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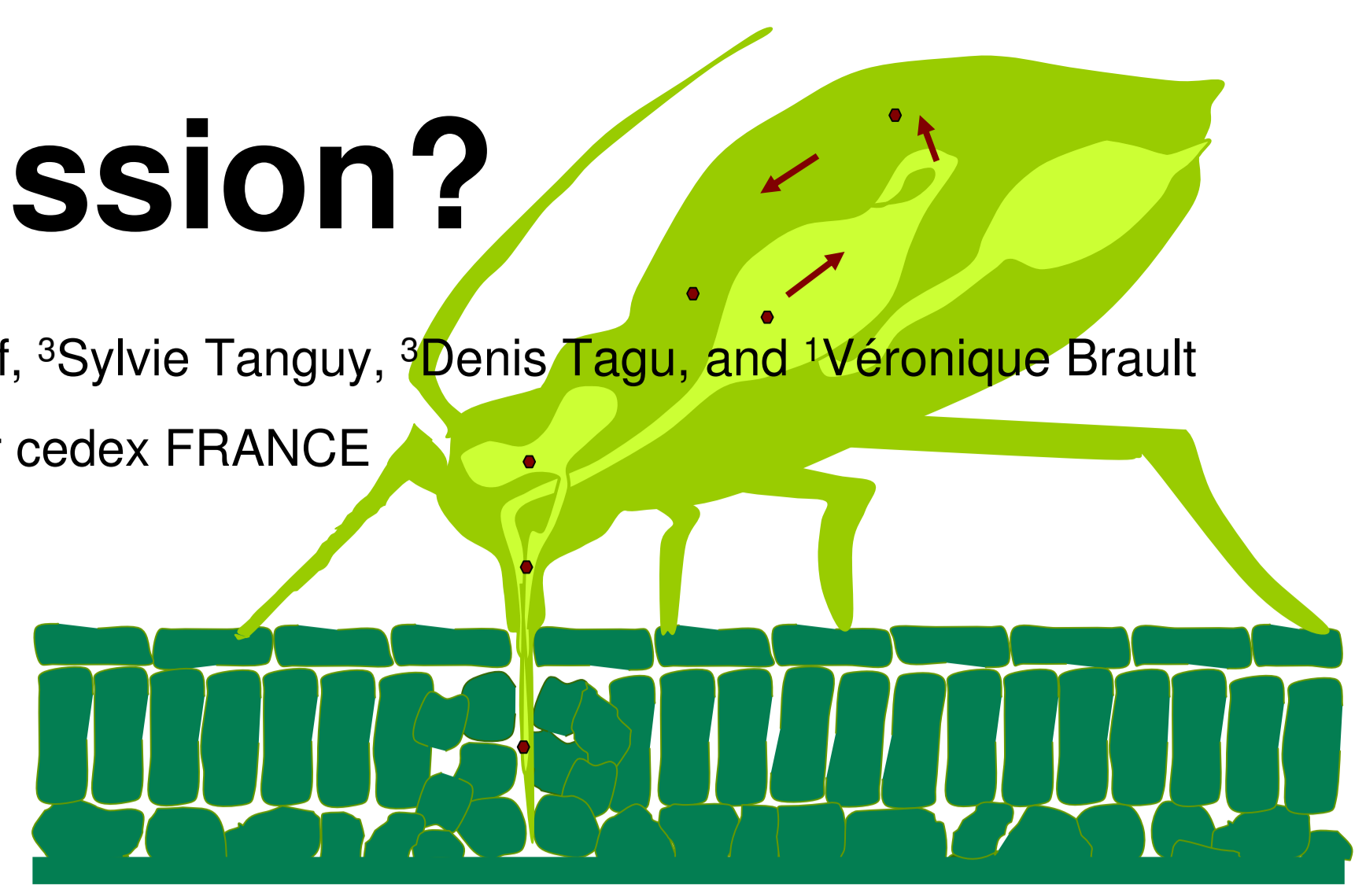
# What's new in polerovirus transmission?

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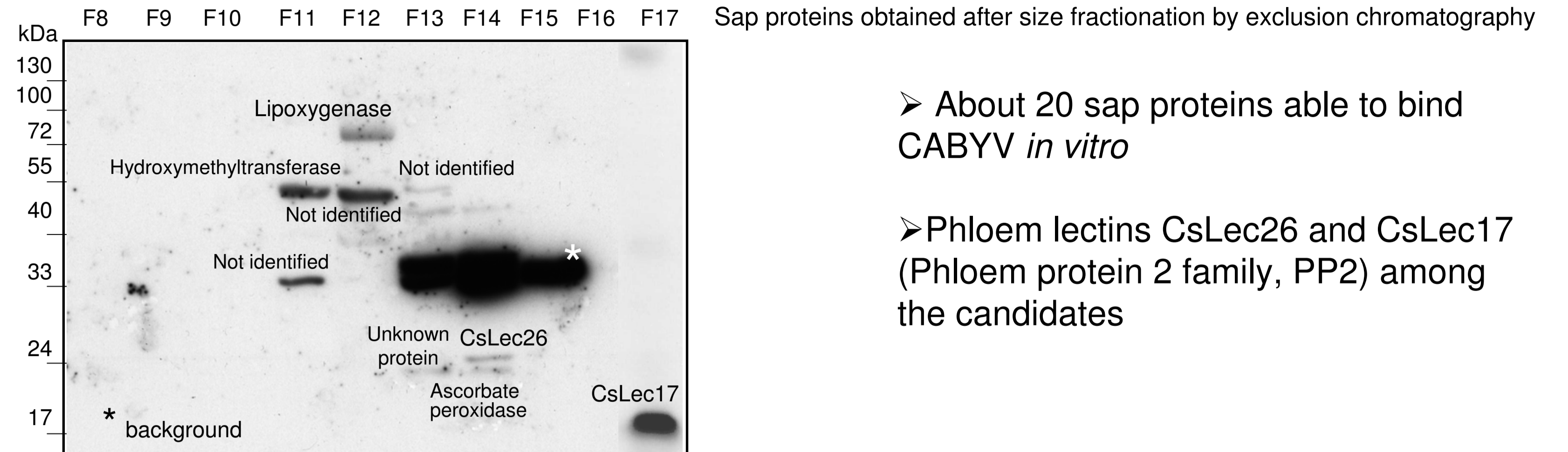
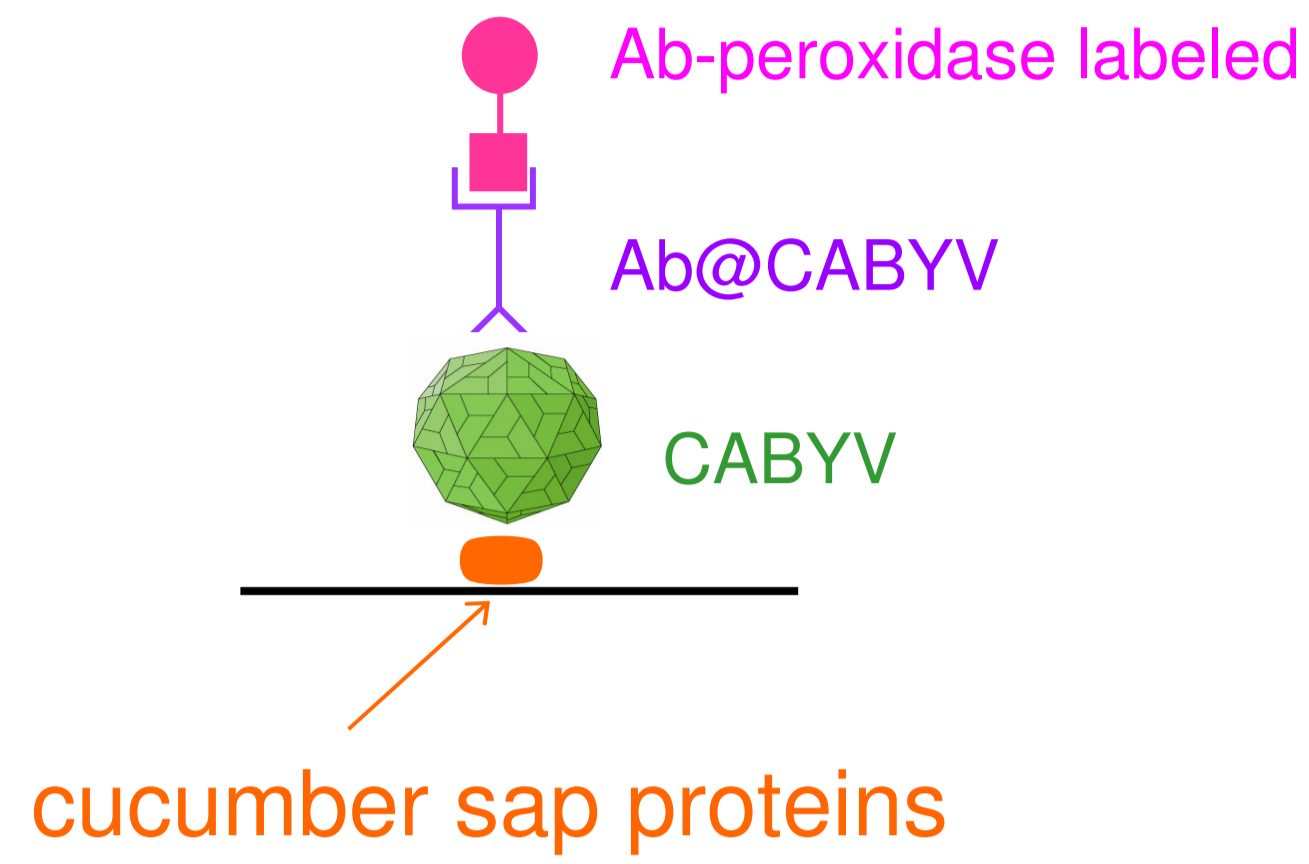
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Poleroviruses (*Luteoviridae* family) are phytoviruses strictly transmitted by phloem-feeding aphids in a circulative and non propagative mode. In the transmission cycle, the three partners, the plant, the virus and the aphid, play a crucial role. 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*).



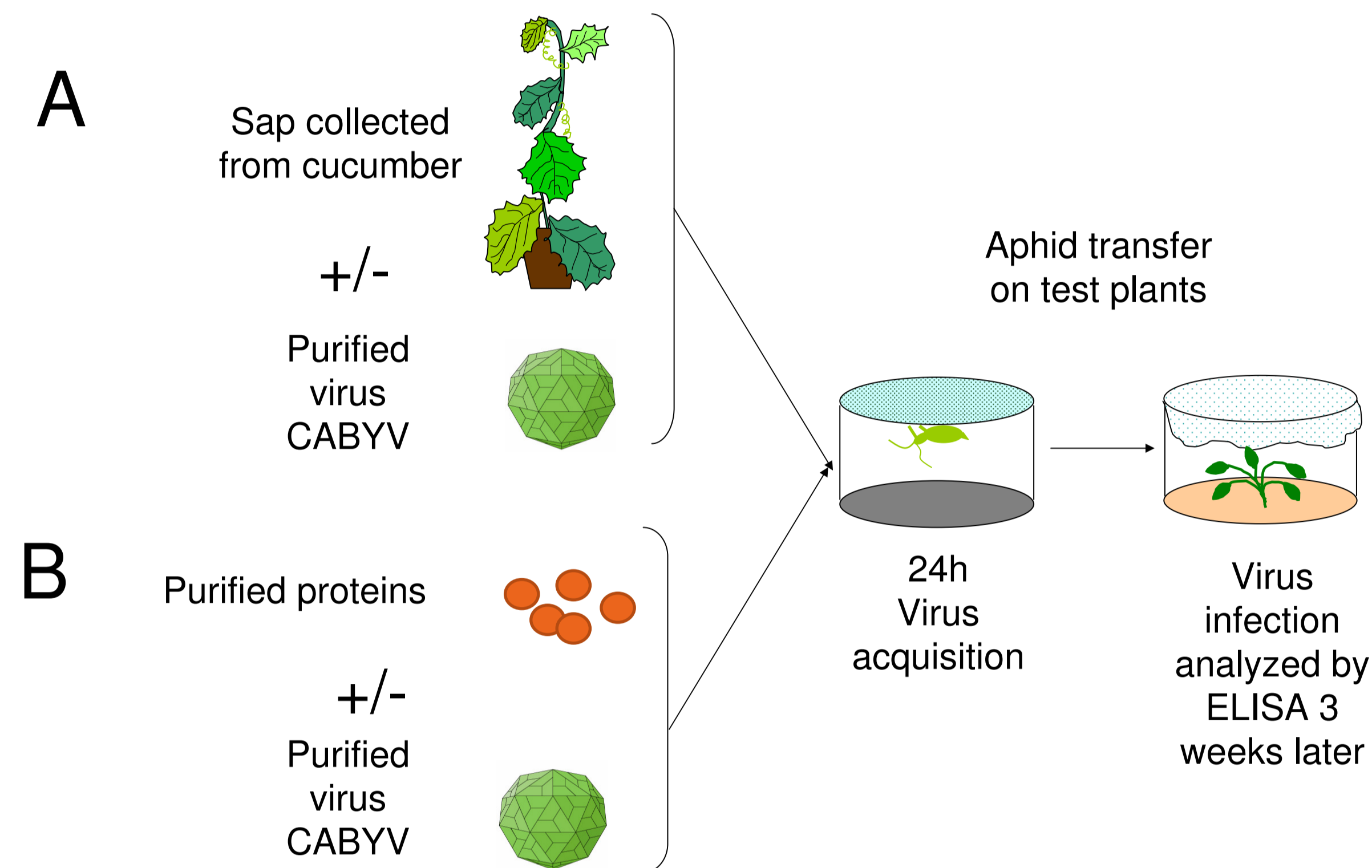
➤ About 20 sap proteins able to bind CABYV *in vitro*

➤ 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.



	Nb. of inf. pl. / total Nb of pl. inoc
CABYV	2/72 (3%)
<b>A CABYV + sap</b>	<b>31/52 (60%)</b>
<b>B CABYV + AtPP2-A1<sup>a</sup></b>	<b>50/75 (71%)</b>
<b>B CABYV + AtPP2-A2<sup>b</sup></b>	<b>18/20 (90%)</b>
<b>B CABYV + BSA</b>	<b>28/42 (67%)</b>
<b>CABYV + Casein</b>	<b>10/10 (100%)</b>
<b>CABYV + Lyzosome</b>	<b>9/10 (90%)</b>
<b>CABYV + Cyto C<sup>c</sup></b>	<b>8/10 (80%)</b>
<b>CABYV + Carbonic anhydrase</b>	<b>10/10 (100%)</b>
<b>CABYV + LcH<sup>d</sup></b>	<b>0/9 (0%)</b>
<b>CABYV + MPA<sup>e</sup></b>	<b>0/9 (0%)</b>

➤ 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

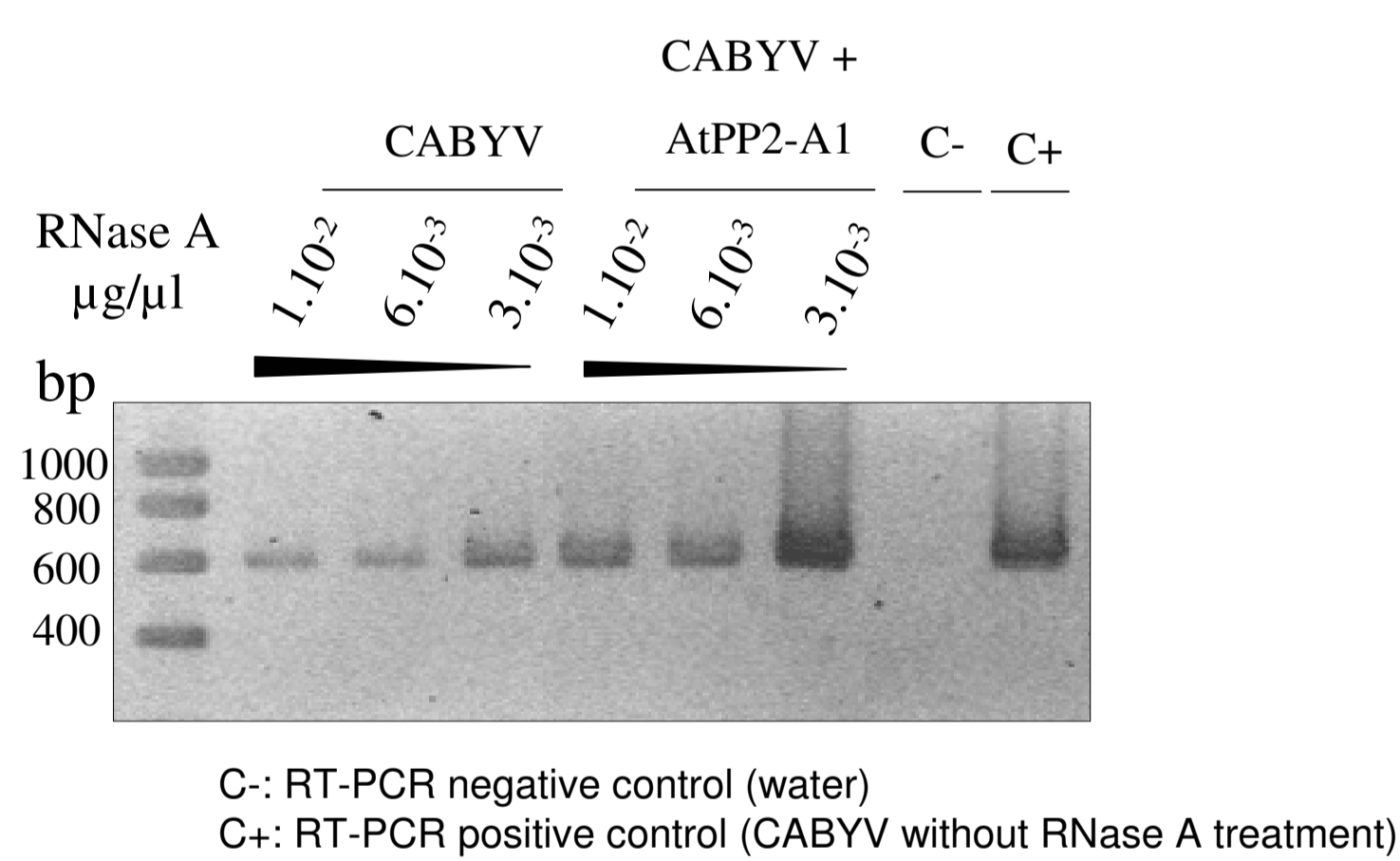
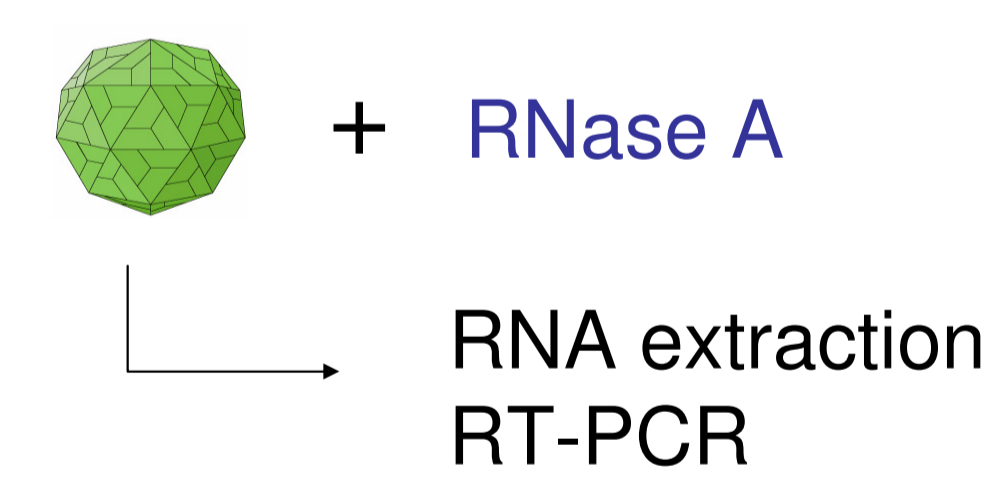
➤ Stimulatory effect not limited to phloem proteins

<sup>a</sup>lectin from *A. thaliana*, ortholog of CsLec26  
<sup>b</sup>lectin *A. thaliana*, ortholog of CsLec17  
<sup>c</sup>Cytochrome C  
<sup>d</sup>lectin from *Lens culinaris*  
<sup>e</sup>lectin from *Maclura pomifera*

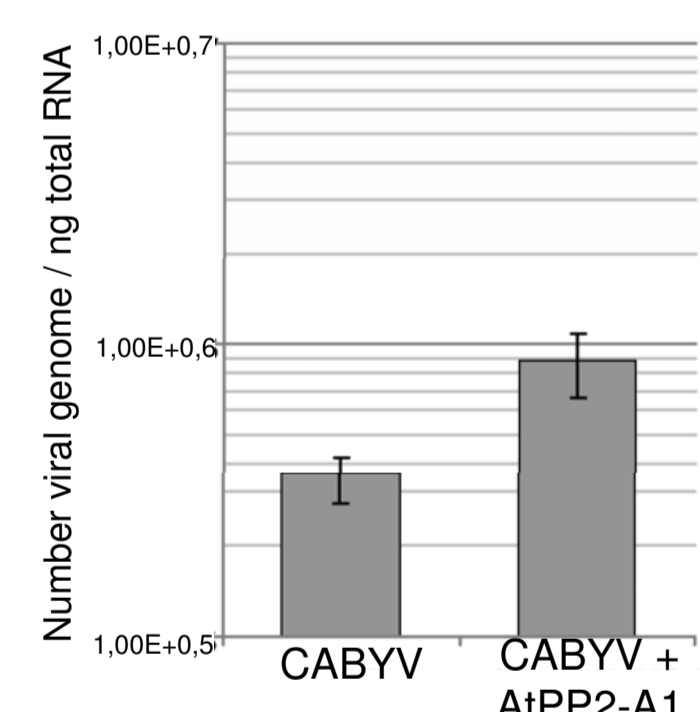
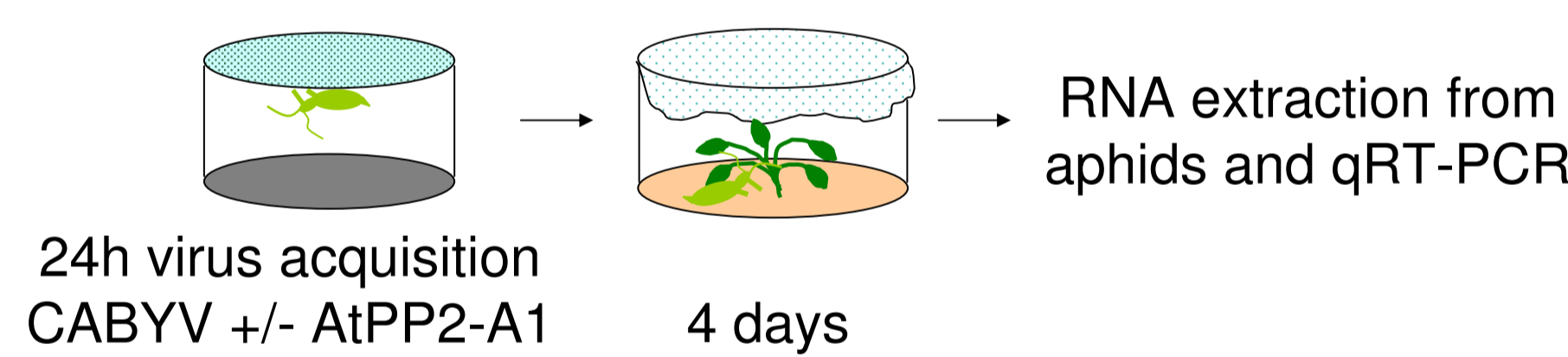
Bencharki B., Mol Plant Microbe Interact. 2010

## 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



➤ Virions protected against degradation when AtPP2-A1 is present



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

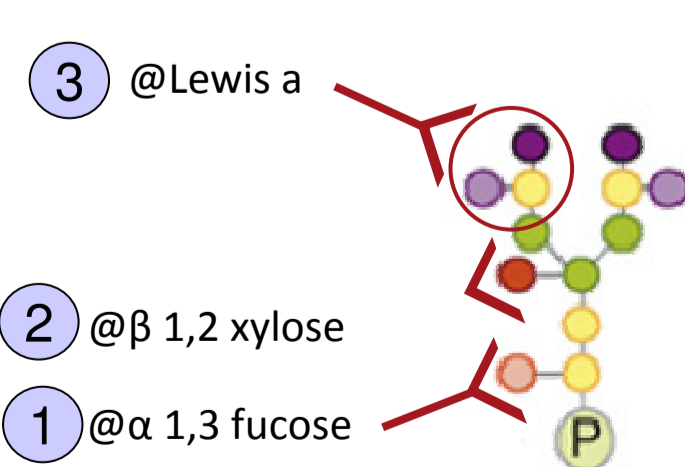
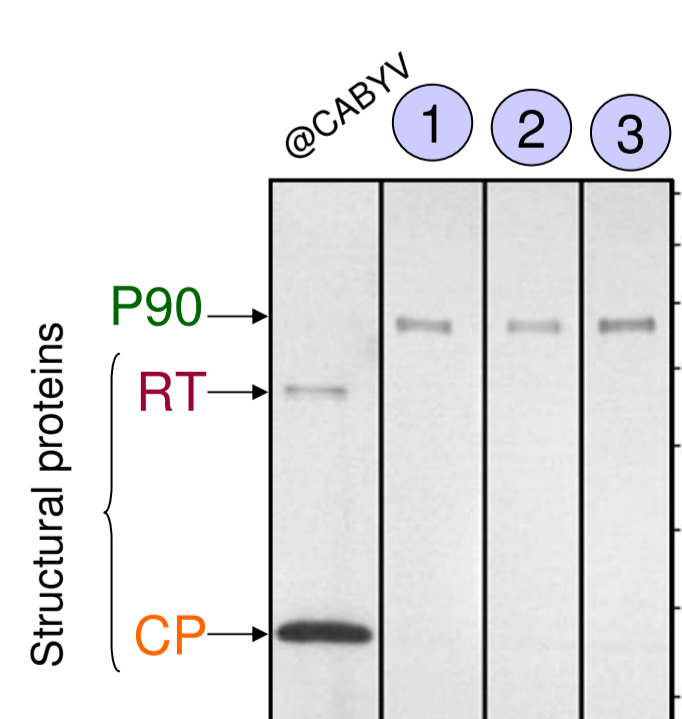
➤ Ingestion of phloem lectins together with virions: protection against protease activity in aphid digestive tube

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 this modification was supposed to be involved in virus transmission by aphids. Several techniques were developed to more precisely assay the glycosylation status of CABYV (lectin binding assay, immunodetection of glycans, mass spectrometry, site directed mutagenesis of N-glycosylation sites. Only some of them are illustrated.

Immunodetection of structural proteins with Ab@complex glycans



➤ No complex glycan on CP and RT

➤ A plant protein of 90 kDa, reproducibly present with purified virions, is glycosylated

Mass spectrometry on CABYV structural proteins

CP  
MNTVAARNQN AGRRRRRNQR PARRDRVVV NPIGGPPRGR RQRNRRRPN  
RGRARRGSP GETFVFSKDN LIGSSTGSIIT FGPSSLESPA FSSGILKAYH  
EYKIIIMVQLE FISEASSTSS GSISYELDPH CKLSSLQSTI NKFGITKSLG  
RRWTAKQNG MEWHATEDQK FKILYKNGS SSVAGSFRIT IKCQVQNPYK  
VDGSSPPPPS PPTPPPPP PQPQPQCAQ RFWYEGNPQ NKLLTAENSR  
NIDSRPLNFV QMYWDEKWK DVLNLAQAYS RNDRCMETY LTIADKGFK  
HVYLEADGEF VVKHIGDELG GSWLGNIAIV VSQRGNVGN YKGGKITNYQ  
SNTVFVAGHP DATMNGKSF TARAVEVDWF ASFELECDDE EGSWAIYPPP  
IQKDSYNYI VSYGNYTEKY CEWGATSYSI DEDNNGNEPR RIPRRGVMAW  
STPEPSFGD DSQRQFNTP SLEERGSAL ESEKKEEDN LLDLEENIP  
DVDDDLKWK ISRASEAGTA EDRRASTSSR LRGNLKPGL PKQPRTIIT  
EFNPKDLIE VWRPDLAPGY SKADVAATV LAGGSVHEGR DMLERREAVK  
MDSRKKWGL SSTSLSLTSGA LKLSAQSEK LATLTGTERV QYQRLKNSMG  
STVAAYLEK VLADKTS

In blue: potential O-glycosylation sites

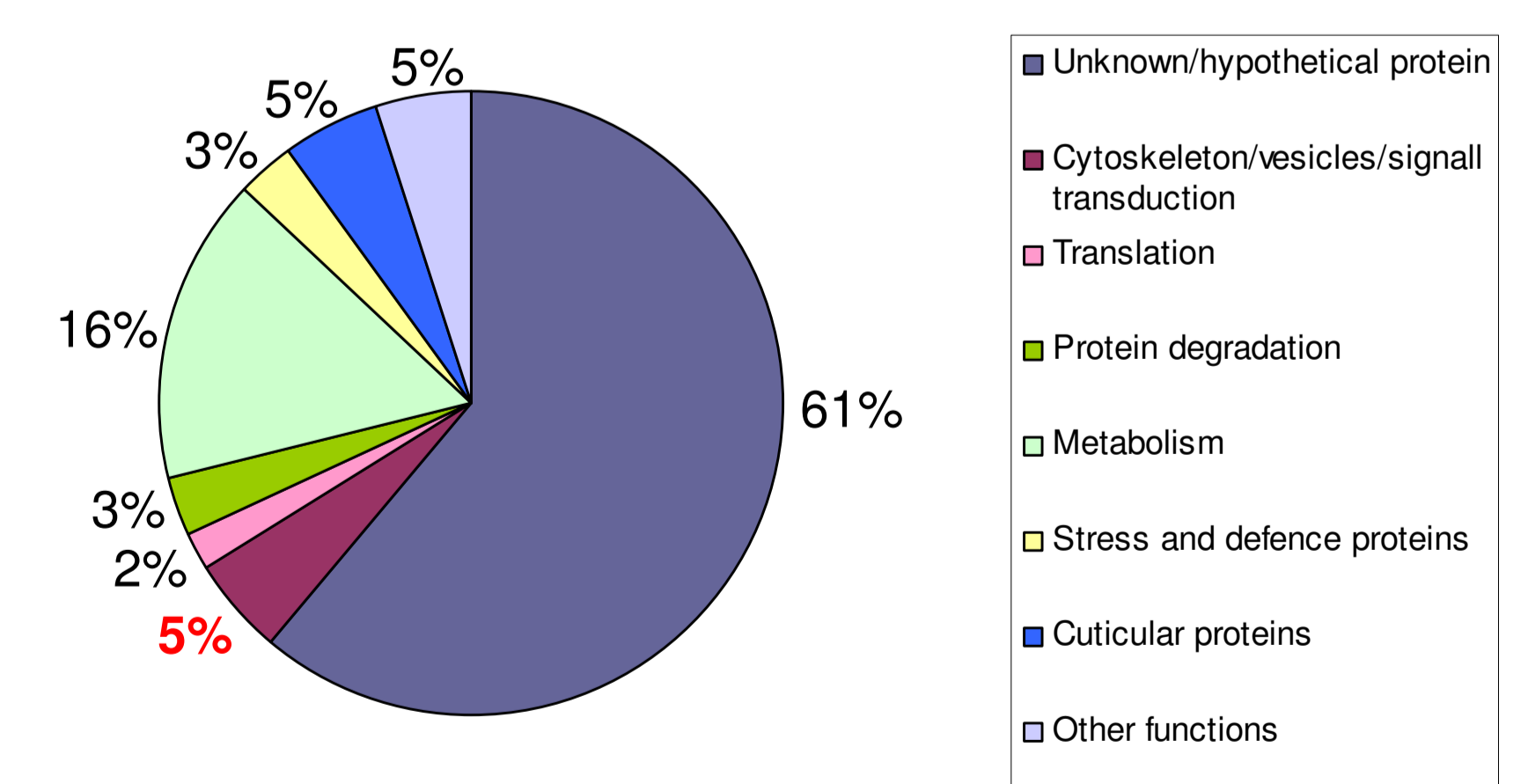
XXXX: Peptides identified by MALDI-TOF and by Nano LC-MS/MS on CABYV structural protein sequences. Potential N-glycosylation consensus sites are underlined

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

Revillon 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 salivary cells before being released in the plant. We conducted a transcriptomic analysis of intestinal genes of the pea aphid *Acyrtosiphon pisum* following uptake of *Pea enation mosaic virus* (*Enamovirus*, *Luteoviridae*).



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