Assessment of resistance/tolerance for sugar beet varieties to virus yellows transmitted by aphids

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

Context & aim

In order to find alternative solutions after the ban of neonicotinoids in the fight against viruliferous aphids, the **YELLOWS RESISTBEET** project (2021-2024), led by GEVES, in partnership with ITB, aims to develop **protocols for assessing varietal resistance/tolerance to 4 virus yellows prevalent in Europe, transmitted by aphids, mainly by the green peach aphid (*Myzus persicae*):**

- **in field : for 3 viruses: BYV, BChV and BMYV**
- **in green house: for a 4th virus less frequent: BtMV (more details in the CIRAA paper)**

BChV	BMYV	BYV	BtMV
beet chlorosis virus	beet mild yellowing virus	beet yellows virus	beet mosaic virus
Polerovirus		Closterovirus	Potyvirus
Persistent acquisition: 12-72h - retention: aphid all life		Semi-persistent acq.: few hours - ret.: 48h-72h	Non-persistent acq.: few min. - ret.: few min.
around 30 % yield loss		40-50 % yield loss	low yield loss
Moderate beet yellowing		Severe beet yellowing	Beet mosaic

Development of inoculation methods for Polerovirus and BYV in field

Optimization of viruliferous aphid production

Sowing	Transplantation	Inoculation	Check	Use of inoculum
• Susceptible variety • In tray	• 14 days post sowing • In pots	• At 4 leaf stage • Deposit of 20 aphids per plant	• 28 dpi • ELISA or RT-qPCR multiplex on aphids and plants	• 35 dpi • Leaf pieces • Pot

Parameters tested for a discriminant & uniform inoculation

Modalities

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

100 plants/plot
4 rep./modality
on a susceptible variety

Inoculation (2-4 leaf stage)

- **0%** Check
- **3%** inoculated **plants**
- **9%** inoculated **plants**
- **1 pot** by **plot**

leaf pieces

Date of aphicides in post inoculation (pi):

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

Measurements:

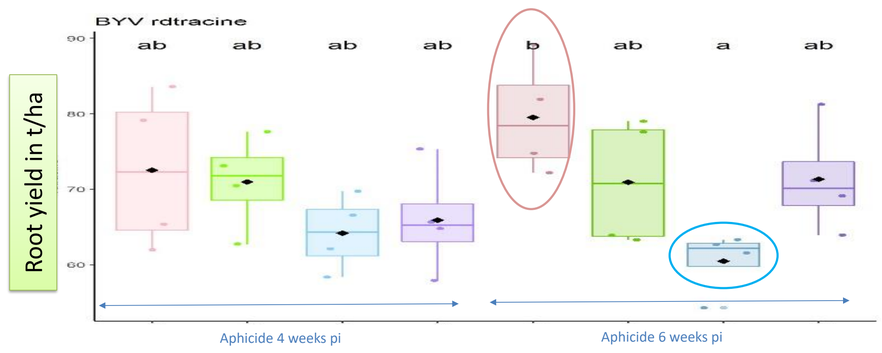
- **Virus identification by ELISA tests**
- **Virus dispersion by ELISA & Visual scoring (more details in the paper)**
- **Sugar yield parameters: root yield, sugar yield, …**

Results:

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 date at 6 weeks pi



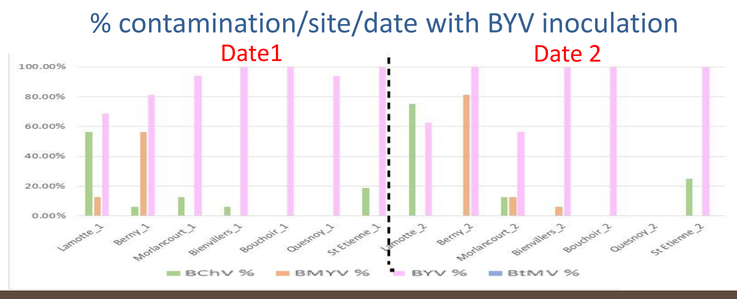
Legend: Non inoculated; 3%; 9%; 1 Pot

Inoculation of BYV or BChV on 9 % plants/plot by leaf pieces at stage 2-4 stage enables the best discriminant loss of yield

Control of virus identification by RT-qPCR multiplex

Multiplex RT-qPCR development by GEVES, in collaboration with INRAE, to detect 4 viruses (BYV, BChV, BMYV, BtMV) in one analysis *(Ruh et al., 2023)*

Analyses of all inoculated sites on controls, at 2 dates:
in July, at the≈ 14 leaves stage & in September before harvest



As we got similar results on 2 dates during 2 years, we decided to keep the first early date in July because of the highest impact on yield.

Current CTPS protocol in field to assess variety performance & first decision rules

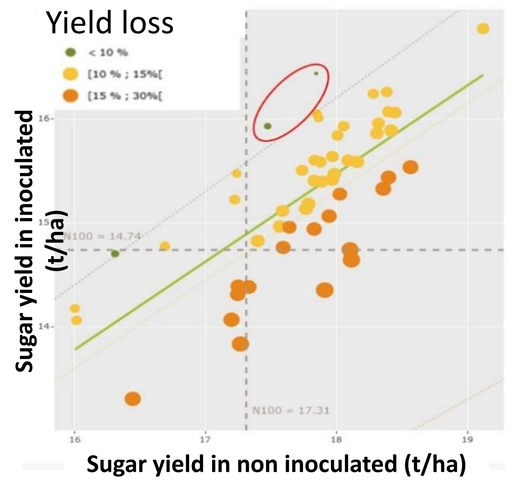
Current CTPS protocol for registration in France

- **Experimental design**:
  - 4 sites /year for 2 years
  - 4 non-inoculated alpha plan replicates
  - 2 complete randomised replicates for BChV, BMYV and BYV
- **Production of viruliferous aphids**, with inoculation at 4-6 leaf stage: Deposition of viruliferous aphids by leaf fragments (or brushes).
  % of inoculated plants: for BChV & BMYV: 7% ; for BYV: 10%
- **Aphicids:** Pre-inoculation & post-inoculation (2 to 4 weeks later)
- **RT-qPCR analysis:** sampling of controls to check identification of present viruses
- **Visual scorings** of yellowing symptoms on leaves
- **Harvest: root yield, sugar content, sugar yield, SM/POL**

First decision rules based on sugar yield performance

Main criteria of decision for registration to the French Catalogue:

- the variety **sugar yield performance** in application for registration must be **at the level of the CTPS controls in both non-inoculated and inoculated conditions.**
- **The average yield loss for all the viruses among the variety in application between the non-inoculated and inoculated conditions must be inferior to thresholds:**
- **for all the 3 viruses < 15% (to be validated)**
- **for each virus < specific thresholds.**



Conclusion & prospect

- **This project has enabled the production optimization of viruliferous aphids and the identification of inoculation parameters to ensure uniform infestation and significant yield discrimination between inoculated and non-inoculated conditions.**
- **A multiplex RT-qPCR method for the detection and identification of these 4 viruses was developed and is used to control the inoculated virus and detect other natural contaminations.**
- **Some graphic tools were built that enable us to define the first decision rules for identifying varieties with high sugar yields under inoculated and healthy conditions. In the short term, we expect to register varieties which are able to fulfil these criteria. This genetic lever should be introduced in the future as part of integrated pest management.**

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