

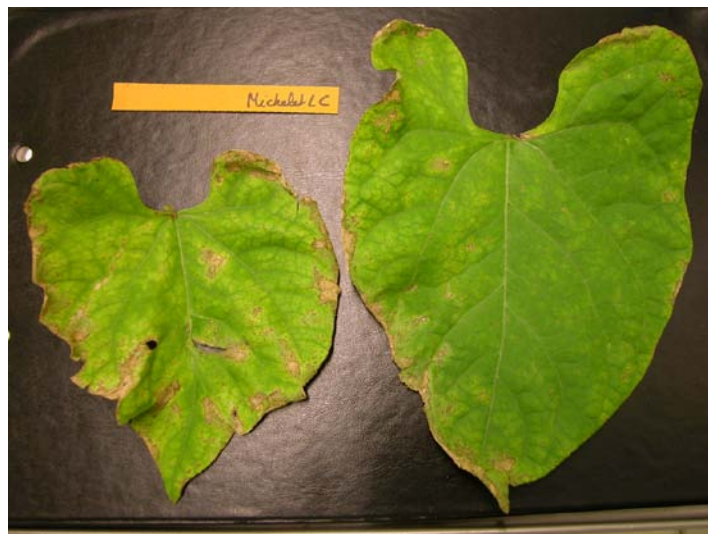


Community Plant Variety Office (CPVO)  
Research and Development Section

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

### Bean resistance to *Pseudomonas savastanoi* pv. *phaseolicola*

Final report



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## **I. Introduction**

In the present program, three national variety examination offices in France (FR), Spain (SP) and the Netherlands (NL) aimed to harmonize the resistance test of *Pseudomonas savastanoi* pv. *phaseolicola* in bean.

2 ring tests were carried out in 2005 and 2006.

The results obtained gave progress on race identification, on notation scale, but still need confirmation about reproducibility in greenhouse or climatic chamber.

It was a too complicated model for a 2 years ring test. It was decided to carry on more experimentation for one year before concluding about protocol.

This report presents the results of ring test obtained in 2007 which enabled to propose a protocol reference with standard for control and isolates.

## **II. Background on the two ring tests in 2005 and 2006**

It was difficult to conclude about conformity for many reasons:

- Countries did not use the same isolates.
  - The notation scale was not harmonized.
  - The controls are homogenous in the 3 countries: Masaï was found resistant and Michelet LC susceptible.
  - Tests are realized in different conditions. In France the test is performed in field, it was in conformity in 2005 and 2006; but the reproducibility in different countries was not tested. In the Netherlands the test is performed in greenhouse, it needs confirmation; the control of environmental parameters (lights, humidity, and temperature) is a limit. In Spain the test is performed in growth chamber, it seems the more reproducible.
- For ring test 2007, France decided to test in climatic chamber.

### III. Ring test plan 2007:

The objectives for a harmonized protocol were:

- Selection of a race or an isolate.
- Determination of the race of the four isolates.
- Definition of a notation scale.

#### 1) *Material and methods*

Four isolates were compared:

- 7722a from FR (race 6 to be confirmed),
- PRI113 from NL (race not yet identified),
- INRA 1429 from NL (new isolate proposed by companies to replace isolate PRI113),
- HRI 1299A from SP (race 6 tested by HRI).

Controls varieties:

- S: Michelet LC,
- R: Masai.

These varieties were the best cultivars of the ring test 2006. They gave uniform results in France and in the Netherlands.

The protocol is defined with a principle of flexibility on different steps:

- Growth stage of plants: first leaves.
- Temperature: 18 to 22°C, 100% relative humidity (depend of the equipment).
- Determination of the race of the four isolates.
- Inoculation:
  - o By spraying on the whole plant (FR),
  - o By rubbing the leaves (NL),
  - o By spraying with impact (SP).
- Inoculum:  $10^8$  cfu/mL.
- Number of plants tested: 20 plants.
- Symptoms (CPVO-TP 012/2)
  - o S: water-soaked lesions, with or without halo and with or without systemic chlorosis
  - o R: necrotic spots, with or without halo and without water-soaked lesions or no symptom.
- 3 notation scales tested.

Temperature and humidity are depending of the equipment of each laboratory. Duration of tests and time between inoculation and notation are depending of these conditions.

Ring test 2007-1:

- The four strains are tested on controls (Michelet LC and Masai) in each laboratory.
- Three notation scales (FR, NL and SP) are compared for each strain in each laboratory.

Ring test 2007-2:

- At least, one strain is tested on a set of 10 varieties in each laboratory.
- Three notation scales (FR, NL and SP) are compared for each strain in each laboratory.

## 2) Notation scales

### France scale:



0: no symptoms (R)



1: a lesion with halo (R)



2: few lesions with halo (S)



3: several lesions with halo (S)

### Dutch scale:



0: resistant, pinpoint lesions with or without halo (R)



1: very few lesions, lesions bigger than pinpoint (R)



2: susceptible, plant continue to grow and sizeable leaf symptoms continue to develop on higher part (S)



3: very susceptible, plant are dying (S)

### Third scale:



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



1: necrosis largely confined to the area of maximum inoculation and few systemic necrosis points over the leaf undersurface (R)



2: water-soaked lesions with few necrosis points distributed over the leaf undersurface (S)

3: water-soaked lesion with several necrosis points distributed over the leaf undersurface (S)

## IV. Results on the ring test in 2007

### 1) Results of race determination

Differentials were acquired in France to identify the four isolates:

	Isolates			
	7722a	PRI113	INRA 1429	HRI1299A
Canadian Wonder	S	S	S	S
A 52	S	S	S	S
Tendergreen	S	S	S	S
Red Mexican UI3	S	S	S	T
1072 (Mesunka)	S	S	S	S
A 53	S	S	S	T
A 43	S	S	T	T
Guatemala	S	S	S	S
Interpretation	Race 6	Race 6	Races 2-6-8 ?	?

This table summarizes the results of 5 tests on differentials. There are certain doubts as for the identification of races INRA1429 and HRI1299A because of A43, A53 and Red Mexican UI3. 7722a and PRI113 are confirmed race 6.

HRI1299A was supplied by HRI in 2005 under a Material Transfer Agreement. The characterization of the isolate is mentioned in the paper Taylor *et al.* 1996, Plant Pathology. This isolate was tested on Guatemala and Canadian wonder by INIA, it was susceptible for all plants tested.

Red Mexican UI3 results are difficult to interpret. No symptoms are observed on inoculated leaves, but there are susceptible symptoms on the other leaves. Plant can dye. In some tests (for 7722a) Red Mexican UI3 is tolerant. All races with Red Mexican UI3 resistant are always resistant for Guatemala. Here Guatemala is susceptible for 7722a. Therefore Red Mexican cannot be sufficient for conclusion.

Seeds of A43 and A53 used for test with PRI113 are not the same seeds used for tests with 7722a and INRA 1429. We are testing seeds from other multiplication to check if there is not a problem with our batch of A43 and A53.

### 2) Ring test 2007-1: on controls

In France

		Notation		
		FR scale	NL scale	Third scale
<b>7722a</b>	Michelet LC	S	S (esc)	S (esc)
	Masai	R	R	R
<b>PRI113</b>	Michelet LC	S	S	S
	Masai	R (dis)	R	R
<b>INRA 1429</b>	Michelet LC	S	S	S
	Masai	R	R	R
<b>HRI 1299A</b>	Michelet LC	S	S (esc)	S (esc)
	Masai	R	R	R

S: susceptible; S(esc): susceptible with escape; R: resistant; R(dis): resistant with disease plants.

## In Netherlands

		Notation		
		FR scale	NL scale	Third scale
<b>7722a</b>	Michelet LC	S	S	Not usable. Leaves damaged.
	Masaï	R	R	Not usable. Leaves damaged.
<b>PRI113</b>	Michelet LC	No symptom	No symptom	Not usable. Leaves damaged.
	Masaï	No symptom	No symptom	Not usable. Leaves damaged.
<b>INRA 1429</b>	Michelet LC	No symptom	No symptom	Not usable. Leaves damaged.
	Masaï	No symptom	No symptom	Not usable. Leaves damaged.
<b>HRI 1299A</b>	Michelet LC	No symptom	No symptom	Not usable. Leaves damaged.
	Masaï	No symptom	No symptom	Not usable. Leaves damaged.

## In Spain

		Other notation scale
<b>7722a</b>	Michelet LC	S
	Masaï	R
<b>PRI113</b>	Michelet LC	S
	Masaï	R
<b>INRA 1429</b>	Michelet LC	S
	Masaï	R
<b>HRI 1299A</b>	Michelet LC	S
	Masaï	R

### Isolates:

The four isolates give expected results. In France PRI113 is more aggressive.

### Control varieties:

Controls give expected results (R: Masaï; S: Michelet LC).

Vaillant can be used too as resistant control. In aggressive tests, Vaillant was more resistant than Masaï.

### Notation scales:

Notation scales depend of the method of inoculation:

- FR scale is not appropriate, it was used in greenhouse or field where no water-soaked lesions were observed. The symptoms observed could be read with another notation scale which takes into account water-soaked lesions.

- NL scale give expected results, note 3 seldom observed.

- Third scale is not adapted with the method of inoculation by spraying or by rubbing. The notion of “area of maximum inoculation” is not usable in the test with inoculation by rubbing or spraying on the whole plant. It can be replaced by “on the leaves inoculated”. “No symptom” must be added to the note 0, because there are plants without symptom.

The tests were realized at 22°C in France and in Netherlands. This can explain why the tests are less aggressive. Abnormal low symptom development or necrotic symptoms can occur from temperatures higher 20°C.

Third notation scale was modified in Spain to make correspond varieties to very aggressive test. The other notation scales (FR, NL and Third scales) were no tested.

### 3) Ring test 2007-2: on a set of 10 varieties

In France

	Expected	PRI113			INRA 1429			7722a		
		FR scale	NL scale	Third scale	FR scale	NL scale	Third scale	FR scale	NL scale	Third scale
Michelet	S	S	S	S	S	S	S	S	S	S
Masai	R	R	R	R	R	R	R	R	R	R
Asterix	R	R	R	R	R (dis)	R	R	R	R	R
Lannion	R	R	R	R	R (dis)	R	R	R	R	R
Bousca	R	R	R	R	R (dis)	R	R	R	R	R
Discover	R	R	R	R	R (dis)	R	R	R	R	R
Nonvert	R	S	S	S	S (esc)	S	S	S	S	S
Ducato	S	S	S	S	S (esc)	S	S	S	S	S
Villeron	S	S	S	S	S (esc)	S	S	S	S	S
Impact	S	S	S	S	S (esc)	S	S	S	S	S
Cruiser	S	S	S	S	S (esc)	S	S	S	S	S
Blondor	S	S	S	S	S (esc)	S	S	S	S	S

#### Isolates:

The three isolates tested give expected results.

#### Set of varieties:

Varieties give expected results, except for Nonavert. This variety was tested resistant during two years in field with a mixture of isolates. This difference can be explained by isolates present in field or particular climatic conditions. Results of other laboratories are waited to interpret results of Nonavert.

#### Notation scales:

- FR are not interpretable.
- NL give expected results.
- Third: the modified notation scale was adapted for inoculation by spraying.



## V. Proposal for modification of CPVO protocol

### 1) *Determination of a race or a isolate to use*

CPVO proposes two US races: race 1 and race 2. We propose to drop race US entirely. Bibliography indicates the predominance of the race 6 in EU. Race 6 (represented by PRI113, 7722a and HRI1299A) is chosen for the harmonized protocol.

Breeders use partial resistance and not specific resistance. The use of an isolate of race 6 will protect them because that confirms broad resistance of their variety.

INRA 1429 is not conserved by Netherlands because this isolate is not more interesting than PRI113.

### 2) *Notation scale*

We cannot do a harmonized notation scale which corresponds of the different conditions and methods of inoculation. We propose therefore a notation with the symptoms observed on susceptible (resistance absent) and resistant (resistance present):

Susceptible:

- halo,
- water-soaked (looking oily) lesions,
  - o Few
  - o Many
- water-soaked lesions becoming necrotic in time (larger than pinpoint),
- deformation and chlorosis on first trifoliolate leaves (phaseolotoxin),
- wounding by inoculation,
- necrosis on stems,
- plants dying.

Resistant:

- no symptoms,
- necrotic pinpoint,
- wounding by inoculation.

Analysis of result should be calibrated with results of susceptible and resistant controls.

### 3) *Conclusion*

We have validated controls and isolates.

We have decided to do extra work to validate isolates on a set of 10 varieties, to give more information. Tests are under progress.

## **Executive Summary of Recommendations to CPVO**

We propose to use the updated protocol (protocol given in annex) for *Pseudomonas* in bean. This protocol has now been validated in three laboratories.