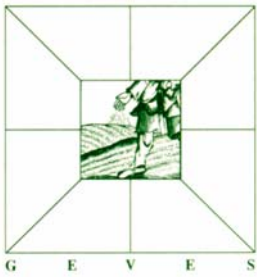
- R: Monalbo, Tradiro, Elias expected results
- S: Marmande Verte, Flix expected results, Clarion less susceptible, could help to monitor aggressiveness of test
- Heterozygous R: Monalbo x Marmande verte expected results, interesting to include to help interpretation of results, could be optional in case of aggressive test

◆ Strains

- FR and NL expected results
- FR give more reproducible results
- ES not expected results on R plants (more disease) : different from 2005

◆ Notation scale:

- Diseased/not diseased: expected results



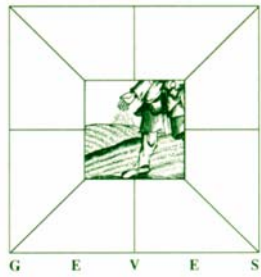
Results Verticillium on tomato

◆ Test:

- Reproducible results with FR strain in all labs
- Inoculation: soaking 4 to 10 min
- Stage of inoculation: cotyledons to first leaf: 10 to 18 days
- Temperature: 20 to 22°C
- Inoculation to reading: 25-30 days

◆ Comparison with 2005:

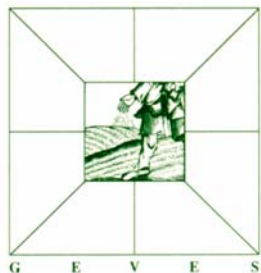
- Good concordance between labs
- Isolates: Strain ES and FR
- Cultivars:
 - R: Clairvil, Marmande VR, Elias OK. Tradiro not OK in SP with NL strain
 - S: Marmande verte, Flix OK. Clarion ?
- Protocols: OK for all labs but necessity to harmonize scale of notation
- ➔ progress 2006: notation scale OK, confirm S controls, no comparison possible on R controls except for Elias and Tradiro



Proposals for modification of CPVO¹⁰⁰ protocol : Verticillium on tomato

- ◆ R and S controls
 - New R: Monalbo or Elias,
 - New S: Marmande Verte or Flix. Clarion could be interesting to add because it is less susceptible and could help to control the aggressiveness of the test
 - HF1: Monalbo x Marmande verte expected results, interesting to include to help interpretation of results, could be optional in case of aggressive test
- ◆ Define strains
 - *V. dahliae* Toreilles
- ◆ Protocol:
 - Number of plants: 10 to 20 in CPVO, quite homogeneous results, at least 20
 - Temperature: 20 to 22°C
 - Sowing to inoculation: 15 to 20 in CPVO, propose 10-20 (cotyledons to first leaf stage)
 - Notation: 25-30 days after inoculation in CPVO protocol,
 - Notation scale:
 - R: no symptoms
 - S: diseased growth reduced and brown vessels or growth not reduced and brown vessels. Analysis of results calibrated with results on R and S controls.

Ring test 2006: ToMV on tomato



◆ Strains used:

- FR: ToMV 0 INRA Avignon
- NL: ToMV 0 strain PRI MA
- Ref CPVO:ToMV strain 0
- ➔OK all ToMV 0

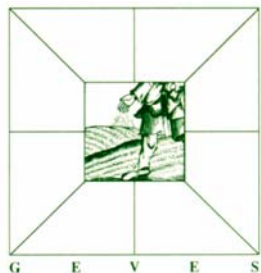
◆ Varieties:

- S:Marmande, Monalbo, Santa
- R: Dorina, Momor, MonalboxMomor, Moperou, Gourmet

◆ Protocols:

- Inoculation: by mechanical inoculation of cotyledons (FR) or first leaves (NL), or first and second leaves (ES)
- Plants:30
- T°C: 24-26°C
- Notation: 12 to 20 days days after inoculation
- Symptoms recorded: mosaics, necrosis, no symptoms

Notation scale: ToMV on tomato



mosaics



mosaics with an aucuba strain

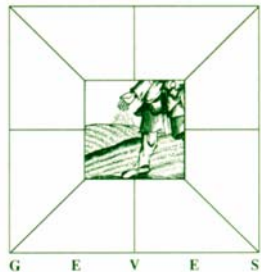


no symptoms

necrosis



ToMV symptoms

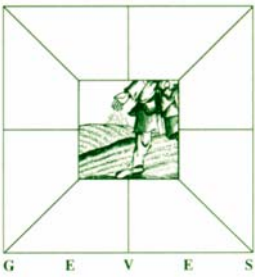


**Susceptible plant with mosaics
ToMV NL strain**



**Resistant HF1 plant with beginning
of necrosis looking to mosaics
ToMV NL strain**

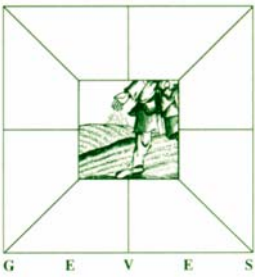




Results ToMV on tomato

			Country					
			FR		NL		ES	
			isolate		isolate		isolate	
Expected result	Standard	Name	FR	NL	FR	NL	FR	NL
S	ES	Marmande	S	S	S	S	S	S
S	CPVO, FR	Monalbo	S	S	S	S	S	S (esc)
S	NL	Santa	S	S	S	S	S	S
R	CPVO, FR	Moperou	R	R	R	R	R	R
R	NL	Gourmet	R	R	R	R	R	R
R	FR	Momor	R	R	nt	nt	R	R
R	ES	Dorina	R*	R*	R	R*	R	R*
R	CPVO, FR	Monalbo X Momor	R*	R*	R*	R*	R*	?

R*: symptoms of necrosis that show that variety is perhaps heterozygous for R gene



Results ToMV on tomato

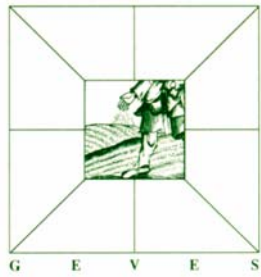
- ◆ R and S controls
 - R: Dorina, Momor, MonalboxMomor, Moperou, Gourmet : expected results, Dorina perhaps heterozygous for R gene
 - S: Marmande, Monalbo, Santa : expected results
- ◆ Strains
 - ToMV I NRA Avignon expected results
 - ToMV PRI MA: expected results on controls because Mobaci was not included but not ToMV O
- ◆ Notation scale:
 - Mosaics: only in S controls
 - no symptoms: only in R controls
 - necrosis: in R heterozygous controls, be careful of possible confusion of symptoms between beginning of necrosis and mosaics with PRI MA strain
- ◆ Test:
 - OK in all labs with the I NRA Avignon strain
 - very homogeneous results



Results ToMV on tomato

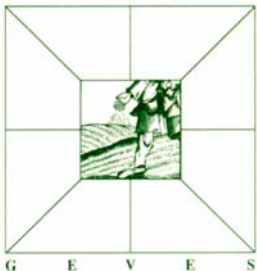
◆ Comparaison 2005

- Isolates: FR strain expected results.
- Cultivars:
 - R: Momor, Gourmet, Mopérrou expected results.
 - S: Monalbo, Marmande, Santa expected results.
 - Monalbo* Momor (resistant) ?
- Protocols:
 - FR lab OK. Pb of interpretation of symptoms for NL and ES
 - NL found as susceptible the resistant cultivars: by not taking account for heterozygotes a necrosis as a mechanism of resistance by hypersusceptibility?
 - ES had some scapes on susceptibles varieties with NL isolate because mosaic is not clear enough with that isolate. Light necrosis has been interpreted as resistant.
 - ➔ progress 2006: notation scale homogeneized, strains better defined, homogeneous results: test OK



Proposals for modification of CPVO¹⁰⁷ protocol : ToMV on tomato

- ◆ R and S controls
 - New R: Momor or Gourmet (R to TMV 0, 1, 2) and Moperou (R to TMV 0 and 1) and MonalboxMomor (heterozygous for resistance gene) and Mobaci (R to TMV 0 and 2 which could help to control isolate identity): include a table in the new protocol
 - New S: Marmande or Monalbo
- ◆ Define isolate
 - ToMV 0 INRA Avignon (aucuba isolate)
- ◆ Protocol:
 - ToMV 0
 - Number of plants: 15 to 30 in CPVO, homogeneous results, propose at least 20
 - Stage of inoculation: 12-14 days in CPVO, propose cotyledons (first leaves emerging) to 2 expanded leaves
 - Temperature: 30-35°C in CPVO, very high, propose 24-26°C. At high temperatures, resistance can break.
 - Light important for symptom expression
 - Notation: 10-12 days after inoculation in CPVO protocol, short time for symptom expression, propose 12-21
 - Notation scale: R: no symptoms, S: mosaics. Necrosis on plants heterozygous for resistance gene
 - Cancel ToMV 1-2 as it does not exist
 - Delete asterisk for ToMV 1 and ToMV 2



Strains validated after 2005 and 2006 ring tests ¹⁰⁸

Species	Pathogen	Isolate	Comments
Bean	BCMNV	<ul style="list-style-type: none"> NL5 NL3 	To be verified on differentials and for temperature
	Psp	<ul style="list-style-type: none"> 7722 (FR race 6) PRI 113 new race 6 (ES) 	To be verified on differentials, select an aggressive race 6 isolate
	CI	<ul style="list-style-type: none"> race 6 	
Tomato	Fol 0	<ul style="list-style-type: none"> Orange 71 PRI 20698 Fol 071 	Aggressiveness: PRI 20698 > Fol 071 > Orange 071. Range of aggressiveness of isolates could be adapted to aggressiveness of test
	Fol 1	<ul style="list-style-type: none"> Fol 1 FR 	
	Vd and Va	<ul style="list-style-type: none"> Toreilles 	
	ToMV	<ul style="list-style-type: none"> I NRA Avignon 	

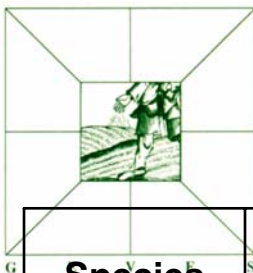


Follow up for isolates

- ◆ isolates should be available in 2 places
- ◆ For testing on differentials, everybody will check the amount of seeds available for differentials and share information: excel table to circulate.

R and S controls validated after 2005 and 2006 ring tests

110



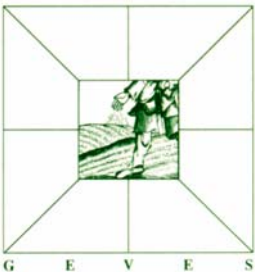
Species	Pathogen	Resistant control	Heterozygous Resistant control	Susceptible control
Bean	BCMV	Top necrosis: Booster, Odessa No symptoms: Bizet		Dufrix Flandria
	Psp	Masai		Michelet à longue cosse
	CI	Booster Pastoral optional (less resistant)		Goldrush Michelet Masai
Tomato	Fol 0	Motelle Gourmet Mowhawk Marporum Larissa	Marporum x Marmande verte	Marmande Marmande verte Resal
	Fol 1	Mohawk Odisea Tradiro Motelle	Motelle x Marmande verte (optional)	Marmande verte Roma Cherry Belle Marporum Ranco
	Vd and Va	Monalbo Tradiro Elias		Marmande verte Flix
	ToMV	R to 0,1,2: Momor or Gourmet R to 0,1: Moperou R to 0 and 2: Mobaci	Momor x Monalbo	Marmande Monalbo



R and S controls validated after¹⁴¹ 2005 and 2006 ring tests

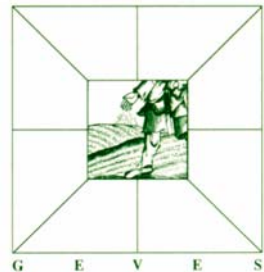
◆ In protocols

- the different classes of resistance will be explained
- Indicate which R and S controls are important for race or pathotype identification



Proposals of modifications for CPVO protocols

- ◆ Compulsory characteristic or not?
 - Compulsory
 - Fol 0 and 1, V, ToMV 0,1,2,1-2,
 - CI race lambda, BCMV
 - Keep compulsory based on our technical results? If result enough repeatable to use this character for DUS
 - Fol 0: OK
 - Fol 1: OK
 - Ve: OK
 - ToMV 0: OK
 - CI: OK 1 year more to confirm race to be used
 - BCMV: OK 1 year more to confirm isolates to be used
 - Possible to be new compulsory?
 - Psp: 1 year more for harmonization (and more than one test a year) to give an advice
 - Proposals to cancel compulsory
 - ToMV 1 and ToMV 2
 - Proposals to cancel characteristic
 - ToMV 1-2



Preparation final report

◆ Planning:

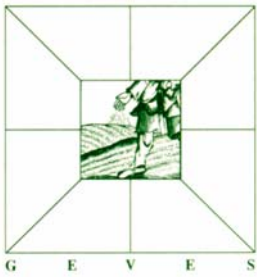
- 15th December: sent to CPVO
- 30th November: send financial justification
- 30th November: return comments to GEVES
- 15th November: send to all the final report



General questions about CPVO¹¹⁴ protocols

◆ Strains/isolates

- Use correct terminology in protocols: species, pathotypes, races, strains/isolates which can mean the same thing and need a reference number
- More than one possible depending of the protocols, if identified and give the same results on controls
- Control identity of isolates on differentials to verify race or pathotype identification,
- Are all differentials described, same as R or S controls? Indicate in protocols the references of differentials



Controls for strain identification

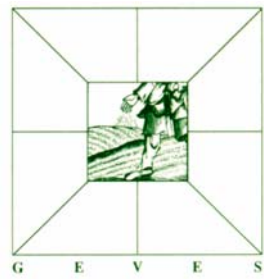
species	pathogen	differentials described?	same as some of the R and S controls
Bean	BCMNV	yes I SF	no
	Psp	yes Taylor, 1996 and I SF	no
	CI	yes, Balardin et al 1997 and I SF	no
Tomato	Fol 0	yes, I SF	most of them: Marmande verte, Marporum, Motelle
	Fol 1	yes, I SF	most of them: Marmande verte, Marporum, Motelle
	Vd and Va	No	yes for Vd race 0, we can use our R and S controls
	ToMV	yes, I SF	most of them: Monalbo, Mobaci, Moperou, Momor



General questions about CPVO¹¹⁶ protocols

◆ Strains

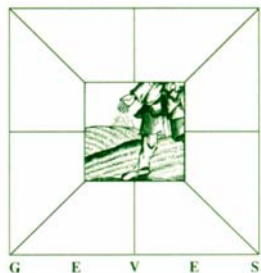
- Are all differentials available? (discuss with R and S controls)
- Possibility to include in a test some extra controls critical for isolates identification
- Maintenance:
 - long term storage/medium. CPVO: maintenance on medium and example of media to be used, this is more an indication for multiplication.
 - Long term storage ex: -80°C or liquid N2 to avoid mutation
 - Available in 2 places to have a ref strain, available for the others. Ex of NL: NAKT has a backup of companies strains, ex of FR: companies have a backup of SNES strains used for DUS. F and NL: free collaborative network, a fee for isolates for non members
- Set up a list of reference isolates separated from protocols, ex: race x is well represented by isolate y and indicate maintainers, identity...
- Control identity: at least test isolates 2 times each 5 years (share between the two maintainers laboratories)
- Question to CPVO: how to manage the availability of strains:
 - Grant from CPVO and free isolates for companies and other laboratories or
 - Each laboratory or company pay a fee to the maintainer when they ask for an isolate?



General questions about CPVO¹¹⁷ protocols

◆ R and S controls

- More than one possible depending of the protocols, if give the same results
- Are all R and S controls available?



Available Resistant and Susceptible controls validated after 2005 and 2006 ring tests

◆ R and S controls

- Example of french network for R and S controls on vegetables and of IBEB for Bremia of lettuce

Species	Pathogen	R control	Heteroz. control	Lower partial resistance	S control
Bean	BCMNV	Booster			Dufrix
	Psp	Masai			Michelet
	Cl	Booster			Michelet or Masai
Tomato	Fol 0	Motelle + Marporum			Marmande or Marmande verte
	Fol 1	Mohawk and Motelle			Marmande verte Marporum
	Vd and Va	Monalbo			Marmande verte
	ToMV	Momor + Moperou + Mobaci	Momor x Monalbo		Marmande or Monalbo

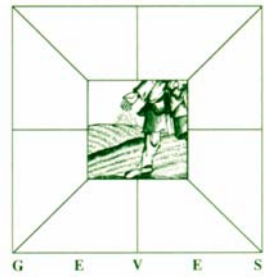
In red: available controls in F network: not all R and S controls validated



Availability of Resistant and Susceptible controls validated

119

- ◆ FR network: controls free for members, a fee for non members
- ◆ I BEB (FR and NL): free for all as far as results come back to I BEB
- ◆ Controls need to be available: how to make them available?
 - Discuss with ESA for an european system for maintainance and distribution of reference R and S controls more or less accorded to the FR system?
 - How does CPVO want to manage the officies in charge of reference collections?



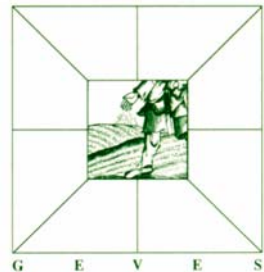
General questions about CPVO¹²⁰ protocols

◆ R and S controls

- What does it include: cost, seed health status of R and S controls for circulation in EU and exports, storage...
- Perspectives?
 - Tomato and bean:
 - the controls are available only for FR varieties with the french R and S controls network
 - Not available seeds in large quantities for other R and S controls: future?

◆ Discussion at CPVO : how to organize reference collections for differentials, R and S controls and strains

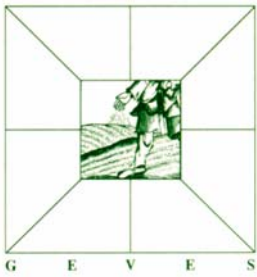
- Availability
- Seed health status for phytosanitary passeports or certificates
- Cost for production and maintenance of R and S controls, differentials and strains: integrate in costs of DUS?



General questions about CPVO¹²¹ protocols

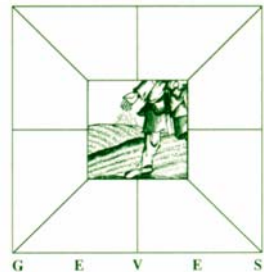
◆ Protocols

- Obligation to apply the protocol or guideline? One only protocol without latitude is not possible because a protocol is dependant of lab conditions and strain choice : propose to have protocols with some flexibility in different steps
 - add one or several contact lab, list of strains
 - Better if a protocol is not one lab protocol but a protocol validated by several laboratories with ring tests
 - Make more validated protocols which would be robust in different laboratories
- ◆ Possibility to share/centralize some tests when not carried out in one country (already possible)
- ◆ Propose a resistance testing group in UPOV



General conclusion on this project

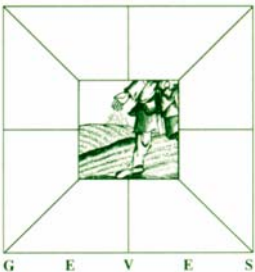
- ◆ This project allowed to:
 - define isolates
 - define R and S controls
 - define notation scales and classes of resistance which would not be identified by markers
 - Propose updated robust protocols validated in different laboratories
- ◆ A lot of questions arised from this project:
 - I solate identification
 - Varieties availability
 - Differentials identification and availability
- ◆ Many things to discover on other protocols not yet harmonized



Future of harmonization of resistance tests in EU?

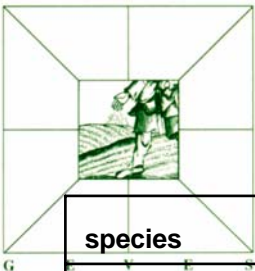
123

- ◆ 2 species and 7 pathogens tested
 - 4 harmonized
 - 2 need confirmation for isolate identification
 - 1 need one year more harmonization
- ◆ An harmonization of protocols is necessary to obtain validated protocols and more robust methods
 - Ex of seed health testing protocols validated by ISHI and ISTA
- ◆ Other interesting?



Possible models

species	pathogen	exist CPVO?	Compulsory?	Comments	interest to harmonize ?
bean	CI race Kappa	yes	no		
	Xap	yes	no		
tomato	Meloidogyne	yes	yes	different notation scale possible, intermediate levels	
	FORL	yes	no		
	Ff	yes	no	different races	
	Pi	yes	no		
	PI	yes	no		
	S	yes	no		
	Pst	yes	no		
	Rs	yes	no		
	TYLCV	yes	no		
	TSWV	yes	no		
	Lt	yes	no		
	OI	yes	no		



Possible models

species	pathogen	exist CPVO?	Compulsory?	Comments	interest ?
Lettuce	Bl	yes	yes not all races	strains and differentials OK, differences in notation dates, number of plants	
	LMV	yes	no		
	Nr	yes	no		
Pea	Fop	yes	yes for race 1		
	Ep	yes	no	field, many races	
	Ap	yes	no		
	Psp	yes	no		
	PSbMV	yes	no		
	BYMV	yes	no		
	PEMV	yes	no		
Melon	Fom	yes	yes for races 0,1,2		
	Ag	yes	no		
	ZYMV	yes	no		
	PRSV	yes	no		
	MNSV	yes	no		
	Sf (Px)	yes	no		
	Ec (Gc)	yes	no		
Pepper	Tobamoviruses	yes	yes for ToMV 0		
	PVY	yes	no		
	Pc	yes	no		



Future of harmonization of resistance tests in EU?

126

◆ Proposals for 2007:

- Follow up 2006
 - Confirm CI, BCMNV and Psp strains
 - New ring test for Psp
- New projects
 - Workshop to explain our work on resistance tests
 - Ring test with more members states with 7 pathogens
 - New project with a maximum of 6 pathogens
 - We would like CPVO to support these new project

Detailed results FOL race 0 / tomato

127

Species: TOMATO

Country : France

Year : 2006

Isolate: FR

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total number of plants			Conclusion	Number of plants with > 3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	11/15	8/14	19/29	4/15	6/14	10/29	S	3/15	6/14	9/29	S	4	3,75		?
2	Larissa	0/14	0/15	0/29	0/14	0/15	0/29	R	0/14	0/15	0/29	R				?
3	Marmande verte	11/14	9/13	20/27	3/14	4/13	7/27	S	3/14	0/13	3/27	S	3,5	2,5		?
4	Marporum	0/15	0/15	0/30	2/15	1/15	3/30	R?	0/15	0/15	0/30	R	2	1		?
5	Motelle	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R				?
6	MarporumXMarmande verte	2/15	0/15	2/30	5/15	6/15	11/30	S?	2/15	1/15	3/30	R?	2,7	2,5		?
7	Resal	13/14	13/15	26/29	1/14	2/15	3/29	S	1/14	2/15	3/29	S	4	4,5		?
8	Ranco	3/15	3/14	6/29	13/15	6/14	19/29	S?	10/15	5/14	15/29	S?	4,9	3,9		?
9	Gourmet	0/15	0/14	0/29	0/15	0/14	0/29	R	0/15	0/14	0/29	R				?
10	Mohawk	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R				?

Isolate: NL

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total number of plants			Conclusion	Number of plants with > 3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	14/14	11/14	25/28	0/14	2/14	2/28	S	0/14	1/14	1/28	S		2,25		
2	Larissa	3/15	0/15	3/30	4/15	1/15	5/30	R?	1/15	0/15	1/30	R?	2,3	1		
3	Marmande verte	15/15	15/15	30/30	0/15	0/15	0/30	S	0/15	0/15	0/30	S				
4	Marporum	6/15	7/15	13/30	0/15	0/15	0/30	R?	0/15	0/15	0/30	R?		0,5		
5	Motelle	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R				
6	MarporumXMarmande verte	7/15	6/15	13/30	7/15	5/15	12/30	S?	2/15	1/15	3/30	S?	3,07	2		
7	Resal	14/14	12/14	26/28	0/14	2/14	2/28	S	0/14	0/14	0/28	S		2,25		
8	Ranco	14/14	12/14	26/28	0/14	2/14	2/28	S	0/14	0/14	0/28	S		2		
9	Gourmet	0/14	0/14	0/28	0/14	0/14	0/28	R	0/14	0/14	0/28	R		0,5		
10	Mohawk	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R				

Isolate: ES

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total number of plants			Conclusion	Number of plants with > 3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	14/14	14/15	28/29	0/14	1/15	1/29	S	0/14	1/15	1/29	S		4,5		?
2	Larissa	0/15	0/13	0/28	0/15	0/13	0/28	R	0/15	0/13	0/28	R				?
3	Marmande verte	15/15	13/14	28/29	0/15	1/14	1/29	S	0/15	1/14	1/29	S		4		?
4	Marporum	3/15	0/15	3/30	0/15	0/15	0/30	R?	0/15	0/15	0/30	R?				?
5	Motelle	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R				?
6	MarporumXMarmande verte	2/15	1/15	3/30	11/15	7/15	18/30	S?	0/15	1/15	1/30	R?	1,77	2,4		?
7	Resal	11/15	10/15	21/30	4/15	4/15	8/30	S	2/15	3/15	5/30	S?	2,75	5		?
8	Ranco	6/15	10/14	16/29	4/15	3/14	7/29	S	0/15	1/14	1/29	S?	1,25	3,17		?
9	Gourmet	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R				?
10	Mohawk	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R				?

...Detailed results FOL race 0 / tomato

128

Country : The Netherlands

Year : 2006

Isolate: FR

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total number of plants			Conclusion	Number of plants with > 2 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	nt	nt	nt	nt	nt	nt	nt	23	22	45/46	S	nt	nt	nt	nt
2	Larissa	nt	nt	nt	nt	nt	nt	nt	0	0	0/43	R	nt	nt	nt	nt
3	Marmande verte	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
4	Marporum	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
5	Motelle	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
6	MarporumXMarmande verte	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
7	Resal	nt	nt	nt	nt	nt	nt	nt	21	23	44/46	S	nt	nt	nt	nt
8	Ranco	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
9	Gourmet	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
10	Mohawk	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt

Isolate: NL

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total number of plants			Conclusion	Number of plants with > 2 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	nt	nt	nt	nt	nt	nt	nt	23	22	46/46	S	nt	nt	nt	nt
2	Larissa	nt	nt	nt	nt	nt	nt	nt	0	0	0/40	R	nt	nt	nt	nt
3	Marmande verte	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
4	Marporum	nt	nt	nt	nt	nt	nt	nt	0	2	2/46	R?	nt	nt	nt	nt
5	Motelle	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
6	MarporumXMarmande verte	nt	nt	nt	nt	nt	nt	nt	4	2	6/46	R?	nt	nt	nt	nt
7	Resal	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
8	Ranco	nt	nt	nt	nt	nt	nt	nt	1	2	3/46	R?	nt	nt	nt	nt
9	Gourmet	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
10	Mohawk	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt

Isolate: ES

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total number of plants			Conclusion	Number of plants with > 2 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
2	Larissa	nt	nt	nt	nt	nt	nt	nt	0	0	0/36	R	nt	nt	nt	nt
3	Marmande verte	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
4	Marporum	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
5	Motelle	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
6	MarporumXMarmande verte	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
7	Resal	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
8	Ranco	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
9	Gourmet	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
10	Mohawk	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt

...Detailed results FOL race 0 / tomato

129

Country : Spain

Year : 2006

Isolate: FR

Varieties		Dead plants/ Total			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with >3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	8/15	11/15	19/30	nt	nt	nt	nt	7/15	4/15	11/30	S	nt	nt	nt	nt
2	Larissa	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
3	Marmande Verte	8/15	12/15	20/30	nt	nt	nt	nt	7/15	3/15	10/30	S	nt	nt	nt	nt
4	Marporum	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
5	Motelle	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
6	Marporum x Marmande Verte	0/15	0/15	0/30	nt	nt	nt	nt	0/15	3/15	3/30	R?	nt	nt	nt	nt
7	Resal	4/15	12/15	16/30	nt	nt	nt	nt	11/15	2/15	13/30	S	nt	nt	nt	nt
8	Ranco	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
9	Gourmette	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
10	Mohawk	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt

resistant:plants with red vessels under cotyledons or plants with < 3 cm red vessels

Isolate: NL

Varieties		Dead plants/ Total			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with >3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	14/15	13/15	27/30	nt	nt	nt	nt	1/15	2/15	3/30	S	nt	nt	nt	nt
2	Larissa	0/14	0/15	0/29	nt	nt	nt	nt	1/14	2/15	3/29	R?	nt	nt	nt	nt
3	Marmande Verte	13/15	14/15	27/30	nt	nt	nt	nt	1/15	1/15	2/30	S	nt	nt	nt	nt
4	Marporum	0/15	0/15	0/30	nt	nt	nt	nt	3/15	3/15	6/30	R?	nt	nt	nt	nt
5	Motelle	0/14	0/15	0/29	nt	nt	nt	nt	0/14	0/15	0/29	R	nt	nt	nt	nt
6	Marporum x Marmande Verte	3/15	0/15	3/30	nt	nt	nt	nt	9/15	8/15	17/30	S?	nt	nt	nt	nt
7	Resal	15/15	15/15	30/30	nt	nt	nt	nt	0/15	0/15	0/30	S	nt	nt	nt	nt
8	Ranco	7/15	5/15	12/30	nt	nt	nt	nt	5/15	5/15	10/30	S?	nt	nt	nt	nt
9	Gourmette	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
10	Mohawk	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt

Isolate: ES

Varieties		Dead plants/ Total			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with >3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Marmande	15/15	14/15	29/30	nt	nt	nt	nt	0/15	1/15	1/30	S	nt	nt	nt	nt
2	Larissa	0/13	0/15	0/28	nt	nt	nt	nt	2/13	1/15	3/28	R?	nt	nt	nt	nt
3	Marmande Verte	7/15	13/15	20/30	nt	nt	nt	nt	7/15	2/15	9/30	S	nt	nt	nt	nt
4	Marporum	0/15	0/15	0/30	nt	nt	nt	nt	8/15	3/15	11/30	S?	nt	nt	nt	nt
5	Motelle	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
6	Marporum x Marmande Verte	0/15	0/15	0/30	nt	nt	nt	nt	10/15	11/15	21/30	S?	nt	nt	nt	nt
7	Resal	0/15	12/15	12/30	nt	nt	nt	nt	14/15	3/15	17/30	S	nt	nt	nt	nt
8	Ranco	0/15	0/15	0/30	nt	nt	nt	nt	14/15	10/15	24/30	S?	nt	nt	nt	nt
9	Gourmette	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
10	Mohawk	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt

: result different from what expected

Detailed results FOL race 1 / tomato

130

Species: TOMATO

Country : France

Isolate: FR

Year : 2006

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with > 3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	9/15	13/15	22/30	6/15	2/15	8/30	S	1/15	1/15	2/30	S?	1,69	2,7	2,195	
2	Odisea	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
3	Marporum	6/15	4/15	10/30	9/15	10/15	19/30	S	4/15	5/15	9/30	S?	3,12	3,34	3,23	
4	Marmande verte	5/15	7/15	12/30	9/15	8/15	17/30	S	4/15	2/15	6/30	S?	3,27	2,71	2,99	
5	Motelle	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	8/15	10/15	18/30	6/15	5/15	11/30	S	2/15	1/15	3/30	S?	2,44	2,234	2,337	
8	Ranco	12/15	13/15	25/30	3/15	2/15	5/30	S	1/15	1/15	2/30	S?	2,57	2,75	2,66	
9	Mohawk	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
10	Tradiro	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	

Isolate: NL

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with > 3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	8/15	11/15	19/30	6/15	1/15	7/30	S?	1/15	1/15	2/30	S?	2,03	4,5	3,27	
2	Odisea	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
3	Marporum	2/14	5/14	7/28	9/14	8/14	17/28	S?	1/14	2/14	3/28	S?	2,15	2,35	2,25	
4	Marmande verte	4/14	4/13	8/27	9/14	8/13	17/27	S?	1/14	3/13	4/27	S?	2,43	2,91	2,67	
5	Motelle	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	5/14	3/15	8/29	7/14	6/15	13/29	S?	0/14	1/15	1/29	S?	1,63	1,66	1,65	
8	Ranco	9/15	5/14	14/29	4/15	4/14	8/29	S?	2/15	4/15	6/30	S?	3,34	4,3	3,82	
9	Mohawk	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
10	Tradiro	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	

Isolate: ES

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with > 3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	12/15	9/15	21/30	2/15	4/15	6/30	S?	0/15	2/15	2/30	S?	2,75	3,38	3,065	
2	Odisea	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
3	Marporum	1/15	8/15	9/30	10/15	2/15	12/30	S?	2/15	0/15	2/30	S?	2,49	2,9	2,695	
4	Marmande verte	0/13	5/15	5/28	11/13	8/15	19/28	S?	0/13	5/15	5/28	S?	2,21	3,18	2,695	
5	Motelle	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	0	0	0	
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	0/15	2/15	2/30	8/15	4/15	12/30	S?	2/15	0/15	2/30	S?	2,73	1,16	1,945	
8	Ranco	8/15	7/15	15/30	4/15	6/15	10/30	S?	2/15	4/15	6/30	S?	2,1	3,62	2,86	
9	Mohawk	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	1/15	1/30	R	0	3,5	1,75	
10	Tradiro	0/15	0/15	0/30	0/15	0/15	0/30	R	0/15	0/15	0/30	R	2,5	0	1,25	

...Detailed results FOL race 1 / tomato

131

Country : The Netherlands

Year : 2006

Isolate: FR

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with > 2 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
2	Odisea	nt	nt	nt	nt	nt	nt	nt	0	0	0/29	R	nt	nt	nt	nt
3	Marporum	nt	nt	nt	nt	nt	nt	nt	21	23	44/46	S?	nt	nt	nt	nt
4	Marmande verte	nt	nt	nt	nt	nt	nt	nt	23	20	43/46	S?	nt	nt	nt	nt
5	Motelle	nt	nt	nt	nt	nt	nt	nt	0	0	0/45	R	nt	nt	nt	nt
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	nt	nt	nt	nt	nt	nt	nt	23	23	45/46	S?	nt	nt	nt	nt
8	Ranco	nt	nt	nt	nt	nt	nt	nt	23	21	44/46	S?	nt	nt	nt	nt
9	Mohawk	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
10	Tradiro	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt

Isolate: NL

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with > 2 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	nt	nt	nt	nt	nt	nt	nt	23	23	46/46	S	nt	nt	nt	nt
2	Odisea	nt	nt	nt	nt	nt	nt	nt	0	0	0/31	R	nt	nt	nt	nt
3	Marporum	nt	nt	nt	nt	nt	nt	nt	14	21	35/46	S?	nt	nt	nt	nt
4	Marmande verte	nt	nt	nt	nt	nt	nt	nt	21	22	43/46	S?	nt	nt	nt	nt
5	Motelle	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	nt	nt	nt	nt	nt	nt	nt	22	23	45/46	S?	nt	nt	nt	nt
8	Ranco	nt	nt	nt	nt	nt	nt	nt	18	19	37/46	S?	nt	nt	nt	nt
9	Mohawk	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
10	Tradiro	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt

Isolate: ES

Varieties		Dead plants/Total number of plants			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with > 2 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	nt	nt	nt	nt	nt	nt	nt	8	20	28/46	S?	nt	nt	nt	nt
2	Odisea	nt	nt	nt	nt	nt	nt	nt	0	0	0/27	R	nt	nt	nt	nt
3	Marporum	nt	nt	nt	nt	nt	nt	nt	15	17	32/46	S?	nt	nt	nt	nt
4	Marmande verte	nt	nt	nt	nt	nt	nt	nt	7	20	27/46	S?	nt	nt	nt	nt
5	Motelle	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	nt	nt	nt	nt	nt	nt	nt	12	20	32/46	S?	nt	nt	nt	nt
8	Ranco	nt	nt	nt	nt	nt	nt	nt	3	12	15/46	S?	nt	nt	nt	nt
9	Mohawk	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt
10	Tradiro	nt	nt	nt	nt	nt	nt	nt	0	0	0/46	R	nt	nt	nt	nt

...Detailed results FOL race 1 / tomato

132

Country : Spain

Year : 2006

Isolate: FR

Varieties		Dead plants/ Total			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with >3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	15/15	14/15	29/30	nt	nt	nt	nt	0/15	1/15	1/15	S	nt	nt	nt	nt
2	Odisea	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/15	R	nt	nt	nt	nt
3	Marporum	15/15	15/15	30/30	nt	nt	nt	nt	0/15	0/15	0/30	S	nt	nt	nt	nt
4	Marmande verte	15/15	15/15	30/30	nt	nt	nt	nt	0/15	0/15	0/30	S	nt	nt	nt	nt
5	Motelle	0/13	0/15	0/28	nt	nt	nt	nt	0/13	0/15	0/28	R	nt	nt	nt	nt
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	15/15	14/15	29/30	nt	nt	nt	nt	0/15	1/15	1/30	S	nt	nt	nt	nt
8	Ranco	15/15	15/15	30/30	nt	nt	nt	nt	0/15	0/15	0/30	S	nt	nt	nt	nt
9	Mohawk	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
10	Tradiro	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt

Isolate: NL

Varieties		Dead plants/ Total			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with >3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	15/15	13/15	28/30	nt	nt	nt	nt	0/15	2/15	2/30	S	nt	nt	nt	nt
2	Odisea	0/13	0/14	0/27	nt	nt	nt	nt	0/13	1/14	1/27	R	nt	nt	nt	nt
3	Marporum	11/15	8/15	19/30	nt	nt	nt	nt	4/15	7/15	11/30	S	nt	nt	nt	nt
4	Marmande verte	9/15	11/15	20/30	nt	nt	nt	nt	6/15	4/15	10/30	S	nt	nt	nt	nt
5	Motelle	0/11	0/15	0/26	nt	nt	nt	nt	0/11	0/15	0/26	R	nt	nt	nt	nt
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	13/15	13/15	26/30	nt	nt	nt	nt	2/15	2/15	4/30	S	nt	nt	nt	nt
8	Ranco	0/15	0/14	0/29	nt	nt	nt	nt	0/15	0/14	0/29	R	nt	nt	nt	nt
9	Mohawk	0/13	0/14	0/27	nt	nt	nt	nt	0/13	0/14	0/27	R	nt	nt	nt	nt
10	Tradiro	9/15	4/15	13/30	nt	nt	nt	nt	6/15	11/15	17/30	S	nt	nt	nt	nt

Isolate: ES

Varieties		Dead plants/ Total			Number of plants with red vessels above cotyledons/ Total			Conclusion	Number of plants with >3 cm of red vessels / Total number of plants			Conclusion	mean length of red vessels in cm			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL		REP 1	REP 2	TOTAL	
1	Roma	15/15	15/15	30/30	nt	nt	nt	nt	0/15	0/15	0/30	S	nt	nt	nt	nt
2	Odisea	0/13	0/15	0/28	nt	nt	nt	nt	0/13	0/15	0/28	R	nt	nt	nt	nt
3	Marporum	8/15	15/15	23/30	nt	nt	nt	nt	7/15	0/15	7/30	S	nt	nt	nt	nt
4	Marmande verte	14/15	15/15	29/30	nt	nt	nt	nt	1/15	0/15	1/30	S	nt	nt	nt	nt
5	Motelle	0/15	0/13	0/28	nt	nt	nt	nt	0/15	0/13	0/28	R	nt	nt	nt	nt
6	Motelle x Marmande verte	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt
7	Cherry belle	10/15	6/15	16/30	nt	nt	nt	nt	5/15	9/15	14/30	S	nt	nt	nt	nt
8	Ranco	13/15	14/15	27/30	nt	nt	nt	nt	2/15	1/15	3/30	S	nt	nt	nt	nt
9	Mohawk	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt
10	Tradiro	0/15	0/15	0/30	nt	nt	nt	nt	0/15	0/15	0/30	R	nt	nt	nt	nt

resistant:plants with red vessels under cotyledons or plants with < 3 cm red vessels

: result different from what expected

Pathogen: ToMV race 0

Country : France
Isolate: FR

Year : 2006

		Number of plants with mosaic / Total number of plants			Number of plants with necrosis / Total number of plants			Number of plants with mosaics and necrosis / Total number of plants			Conclusion
Varieties	Name	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	
1	Marmande	14/14	14/14	28/28	0/14	0/14	0/28	0/14	0/14	0/28	S
2	Dorina	0/13	0/15	0/28	9/13	8/15	17/28	0/13	0/15	0/28	R Heteroz
3	Monalbo	14/14	14/14	28/28	0/14	0/14	0/28	0/14	0/14	0/28	S
4	Momor	0/12	0/14	0/26	1/12	0/14	1/26	0/12	0/14	0/26	R
5	MonalboXMomor	0/12	0/12	0/24	11/12	10/12	21/24	0/12	0/12	0/24	R Heteroz
6	Moperou	0/15	0/14	0/29	0/15	0/14	0/29	0/15	0/14	0/29	R
7	Santa	15/15	13/13	28/28	0/15	0/13	0/28	0/15	0/13	0/28	S
8	Gourmet	0/15	0/14	0/29	0/15	0/14	0/29	0/15	0/14	0/29	R

Isolate: NL

		Number of plants with mosaic / Total number of plants			Number of plants with necrosis / Total number of plants			Number of plants with mosaics and necrosis / Total number of plants			Conclusion
Varieties	Name	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	
1	Marmande	13/13	12/12	25/25	0/13	0/12	0/25	0/13	0/12	0/25	S
2	Dorina	0/16	0/14	0/30	16/16	14/14	30/30	0/16	0/14	0/30	R Heteroz
3	Monalbo	15/15	15/15	30/30	0/15	0/15	0/30	0/15	0/15	0/30	S
4	Momor	0/15	0/14	0/29	0/15	0/14	0/29	0/15	0/14	0/29	R
5	MonalboXMomor	0/12	0/15	0/27	12/12	15/15	27/27	0/12	0/15	0/27	R Heteroz
6	Moperou	0/14	0/14	0/28	0/14	0/14	0/28	0/14	0/14	0/28	R
7	Santa	15/15	15/15	30/30	0/15	0/15	0/30	0/15	0/15	0/30	S
8	Gourmet	0/13	0/13	0/26	0/13	0/13	0/26	0/13	0/13	0/26	R

Country : The Netherlands
Isolate: FR

Year : 2006

[illegible]Isolate: NL[illegible]

...Detailed results ToMV race 0 / tomato

134

Country : Spain
Isolate: FR

Year : 2006

Varieties	Name	Number of plants with mosaic / Total number of plants			Number of plants with necrosis / Total number of plants			Number of plants with mosaics and necrosis / Total number of plants			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	
1	Marmande	15/15	15/15	30/30	0/15	0/15	0/30	0/15	0/15	0/30	S
2	Dorina	0/15	0/15	0/30	0/15	0/15	0/30	0/15	0/15	0/30	R
3	Monalbo	15/15	14/15	29/30	0/15	0/15	0/30	0/15	0/15	0/30	S
4	Momor	0/14	0/15	0/29	0/14	0/15	0/29	0/14	0/15	0/29	R
5	MonalboXMomor	0/15	0/15	0/30	0/15	3/15	3/30	0/15	0/15	0/30	R Heteroz
6	Moperou	0/15	0/15	0/30	0/15	0/15	0/30	0/15	0/15	0/30	R
7	Santa	15/15	15/15	30/30	0/15	0/15	0/30	0/15	0/15	0/30	S
8	Gourmet	0/16	0/15	0/31	0/16	0/15	0/31	0/16	0/15	0/31	R

Isolate: NL

Varieties	Name	Number of plants with mosaic / Total number of plants			Number of plants with necrosis / Total number of plants			Number of plants with mosaics and necrosis / Total number of plants			Conclusion
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	
1	Marmande	12/15	15/15	27/30	0/15	0/15	0/30	0/15	0/15	0/30	S
2	Dorina	0/15	0/15	0/30	9/15	6/15	15/30	0/15	0/15	0/30	R Hereroz
3	Monalbo	10/15	8/15	18/30	0/15	0/15	0/30	0/15	0/15	0/30	S
4	Momor	0/15	0/15	0/15	0/15	0/15	0/30	0/15	0/15	0/30	R
5	MonalboXMomor	3/15	0/15	3/30	0/15	0/15	0/30	12/15	6/15	18/30	?
6	Moperou	0/15	0/15	0/15	0/15	0/15	0/30	0/15	0/15	0/30	R
7	Santa	13/15	15/15	28/30	0/15	0/15	0/30	0/15	0/15	0/30	S
8	Gourmet	0/15	0/15	0/30	0/15	0/15	0/30	0/15	0/15	0/30	R

Detailed results *Verticillium dahliae* and *V. albo atrum* / tomato

135

Species: TOMATO

Country : France

Isolate: FR

Year : 2006

Verticillium dahliae

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	10/15	9/15	19/30	S (esc)
2	Monalbo	0/15	0/15	0/30	R
3	Monalbo x Marmande verte	1/14	0/15	1/29	R
4	Clarion	13/15	15/15	28/30	S
5	Tradiro	0/15	0/14	0/29	R
6	Clairvil	nt	nt	nt	nt
7	Elias	0/14	0/15	0/29	R
8	Flix	7/9	9/9	16/18	S

Isolate: NL

Verticillium albo atrum

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	8/14	9/15	17/29	S (esc)
2	Monalbo	0/15	0/15	0/30	R
3	Monalbo x Marmande verte	0/15	0/15	0/30	R
4	Clarion	5/15	5/15	10/30	S (esc)
5	Tradiro	0/15	0/15	0/30	R
6	Clairvil	nt	nt	nt	nt
7	Elias	0/15	0/15	0/30	R
8	Flix	9/12	10/12	19/24	S (esc)

Isolate: ES

Verticillium dahliae

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	8/15	10/15	18/30	S (esc)
2	Monalbo	3/15	2/15	5/30	R?
3	Monalbo x Marmande verte	7/13	9/15	16/28	S (esc)
4	Clarion	15/15	12/15	27/30	S
5	Tradiro	4/15	1/15	5/30	R?
6	Clairvil	nt	nt	nt	nt
7	Elias	3/15	3/15	6/30	R?
8	Flix	6/8	4/4	10/12	S (esc)

Country : The Netherlands

Year : 2006

Isolate: FR

Verticillium dahliae

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	23/23	23/23	46/46	S
2	Monalbo	0/23	0/23	0/46	R
3	bo x Marmande	0/23	0/23	0/46	R
4	Clarion	21/23	18/23	39/46	S (esc)
5	Tradiro	0/23	0/23	0/46	R
6	Clairvil	nt	nt	nt	nt
7	Elias	0/23	0/23	0/46	R
8	Flix	11/11	-	11/11	S

Isolate: NL

Verticillium albo atrum

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	22/23	23/23	45/46	S
2	Monalbo	0/23	0/23	0/46	R
3	bo x Marmande	0/23	0/23	0/46	R
4	Clarion	23/23	23/23	46/46	S
5	Tradiro	0/23	0/23	0/46	R
6	Clairvil	nt	nt	nt	nt
7	Elias	0/23	0/23	0/46	R
8	Flix	13/13	-	13/13	S

Isolate: ES

Verticillium dahliae

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	22/23	22/23	44/46	S
2	Monalbo	23/23	23/23	46/46	S
3	bo x Marmande	23/23	23/23	46/46	S
4	Clarion	21/23	21/23	42/46	S
5	Tradiro	19/23	21/23	40/46	S
6	Clairvil	nt	nt	nt	nt
7	Elias	23/23	23/23	46/46	S
8	Flix	20/20	-	20/20	S

Country : Spain

Year : 2006

Isolate: FR

Verticillium dahliae

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	15/15	15/15	30/30	S
2	Monalbo	0/15	0/15	0/30	R
3	Monalbo x Marmande verte	0/15	0/15	0/30	R
4	Clarion	15/15	15/15	30/30	S
5	Tradiro	0/15	0/15	0/30	R
6	Clairvil	nt	nt	nt	nt
7	Elias	0/15	0/15	0/30	R
8	Flix	15/15	15/15	30/30	S

Isolate: NL *Verticillium albo atrum*

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	14/15	15/15	29/30	S
2	Monalbo	13/15	4/15	17/30	S
3	Monalbo x Marmande verte	6/15	1/13	7/28	R?
4	Clarion	14/15	15/15	29/30	S
5	Tradiro	0/15	12/15	12/30	S
6	Clairvil	nt	nt	nt	nt
7	Elias	0/15	0/15	0/30	R
8	Flix	10/10		10/10	S

Isolate: ES

Verticillium dahliae

Varieties	Name	Number of diseased plants/ Total number of plants			Conclusion
		Notation :			
		REP 1	REP 2	TOTAL	
1	Marmande verte	15/15	12/15	27/30	S
2	Monalbo	15/15	14/15	29/30	S
3	Monalbo x Marmande verte	2/15	3/14	5/29	R?
4	Clarion	11/15	3/15	14/30	S (esc)
5	Tradiro	3/15	5/16	8/31	R?
6	Clairvil	nt	nt	nt	nt
7	Elias	2/15	3/15	5/30	R?
8	Flix	13/15	8/11	21/26	S (esc)

Detailed results *Colletotrichum lindemuthianum* / bean

137

Species: Bean
Country : France

Year : 2006

Isolate: FR Delta + Gamma

		Inoculation by soaking seeds									Inoculation by spraying cotyledons			
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion
		Notation scale:				Notation scale:						REP 1	REP 2	
		0	1	2	3	0	1	2	3					
1	Goldrush			7	7				15	92	S	14/14	15/15	S
2	Pastoral	15				15				0	R	0/16	0/15	R
3	Michelet			15				14		67	S	15/15	12/12	S
4	Booster	8				15				0	R	0/15	0/13	R
5	Tuf		2	13			3	12		61	S	15/15 *	15/15 *	S
6	Talisman	15				15				0	R	0/15	0/15	R
7	Masai			14	1			15		68	S	15/15	15/15	S

Notation scale:

0: no symptoms

1: weak reaction with small superficial necrosis (dots or stripes)

2: deeply sunken necrotic flecks on hypocotyl or stem or strong reaction with necrosis larger

3: dying plants

* weak symptoms

Resistant: note 0, 1, disease index <50%

Susceptible: note 2,3, disease index >50%

Disease index: ((nb plants 0 *0)+(nb plants 1*1)+(nb plants 2*2)+(nb plants 3*3)/(nb tot plants*3))*100

Isolate: NL Lambda 418

		Inoculation by soaking seeds										Inoculation by spraying cotyledons			
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion	
		Notation scale:				Notation scale:						REP 1	REP 2		
		0	1	2	3	0	1	2	3						
1	Goldrush			4		0		5		67	S	1/15	0/15	S?	
2	Pastoral	15				15				0	R	0/15	0/15	R	
3	Michelet									#DIV/0!	nt	1/15	2/15	S?	
4	Booster	15				15				0	R	0/15	0/15	R	
5	Tuf	13	2				2	1		11	S?	0/15	1/15	S?	
6	Talisman	15				15				0	R	0/15	0/15	R	
7	Masai	12	3			13	1			5	R	0/15	0/15	R	

Isolate: ES Race 6

		Inoculation by soaking seeds									Inoculation by spraying cotyledons			
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion
		Notation scale:				Notation scale:						REP 1	REP 2	
		0	1	2	3	0	1	2	3					
1	Goldrush				9					100	S	15/15	15/15	S
2	Pastoral	15				15				0	R	1/15	0/15	R
3	Michelet			4	8			4	10	90	S	15/15	15/15	S
4	Booster	15				12	1			1	R	0/15	0/15	R
5	Tuf	1		6	6		1	8	5	77	S	11/15	13/15	S
6	Talisman	15				12	3			3	R	0/15	0/15	R
7	Masai				6				3	100	S	15/15	15/15	S

...Detailed results *Colletotrichum lindemuthianum* / bean

138

Country : The Netherlands

Year : 2006

Isolate: FR

		Inoculation by soaking seeds										Inoculation by spraying cotyledons			
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion	
		Notation scale:				Notation scale:						REP 1	REP 2		
		0	1	2	3	0	1	2	3						
1	Goldrush	10		13		8		15		41	S?	nt	nt	nt	
2	Pastoral	23				23				0	R	nt	nt	nt	
3	Michelet	14	3	6		15	1	8		23	S?	nt	nt	nt	
4	Booster	23				23				0	R	nt	nt	nt	
5	Tuf	4		19		9		12		47	S?	nt	nt	nt	
6	Talisman	23				23				0	R	nt	nt	nt	
7	Masai	9	1	13		11	2	10		36	S?	nt	nt	nt	

Isolate: NL

		Inoculation by soaking seeds								Inoculation by spraying cotyledons				
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion
		Notation scale:				Notation scale:						REP 1	REP 2	
		0	1	2	3	0	1	2	3					
1	Goldrush	11	6	6		8	6	7		29	S?	nt	nt	nt
2	Pastoral	23				23				0	R	nt	nt	nt
3	Michelet	22	1			22	1			1	R	nt	nt	nt
4	Booster	22				23				0	R	nt	nt	nt
5	Tuf	23				23				0	R	nt	nt	nt
6	Talisman	23				23				0	R	nt	nt	nt
7	Masai	21	2			18	5			5	R	nt	nt	nt

Isolate: ES

		Inoculation by soaking seeds								Inoculation by spraying cotyledons				
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion
		Notation scale:				Notation scale:						REP 1	REP 2	
		0	1	2	3	0	1	2	3					
1	Goldrush	1		22				23		65	S	nt	nt	nt
2	Pastoral	23				23				0	R	nt	nt	nt
3	Michelet			23		1		22		65	S	nt	nt	nt
4	Booster	24				23				0	R	nt	nt	nt
5	Tuf	7		16		3		20		52	S?	nt	nt	nt
6	Talisman	23				18	5			4	R	nt	nt	nt
7	Masai	11		11		4		19		44	S?	nt	nt	nt

...Detailed results *Colletotrichum lindemuthianum* /bean

139

Country : Spain

Year : 2006

Isolate: FR

		Inoculation by soaking seeds									Inoculation by spraying cotyledons			
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion
		Notation scale:				Notation scale:						REP 1	REP 2	
		0	1	2	3	0	1	2	3					
1	Goldrush			9	6			12	3	77	S	nt	nt	nt
2	Pastoral	9	3			11	1			6	R	nt	nt	nt
3	Michelet			2	11			2	11	95	S	nt	nt	nt
4	Booster	12				12				0	R	nt	nt	nt
5	Tuf			12	3			13		70	S	nt	nt	nt
6	Talisman	8	1	2		10	1	1		12	R?	nt	nt	nt
7	Masai			14				13	2	69	S	nt	nt	nt

Isolate: NL

Isolate. NL		Inoculation by soaking seeds								Inoculation by spraying cotyledons				
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion
		Notation scale:				Notation scale:						REP 1	REP 2	
		0	1	2	3	0	1	2	3					
1	Goldrush		1	2	3	0	1	2	3	88	S	nt	nt	nt
2	Pastoral	11				13				0	R	nt	nt	nt
3	Michelet		1	9	1	1		5	4	70	S	nt	nt	nt
4	Booster	11				11				0	R	nt	nt	nt
5	Tuf	3	2	3			1	4	2	51	S?	nt	nt	nt
6	Talisman	11	1			11	1			3	R	nt	nt	nt
7	Masai		4	5		6	3	1		33	S?	nt	nt	nt

Isolate: ES

		Inoculation by soaking seeds								Inoculation by spraying cotyledons				
Varieties	Name	REP 1				REP 2				Disease index	Conclusion	Number of diseased plants/ Total number of plants		Conclusion
		Notation scale:				Notation scale:						REP 1	REP 2	
		0	1	2	3	0	1	2	3					
1	Goldrush			2	3			2	3	100	S	nt	nt	nt
2	Pastoral	11		1		12				3	R?	nt	nt	nt
3	Michelet			12				1	11	82	S	nt	nt	nt
4	Booster	9	1			12				2	R	nt	nt	nt
5	Tuf			2	10					94	S	nt	nt	nt
6	Talisman	2	3	5			2	7		51	S?	nt	nt	nt
7	Masai				12				13	100	S	nt	nt	nt

: result different from what expected

Detailed results *BCMV BCMNV* /bean

Species: Bean

Country : France

Year : 2006

Isolate: FR BCMNV

Varieties	Name	Total number of observed plants			Number of plants with local necrosis			Number of plants with top necrosis			Number of plants with mosaic			Conclusion	Comments
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		
1	Aneto (SP)	12	17	29	0	0	0	0	0	0	0	0	0	R?	very weak symptoms
2	Dufrix	17	12	29	0	0	0	0	0	0	17	12	29	S	
3	Booster	17	12	29	0	0	0	17	12	29	0	0	0	R	
4	Odessa	18	11	29	0	0	0	18	11	29	0	0	0	R	
5	Aneto (NL)	17	12	29	0	0	0	0	0	0	17	12	29	S	very weak symptoms
6	Flandria	12	18	30	0	0	0	0	0	0	12	18	30	S	curled leaf and mosaic
7	Bizet	17	12	29	0	0	0	1	0	1	0	0	0	R	

Resistant: no symptoms or top necrosis

Susceptible: mosaics

Isolate: NL BCMNV

Varieties	Name	Total number of observed plants			Number of plants with local necrosis			Number of plants with top necrosis			Number of plants with mosaic			Conclusion	Comments
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		
1	Aneto (SP)	18	11	29	0	0	0	0	0	0	18	11	29	S	very weak symptoms
2	Dufrix	18	12	30	0	0	0	0	0	0	18	12	30	S	
3	Booster	17	12	29	0	0	0	0	0	0	0	0	0	R	
4	Odessa	17	12	29	17	12	29	0	0	0	0	0	0	R	
5	Aneto (NL)	18	12	30	0	0	0	0	0	0	18	12	30	S	very weak symptoms
6	Flandria	19	11	30	0	0	0	0	0	0	19	11	30	S	curled leaf and mosaic
7	Bizet	18	11	29	0	0	0	0	2	2	0	0	0	R	

Country : The Netherlands

Year : 2006

Isolate: FR BCMNV

Varieties	Name	Total number of observed plants			Number of plants with local necrosis			Number of plants with top necrosis			Number of plants with mosaic			Conclusion	Comments
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		
1	Aneto (SP)	22	22	44	22	22	44				22	22	44	S	chlorosis, not mosaic
2	Dufrix	22	22	44	22	22	44				22	22	44	S	
3	Booster	22	22	44	0	0	0	22	22	44				R	
4	Odessa	22	22	44				21	19	40	0	0	0	R	NB: escapes have weak TN later
5	Aneto (NL)	--	--	--	--	--	--	--	--	--	--	--	--	--	
6	Flandria	22	22	44							22	22	44	S	
7	Bizet	22	22	44				0	0		0	0		R	

Isolate: NL BCMNV

Varieties	Name	Total number of observed plants			Number of plants with local necrosis			Number of plants with top necrosis			Number of plants with mosaic			Conclusion	Comments
		REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL	REP 1	REP 2	TOTAL		
1	Aneto (SP)	22	22	44	22	22	44				22	22	44	S	chlorosis, not mosaic
2	Dufrix	22	22	44	22	22	44				22	22	44	S	
3	Booster	22	22	44	22	22	44	22	22	44	0	0		R	
4	Odessa	22	22	44				22	22	44	0	0	0	R	
5	Aneto (NL)	--	--	--	--	--	--	--	--	--	--	--	--	--	
6	Flandria	22	22	44							22	22	44	S	
7	Bizet	22	22	44				2	0	2	0	0		R	

...Detailed results *BCMV BCMNV* / bean

141

Country : Spain

Year : 2006

Isolate: FR BCMNV

Varieties	Name	Total number of observed plants				Number of plants with local necrosis				Number of plants with top necrosis			
		REP 1	REP 2	REP 3	TOTAL	REP 1	REP 2	REP 3	TOTAL	REP 1	REP 2	REP 3	TOTAL
1	Aneto (SP)	7	9	9	25	0	0	0	0	0	0	0	0
2	Dufrix	10	9	8	27	0	0	0	0	0	0	0	0
3	Booster	10	11	0	21	1	1	0	2	9	10	0	19
4	Odessa	9	10	0	19	3	6	0	9	6	4	0	10
5	Aneto (NL)	9	8	6	23	0	0	0	0	0	0	0	0
6	Flandria	7	7	7	21	0	0	0	0	0	0	0	0
7	Bizet	9	8	8	25	0	0	0	0	0	0	0	0

Varieties	Name	Number of plants with mosaic				Conclusion	ELISA
		REP 1	REP 2	REP 3	TOTAL		
1	Aneto (SP)	7	9	9	25	S	pos.
2	Dufrix	8	8	7	23	S	pos.
3	Booster	0	0	0	0	R	neg.
4	Odessa	0	0	0	0	R	neg.
5	Aneto (NL)	0	0	1	1	?	pos.
6	Flandria	7	6	6	19	S	pos.
7	Bizet	0	0	0	0	R	neg.

Isolate: NL BCMNV

Varieties	Name	Total number of observed plants				Number of plants with local necrosis				Number of plants with top necrosis			
		REP 1	REP 2	REP 3	TOTAL	REP 1	REP 2	REP 3	TOTAL	REP 1	REP 2	REP 3	TOTAL
1	Aneto (SP)	7	8	9	24	0	0	0	0	0	0	0	0
2	Dufrix	9	9	9	27	0	0	0	0	0	0	0	0
3	Booster	8	8	8	24	8	8	8	24	0	0	0	0
4	Odessa	9	9	9	27	9	8	9	26	0	0	0	0
5	Aneto (NL)	8	10	8	26	0	0	0	0	0	0	0	0
6	Flandria	9	9	7	25	0	0	0	0	0	0	0	0
7	Bizet	9	9	7	25	0	0	1	1	1	0	0	1

Varieties	Name	Number of plants with mosaic				Conclusion	ELISA
		REP 1	REP 2	REP 3	TOTAL		
1	Aneto (SP)	7	8	9	24	S	pos.
2	Dufrix	9	9	9	27	S	pos.
3	Booster	0	0	0	0	R	neg.
4	Odessa	0	0	0	0	R	neg.
5	Aneto (NL)	0	0	0	0	?	pos.
6	Flandria	9	9	7	25	S	pos.
7	Bizet	0	0	0	0	R	neg.

: result different from what expected

Detailed results *Pseudomonas savastanoi* pv *phaseolicola* / bean

142

Species: Bean

Country : France

Year : 2006

Date sowing:

19/05/2006

Date inoculation:

13/06/2006 et 27/06/2006

Date notation:

21/07/2006

Isolate: FR

Field test: on leaves

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	ASTUN	2	2	1	2		11			1		17,11	S (esc)
2	MASAI (SP)	25					27					0,00	R
3	MICHELET				9				1	3		73,08	S
4	MASAI (F)	5					15					0,00	R
5	SLENDERETTE			1	10		2	1	4	8		62,50	S
6	MASAI (NL)	10					12					0,00	R
7	VAILLANT	11					22					0,00	R

Isolate: NL

Field test: on leaves

Date notation:

21/07/2006

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	ASTUN	7	9		3		3	3		3		26,79	S (esc)
2	MASAI (SP)	24					19					0,00	R
3	MICHELET									2		75,00	S
4	MASAI (F)	11					1					0,00	R
5	SLENDRETTE		1		4		1	1		4		59,09	S
6	MASAI (NL)	9										0,00	R
7	VAILLANT	8					6					0,00	R

Isolate: ES

Field test: on leaves

Date notation:

21/07/2006

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	ASTUN	10					11					0,00	R
2	MASAI (SP)	26					27					0,00	R
3	MICHELET	4					1					0,00	R
4	MASAI (F)	5					6					0,00	R
5	SLENDRETTE	15					6					0,00	R
6	MASAI (NL)	7					5					0,00	R
7	VAILLANT	12					3					0,00	R

Notation scale:

0: red-brown necrotic reaction, or no symptom

1: few water soaked lesions on the leaf (weack attack)

2: few and small water soaked lesions (<1mm) distributed over the leaf undersurface

3: several water soaked lesions

4: water soaked lesions on the whole plant (strong attack)

...Detailed results *Pseudomonas savastanoi* pv *phaseolicola* / bean

143

Species: Bean

Country : France

Year : 2006

Date sowing: 19/05/2006

Date inoculation: 13/06/2006 et 27/06/2006

Date notation: 28/07/2006

Isolate: FR

In field : on pods

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	ASTUN			2	3		3	1	2	7		54,2	S*
2	MASAI (SP)	23					23	1	1	2		4,5	R
3	MICHELET	2			5	1				2	2	68,8	S
4	MASAI (F)	5					14	1				1,3	R
5	SLENDRETTE	1			7	2	1		1	11	2	72,0	S
6	MASAI (NL)	9	1				12					1,1	R
7	VAILLANT	8	3				11	8	3			12,9	R

* in a first trial : Astun was less susceptible

Isolate: NL

In field : on pods

Date notation:

28/07/2006

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	ASTUN	16	1	2			8		1			6,3	?
2	MASAI (SP)	23	1				18					0,6	R
3	MICHELET						1	1				12,5	S ?
4	MASAI (F)	11					2					0,0	R
5	SLENDRETTE	6			2		5			2		20,0	S?
6	MASAI (NL)	9					1					0,0	R
7	VAILLANT	8					6					0,0	R

Isolate: ES

In field : on pods

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	ASTUN	11					12					0,0	R
2	MASAI (SP)	26					25		1			1,0	R
3	MICHELET	4					1					0,0	R
4	MASAI (F)	5					6					0,0	R
5	SLENDRETTE	14	2				6					2,3	R
6	MASAI (NL)	8					4					0,0	R
7	VAILLANT	12					3					0,0	R

Slenderette was tested in a previous trial and was found susceptible to the 3 strains,

Species: Bean
Country : France

Year : 2006

Isolate: FR In greenhouse

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	Astun	1		1	3					5		65,00	S
2	Masai (SP)	6					4		1			4,55	R
3	Michelet			1	4					6		72,73	S
4	Masai (F)	6					6					0,00	R
6	Masai (NL)	6					6					0,00	R
7	Vaillant	6					6					0,00	R

Isolate: NL

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	Astun	1		1	2		1			4		55,56	S
2	Masai (SP)	6					6					0,00	R
3	Michelet	1		5					2	3		52,27	S
4	Masai (F)	6					6					0,00	R
6	Masai (NL)	5					6					0,00	R
7	Vaillant	6					6					0,00	R

Isolate: ES

VARIETIES	NAME	rep 1					rep 2					disease index	Conclusion
		0	1	2	3	4	0	1	2	3	4		
1	Astun	1			2		1		3	1		46,88	S
2	Masai (SP)	6					6					0,00	R
3	Michelet			4	2				2	2		60,00	S
4	Masai (F)	6					6					0,00	R
6	Masai (NL)	6					6					0,00	R
7	Vaillant	6					6					0,00	R

Country : The Netherlands

In greenhouse (second trial)

Year : 2006

Expected	Name	isolates		
		FR	NL	ES
S	Astun	S	S	seg
S	Michelet	SS	SS	S
S	Slenderette	SS	SS	S
R	Masai F	R	R	R
R	Masai SP	R	R	R
R	Masai NL	R	R	R
R	Vaillant	R	R	R
diff	Red Mexican UI3	S	SS	S?
diff	Tendergreen	S	S	S

Notes

SS = very susceptible, plants are dying**S = susceptible, plants continue to grow and sizeable leaf symptoms continue to develop on higher parts****S? = very few lesions; lesions bigger than pinpoint****R = resistant, pinpoint lesions with or without halo**

segregation Astun with ES isolate": rep 1 10S/12R; rep2 15 S/7R

Astuns also seemed to segregate for weak resistance within S category with FR and NL isolate

Red Mexican UI3 shows low amount of larger lesions with SP isolate, very small difference with FR isolate

Country: Spain

In climatic chamber

Date sowing: 06/09/2006

Date inoculation: 13/09/2006

Date notation: 25/09/2006

Scale of notation:

0: necrosis in the area of maximum inoculation either side of the leaf midrib (R)

1: some necrosis and water-soaking largely confined to the area of maximum inoculation (S)

2: Water-soaked lesions less than 1 mm distributed over the leaf undersurface (S)

3: Water-soaked lesions more than 1 mm distributed over the leaf undersurface (S)

Isolate: FR

VARIETIES	NAME	rep 1				rep2				OBSERV	R/S
		0	1	2	3	0	1	2	3		
1	ASTUN			4	6		3		8		S
2	MASAI (SP)	10	2			12	3				R(esc)
3	MICHELET	5			5	1			6		S(esc)
4	MASAI (F)	13				13					R
5	SLENDRETTE			3	9		1	7	3		S
6	MASAI (NL)	13	12	1		13					R

Isolate: NL

VARIETIES	NAME	rep 1				rep 2				OBSERV	R/S
		0	1	2	3	0	1	2	3		
1	ASTUN			10	1			9	1		S
2	MASAI (SP)	11				14					R
3	MICHELET			12				7	1		S
4	MASAI (F)	9	4			7	8				R(esc)
5	SLENDRETTE			13			2	10			S
6	MASAI (NL)	13	1			12	1				R

Isolate: ES

VARIETIES	NAME	rep 1				rep2				OBSERV	R/S
		0	1	2	3	0	1	2	3		
1	ASTUN	4		7			1	9			S
2	MASAI (SP)	14				12					R
3	MICHELET	2			9	3			8		S(esc)
4	MASAI (F)	12				14					R
5	SLENDRETTE	1	3		10	4	3	1	2		?

Appendix 3 : Updated protocols

**Tomato resistance
to *Fusarium oxysporum fsp. lycopersici***

Maintenance of strains

Long term storage of strains: at -80°C in 20% glycerol.

Race 0 (ex 1) represented by strains Orange 71 or PRI 20698 or Fol 071 and race 1 represented by strains 4152 (more aggressive) or PRI10195 or RAF 70 (less aggressive) are used.

Strains can be multiplied on PDA or S of Messiaen media.

Execution of test

Growth stage of plants

Plants are grown in greenhouse or growth chamber for 10 to 18 days (cotyledons to first leaf stages).

The following varieties are used as controls. Each line will be represented by at least one variety which can be chosen in the varieties indicated; the resistance phenotype to the two pathotypes of Fol is indicated. The heterozygous variety has a resistance phenotype usually weaker than in homozygous lines. This weak resistance can be used to calibrate the borderline between resistance and susceptibility. The heterozygous control for Fol 1 is optional.

<u>Controls for Fol0:</u>	Fol:0	Fol:1*
Marmande, Marmande verte, Resal	S	S
Marporum x Marmande verte (heterozygous)	R	S
Marporum, Larissa	R	S
Motelle, Gourmet, Mohawk	R	R

* For information

<u>Controls for Fol1:</u>	Fol:0*	Fol:1
Cherry Belle, Roma, Marmande verte	S	S
Ranco**, Marporum	R	S
Motelle x Marmande verte	R	R
Tradiro, Odisea	R	R

* For information

** For Ranco: weak resistance to Fol0 with many escapes

R = resistance present

S = resistance absent

Temperature:

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

Inoculum:

Fusarium oxysporum fsp. *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

Soaking of roots (cutting of roots optional) and of hypocotyls axis for 5 to 15 min in the inoculum suspension and transplantation of inoculated plantlets in soil.

Duration of test

At least 28 days from sowing to notation.

Number of plants tested:

At least 20 plants.

Notation:

At least 21 days after inoculation.

Notation scale:

4 qualitative classes:

- 0: no symptoms,
- 1: external healthy aspect of plant (without growth reduction) with brown vessels (sometimes extending above cotyledons, generally remaining below cotyledons),
- 2: growth reduction and brown vessels above cotyledons,
- 3: dead plant.

Analysis of results:

Generally R: 0 and 1, S: 2 and 3 but analysis of results should be calibrated with results of R and S controls.

Tomato resistance to ToMV race 0

Maintenance of strains

Strains are long term stored as desiccated leaves below 10°C (BOS).

Race 0 represented by isolate INRA Avignon 6-5-1-1 (aucuba strain) is used.

Virus should be multiplied on the susceptible control before being used for inoculation of the test.

Execution of test

Growth stage of plants

Plants are grown in greenhouse or growth chamber until cotyledons (first leaves emerging) to 2 expanded leaves stages.

Within each test at least one resistant and one susceptible standard variety is included.

The following varieties are used as controls. Each line will be represented by at least one variety which can be chosen in the varieties indicated; the resistance phenotype to the 3 pathotypes of ToMV is indicated. Mobaci and Moperou will allow checking the pathotype identity of the virus. Monalbo x Momor will help the interpretation of the distinct resistance phenotype with necrosis.

Controls for ToMV 0, these varieties were not validated as standard varieties for ToMV1 and 2:

Variety	Resistance phenotype		
	ToMV:0	ToMV:1	ToMV:2
Marmande, Monalbo	S	S	S
Mobaci	R	S	R
Moperou	R	R	S
Monalbo x Momor	RN	RN	RN
Momor, Gourmet	R	R	R

R = resistance present; no symptoms

RN = resistance present; a variable proportion of plants showing some or extensive necrosis; all other plants have no symptoms.

S = resistance absent; mosaic symptoms

Temperature:

Test performed in climatic chambers or greenhouse at 24 to 26°C. At higher temperatures, resistance can break.

Inoculum and method of inoculation

Mechanical inoculation by rubbing cotyledons (first leaves emerging) or 2 expanded leaves leaves with an inoculum solution consisting of symptomatic leaves grinded in a buffer with carborundum added. Leaves can be rinsed after inoculation. Light is important for symptom expression.

Duration of test

24 to 42 days from sowing to notation.

Number of plants tested:

At least 20 plants.

Notation:

12-21 days after inoculation when symptoms are well developed on susceptible control.

Notation scale and analysis of results:

R: without symptoms or with necrosis (necrosis can be observed on plants heterozygous for resistance gene, these plants are noted resistant)

S: mosaics.

**Tomato resistance
to *Verticillium dahliae***

Maintenance of strains

Race 0 (ex 1) represented by strain Toreilles 4-1-4-1 is used.
Long term storage of strains: conidia suspended in glycerol solution at -80°C.
Strain can be subcultured on PDA or S of Messiaen media.

Execution of test

Growth stage of plants

Plants are grown in greenhouse or growth chamber. Inoculation can be done from the cotyledon stage (first leaves emerging) to 2 expanded leaves stage.

The following varieties are used as controls. Each line will be represented by at least one variety which can be chosen in the varieties indicated. The heterozygous variety will help interpretation of results in case of aggressive test. Clarion could be interesting to add to susceptible controls as it is less susceptible and could help to control the aggressiveness of the test. These 2 varieties are optional.

Standard variety	Vd:0
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Marmande verte, Flix	S
Monalbo x Marmande verte	RH
Clarion	~
Monalbo, Elias	R

R	resistance present; no symptoms
RH	resistance present; sometimes very weak symptoms
~	resistance absent; weak symptoms
S	resistance absent; clear symptoms

Temperature:

Test performed under controlled conditions at 20 to 22°C .

Inoculum:

Verticillium dahliae is grown on liquid Czapek Dox Broth or S of Messiaen media for 3 to 7 days in the dark, at 20 to 25°C with shaking. Spores are harvested and adjusted to 10⁶sp/ml.

Method of inoculation

Plantlets are harvested, roots are cut and soaked for 5 to 15 min in the inoculum suspension. Plantlets are then transplanted in soil.

Duration of test

At least 33 days from sowing to notation.

Number of plants tested:

At least 20 plants.

Notation:

25-30 days after inoculation.

Notation scale and analysis of results:

R: no symptoms

S: chlorosis in the lower leaves, growth reduced and brown vessels or growth not reduced and brown vessels.

Analysis of results should be calibrated with results on R and S controls.

**Bean resistance
to *Colletotrichum lindemuthianum* (anthracnose)**

Maintenance of strains

Long term storage of strains: at -80°C in 20% glycerol

A race able to differentiate between varieties containing the Are gene, represented by strain Cl.6.A from SERIDA belonging to the race 6 is used.

Strains can be subcultured on PDA or Mathur media.

Execution of test

Growth stage of plants

For inoculation by soaking seeds:

Pregermination of seeds in Petri dishes with moistured filter paper or on vermiculite for 4-5 days.

For inoculation by spraying cotyledons:

Seeds are sown on vermiculite or blotter for 2 days and transplanted in soil for 3 days.

The following varieties are used as controls. Each line will be represented by at least one variety which can be chosen in the varieties indicated. Pastoral can be added as resistant control as it has a weaker resistance and can give an indication on aggressiveness of the test

<u>Variety</u>	<u>Resistance phenotype</u>
Goldrush, Michelet à longue cosse, Masai	S
Booster	R

S = resistance absent; deeply sunken lesions or plant death

R = resistance present; superficial lesions or no symptoms

Temperature:

Test performed in climatic chambers or greenhouse at 20 -22°C. A high humidity is important for symptoms development.

Inoculum:

Colletotrichum lindemuthianum is grown on PDA or Mathur media for 7-20 days at 20 to 25°C. Spores are harvested with a scraper and suspension is adjusted to 10⁶sp/ml .

Method of inoculation

Two methods can be used for inoculation:

By soaking seeds:

Pre-germinated seeds are soaked in the inoculum suspension for 2 min. Seeds are transplanted in soil after inoculation.

By spraying cotyledons:

5 days after sowing cotyledons are sprayed with inoculum suspension.

Duration of test

12-14 days from sowing to notation.

Number of plants tested:

At least 20 plants.

Notation:

When symptoms are well developed on S control (usually after 7 to 14 days post inoculation)

Notation scale:**For soaking seeds: 4 qualitative classes**

0: no symptoms

1: weak reaction with small superficial necrosis (dots or stripes)

2: deeply sunken necrotic flecks on hypocotyl or stem and /or strong reaction with necrosis larger than 3 mm sunk deeply into the tissue

3: dying plants

For spraying cotyledons:

No symptoms

Necrosis observed on plants (hypocotyls, stems, veins)

Analysis of results:**For soaking seeds:**

R: classes 0: no symptoms and 1: superficial lesions

S: classes 2: deeply sunken lesions and 3: plant death

For spraying cotyledons:

R: no symptoms, some flecks of necrosis can occur in the stem and some necrosis in the cotyledons.

S: deep necrosis observed on plants

Bean resistance to BCMNV

Preliminary note on the BCMV/BCMNV complex of virus species

Bean common mosaic (BCM) symptoms may be caused by two distinct virus species (BCMNV and BCMV) corresponding with serotype A (BCMNV) and B (BCMV) (Mink 1992, 1994; McKern 1992). These two viruses have been classified into seven pathogenicity groups based on their virulence pattern on a differential set of 11 varieties. Pathogenicity group VI comprises the BCMNV strains NL3 and NL5. NL3 and NL5 have the ability to induce necrosis on bean varieties with gene I. Some strains have this ability only at high temperatures. The extent of necrosis may vary from local vein necrosis to top necrosis or in extreme cases whole plant necrosis (commonly called blackroot). Higher temperatures (26-32°C) generally enhance necrosis and mosaic symptom expression compared with lower temperatures (20-25°C) (Drijfhout, 1978; Mavric and Sustar-Vozlic, 2004). “In response to NL3 strain, the I+bc1² restricts necrosis to the veins of the inoculated leaves, a symptom referred to as localized vein necrosis; I+bc2² restricts necrosis to small lesions on the inoculated leaf, a symptom referred to as local lesion necrosis” (Miklas et al, 2000).

Maintenance of BCMNV strains

Strains are long term stored as desiccated leaves below 10°C (BOS). The Pathogenicity group PG-06 represented by strains NL5 or NL3 are used. Strains should be multiplied on the susceptible control before being used for inoculation of the test.

Execution of test

Growth stage of plants

Plants are grown in greenhouse or growth chamber until the first expanded leaf stage.

The following varieties are used as controls for NL3 and NL5 strains. Each line will be represented by at least one variety which can be chosen in the varieties indicated. Within each class there may be considerable variation in the phenotypic expression of the symptoms.

Variety	Resistance phenotype NL3 or NL5
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Dufrix, Flandria	S
Booster, Odessa	RN
Bizet	R

R	= resistance present; no symptoms
RN	= resistance present with vein or top necrosis
S	= resistance absent; mosaic; leaf rolling

Temperature:

Test performed in climatic chambers or greenhouse at 25°C with an optional 5-7 days period at 30°C just after inoculation.

Method of inoculation

Mechanical inoculation by rubbing first expanded leaves with an inoculum solution consisting of symptomatic leaves grinded in a buffer with carborundum added. Leaves can be rinsed after inoculation.

Duration of test

At least 21 days from sowing to notation.

Number of plants tested:

At least 20 plants.

Notation:

When mosaic symptoms are well developed on S control (usually after 13-21 days)

Notation scale: 3 qualitative classes

1: mosaics and/or leaf rolling

2: top necrosis, or vein necrosis and/or small necrotic lesions in the leaf. Top necrosis is a systemic necrosis beginning at the apex of the plant whereas vein necrosis is a brown necrotic netting localized on veins.

3: without symptoms

Analysis of results:

S: 1: mosaics or leaf deformation

R: 2: top or vein necrosis. Top necrosis is a systemic necrosis whereas vein necrosis is a brown necrotic netting localized on veins.

R: 3: without symptoms

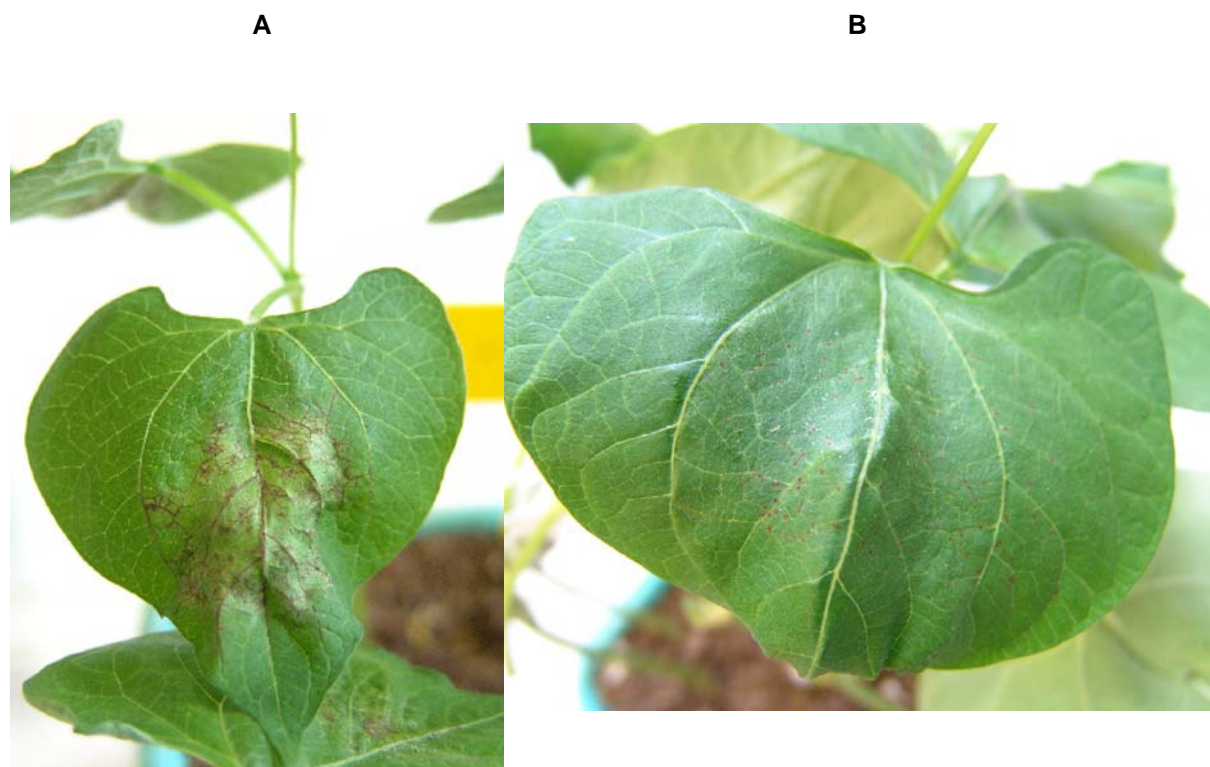
Genetic background

One dominant and several recessive resistance genes have been described. The dominant gene I is responsible for the necrotic response to specific virus strains and absence of symptoms to other strains. Several recessive bc genes may cause resistance without necrotic response. These bc genes need to be combined with one or more other bc genes for being effective. The presence of bc genes or gene combinations may suppress the necrotic response of the I gene partially or completely. In that case the I gene is said to be "protected" by the action of the bc gene or genes (Strausbaugh et al, 2003; Vandemark and Miklas, 2005).

BCMNV Pathogenicity Group VI resistance (example strains: NL3 and NL5)

	Phenotype		Resistance Genes
1	resistance present	healthy	I gene + bc gene(s) or only bc genes
2	resistance present	necrosis	I gene + bc gene(s) or only I gene
3	resistance absent	mosaic; leafroll	

Picture 1: BCMNV: Symptoms of vein necrosis (A) and small necrotic lesions (B)



Picture 2 : Symptoms of BCMNV on resistant and susceptible controls

