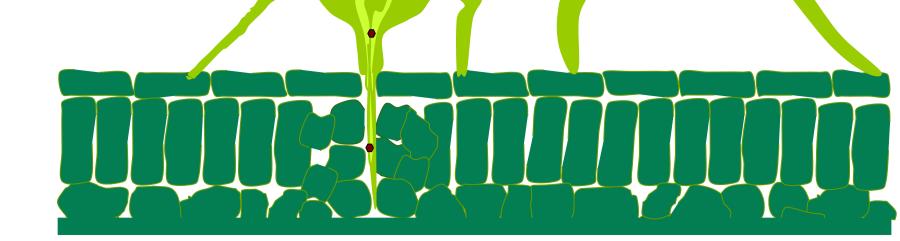
## What's new in polerovirus transmission?

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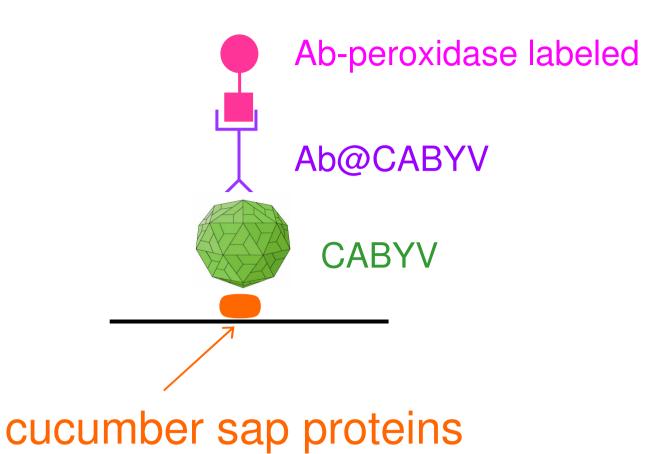
<sup>2</sup>IBMP-CNRS 12 rue du Général Zimmer, 67084 Strasbourg, France <sup>3</sup>INRA, UMR 1099 BiO3P, Domaine de la Motte, 35653 Le Rheu, France

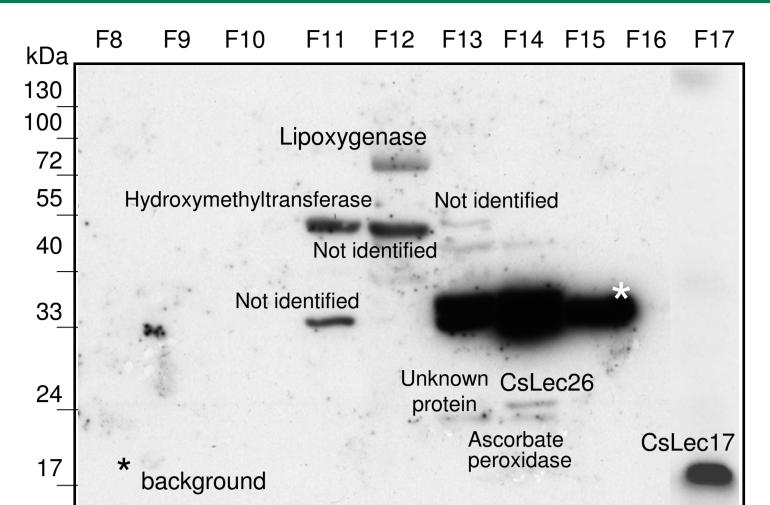


Poleroviruses (*Luteoviridae* family) are phytoviruses strictly transmitted by phloem-feeding aphids in a circulative and non propagative mode. In the transmission cycle, the three partners, the plant, the virus and the aphid, play a crucial role. The results presented thereafter summarize recent data related to polerovirus transmission by aphids.

#### Virus partners in phloem

During ingestion, aphids sample virions in sieve tubes along with sap. Therefore, any sap protein bound to virions will be acquired by the insects and could potentially be involved in the transmission process. We developed Far-western blot to identify sap proteins able to bind purified *Cucurbit aphid borne yellows virus* (CABYV, *Polerovirus*).





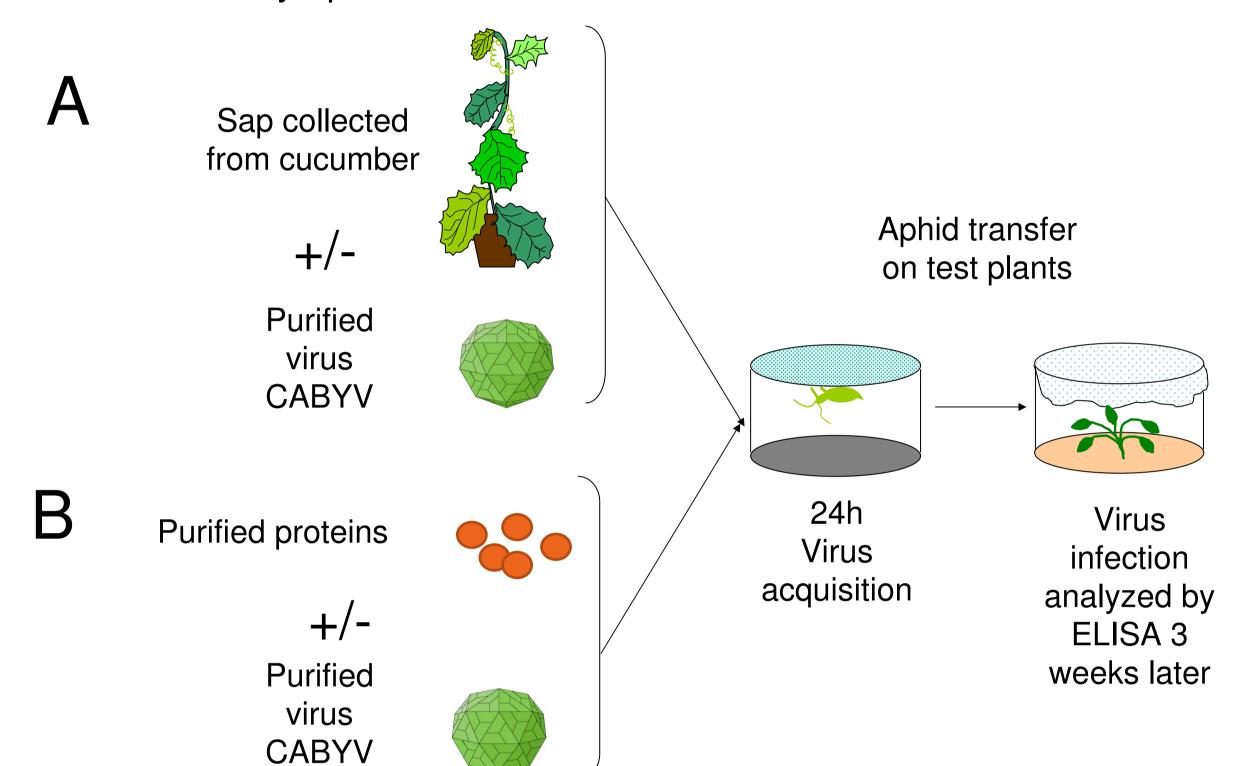
Sap proteins obtained after size fractionation by exclusion chromatography

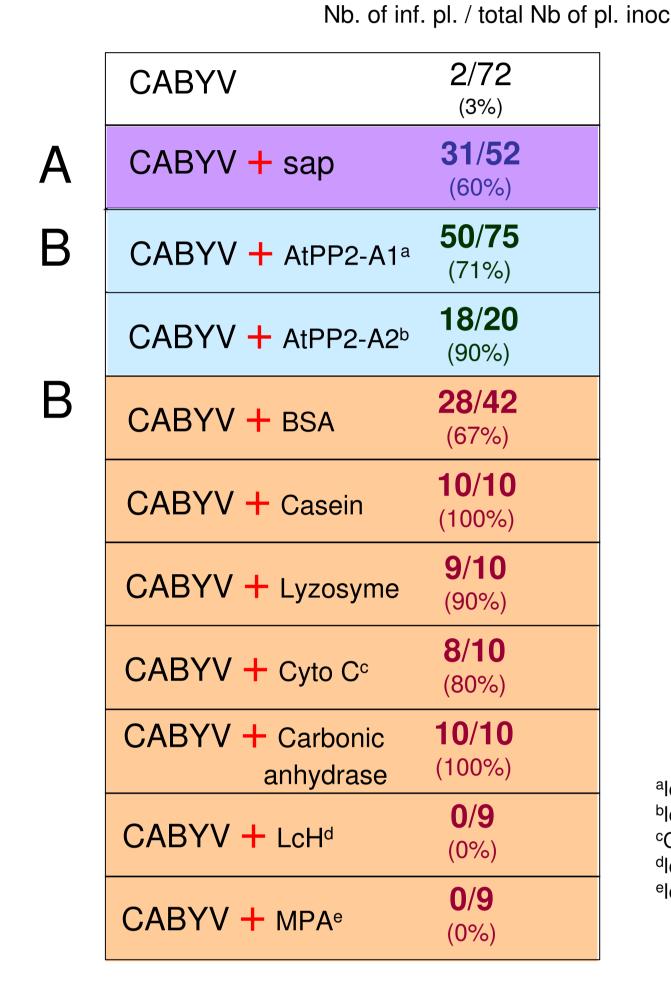
- ➤ About 20 sap proteins able to bind CABYV *in vitro*
- ➤ Phloem lectins CsLec26 and CsLec17 (Phloem protein 2 family, PP2) among the candidates

Bencharki B., Mol Plant Microbe Interact. 2010

### Phloem proteins involved in aphid transmission

Transmission experiments were designed to assess the role of sap extract or purified phloem proteins on virus transmission by aphids.





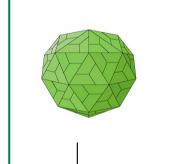
- > Stimulating factor of virus transmission by aphids in cucumber sap
- > Stimulating effect of both lectins from A. thaliana
- Other lectins (LcH, MPA) are toxic for aphids
- > Stimulatory effect not limited to phloem proteins

alectin from *A. thaliana*, ortholog of CsLec26 blectin *A. thaliana*, ortholog of CsLec17 cCytochrome C dlectin from *Lens culinaris* electin from *Maclura pomifera* 

Bencharki B., Mol Plant Microbe Interact. 2010

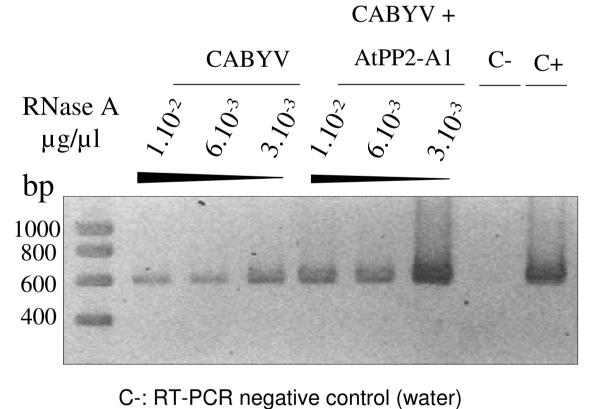
#### Effect of a phloem lectin on virus acquisition and stability

Experiments were designed to assess the role of one *A. thaliana* lectin (AtPP2-A1) on virion stability and on virus internalization in aphid cells



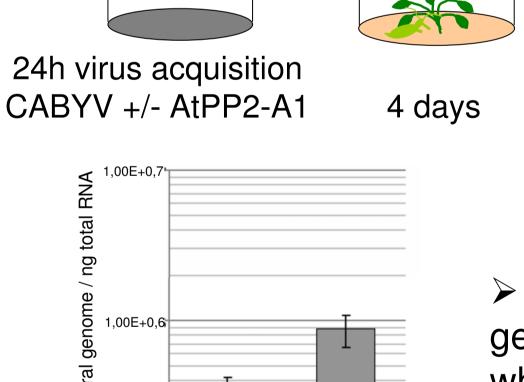
RNase A

RNA extraction RT-PCR



C+: RT-PCR positive control (CABYV without RNase A treatment)

Virions protected against degradation when AtPP2-A1 is present



**CABYV** 

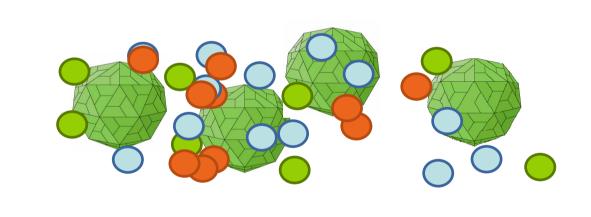
AtPP2-A1

ays

RNA extraction from

aphids and qRT-PCR

➤ 2.5 times more viral genomes internalized when AtPP2-A1 is present in the aphid diet



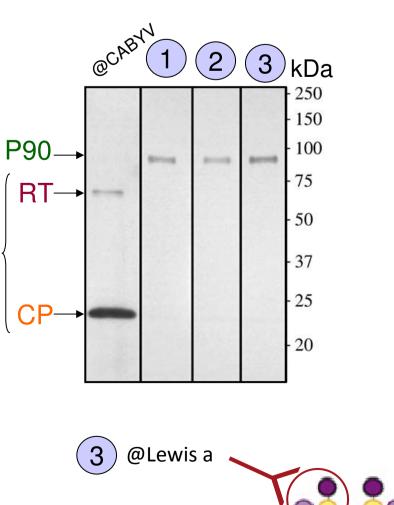
➤ Ingestion of phloem lectins together with virions: protection against protease activity in aphid digestive tube

Bencharki B., Mol Plant Microbe Interact. 2010

# Absence of sugar residues on structural proteins of poleroviruses: glycosylation not involved in aphid transmission

Glycosylation of Turnip yellows virus (TuYV, Polerovirus) was previously reported and modification was supposed to be involved in virus transmission by aphids. Several techniques were developed to more precisely the assay glycosylation status of CABYV (lectin binding assay, immunodetection glycans, mass spectrometry, site directed mutagenesis of N-glycosylation sites. Only some of them are

Immunodectection of structural proteins with Ab@complex glycans



Mass spectrometry on CABYV structural proteins

MNTVAARNQN AGRRRRRNQR PARRDRVVVV NPIGGPPRGR RQRRNRRRPN RGGRARRGSP GETFVFSKDN LTGSSTGSIT FGPSLSESPA FSSGILKAYH EYKIIMVQLE FISEASSTSS GSISYELDPH CKLSSLQSTI NKFGITKSGL RRWTAKQING MEWHDATEDQ FKILYKGNGS SSVAGSFRIT IKCQVQNPKY VDGSSPPPS PSPTPPPPPP PQPQPQPCAQ RFWGYEGNPQ NKILTAENSR NIDSRPLNFV QMYKWEDEKW DKVNLQAGYS RNDRRCMETY LTIPADKGKF HVYLEADGEF VVKHIGDELD GSWLGNIAYD VSQRGWNVGN YKGCKITNYQ SNTVFVAGHP DATMNGKSFD TARAVEVDWF ASFELECDDE EGSWAIYPPP IQKDSSYNYT VSYGNYTEKY CEWGAISVSI DEDNNGNEPR RIPRRGVMAW STPEPSFSGD DSQRQDFNTP SLEERGSDAL ESEEKKEEDN LLDLEEENIP DVDDDDLWKG ISRASEAGTA EDDRASTSSR LRGNLKPKGL PKPQPTRTIT EFNPGPDLIE VWRPDLAPGY SKADVAAATV LAGGSVHEGR DMLERREAKV MDSRKKWGIL SSTSSLTSGA LKKLSAQSEK LATLTTGERV QYQRLKNSMG STVAAEYLEK VLADKTS

3 @Lewis a
2 @β 1,2 xylose
1 @α 1,3 fucose

on CABYV structural protein sequences. Potential N-glycosylation consensus sites are underlined

Trypsic pentides with N-alycosylation

in blue: potential O-glycosylation sites

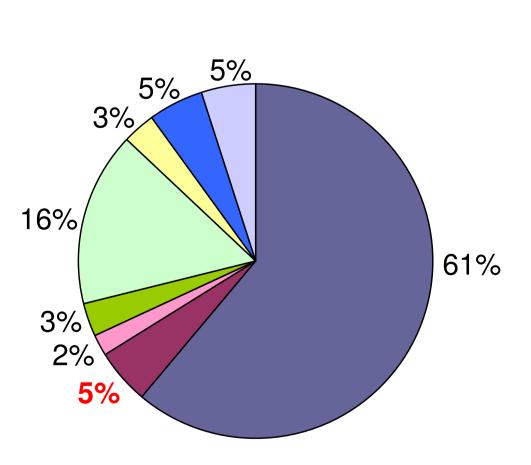
Trypsic peptides with N-glycosylation consensus sites (N-X-S/T) detected as non modified

XXXX: Peptides identified by MALDI-TOF and by Nano LC-MS/MS

- ➤ No complex glycan on CP and RT
- A plant protein of 90 kDa, reproducibly present with purified virions, is glycosylated
  Revollon S., Virology, 2010

## The virus hijacks in aphid a constitutive endocytosis–exocytosis mechanism without heavily perturbing cell metabolism

After virus uptake in the phloem, virions successively cross intestinal and accessory before salivary cells being released plant. We conducted a transcriptomic analysis of intestinal genes of the pea aphid Acyrthosiphon pisum following uptake of Pea enation mosaic (Enamovirus, virus Luteoviridae).



Unknown/hypothetical protein
 Cytoskeleton/vesicles/signall transduction
 Translation
 Protein degradation
 Metabolism
 Stress and defence proteins
 Cuticular proteins
 Other functions

7166 transcripts analysed (20% of the aphid genome): 128 significantly regulated but limited levels of regulation: maximum of downregulation of 3.45 fold and of upregulation 1.37 fold

Only 5% of regulated genes involved in intracellular trafficking, endocytosis or signal transduction

The virus hijacks a constitutive endocytosis-exocytosis mechanism at the intestinal level without heavily perturbing cell metabolism

Brault V., *J. Gen. Vir.*, 2010



illustrated.







