



Specificity in virus-vector interactions

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XXXIV Conference, Plant Virology Section, Committee of Plant Protection,
Polish Academy of Sciences

&

XII Scientific Symposium, Section of Plant Virology Section,
Polish Phytopathological Society

Specificity in virus-vector interactions

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Laboratoire 'Virologie & Vection'

UMR INRA-ULP 'Santé de la Vigne & Qualité du Vin'

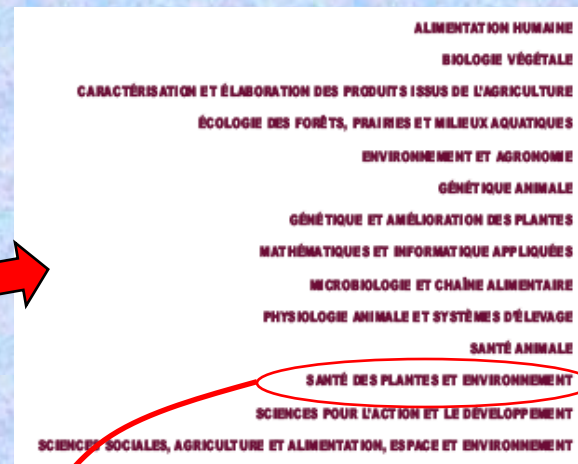
Colmar, France



Presentation of INRA

Institut National de la Recherche Agronomique

8,850 employees - 4,200 scientists & engineers
14 research departments
257 research units
21 research centers



Plant Health and Environment



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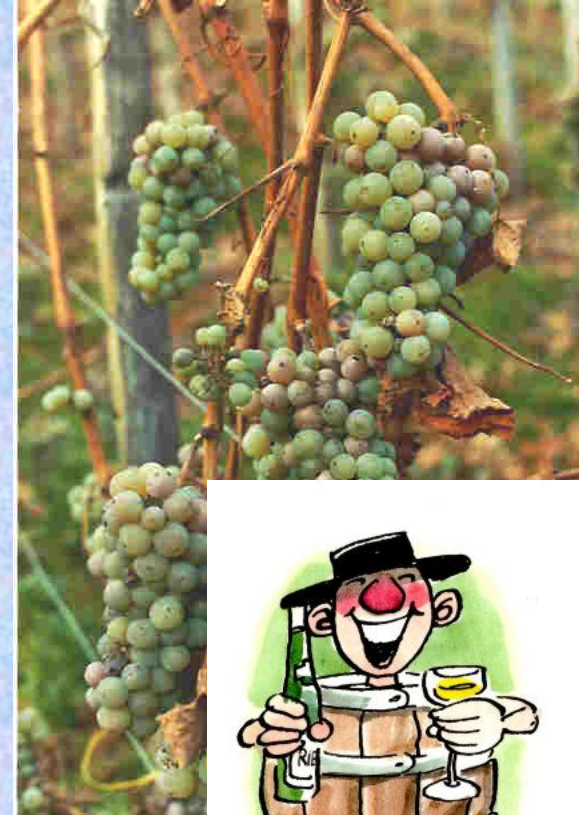
Unit « Santé de la Vigne et Qualité du Vin »
(*Grapevine Health and Wine Quality*)
ca. 50 permanent employees
INRA and Université L. Pasteur, Strasbourg

- 4 research groups:
- Grapevine genetics and breeding
 - Grapevine development
 - Oenology and yeast biology
 - **Virology & Vection**



Virology & Vection
4 scientists, 5 engineers, 5 technicians

- Main topics:*
- grapevine resistance to fanleaf disease
 - etiology and detection methodology
 - virus transmission by invertebrates

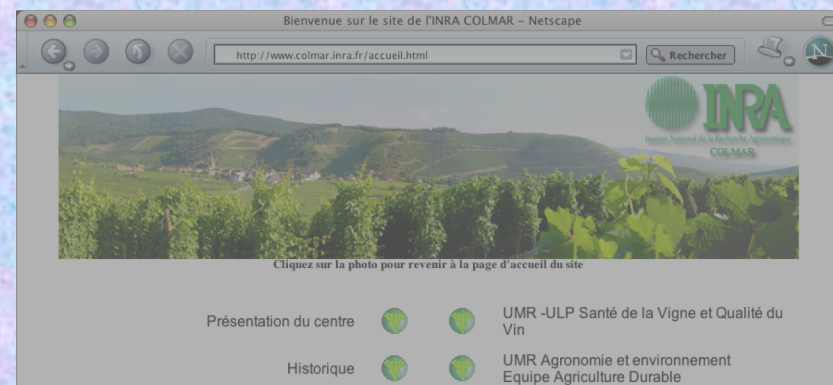


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Virus-vector specificity

what does it mean?

Vector species	Virus species										
	1	2	3	4	5	6	7	8	9	10	...
1			-								
2			-								
3			-								
4			+								
6			-								
7			-								
8			-								
9			-								
10			-								
...			...								

« Species-specificity »

>> two-entry table:
virus species * vector species
relation is compatible (+) or not (-)

a given virus species is transmitted by:

- one vector sp => **'high' specificity** (exclusivity)
- a few related vector spp => **specificity**
- many related vector spp => **'low' specificity,**
but not « unspecificity »

Vector specificity:

>> a continuum ranging from exclusivity to taxon-specificity

Virus-vector specificity

limits of « species-specificity »

- What do «+» and «-» mean?
 - >> vector efficiency *versus* vector competency
- What is a virus species? a vector species?
 - >> taxonomy evolution
- What about intraspecific variability?
 - >> viral strains, vector clones or stages
- What if new observations/data are collected?
 - « evidence for specificity of transmission is generally of a **negative character** and (...) there is always the possibility of additional vectors being discovered » (J.G. Leach, 1940, *Insect transmission of plant diseases*)

Virus-vector specificity

various dimensions of a concept

- Degree of specificity is linked to **intimacy** of virus-vector relations
- Effect of vector- and virus-**plant specificity**
- Modulation of specificity as a result of **co-infection**
e.g. transcapsidation (BYD viruses, W.F. Rochow) ; helper factors
- Mechanisms underlying specificity:
molecular interactions between viral « determinants » or « ligands » and vector « receptors »

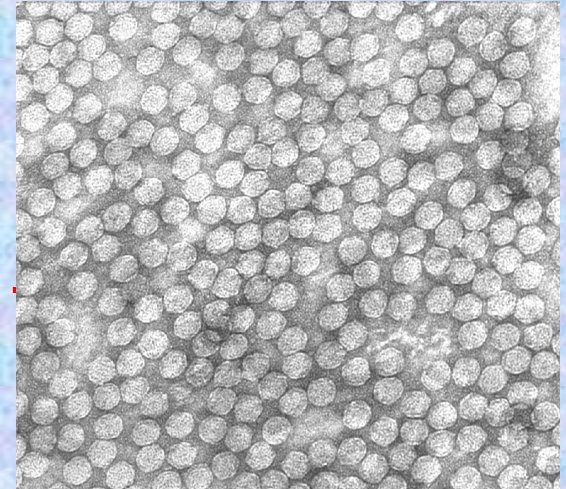
Our biological models of virus transmission by invertebrates

- Aphid-borne **Poleroviruses**
on herbaceous plants
- Nematode-borne **Nepoviruses**
on grapevine
- Scale-borne **Ampeloviruses**
on grapevine

Polerovirus - aphid



© R. Coutin OPIE

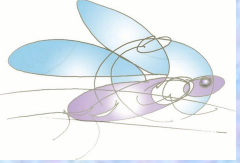


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*Sugar beet
mild yellowing*



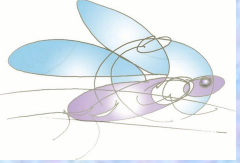
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Polerovirus - aphid

The pathosystem

- *Polerovirus*, *Luteoviridae*
- *Beet western yellows virus* (BWYV) and *Cucurbit aphid-borne yellows virus* (CABYV)
- infect **annual crops** (*Brassicaceae*, *Cucurbitaceae*, ...)
- (+) ss RNA virus, icosahedral particles
- phloem-limited viruses
- transmitted by **aphids** (*Homoptera*, *Aphididae*), in a circulative and persistent mode



Polerovirus - aphid

Vector specificity: high degree



Myzus persicae

Aphis gossypii

Macrosiphum euphorbiae

BWYV

CABYV

+

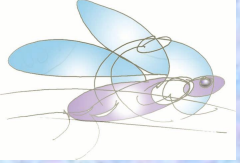
+

-

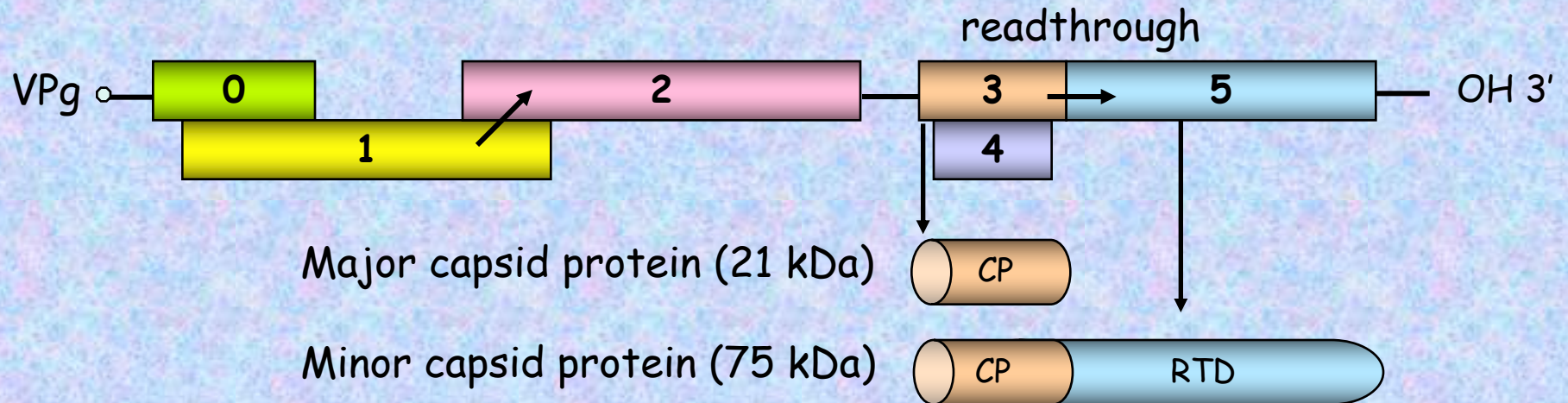
+

+

(+)



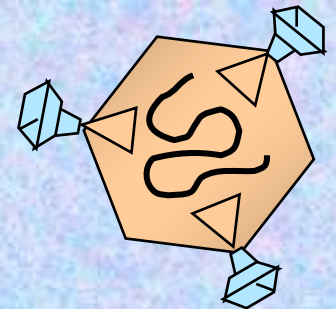
Polerovirus - aphid BWYV genome organization

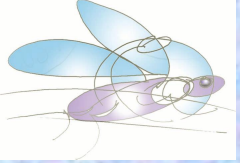


>> Transmission experiments of mutagenized virus revealed that both capsid proteins are indispensable for transmission

Brault *et al.*, 1995, *EMBO J.*; Bruyère *et al.*, 1997, *Virology*.

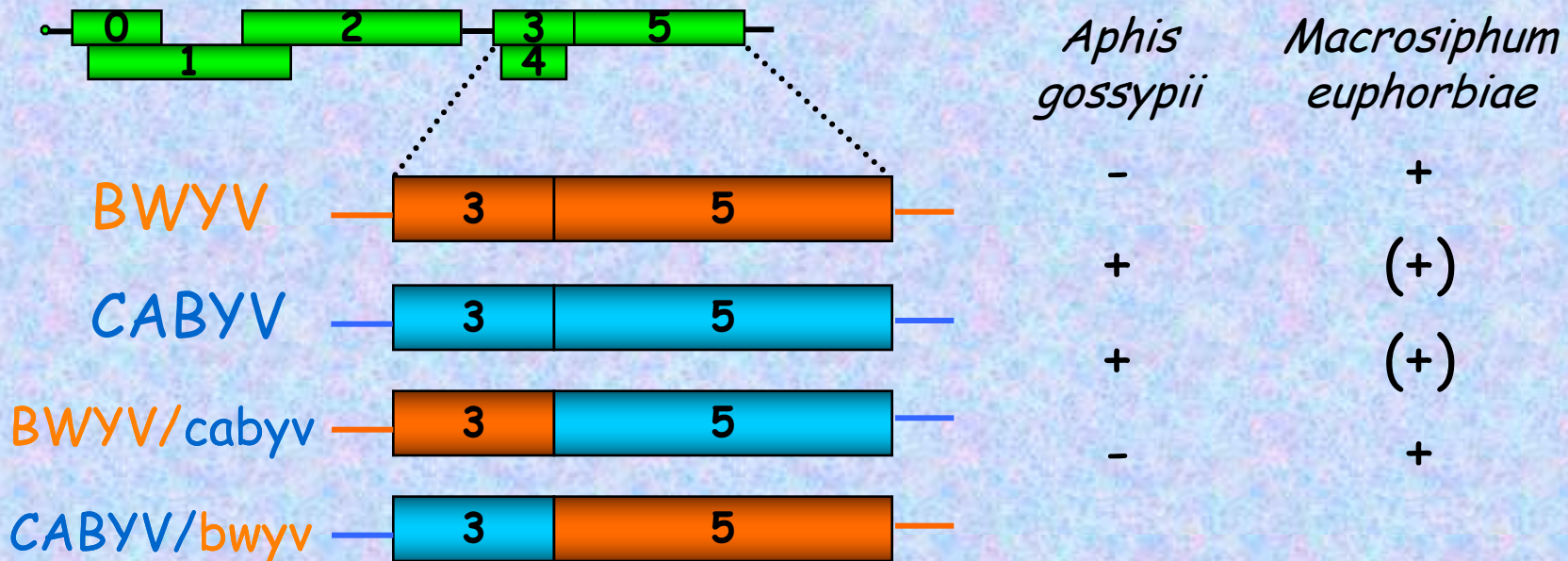
Brault *et al.*, 2000 & 2003, *J. Virol.*





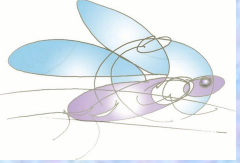
Polerovirus - aphid

Sequence exchange experiments (1)



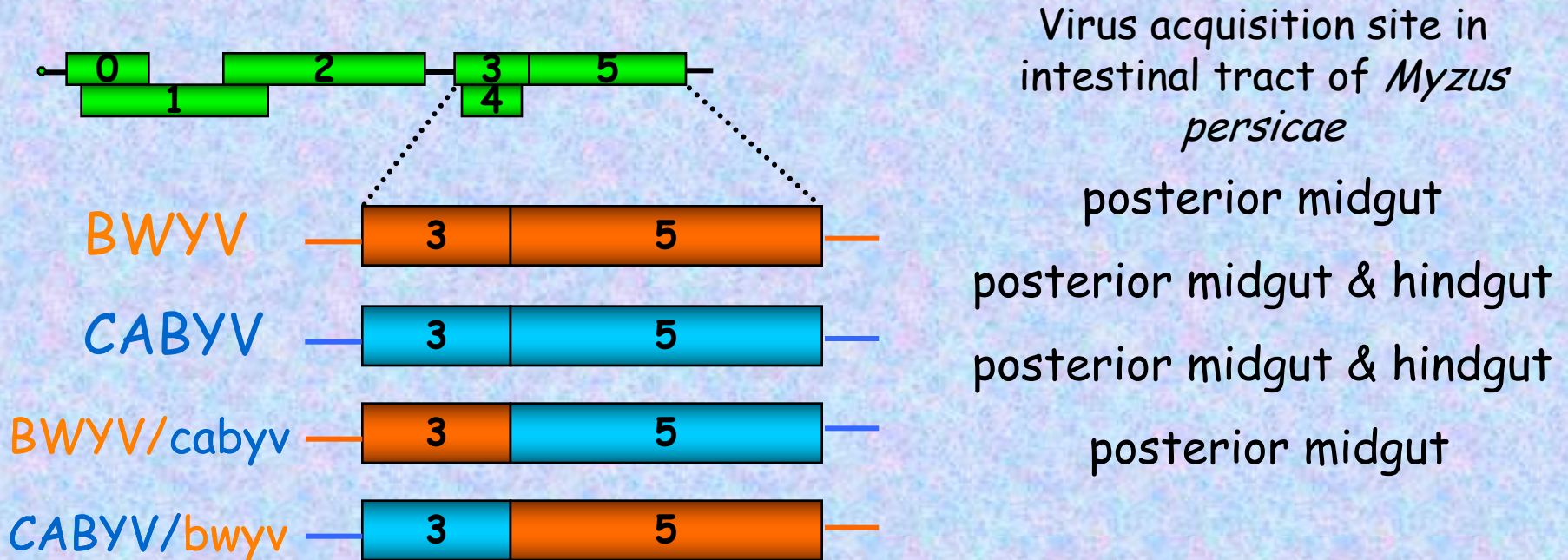
>> Transmission specificity is determined by the readthrough domain

Brault *et al.*, 2005, *J. Virology*



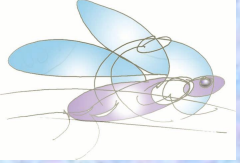
Polerovirus - aphid

Sequence exchange experiments (2)



>> Tissue tropism for acquisition across gut wall is determined by the readthrough domain

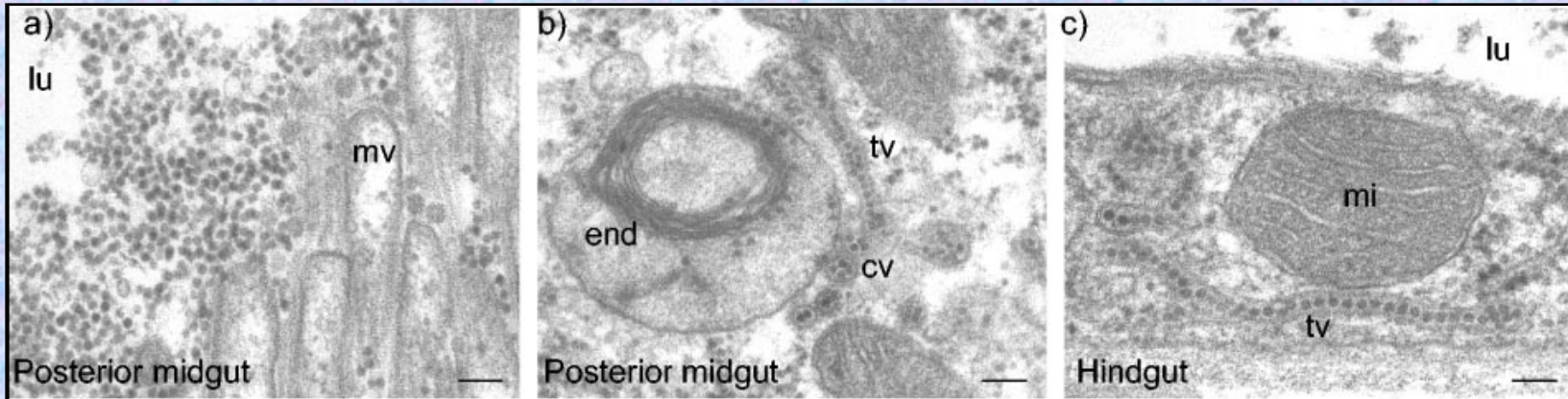
Reinbold *et al.*, 2003, *J. Gen. Virol.*; Brault *et al.*, 2005, *J. Virology*



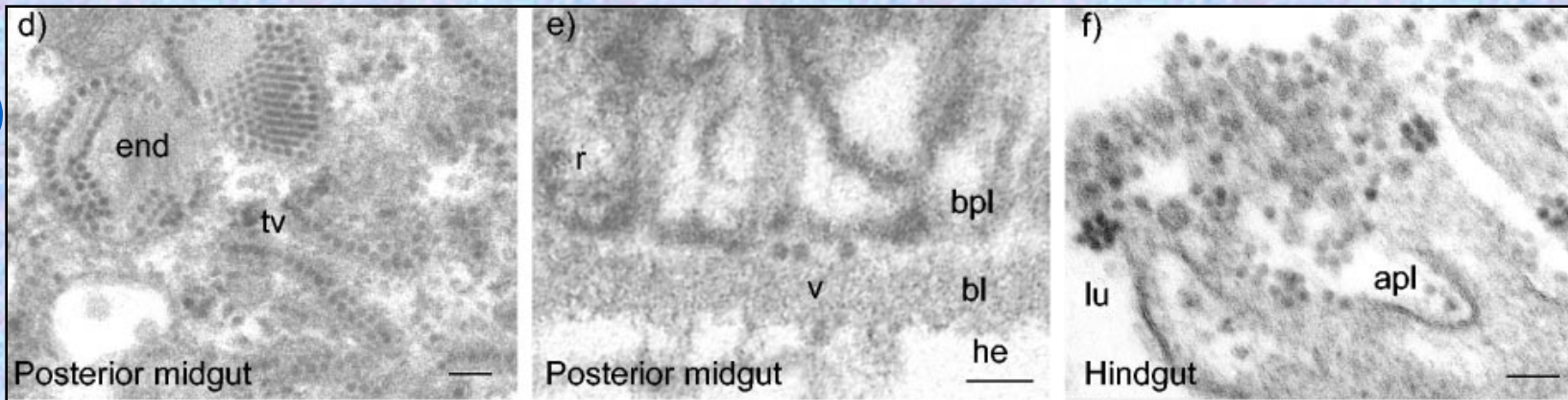
Polerovirus - aphid

Tissue tropism in *Myzus persicae*

BWYV
RTD(CA)



CABYV
RTD(BW)

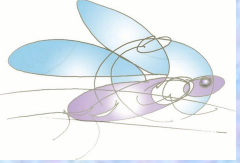


Recombinant virions in gut cells of *Myzus persicae*

Bar = 100 nm

apl = apical plasmalemma, bl = basal lamina, cv = coated vesicles, end = endosome-like vesicle, lu = lumen, he = hemolymph, mi = mitochondrion, mv = microvilli, r = ribosomes. tv = tubular vesicles, v = virions

© Catherine Reinbold, Brault *et al.*, 2005, *J. Virology*

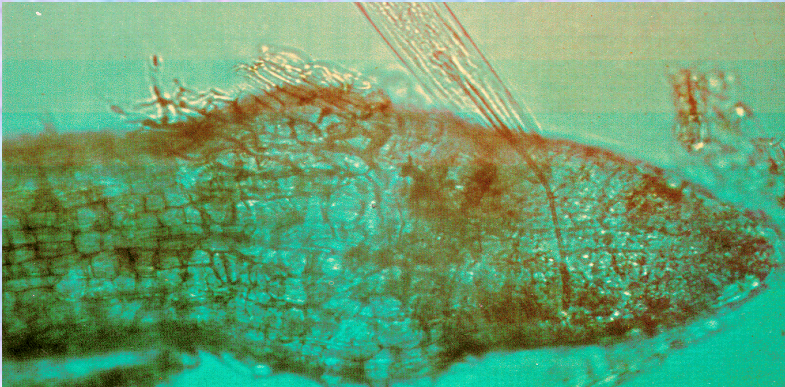


Polerovirus - aphid

Conclusions and prospects

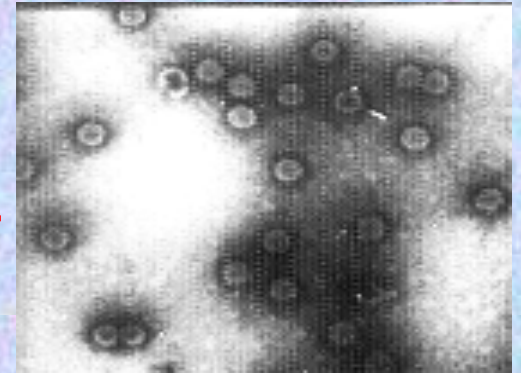
- Viral determinants of vector specificity and gut tropism are borne by **readthrough-domain** of minor capsid protein
- >> Search for **virus receptors** in vector's body (proteomic approach)
- >> Characterization of vector specificity of « **beet poleroviruses** » (*Beet mild yellowing virus*, *Beet chlorosis virus*, 'Beet western yellows virus')

Nepovirus - nematode



© INRA

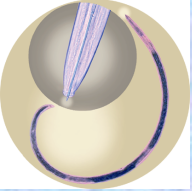
*Grapevine
fanleaf*



© Catherine Reinbold



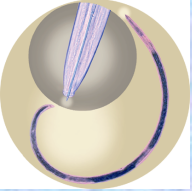
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Nepovirus - nematode

The pathosystem

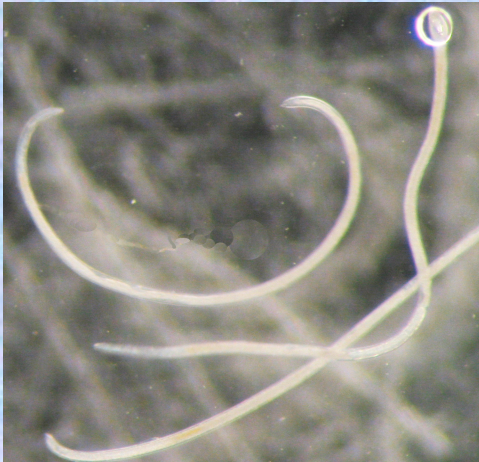
- *Nepovirus*, *Comoviridae*
- *Grapevine fanleaf virus* (GFLV) and *Arabidopsis mosaic virus* (ArMV)
- responsible for **grapevine fanleaf disease**
- 2 (+) ss RNAs, icosahedral particles
- not phloem-limited, invading all plant organs
- transmitted by a **nematode** (*Longidoridae*) in a non-circulative but 'persistent' mode



Nepovirus - nematode

Vector specificity: exclusivity

© G. Demangeat



Xiphinema index

Xiphinema diversicaudatum

GFLV

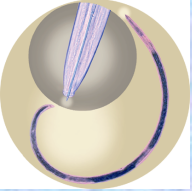
ArMV

+

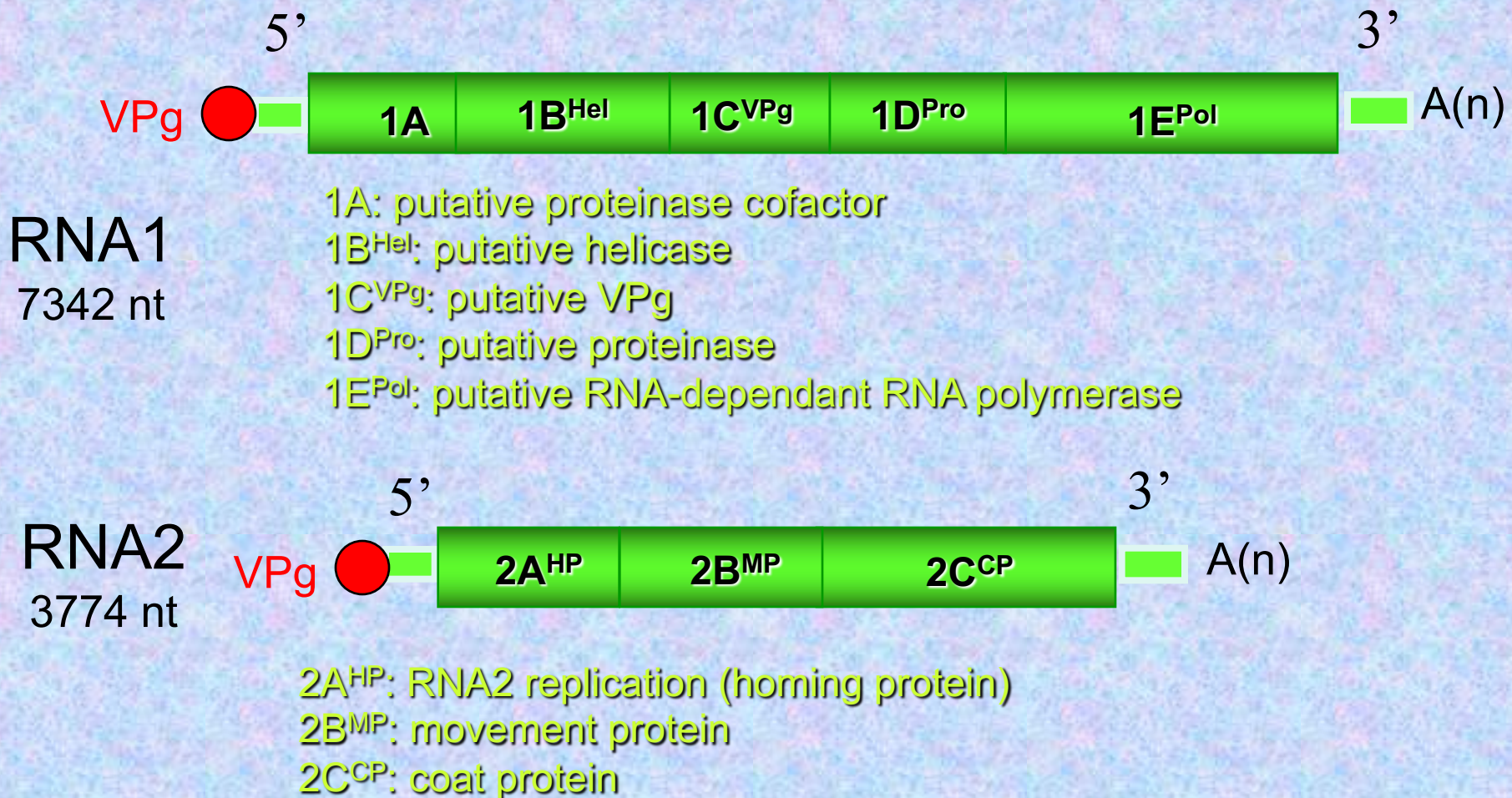
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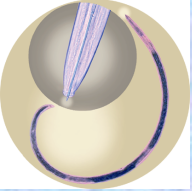
-

+



Nepovirus - nematode GFLV genome organization



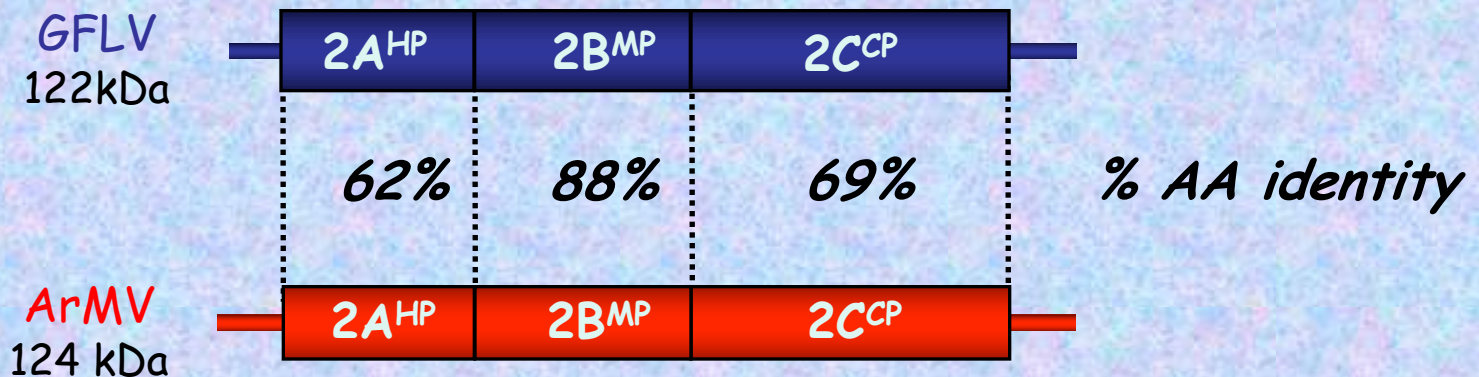


Nepovirus - nematode

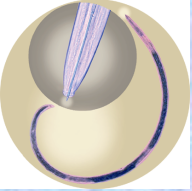
Which viral protein(s) involved?

- Transmission tests using pseudo-recombinant viruses showed that **only RNA2 is involved in transmission**

Scottish virologists, 1970-1980

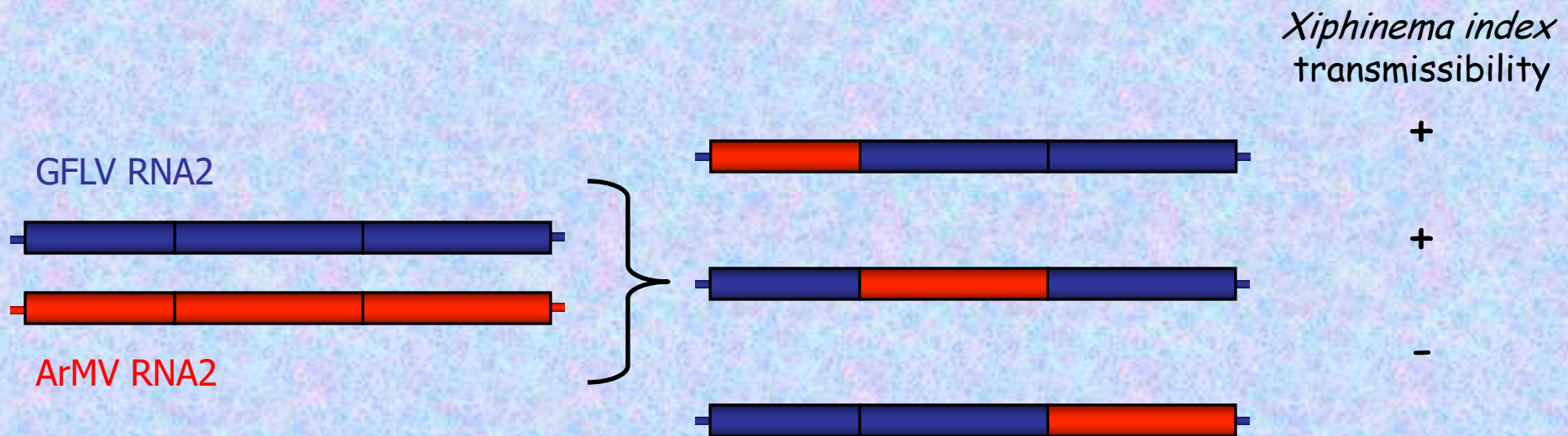


>> *Strategy:* gene exchange between infectious cDNA clones of GFLV and ArMV, and analysis of transmissibility of chimeric viruses



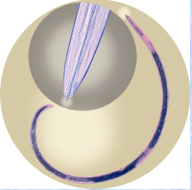
Nepovirus - nematode

Sequence exchange experiments



>> **only the coat protein gene 2C^{CP}** is involved in transmission specificity

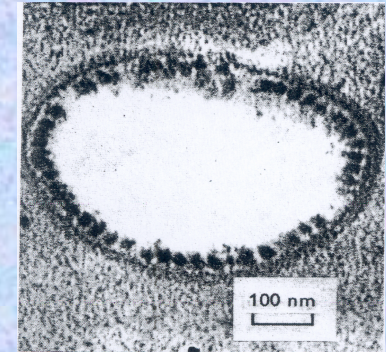
Belin *et al.*, 2001, *Virology*; Andret-Link *et al.*, 2004, *Virology*



Nepovirus - nematode

Prospects: what CP domain(s) involved?

Cross-section of a viruliferous nematode showing retained virions on the inner lining of the odontophorus
© Taylor et Robertson



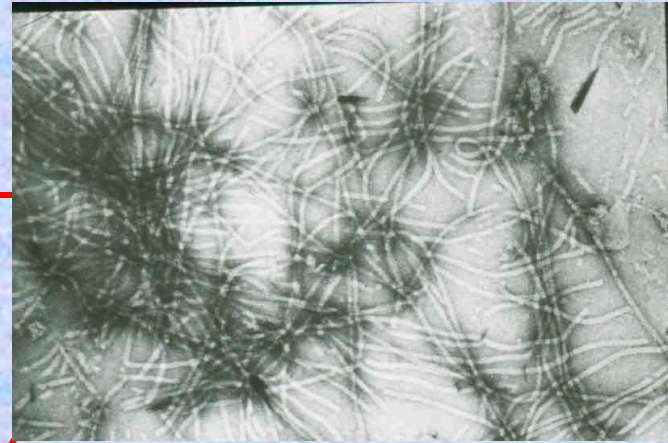
- >> Search for **domains exposed** on the particle surface and to be targetted
3-dimensional structure of GFLV capsid (coll. IBMP-CNRS Strasbourg)
- >> Sequence exchange between GFLV and ArMV and evaluation of **transmissibility of chimeric viruses** by *Xiphinema index* and *X. diversicaudatum*

Ampelovirus - scale insects



© Benoît Nusillard

*Grapevine
leafroll*



© Catherine Reinbold



© INRA



Ampelovirus - scale insects

The pathosystem

- *Ampelovirus*, *Closteroviridae*
- *Grapevine leafroll-associated virus -1 & -3* (GLRaV-1 & -3) in France
- associated with **grapevine leafroll disease**
- (+) ss RNA virus with flexuous particles
- phloem-limited viruses, often associated with *Grapevine virus A* (GVA, *Vitivirus*)
- vectored by **scales** (*Homoptera*, *Pseudococcidae* & *Coccidae*), probably in a non-circulative and semi-persistent mode



Ampelovirus - scale insects

Vector specificity

	Ampelovirus: GLRaV			Vitivirus	
	-1	-3	-5/-9	GVA	GVB
<i>Pseudococcidae</i> (mealybugs)					
<i>Heliococcus bohemicus</i>	+	+			
<i>Phenacoccus aceris</i>	+	+			
<i>Planococcus citri</i>		+		+	+
<i>Pl. ficus</i>	-	+		+	+
<i>Pseudococcus longispinus</i>	-	+	+	+	+
<i>Ps. calceolariae</i>	-	+			
<i>Ps. affinis</i>				+	+
<i>Ps. viburni</i>		+			
<i>Ps. maritimus</i>		+			
<i>Ps. comstocki</i>		+		+	
<i>Coccidae</i> (soft scales)					
<i>Parthenolecanium corni</i>	+			-	
<i>Pulvinaria vitis</i>		+			
<i>Neopulvinaria innumerabilis</i>	+			+	



Ampelovirus - scale insects

Vector specificity: 'northern' vineyard

GLRaV-1 GLRaV-3 GVA

Pseudococcidae

Heliococcus bohemicus

+

+

Phenacoccus aceris

+

+

Coccidae

Parthenolecanium corni

+

-



© B. Nusillard



Ampelovirus - scale insects

Natural infectivity experiments

Objective: to assess **infective potential** of scales sampled on infected vineyard (natural AAP) and transferred onto healthy plants (IAP)

Results:

- *P. corni* nymphs from GLRaV-1-infected vines inoculated healthy vines (36%)
- *P. corni* nymphs from GVA-infected vines inoculated healthy vines (37%)

>> ***P. corni* is naturally infective with GLRaV-1 and GVA**



Ampelovirus - scale insects

Controlled transmission experiments

Objective: to assess **vector efficiency** of non-viruliferous scales confined on infected plant (controlled AAP) and transferred to healthy plants (IAP)

Results:

- *P. corni* nymphs having acquired GLRaV-1 inoculated healthy vines (13%)
- *P. corni* nymphs having acquired GVA & GLRaV-1 inoculated healthy vines (22%)

>> ***P. corni* vectors GLRaV-1 and GVA** in lab conditions



Ampelovirus - scale insects

Conclusions and prospects

- *Parthenolecanium corni* vectors GLRaV-1 and **GVA**
- GVA seems to require **co-infection** with another virus (GLRaV-1 here) to be transmitted
 - >> Effect of **co-infections** of vector specificity
 - >> Characterize **vector competency** of mealybugs *Heliococcus bohemicus* and *Phenacoccus aceris*
 - >> Epidemiological role of scales in **natural spread** of grapevine leafroll



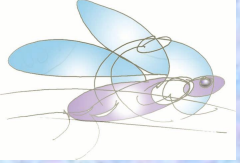
© Babache

A final question: what natural vector for **GLRaV-2**?

- *Grapevine leafroll-associated virus-2* (GLRaV-2):
a member of genus *Closterovirus*, along with:
 - * **Beet yellows virus** vectored by *Aphis fabae* & *Myzus persicae*
 - * **Citrus tristeza virus** vectored by *Aphis gossypii* (among others)
- *Hypothesis*: is GLRaV-2 transmitted by **aphids**?
- Our experiments showed **no transmission** of GLRaV-2
by either *M. persicae*, *A. gossypii*, *A. fabae*
- *Conclusion*:
 - >> vector of GLRaV-2 remains to be identified
or
 - >> GLRaV-2 has lost its transmissibility

General conclusions

- **Diverse biological models** of virus-vector interactions :
 - transmission mode and persistence
 - magnitude of vector specificity
 - nature of host plants (woody *vs* herbaceous)
 - vector biology (aerial *vs* soil-living)
 - underlying mechanisms
- Comparative studies and synergy
- Need for a **multidisciplinary** approach



Acknowledgements

Polerovirus work:

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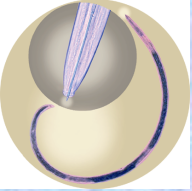
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Dziękuję bardzo!

