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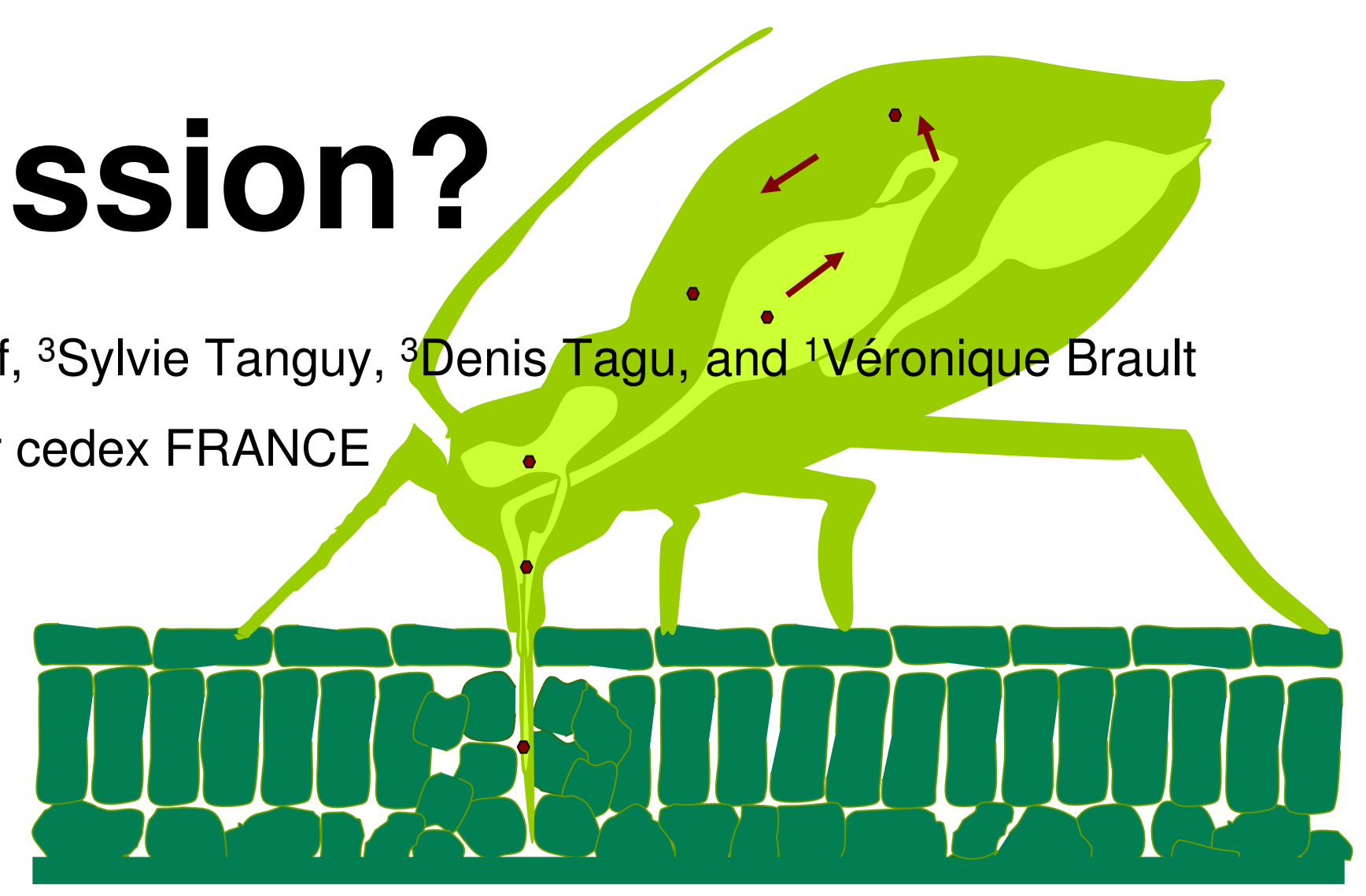
What's new in polerovirus transmission?

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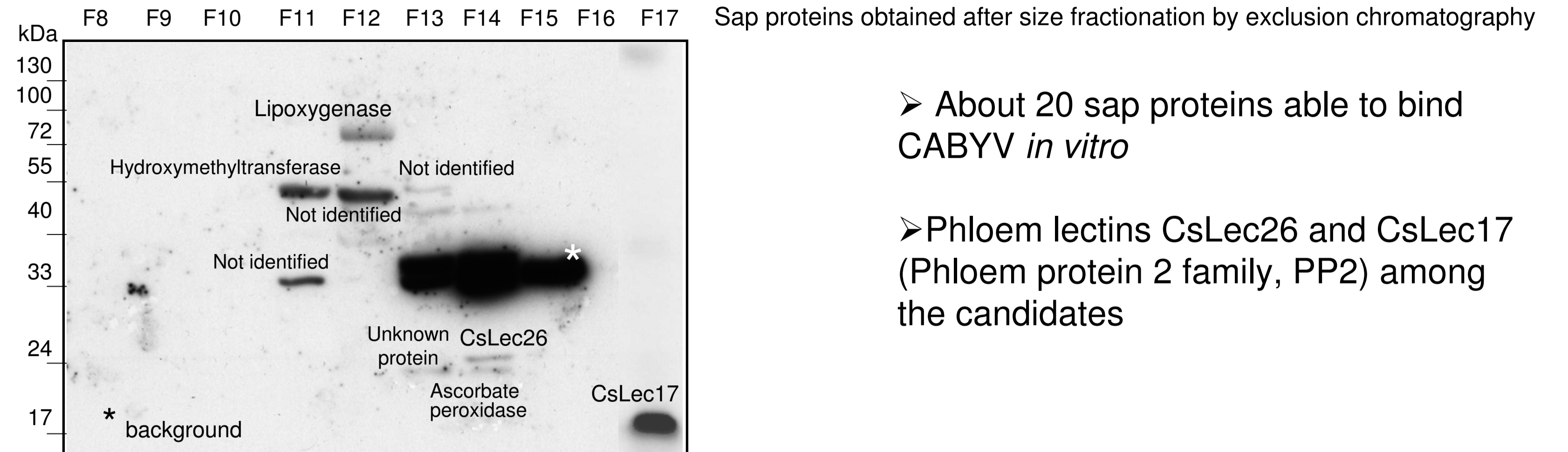
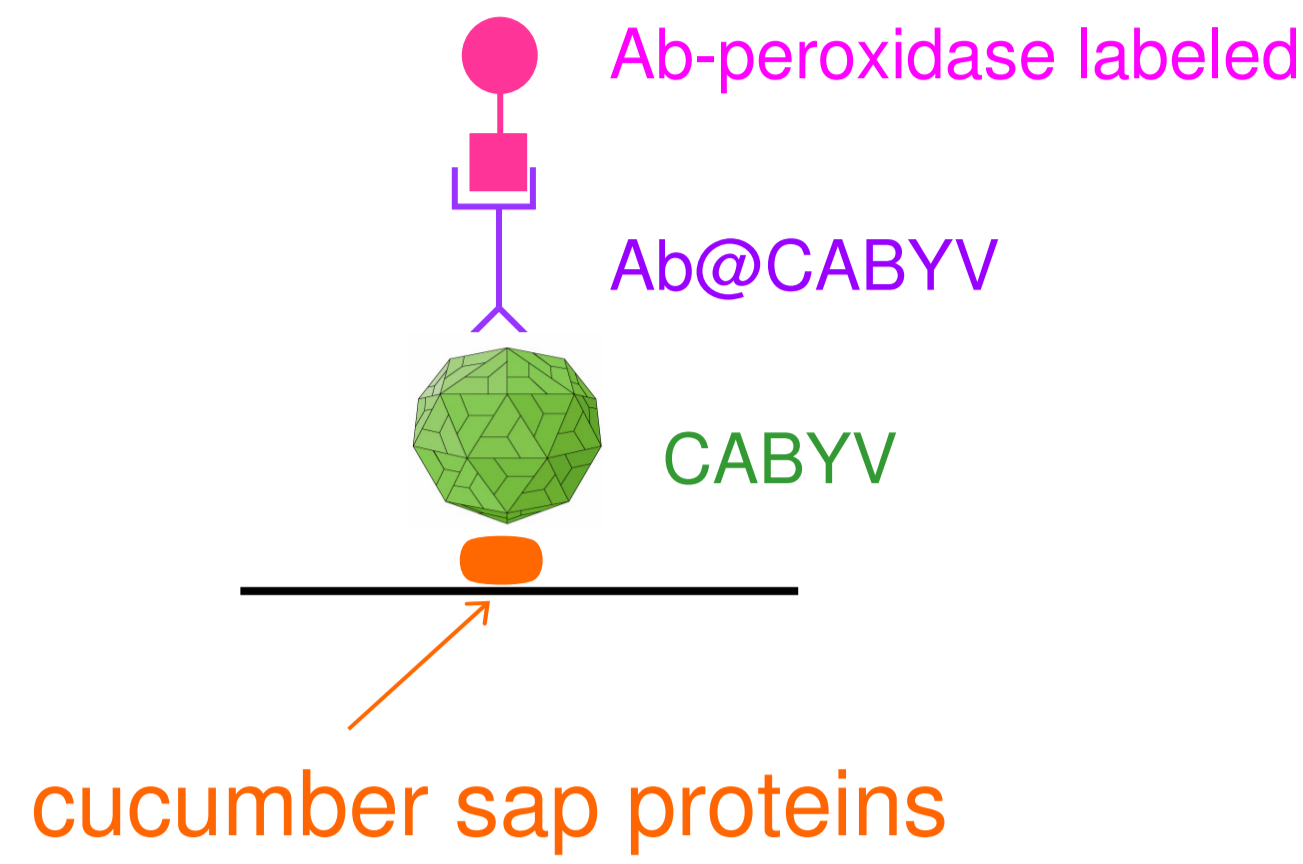
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Poleroviruses (*Luteoviridae* family) are phytoviruses strictly transmitted by phloem-feeding aphids in a circulative and non propagative mode. In the transmission cycle, the three partners, the plant, the virus and the aphid, play a crucial role.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



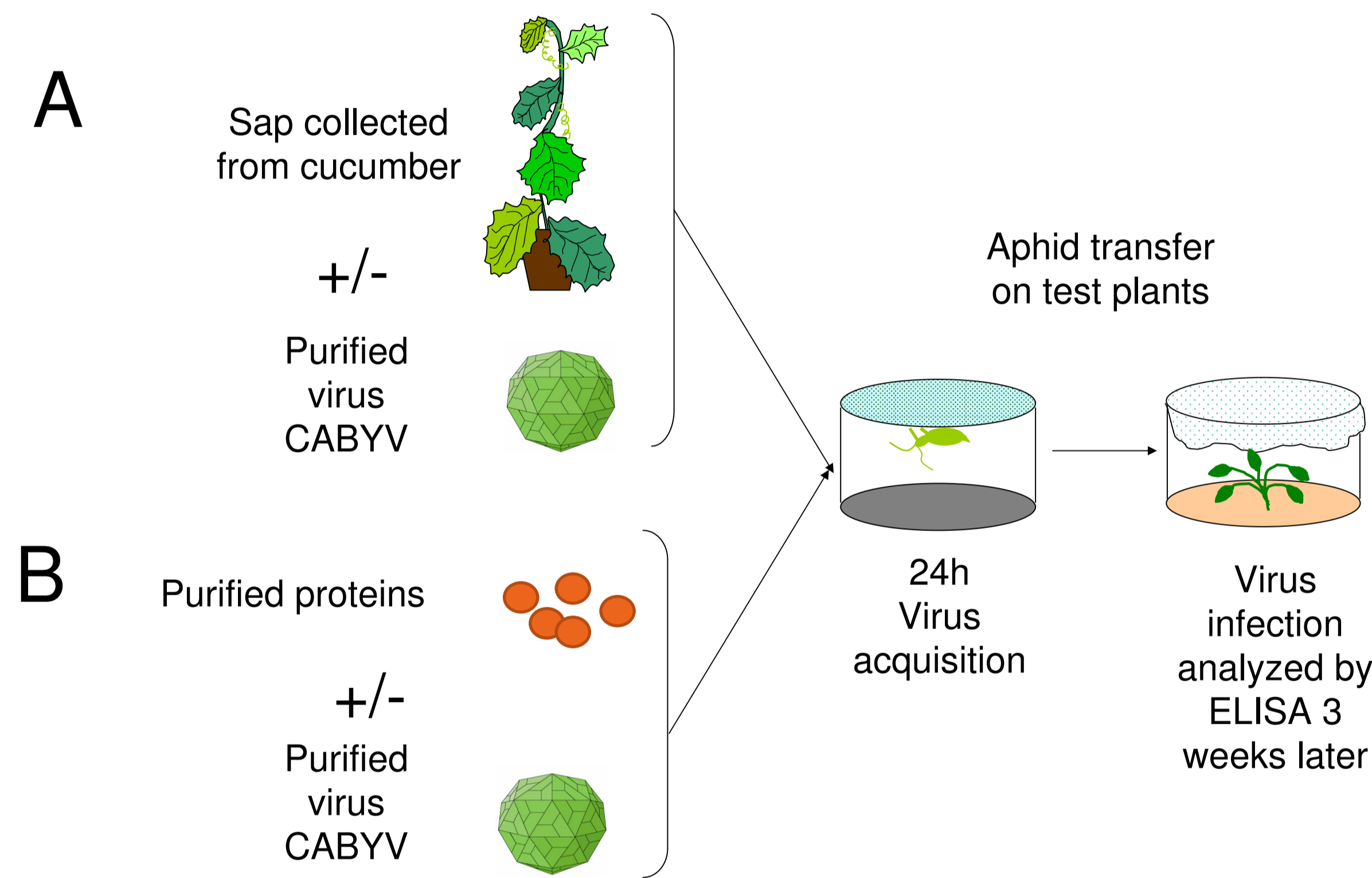
➤ About 20 sap proteins able to bind CABYV *in vitro*

➤ 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%)
A CABYV + sap	31/52 (60%)
B CABYV + AtPP2-A1^a	50/75 (71%)
B CABYV + AtPP2-A2^b	18/20 (90%)
B CABYV + BSA	28/42 (67%)
CABYV + Casein	10/10 (100%)
CABYV + Lyzosome	9/10 (90%)
CABYV + Cyto C^c	8/10 (80%)
CABYV + Carbonic anhydrase	10/10 (100%)
CABYV + LcH^d	0/9 (0%)
CABYV + MPA^e	0/9 (0%)

➤ 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

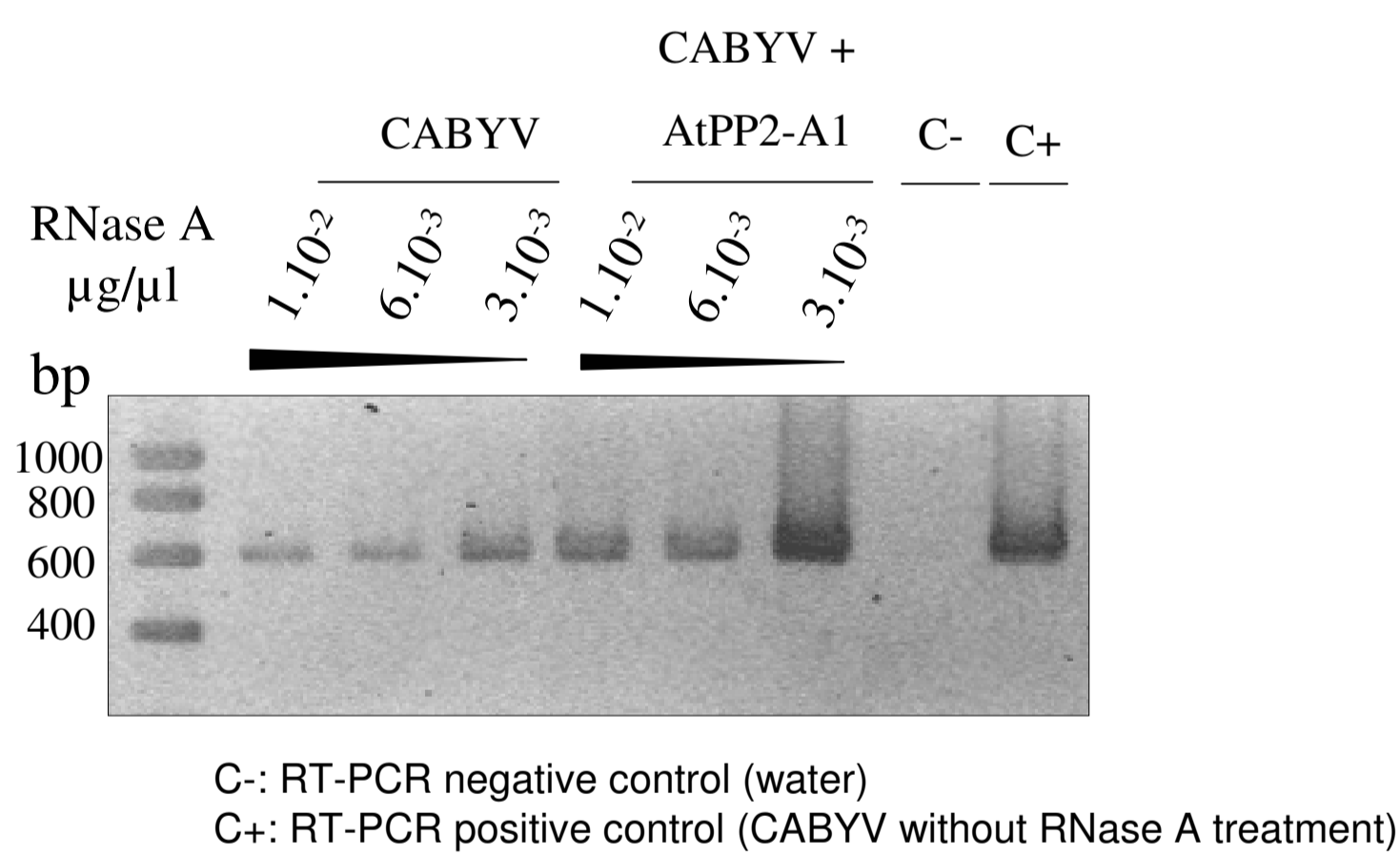
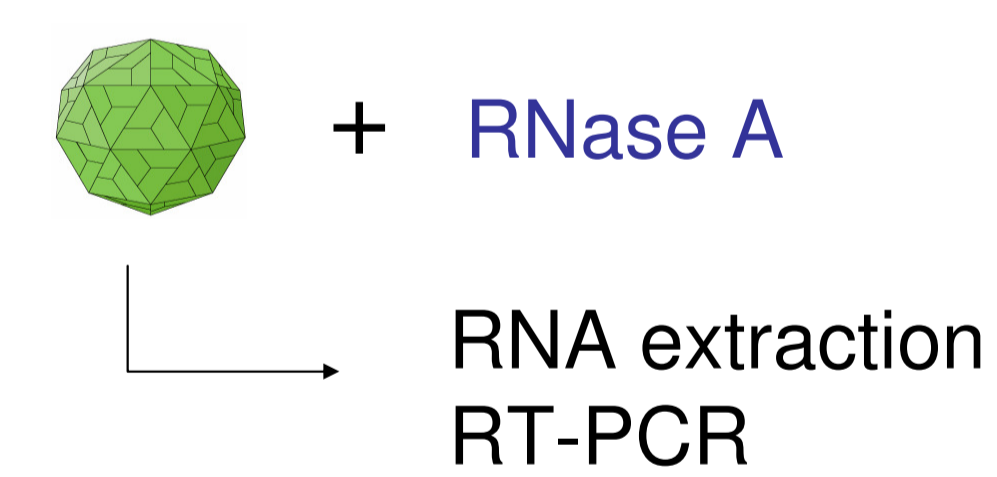
➤ Stimulatory effect not limited to phloem proteins

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

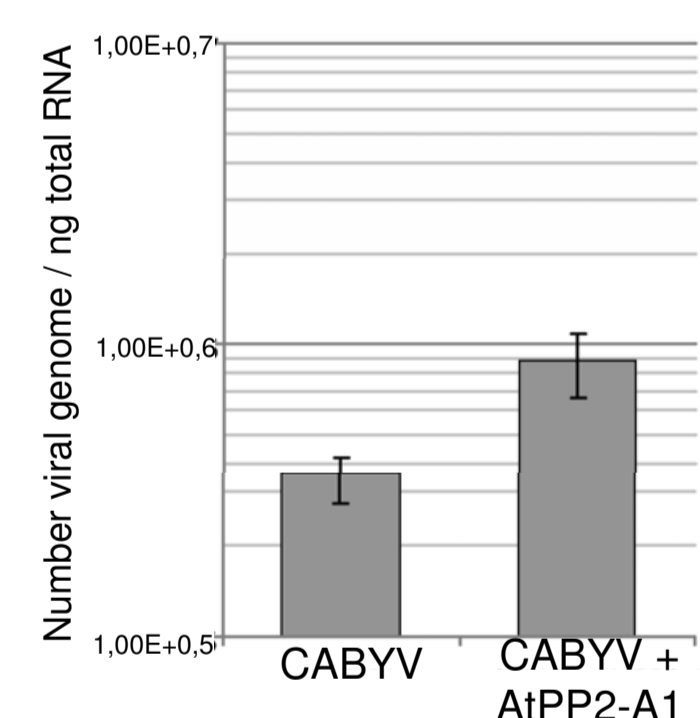
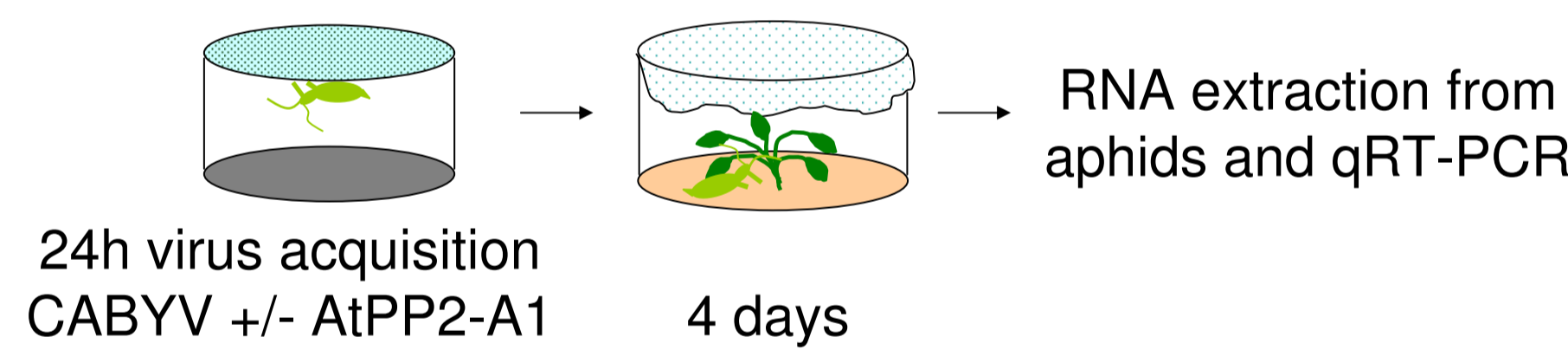
Bencharki B., Mol Plant Microbe Interact. 2010

Effect of a phloem lectin on virus acquisition and stability

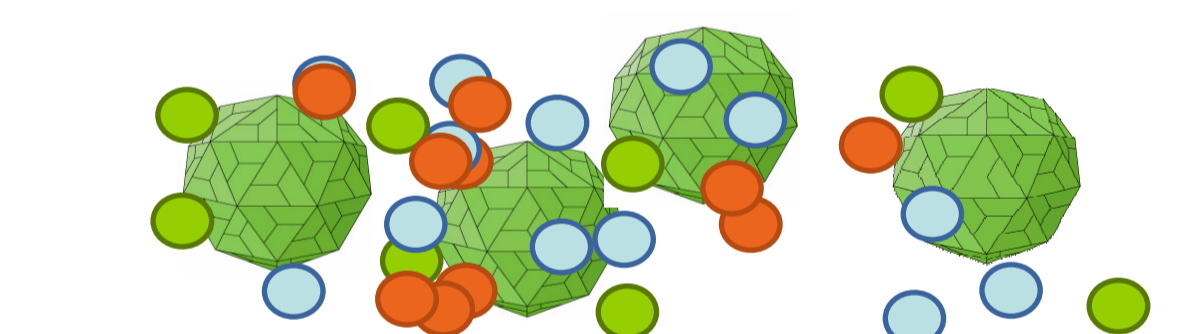
Experiments were designed to assess the role of one *A. thaliana* lectin (AtPP2-A1) on virion stability and on virus internalization in aphid cells



➤ 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



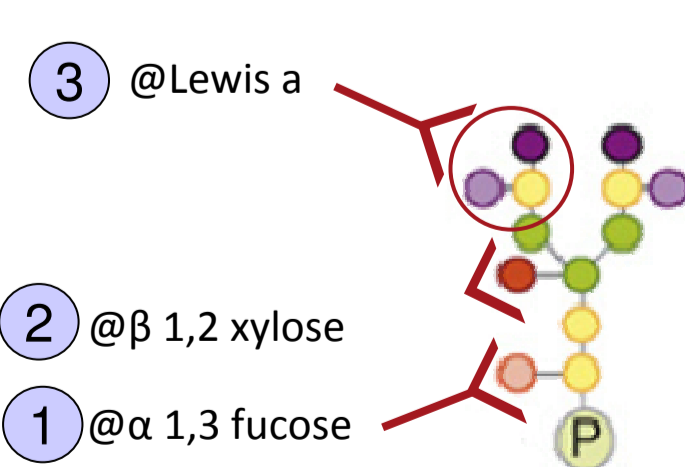
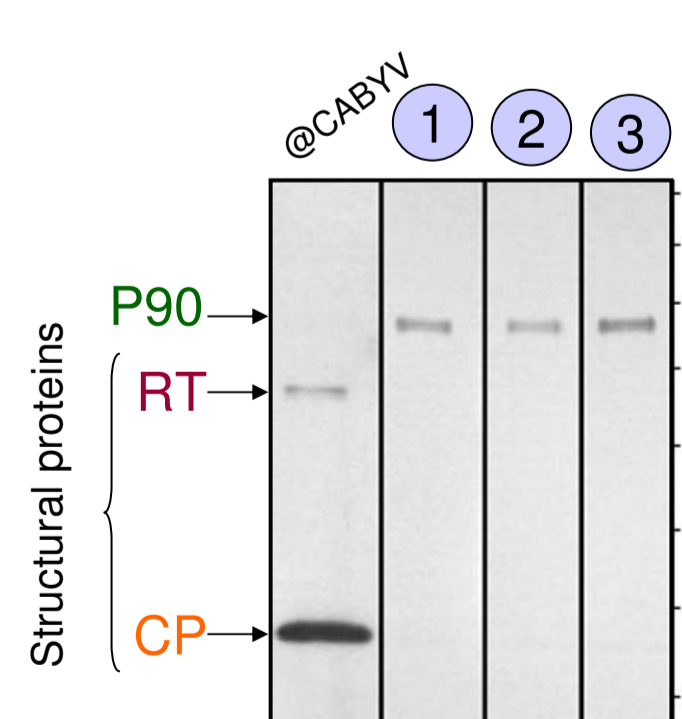
➤ Ingestion of phloem lectins together with virions: protection against protease activity in aphid digestive tube

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 PARRDRVVVY NPIGGPPRGR RQRNRRRPN
RGRARRGSP GETFVFSKDN LIGSSTGSIIT FGPSSLESPA FSSGILKAYH
EYKIIIMVQLE FISEASSTSS GSISYELDPH CKLSSLQSTI NKFGITKSLG
RRWTAKQNG MEWHATEDQK FKILYKNGS SSVAGSFRIT IKCQVQNPYK
VDGSPPPPSPPTPPPPP PQPQPQCAQ RFWYEGNPQ NKLLTAENSR
NIDSRPLNFV QMYKWEDEK DIKVLQAGYS RNDRCMETY LTIADKGFK
HVYLEADGEF VVKHIGDELG GSWLGNIAIV VSQRGNVGN YKGGKITNYQ
SNTVFVAGHP DATMNGKSF TARAVEVDWF ASFELECDDE EGSWAIYPP
IQKDSYNYI VSYGNYTEKY CEWGATSVSI DEDNNGNEPR RIPRRGVMAW
STPEPSFGD DSQRQFNTP SLEERGSAL ESEKKEEDN LLDLEENIP
DVDDDLKKG ISRASEAGTA EDRRASTSR LRGLKPKGL PKQPRTIIT
EFNPKDLIE VWRPDLAPGY SKADVAATV LAGGSVHEGR DMLERREAVK
MDSRKKWGL SSTSLSLTSGA LKLSAQSEK LATLTGTERV QYQRLKNSMG
STVAAYLEK VLADKTS

In blue: potential O-glycosylation sites

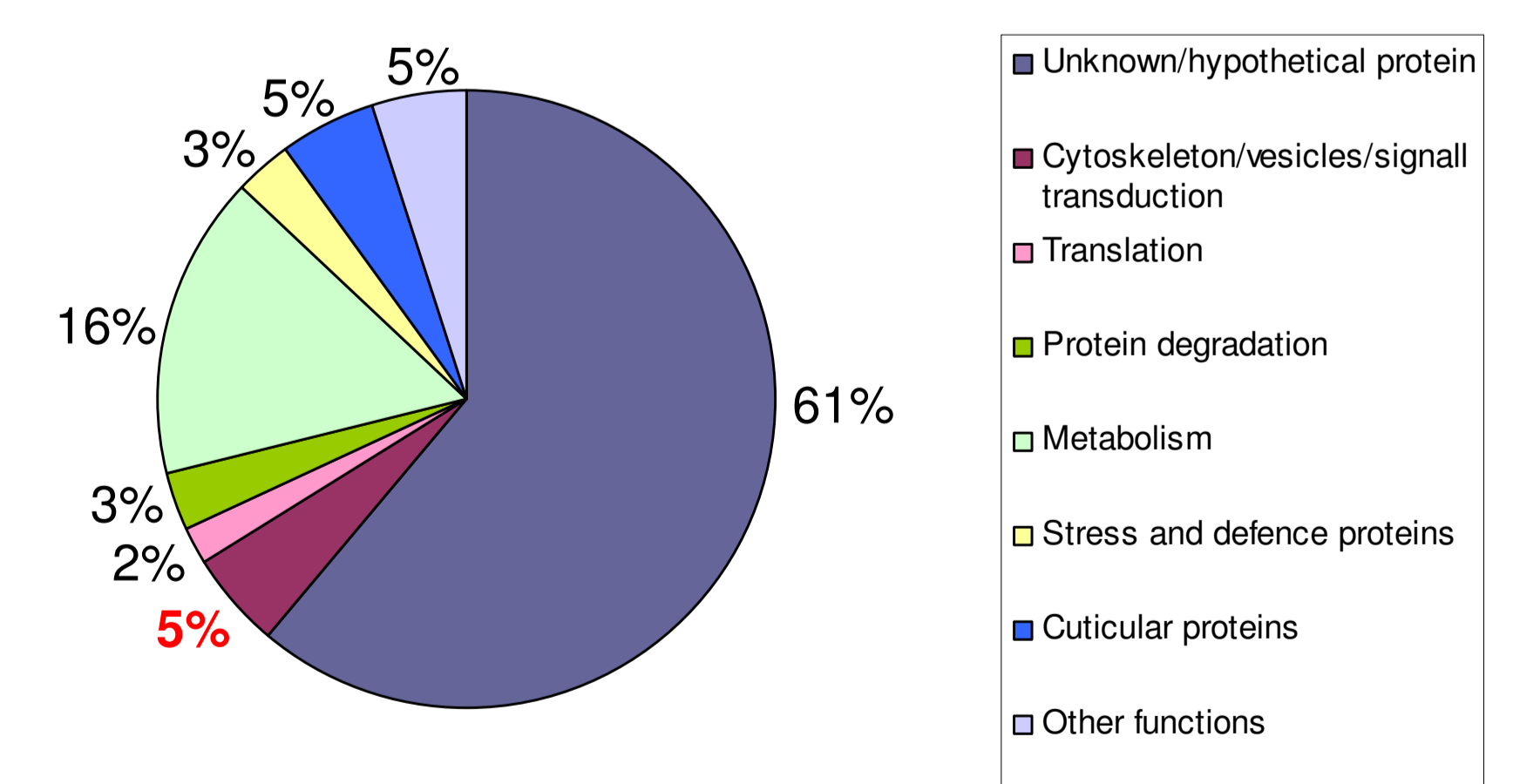
XXXX: Peptides identified by MALDI-TOF and by Nano LC-MS/MS on CABYV structural protein sequences. Potential N-glycosylation consensus sites are underlined

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

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 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