A new protocole to assess tolerance/ resistance for sugar beet varieties to virus yellows

V. CADOT (1), M. RUH (1) ,D. BEGHIN (2), S. PERROT (1), C. MIRGUET (1), T. QUEMAR (1), J. ADRIAN (3), S. HOUDAULT (1), T. LEVEQUE (1), N. MASCHER (1), P. ROULIER (1), G. MALATESTA (4)

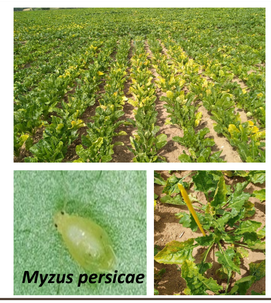
(1) GEVES, 25 rue Georges Morel, 49071 Beaucouzé, FRANCE ;
(2) GEVES, site INRAE Estrées-Mons, 2 Chaussée Brunehaut, 80203 Péronne, FRANCE ;
(3) ITB, 45 rue de Naples - 75008 Paris, FRANCE ;
(4) ITB, 4 rue Denis Papin 02000 Laon , FRANCE

Contact: valérie.cadot@geves.fr

Yellows Resistbeet: PNRI Axis 2 project

Context & aim

- **In order to find alternative solutions after the ban of neonicotinoids (NNI) in the fight against viruliferous aphids, the National Research and Innovation Plan (PNRI**) has launched several projects, which have to be effective and environmentally friendly by 2024.
- The **Yellows Resistbeet** project (2021-2024), led by GEVES, in partnership with ITB, aims to develop **a protocol for assessing varietal resistance/tolerance to Virus Yellows (VY) present in the EU**, transmitted by aphids, mainly *Myzus persicae :* **Beet Yellows Virus (BYV), Beet Chlorosis Virus (BChV), Beet Mild Yellowing Virus (BMYV) and Beet Mosaic Virus (BtMV).**
- The aim is to rapidly promote the inclusion of these tolerant/resistant varieties **in the French Catalogue**.



2021: Development of inoculation methods for Polerovirus and BYV in field

1. Production of plants with viruliferous aphids

Green aphid: Myzus persicae

Sowing
- Susceptible variety
- In tray

Transplantation
- 14 days post sowing
- In pots

Inoculation
- At 4 leaf stage
- Deposit of 20 aphids per plant

Check
- 28 dpi
- ELISA or RT-qPCR multiplex on aphids and plants

Use of inoculum
- 35 dpi
- Leaf pieces
- Pot

Ensured by GEVES, ITB & breeders:

2. Method of inoculation in field

Modalities

- **non inoculated**
- **BYV**
- **BChV**

100 plants/plot
4 reps/modality
on a susceptible variety

Inoculation (2-4 leaf stage)

- **0%** Check
- **3%** inoculated **plants**
- **9%** inoculated **plants**

leaf pieces

- **1 pot** by **plot**

Date of aphicides in post inoculation (pi):

- **4 weeks pi**
- **6 weeks pi**

3. Parameters tested

- **Virus identification and spread by ELISA tests or RT qPCR**
- **Visual notations of symptoms: rate of infected surface area**
- **Sugar yield parameters**

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4. Virus identification and spatio temporal spread in a plot

Method of inoculation	Inoculum density	BYV : % detection by ELISA		BChV : % detection by ELISA	
		Aphicide position		Aphicide position	
		4 weeks pi	6 weeks pi	4 weeks pi	6 weeks pi
% plants inoculated/plot	0%	0%	0%	8%	22%
	3%	8%	12%	65%	61%
	9%	33%	46%	93%	68%
Pot/plot	1 pot/plot	18%	26%	93%	61%

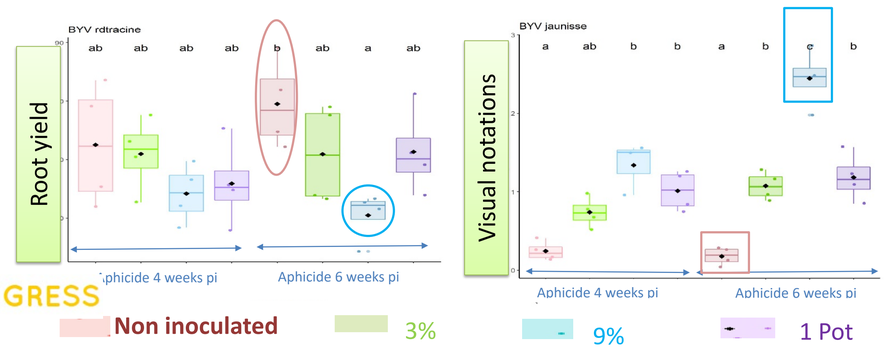
- **BChV : better detection & spread** with **leaf pieces at 9% & 1 pot**
- **BYV: only with 9%**

5. Impact of BYV and BChV virus concentration on sugar yield

Method of inoculation	Inoculum density	BYV : Loss of sugar yield		BChV : Loss of sugar yield	
		Aphicide position		Aphicide position	
		4 weeks pi	6 weeks pi	4 weeks pi	6 weeks pi
% plants inoculated/plot	0%	0%	0%	0%	0%
	3%	3%	13%	-5%	21%
	9%	16%	30%	9%	29%
Pot/plot	1 pot/plot	12%	12%	15%	24%

For BYV & BChV: significant higher sugar yield loss (30%) with leaf pieces on 9% inoculated plants, with an aphicide at 6 weeks pi

6. Correlations between yellow notations and root yield for BYV



- **BYV: variable partial negative correlation between root yield & yellow notations:** up to -0.60
- **Idem for BChV**: up to -0.77

Conclusion: 9 % plants inoculated by leaf pieces, at stage 2-4 stage

2022: Evaluation of varietal resistance /tolerance (R/T) in tunnels and in field

1. Inoculation in tunnels & field

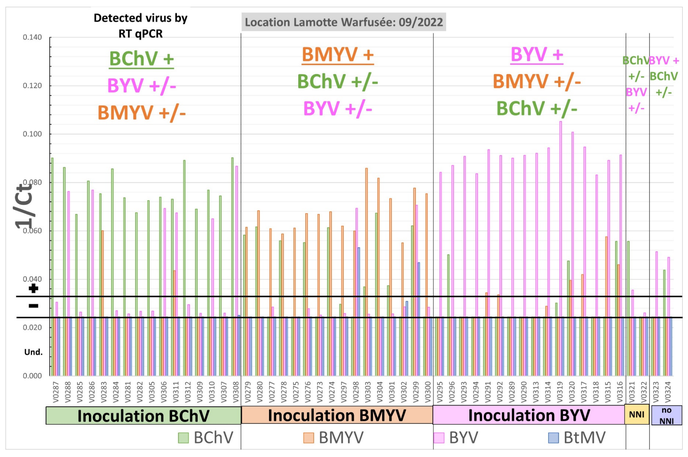
5 tunnels :
1) non inoculated
2) BYV
3) BChV
4) BMYV
5) Co-inoc BChV+BYV

6 VCU trials :
1) non inoculated
2) BYV
3) BChV
4) BMYV

77 varieties inoculated with the modality 10% plant infected by leaf pieces

- **Control of virus identification by RT qPCR multiplex to 4 viruses:**
  BYV, BChV, BMYV, BtMV at 2 dates: July & september **(more details on P4.2-012)**

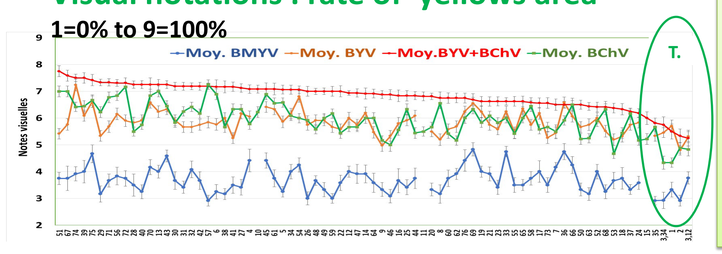
Tunnel inoculation could be complementary to field inoculation, in the case of secondary cross-contamination in the field



2. Evaluations

- **Visual notations : rate of yellows area**

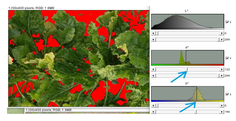
1=0% to 9=100%



- Significant differences in yellow symptoms,
- Less yellows in the 4 tolerant controls (T.)
- More symptoms with BYV+BChV co-inoculation than with BYV or BChV alone

- **RGB imaging with the « Phenoman » perch, by using Image J**

1. **Leaf segmentation**



2. **Yellows quantification**

→ **Algorithm on going**

Main criteria of decision for breeders & registration: Sugar yield performance in both inoculated & non inoculated conditions

Conclusion & prospect

- This project has enabled the development of a method for producing inoculum from viruliferous aphids, to define the parameters of inoculation ensuring a homogeneous virus infestation and significant discrimination of symptoms and yield between inoculated and non-inoculated modalities, to develop a multiplex RT-qPCR method for detecting and identifying these 4 viruses, and to study the most relevant criteria for assessing varietal tolerance, based mainly on productivity data.
- In 2023, R/T varietal evaluation in the field is ongoing, with a greater number of trials inoculated with BChV, BYV & BMYV, in order to identify new varieties with high sugar yield performance, under both inoculated and non-inoculated conditions. The development of this varietal tolerance/resistance assessment protocol will provide a genetic solution to NNI ban. This genetic lever will be offered to experimental pilot farms to develop an integrated pest management method in an agro-ecological context.

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