UPOV
International Union for the Protection of New Varieties of Plants

E

Technical Working Party for Vegetables

TWV/52/13

Fifty-Second Session
Beijing, China, September 17 to 21, 2018

Original: English
Date: September 11, 2018

NEW ISSUES ARISING FOR DUS EXAMINATION

Document prepared by the Office of the Union

Disclaimer: this document does not represent UPOV policies or guidance

This document contains presentations to be made at the fifty-second session of the Technical Working Party for Vegetables (TWV) by an expert from France, as follows:

Annex I "MILAROM project: Study of Downy Mildew caused by a *Peronospora belbahrii* on Basil in France"
Annex II "Pathotyping Melon necrotic spot virus (MNSV) in Melon"
Annex III "*Meloidogyne incognita* disease resistance test protocol on Pepper"
Annex IV "*Pyrenochaeta lycopersici*, causal agent of the corky root disease of Tomato / RT Tomato - FR test protocol"

[Annexes follow]

ASTREDHOR projet

(co- funded by French regional founds)

MILAROM project: Study of Downy Mildew caused by a *Peronospora belbahrii* on basil in France

Presentation at the TWV 52 – September 2018

GEVES
Expertise & Performance

MILAROM PROJECT

- **6 FR Partner** of the project (2014-2016)

GEVES · BHR · ASTREDHOR LOIRE-BRETAGNE · iteipmai · ASTREDHOR LOIRE-BRETAGNE · Vegenov

Background

- **Since 2001**, **basil crops** are attacked by ***Peronospora belbahrii*** *(agent of the Downy mildew)*
- Symptoms on the leaves of fresh plants **(Fig.1),** which make them unfit for consumption or processing (deep freezing, dehydration).
- Affect all the FR and EU production regions causing significant economic losses (Belbahrii et al., 2005).

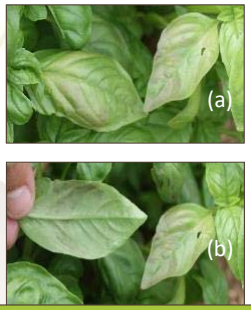


Fig. 1. **Chlorosis on the adaxial surface** (a) and **sporulation on the abaxial surface** (b) of the leaves in fields

GEVES

CHARACTERIZATION OF THE PATHOGEN ISOLATES

P. belbahrii is an obligatory parasite belonging to the class Oomycetes. Symptoms are due to the presence of sporangiophores on the abaxial surface of leaves (Fig. 2).

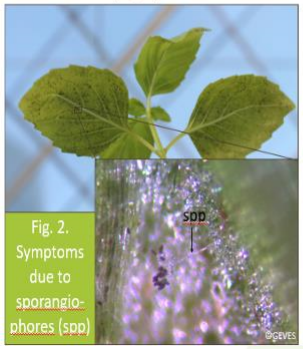


Fig. 2. Symptoms due to sporangio-phores (spp)

Their branches bears, at their tips, airborne spores in humid condition. These spores can infect the leaves and contaminate a new plant (Fig. 3).

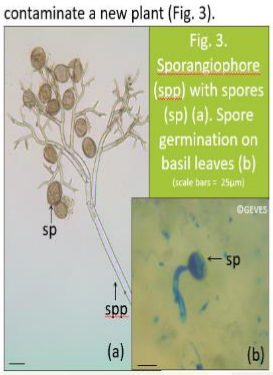


Fig. 3. Sporangiophore (spp) with spores (sp) (a). Spore germination on basil leaves (b) (scale bars = 25µm)

A protocol of leaves discoloration and mycelium stainingconfirmed the emergence of sporangiophores from stomata of basil leaves contaminated for 21 days (Fig.4.), creating a new source of contamination.

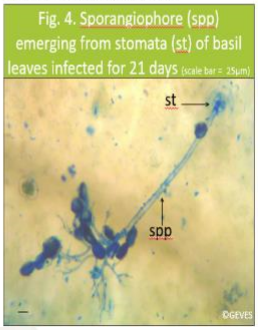


Fig. 4. Sporangiophore (spp) emerging from stomata (st) of basil leaves infected for 21 days (scale bar = 25µm)

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

PATHOGENICITY OF STRAINS AND SUSCEPTIBILITY OF VARIETIES

18 different varieties were separately inoculated with **8 strains** of *P.belbahrii* (Fig.5).

For **inoculation**, a solution of 4.10^4 spores.ml^{-1} in distilled water+Tween 20 (1%) were sprayed on the adaxial surface of basil leaves at two leaf stages. **Symptoms notation** was realized at 21 days post-inoculation with a rating scale from 0 to 5 .

Each strain and variety were compared according to the **observed symptoms** (Fig.6).

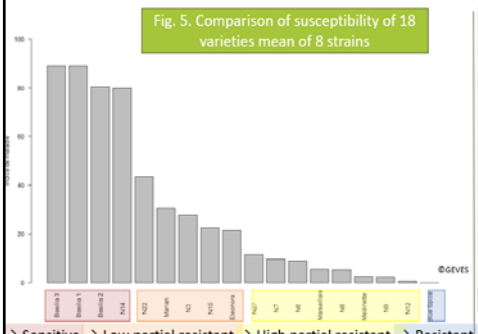


Fig. 5. Comparison of susceptibility of 18 varieties mean of 8 strains

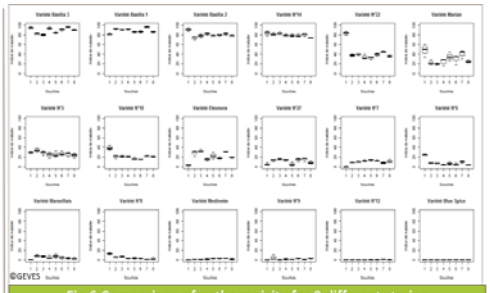


Fig.6 Comparison of pathogenicity for 8 different strains

→ Sensitive → Low partial resistant → High partial resistant → Resistant → 3 strains selected (1, 7 and 8) with different pathogenicity for breeding.

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

SEED DETECTION

A **protocol of detection** has been developed for the detection of *P. belbahrii* on seeds.

To detect **if a seed lot is contaminated**, 400 seeds/lot are sown and grown with high humidity **(Fig. 7).**

Weekly reads are realized to quantify the percent of contamination.
Symptoms appears **after 20 to 30 days post-sowing (Fig. 8).**

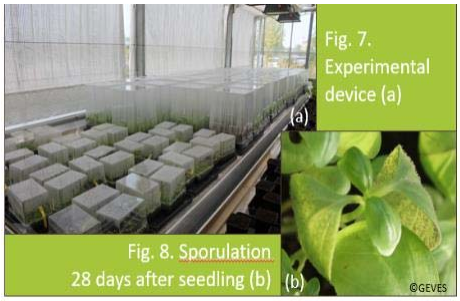


Fig. 7. Experimental device (a)

Fig. 8. Sporulation 28 days after seedling (b)

➢ In 2015, **109 lots were analyzed** and 7 were positive to *P. belbahrii*. The percentage of seed contamination of these lots is estimated to 1% -5%.

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

MILAROM DELIVERABLES

- Protocol of the artificial inoculation to **maintain** and **characterize** the *P. belbahrii* strains.
- Protocol of *P. belbahrii* **detection on seed lots**
- Definition of a **sensibility range of Basil varieties** for each strain of *P. belbahrii*.
- Identification of resistant materials, **first step of potential breeding programs.**

Do you agree a partial revision of the Basil UPOV Test Guidelines (TG/200/2), including a new characteristic: "Resistance to *Peronospora belbahrii* (Downy Mildew)"?

GEVES could propose a protocol for the 2019 TWV.

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

[Annex II follows]

2016-2017 UFS projet

co- funded by SNES- GEVES and 8 breeding companies

Sakata Vegetables Europe, Gautier Semences, HM.Clause,
Takii France, Monsanto, Syngenta France, Rijk-ZwaanFrance

Pathotyping *Melon necrotic spot virus* (MNSV) in Melon

Presentation at the TWV 52 – September 2018

GEVES
Expertise & Performance

BACKGROUND - OBJECTIVES OF THE PROJECT

The *Carmovirus* ***Melon necrotic spot virus*** (MNSV) is seriously affecting **melon green house production** in Spain.

Since the early 2000's, recessive resistance **gene *nsv*** has been deployed in melon commercial varieties and MNSV resistance declaration has been included in European melon variety registration.

In the last few years, an **increasing number of MNSV symptoms** has been reported in melon commercial fields grown with **MSNV resistant varieties** in Almeria region, suggesting the spreading of **resistance breaking MNSV**.

A characterization of virulence of isolates from commercial field was launched in order to **confirm the occurrence of resistance breaking** and **support the pathotype description**.

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

MATERIAL & METHOD

- **MNSV isolates**
  - with MNSV **typical symptoms**
  - collected on **commercial resistant varieties**
  - in Almeria region from 2011 to 2013.

- **Virulence comparison** of:

6 representative isolates (A to F)	the **official reference isolate (9-32)**	**on a varieties panel:** - VIRGOS (R control) - VEDRENTAIS (S control) - 7 varieties carring the *nsv* resistance gene.

- **Virulence characterization**
  - carried out through an **inter-laboratory ring-test**
  - according to the **official protocol** used for the DUS disease resistance test.

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

RESULTS OF THE PROJECT

6 representative isolates (A to F)	Official reference isolate (9-32)
infect(s)	
ALL the tested panel (including the S and R controls)	ONLY VEDRANTAIS (S control)
with MNSV typical symptoms.	

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

RESULTS OF THE PROJECT

Figure 1: Symptom on cotyledon with MNSV reference isolate 9-32 (A) and resistance breaking isolate (B) inoculated on susceptible control Vedrantais (1), resistant commercial variety carrying *nsv* gene (2) and resistant control Virgos (3)

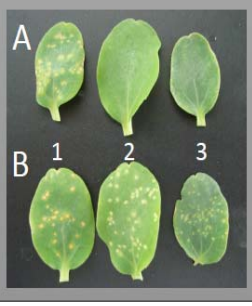


Figure 2: Systemic leaf symptom of MNSV reference isolate 9-32 inoculated on susceptible control Vedrantais

Table 1: Virulence (+) and avirulence (-) of the reference MNSV isolate 9-32 and six MNSV field isolates coded A to F inoculated at cotyledon stage on susceptible control Vedrantais, resistant control Virgos, and seven commercial variety carrying *nsv* gene

Variety	Isolate						
	9-32	A	B	C	D	E	F
Vedrantais	+	+	+	+	+	+	+
Virgos	-	+	+	+	+	+	+
Monzon	-	+	+	+	+	+	+
Quijano	-	+	+	+	+	+	+
Yoga	-	+	+	+	+	+	+
Homer	-	+	+	+	+	+	+
Mendoza	-	+	+	+	+	+	+
Mabel	-	+	+	+	+	+	+
Delsol	-	+	+	+	+	+	+

CONCLUSION

- These results confirm **a virulence of some MSNV field isolates** on *nsv* resistance gene.

- It is then proposed **<u>to define as</u>**
  - **<u>MNSV pathotype 0 (MNSV:0)</u>**
    - isolates avirulent to *nsv* gene (in VIRGOS)
      - VIRGOS R control
      - VEDRENTAIS S control
  - **<u>MNSV pathotype 1</u>**
    - isolates virulent to nsv gene
      - VIRGOS S control
      - **** *R control not yet identified).*

➢ **A more transparent <u>MNSV carried out resistance</u> and claimed by commercial varieties.**

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

AND FOR US…NEXT STEP:

- **FR proposal for TWV2019:**

Partial revision of the Melon TG/104/5 Rev.,
to update the title of the **characteristic 75:**

TG/104/5 Rev.
Melon, Melone, Melón, 2006-04-05 + 2014-04-09
- 30 -

		English	français	deutsch	español	Example Varieties/ Exemples/ Beispielssorten/ Variedades ejemplo	Note/ Nota
75. (+)	VG	**Resistance to *Melon necrotic spot virus* (MNSV) E8 strain**	**Résistance au virus de la criblure du melon (MNSV) Souche E8**	**Resistenz gegen Netzmelonen-nekrosefleckenvirus (MNSV) Pathotyp E8**	**Resistencia al virus del cribado del melón (MNSV) Raza E8**		
QL		absent	absente	fehlend	ausente	Védrantais	1
		present	présente	vorhanden	presente	Cyro, Primal, Virgos, Yellow Fun	9

Resistance to *Melon necrotic spot virus* Pathotype 0 (MNSV:0)

Strain 9-32 or E8**.**

with the **related updates in the Ad.75.**

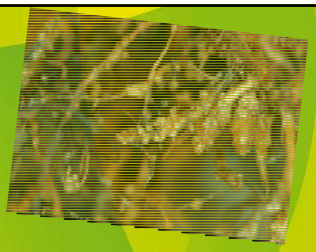
GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

WWW.GEVES.FR

GEVES
Expertise & Performance

[Annex III follows]

2016-2017 GEVES –SNES
Test development



Meloidogyne incognita disease resistance test protocol on Pepper

Presentation at the TWV 52 – September 2018

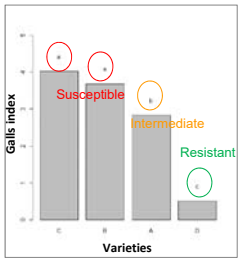
GEVES
Expertise & Performance

BACKGROUND - OBJECTIVES OF THE PROJECT

- Root-knot nematodes are an **active pest** on Pepper (France, Mediterranean basin, ...)
- The resistance to *Meloidogyne* spp. is a **goal of pepper breeding.** *(Announced DUS applications)*
- To be an operating laboratory with a test protocol at SNES in 2017.

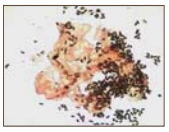
GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

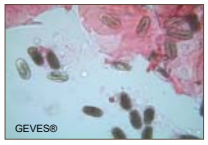
Test SNES Pepper test / *Meloidogyne spp.*



egg-laying colored by eosin on pepper roots

Control of the presence of mature eggs in the ponds





Microscopic observation of eggs (x200)

11 weeks of incubation in greenhouse (19-23 ° C)

Multiplication on tomatoes

Inoculation by sections of roots

7 weeks of incubation in greenhouse (19-23 ° C)

Rating of gall indices

CONCLUSION

- This **protocol** and **its interest** in **Pepper DUS test**
  - was **validated by the Vegetables species National Listing Commitee (Section CTPS) in December 2017**
    - for the new Pepper applications to receive in 2018.

Each variety **declared resistant** will be **officially tested** by GEVES- SNES phytopathogy lab according to the established protocol.

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

AT A UPOV LEVEL…

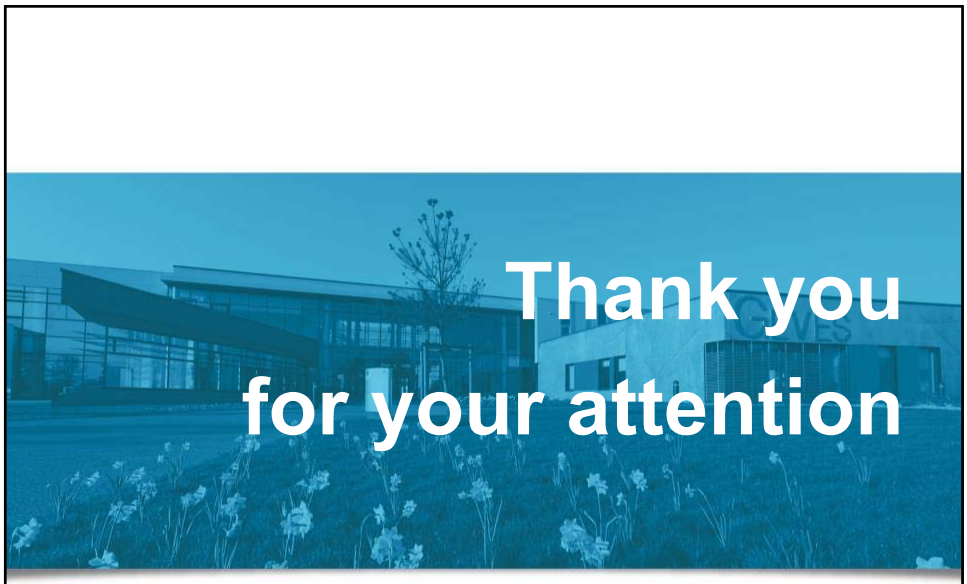
GEVES propose :

- **to add at the 2019 planned Pepper partial revision of the TG/76/8 Rev.**

  ➢the introduction of a **new characteristic**:

  Resistance to *Meloidogyne incognita*

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences



WWW.GEVES.FR

GEVES
Expertise & Performance

[Annex IV follows]

Following the CASDAR project (2013-2016)

Pyrenochaeta lycopersici, *causal agent of the* ***corky root disease*** *of Tomato / RT Tomato*

FR test Protocol

Presentation at the TWV 52 – September 2018

WWW.GEVES.FR

GEVES
Expertise & Performance

Maintenance of strains | Medium used | Test Method | Notations | Analysis of results

The strains of *Pyrenochaeta lycopersici* are maintained at -80°C or in liquid nitrogen.

These strains are available in the MATREF network:

MAT/REF/04-01-06-01

(contact: valerie.grimault@geves.fr)

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

JC2 Maintainance of strain | Medium used | Test Method | Notations | Analysis of results

Substratum of Messiaen PS2 JC1 JC3

PRODUCTS	1l
Calcium nitrate $Ca(NO_3)_2, 4H_2O$	1.0g
Potassium nitrate KNO_3	0.250g
Magnesium sulphate $MgSO_4, 7H_2O$	0.250g
Potassium dihydrogen phosphate KH_2PO_4	0.125g
Di-Potassium hydrogen phosphate K_2HPO_4	0.125g
Malt extract	1.0g
Saccharose	5.0g
Citric acid	0.050g
Agar	25.0g
Water	1 L

The medium is autoclaved for **20 min at 120°C**

GEVES Groupe d'Étude et de contrôle des Variétés Et des Semences

Maintainance of strain | Medium used | Test Method | Notations | Analysis of results

Duration of test
At least 61 days from sowing to notation.

Controls
The following varieties are used as controls. These varieties are available in the MATREF network (contact: MATREF@geves.fr).

S = susceptible / IR= Intermediate resistant

Varieties	Phenotype of resistance
Montfavet	S
Emperador	IR
Garance	IR

Number of plants tested
30 plants per variety.

Growth stage of plants
Plants are grown in greenhouse or in climatic chamber until 3 or 4 leaves stage (generally 21 days after sowing) around 20°C with 80% hygrometry, 12h light.

GEVES Groupe d'Étude et de contrôle des Variétés Et des Semences

Maintainance of strain | Medium used | Test Method | Notations | Analysis of results

Inoculum

Pyrenochaeta lycopersici is first grown on Messiaen and plates are incubated at 20°C in the dark for about 19 days. Then, *Pyrenochaeta lycopersici* is multiplied on autoclaved seeds for 21 days.

Method of inoculation

Plantlets are transplanted in contaminated soil with a mix of contaminated seeds and soil (1:5).

Temperature

Test performed in greenhouse or climatic chamber at 20°C day, 80% hygrometry, 12h light.

GEVES Groupe d'Étude et de contrôle des Variétés Et des Semences

Maintainance of strain | Medium used | Test Method | Notations | Analysis of results

40 days after inoculation

A quantitative notation scale is used from 0 to 4 on roots.

0: no necrosis
1: Small necrotic lesions or with light browning compared to susceptible control
2: Some visible brown necrotic lesions (less than half of the pivot surface)
3: Many visible brown necrotic lesions (more than half of the pivot surface)
4: Complete necrosis or destruction of the pivot

GEVES Groupe d'Étude et de contrôle des Variétés Et des Semences

Maintainance of strain | Medium used | Test Method | Notations | Analysis of results

Analysis of results

Analysis of results should be calibrated with results of RI and S controls. Varieties tested with the same level or with a better behavior than Garance will be judged intermediate resistant.

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

Our proposal, before the TWV(53), in 2019…

In addition to this protocol…

we could provide you, if you are interested in:

- the **reference strain PI21**, used and validated by GEVES – SNES during this project
- a **seed sample of the 3 example varieties**

to allow you to transfer this test in your own laboratory conditions.

Your GEVES – SNES contact:
Mrs Sophie Perrot :
sophie.perrot@geves.fr

GEVES | Groupe d'Étude et de contrôle des Variétés Et des Semences

[End of Annex IV and of document]