

# Vírus e Viroides

Jorge A. M. Rezende

# Vírus - histórico



Hieroglifo egípcio  
3700 a.c.

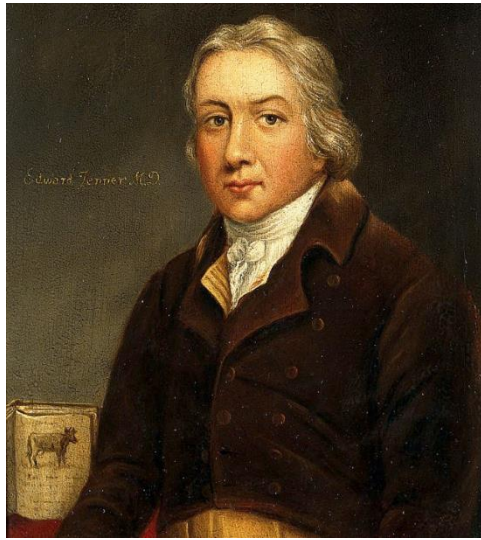
Vacinas Sabin e Salks



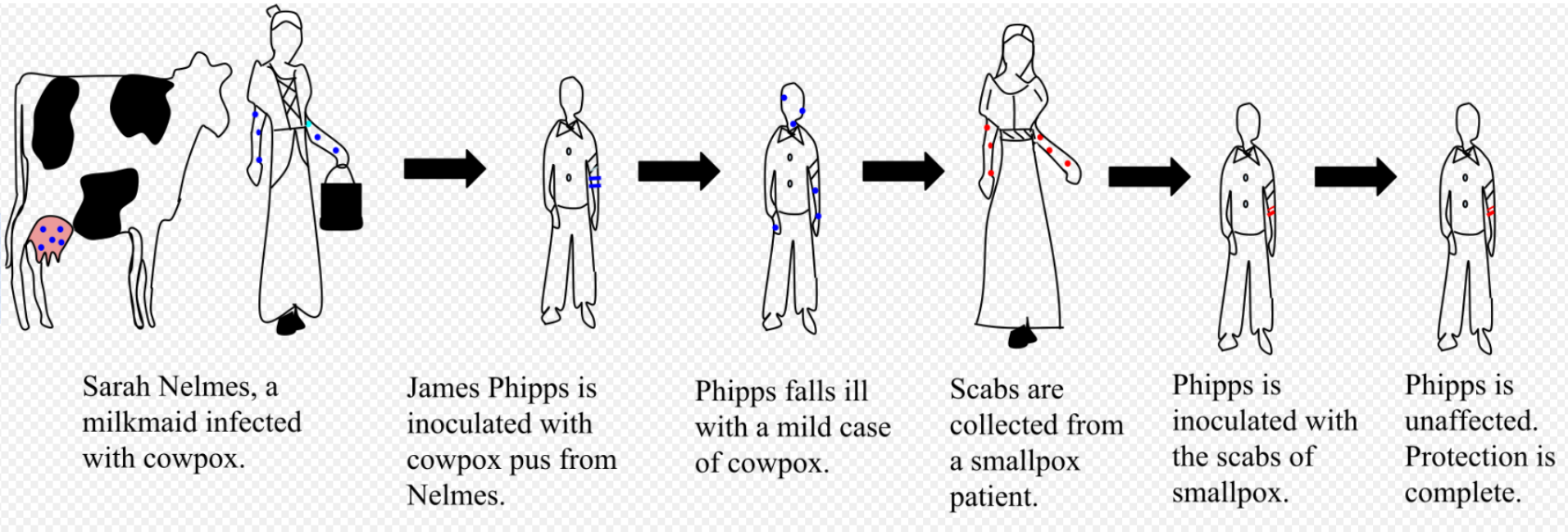
Rhamses V  
Egito, 1146 a.c.

Edward Jenner, 1789: primeira vacina  
Erradicada década de 1970





## Edward Jenner, 1789: primeira vacina contra variola



Sarah Nelmes, a milkmaid infected with cowpox.

James Phipps is inoculated with cowpox pus from Nelmes.

Phipps falls ill with a mild case of cowpox.

Scabs are collected from a smallpox patient.

Phipps is inoculated with the scabs of smallpox.

Phipps is unaffected. Protection is complete.

752: Poema amarelo do *Eupatorium lindleyanum*  
"Tobacco leaf curl virus"



**Asteraceae**



# 1600 - 1660: "Tulipomania" na Holanda ("Tulip breaking virus", 1926)



Fig. 1. *Tulip Planting in Spring* by Pieter Brueghel, the Younger. Reproduced with permission from Scala/Art Resource, New York.

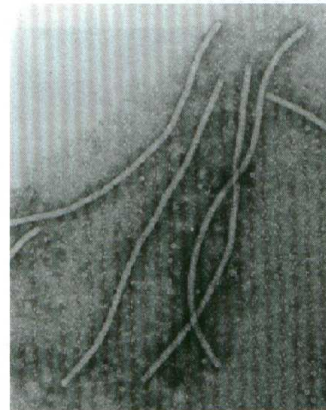


Fig. 9. Negatively stained virions of *Tulip breaking virus*. The particles are filamentous, usually flexuous, 750 to 775 nm long and 14 nm wide. Reproduced from <http://www.ncbi.nlm.nih.gov/CTVdb/Wintkey/images/a1.gif>



Fig. 10. Flower-breaking symptoms in *Tulip breaking virus*-infected tulips (Madame Spoor). Light and dark breaking symptoms can be observed. Reproduced from Brunt et al. (9).

# VALORES DE MECADORIAS = TULIPA RARA (Semper Augustus) Panfleto de 1636

**Guilders (Dutch florins)**

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<b>8 porcos gordos</b>	<b>240</b>
<b>4 bois gordos</b>	<b>480</b>
<b>12 carneiros gordos</b>	<b>120</b>
<b>24 ton. de trigo</b>	<b>448</b>
<b>48 ton. de cevada</b>	<b>558</b>
<b>2 barris de vinho</b>	<b>70</b>
<b>4 barris de cerveja</b>	<b>32</b>
<b>2 ton. de manteiga</b>	<b>192</b>
<b>~500 Kg de queijo</b>	<b>120</b>
<b>Um copo de prata</b>	<b>60</b>
<b>Um “pacote” de roupas</b>	<b>80</b>
<b>Uma cama, colchão e roupa de cama</b>	<b>100</b>
<b>Um barco</b>	<b>500</b>
<b>TOTAL</b>	<b>3000</b>

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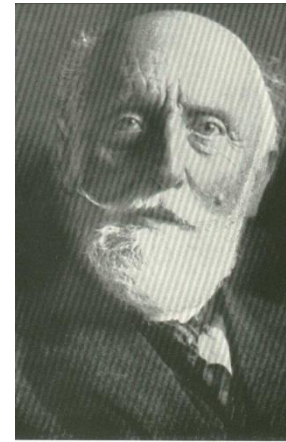
Lesnaw & Ghabrial, 2000

**3000 Florins = US\$ 900.000,00**

(Hull, R., 2009)

## 1886: ADOLF MAYER

Transmissão agente causal  
mosaico do fumo por injeções



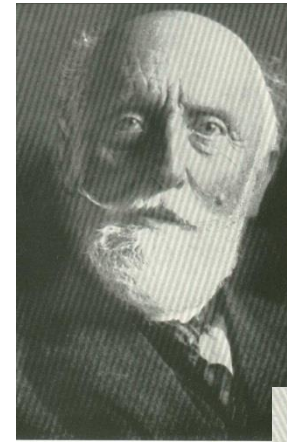
ADOLF MAYER



- “Mosaic disease of tobacco”.
- Causa não nutricional.
- Nematoides excluídos.
- Não estava associada às mudas.
- **TRANSMISSÃO (90%)**.
- Postulado de Koch falhou.
- Passou por filtro de papel
- Aquecimento 65-75°C não altera infecção.
- Aquecimento acima de 80 °C abole infecção.
- **CONCLUSÃO: doença bacteriana.**

## **1886: ADOLF MAYER**

Transmissão agente causal  
mosaico do fumo por injeções



ADOLF MAYER

## **1892: DMITRI IVANOWISKI**

Agente do mosaico do fumo passa  
por filtro que retém bactéria  
**CONCLUSÃO: doença bacteriana.**

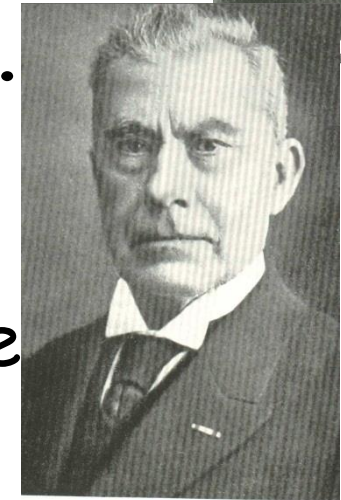


IVANOWSKI

## **1898: MARTINUS BEIJERINK**

Repetiu testes. Agente difundiu-se  
em gel de agar.

**FLÚIDO VIVO CONTAGIOSO**



MARTINUS WILLEM BEIJERINCK

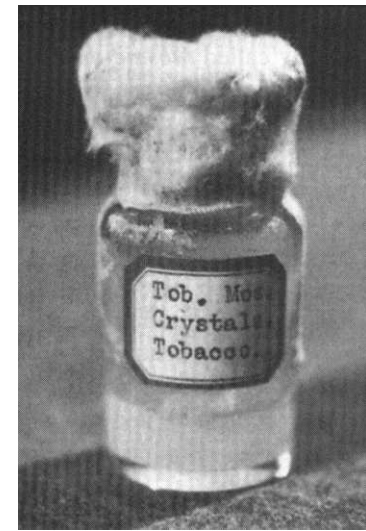
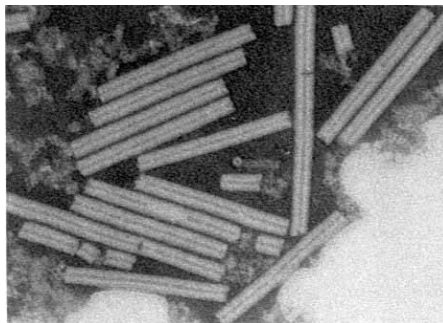


**1935: STANLEY**

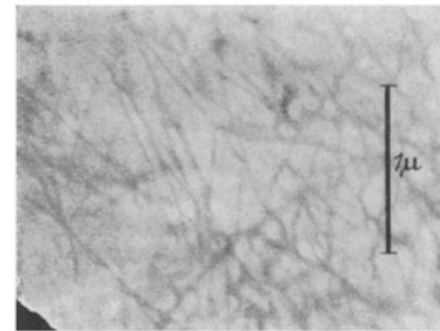
**Purificou TMV,  
continha proteína  
PRÊMIO NOBEL**

**1935: BAWDEN & PIRIE  
TMV continha RNA**

**1939: KAUCHE ET AL  
TMV no Micros-  
cópio eletrônico**



**Na TV - 1960  
Infeccioso**



II 263/38.

Fig. 3. Kollodiumfilm mit niedermolarem Eiweiß und  
Tabakmosaikvirus beschickt. el.opt. 19000 : 1.

# Vírus - importância

- International Committee on Taxonomy of Viruses - 2022:
  - ~ 10.434 espécies de vírus (290 em 1971);
  - 42 espécies de viroides
  - ~ Milhares de vírus ainda não caracterizados

[https://talk.ictvonline.org/taxonomy/p/taxonomy\\_releases](https://talk.ictvonline.org/taxonomy/p/taxonomy_releases)

## - Infectam:

- Vertebrados: homem e outros animais

- Invertebrados



*Anticarsia gemmatalis*  
*multiple nucleopolyhedrovirus*

- Bactérias, fungos, algas, leveduras e protozoários

- Plantas

# Vírus - o vírion

**Vírion** = partícula viral em sua forma extracelular

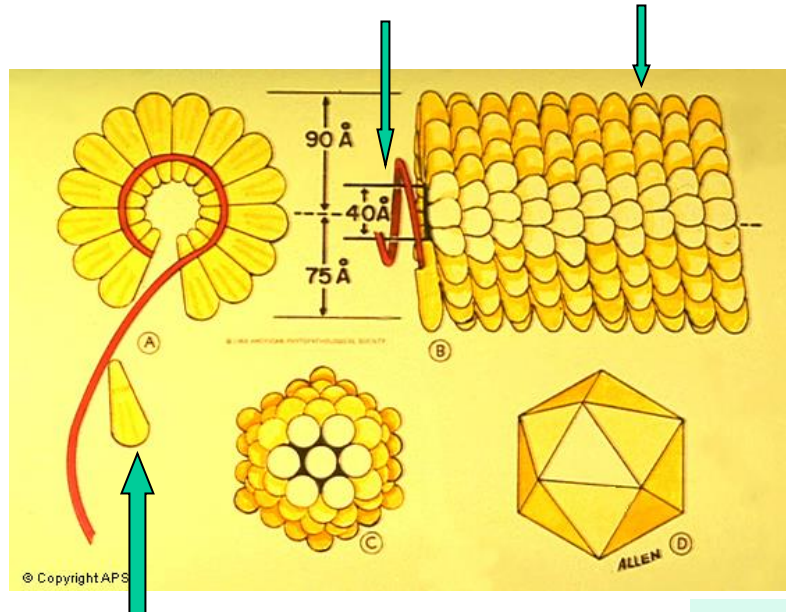
Ácido nucléico

RNA ou DNA

Fita simples

Fita dupla

Ácido nucléico    Capa protéica

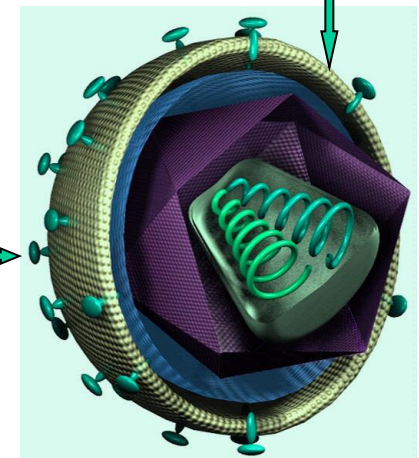


Capsômero

Envelope

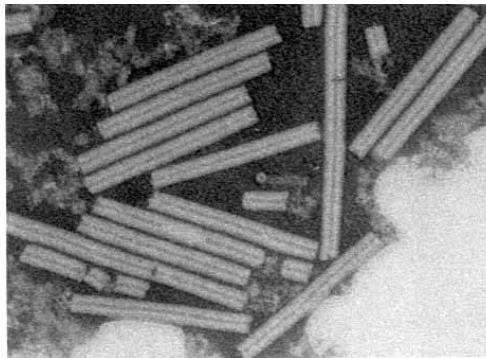
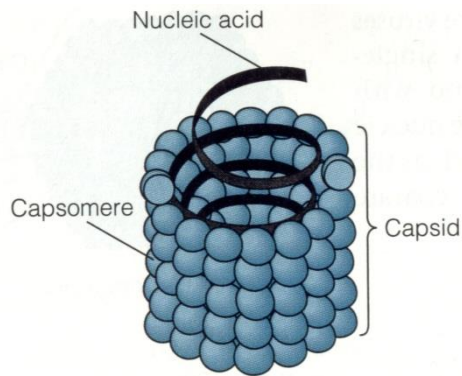
Espículas

- Capa protéica
- Envelope (alguns)
- Espículas (alguns)
- Replicação na hospedeira
- Parasitas obrigatórios
- Visualizados somente no microscópio eletrônico

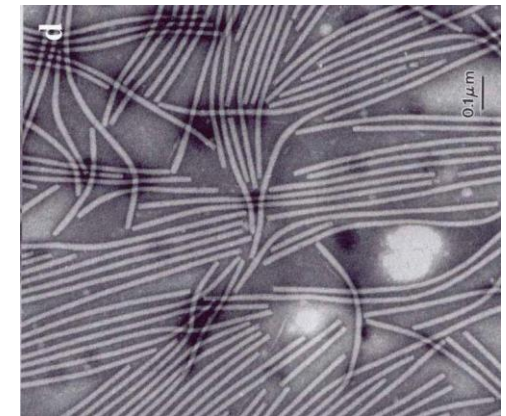


# Vírus - Formas Básicas

## 1. Helicoidal: rígido ou flexuoso com ou sem envelope

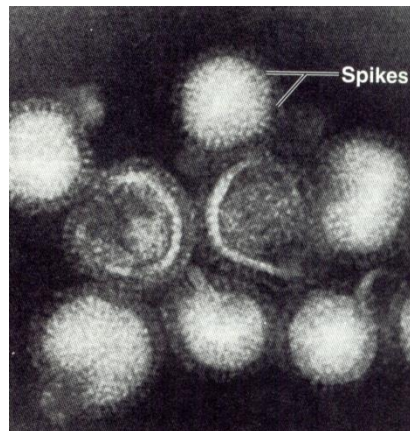
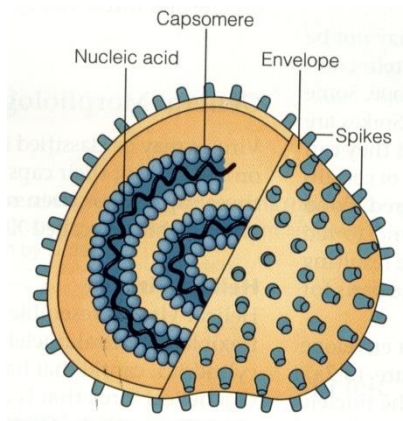


RÍGIDO (TMV)



Potyvirus

FLEXUOSO



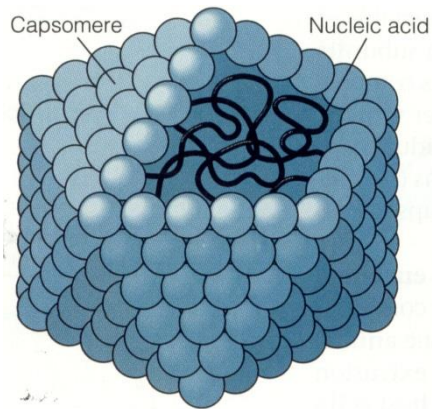
Influenzavirus

ISOMÉTRICO (Esférico)

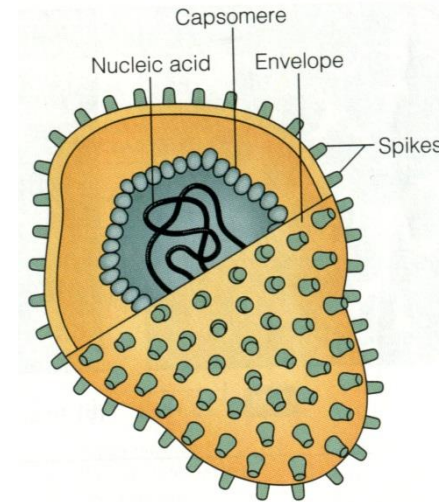
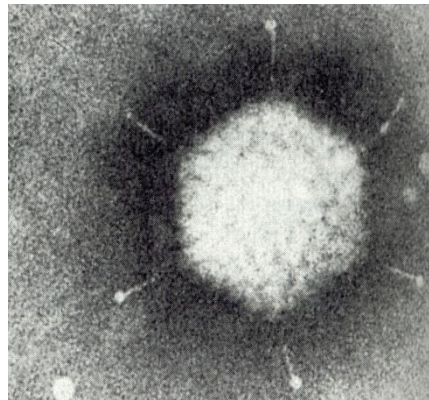


# Vírus - Formas Básicas

## 2. Poliédrico: Icosaédrico (maioria)

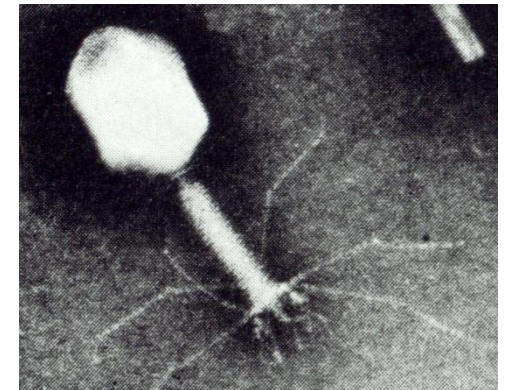
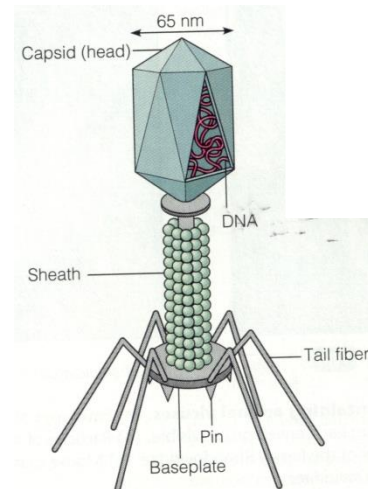


Sem envelope



Com envelope

## 3. Complexa



# Características do genoma



- o Ácido nucléico pode ser fita dupla ou simples  
RNAs; RNAfd; DNAs e DNAfd
- o RNAs: positiva ou negativa
- o O ácido nucléico pode estar segmentado
- o Genoma variável
- o Genes codificam proteínas:
  - o Estruturais = capa protéica
  - o Não estruturais = RNA polimerase;  
transcriptase reversa (HIV);  
lisosima (bacteriófagos); etc

# Vírus - entrada na célula

## A. Vírus de animal

a) Adsorção: contacto com a célula

Proteínas ou glicoproteínas da partícula.

Receptores na superfície da célula.

(glicoproteínas ou lipoproteínas)

b) Penetração: entrada no citoplasma

- Vírus sem envelope: translocação por **endocitose**

- Vírus com envelope:

**Endocitose**: Partícula é presa numa invaginação da célula, e liberada dentro desta na forma de vesícula.

**Fusão**: envelope viral funde-se com a membrana e o virion passa diretamente para dentro.

# Poliovirus

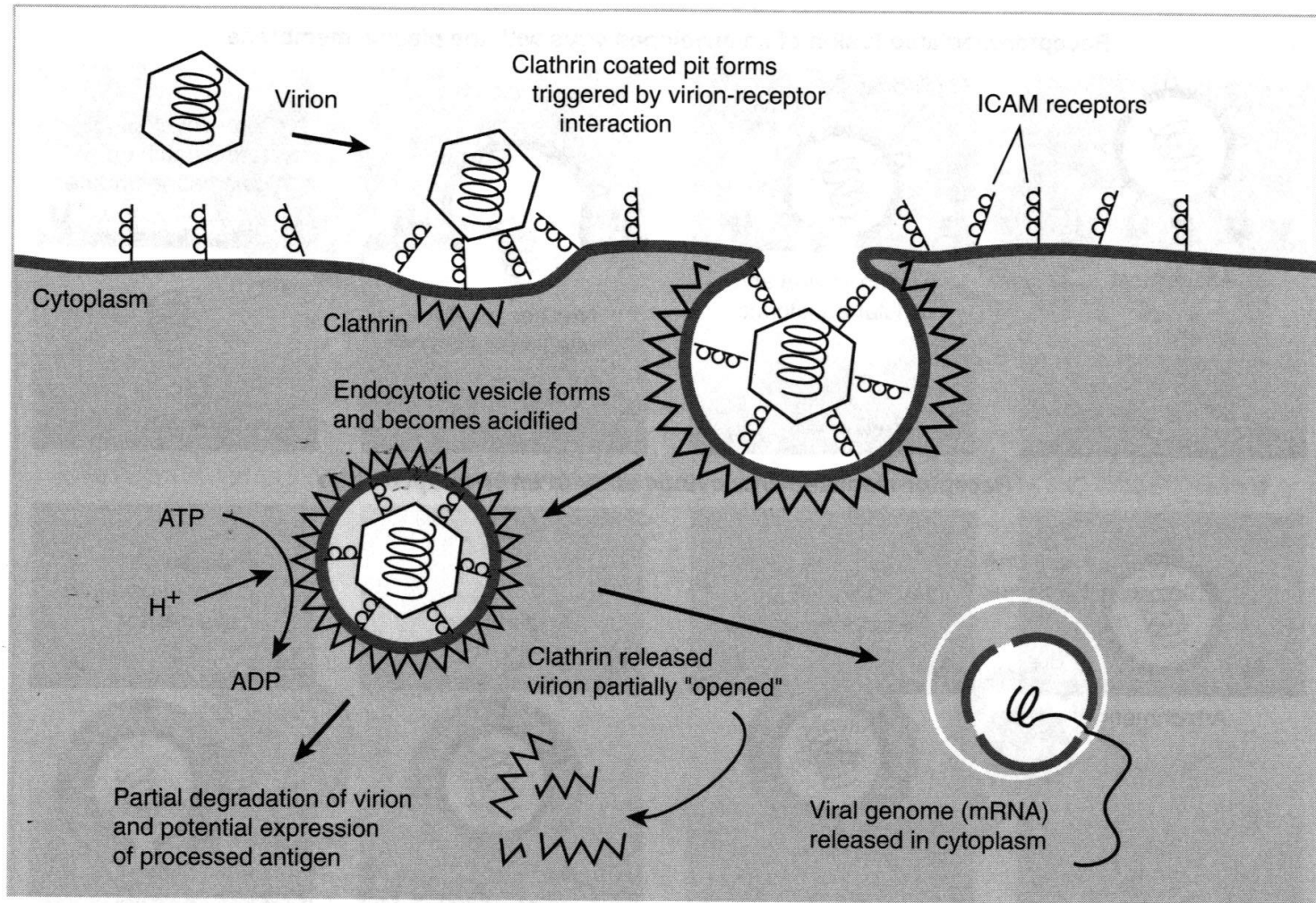


Fig. 6.2 Schematic of receptor-mediated endocytosis utilized by poliovirus for entry into the host cell. The endocytotic vesicle forms as a consequence of close association between the poliovirus-receptor complex and the plasma membrane.



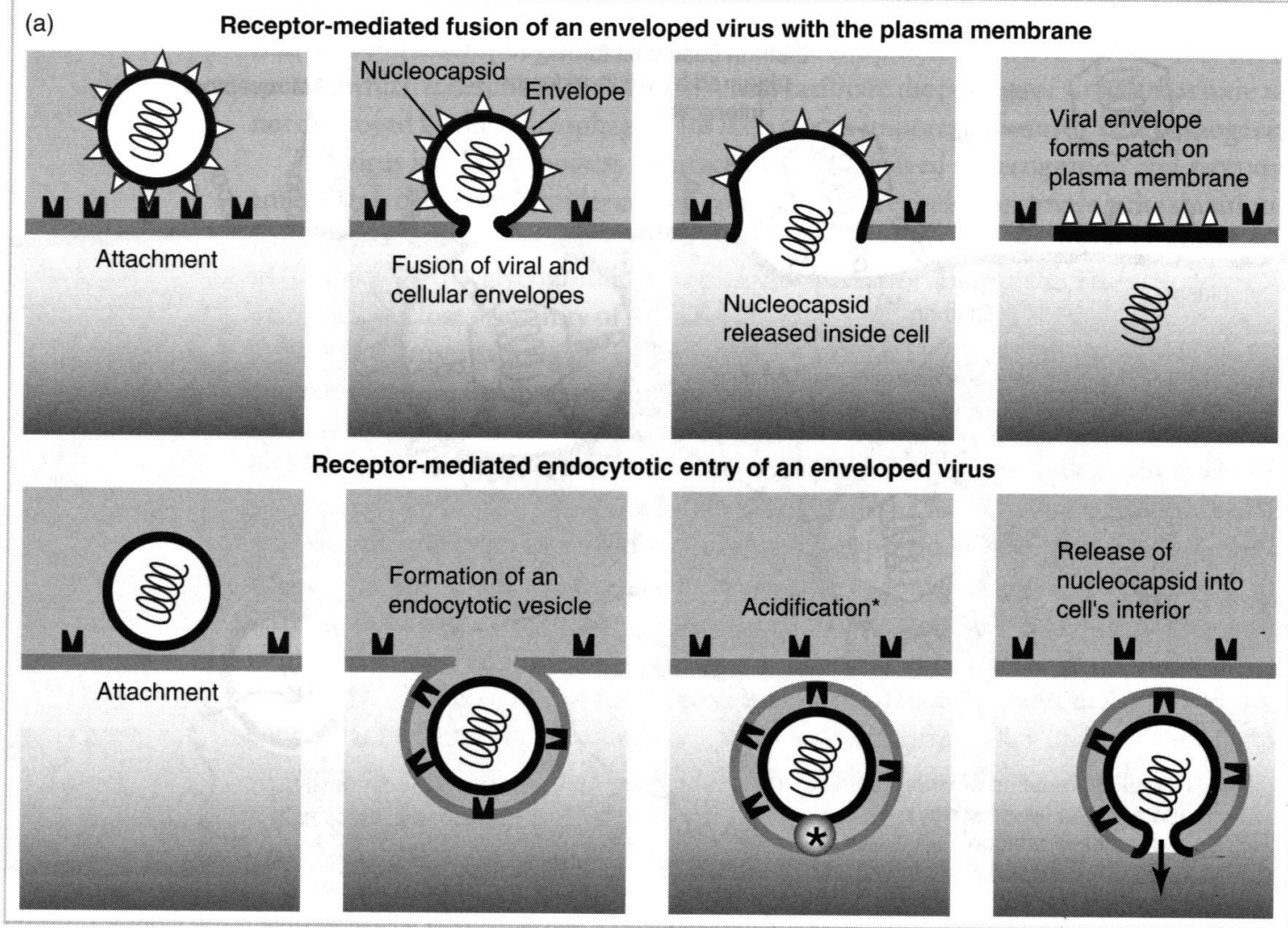


Fig. 6.3 *a*. The two basic modes of entry of an enveloped animal virus into the host cell. Membrane-associated viral glycoproteins either can interact with cellular receptors to initiate a fusion between the viral membrane and the cell plasma membrane, or can induce endocytosis. The fate of the input virus membrane differs in the two processes. *b*. The fusion of pseudorabies virus with the plasma membrane of an infected cultured cell is shown in this series of electron micrographs (the bars represent 150 nm). Although each electron micrograph represents a single event “frozen in time,” a logical progression from the initial association between viral envelope glycoproteins and the cellular receptor on the plasma membrane through the fusion event is shown. The final micrograph contains colloidal gold particles bound to antibodies against the viral envelope glycoproteins (dense dots). With them, the envelope can be seen clearly to remain at the surface of the infected cell. (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow, H., Weiland, F., Jöns, A., Klupp, B., Karger, A., and Mettenleiter, T. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: a reassessment. *Journal of Virology* 1997;71:2072–82.)

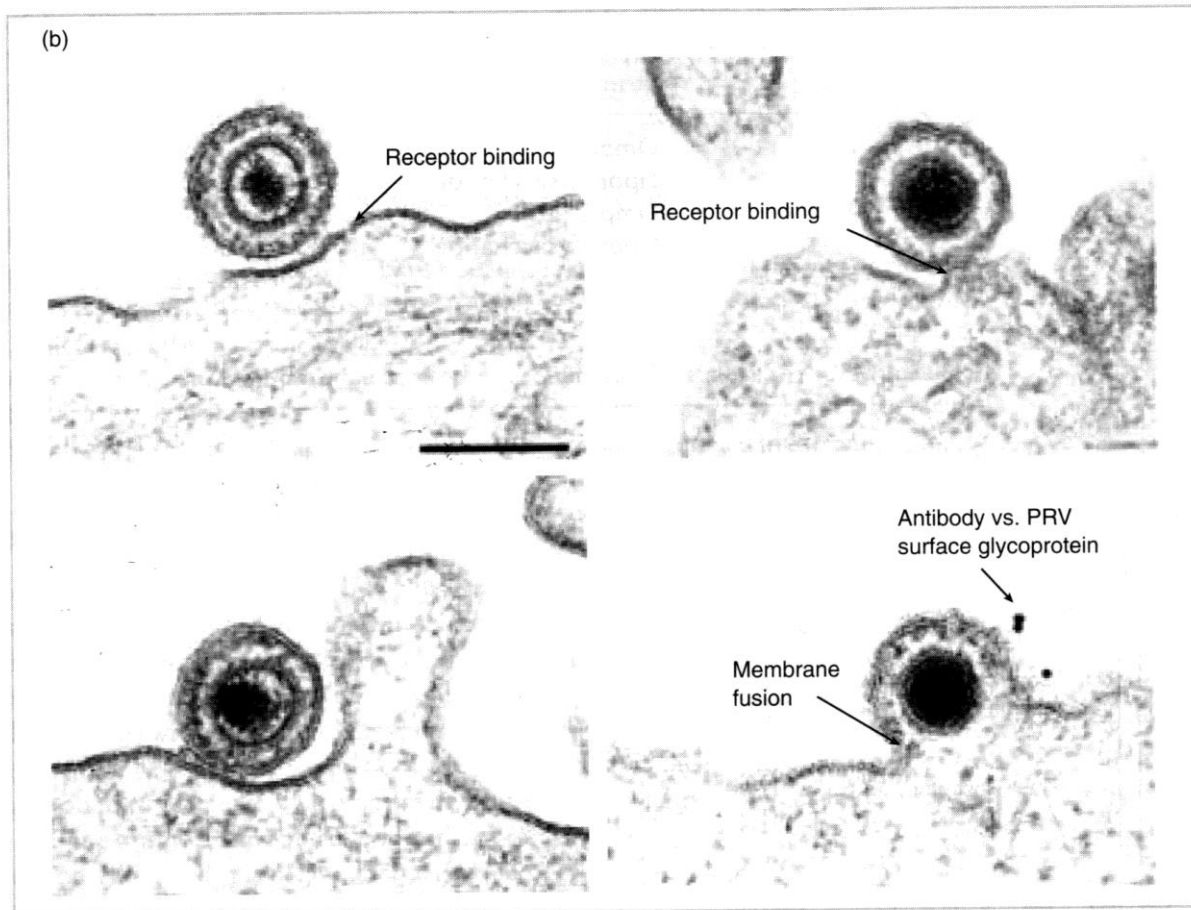


Fig. 6.3 *Continued*

**Fig. 6.3** *a.* The two basic modes of entry of an enveloped animal virus into the host cell. Membrane-associated viral glycoproteins either can interact with cellular receptors to initiate a fusion between the viral membrane and the cell plasma membrane, or can induce endocytosis. The fate of the input virus membrane differs in the two processes. *b.* The fusion of pseudorabies virus with the plasma membrane of an infected cultured cell is shown in this series of electron micrographs (the bars represent 150 nm). Although each electron micrograph represents a single event “frozen in time,” a logical progression from the initial association between viral envelope glycoproteins and the cellular receptor on the plasma membrane through the fusion event is shown. The final micrograph contains colloidal gold particles bound to antibodies against the viral envelope glycoproteins (dense dots). With them, the envelope can be seen clearly to remain at the surface of the infected cell. (Micrographs reprinted with the kind permission of the American Society for Microbiology from Granzow, H., Weiland, F., Jöns, A., Klupp, B., Karger, A., and Mettenleiter, T. Ultrastructural analysis of the replication cycle of pseudorabies virus in cell culture: a reassessment. *Journal of Virology* 1997;71:2072–82.)

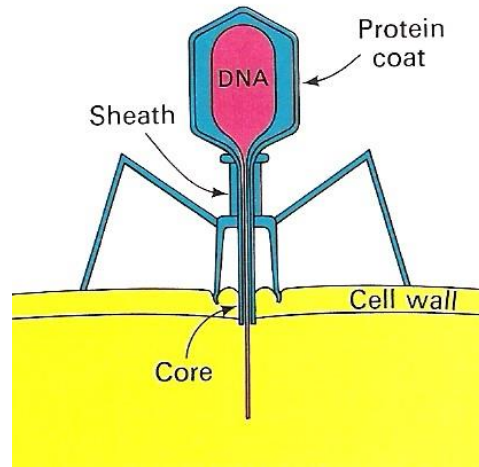
## B. Vírus de bactéria (bacteriófagos)

### a) Adsorção

Fibras longas: reconhecimento e aderência na célula.  
Glicoproteínas, lipopolissacarídeos,

### b) Penetração

### c) Retirada da capa protéica



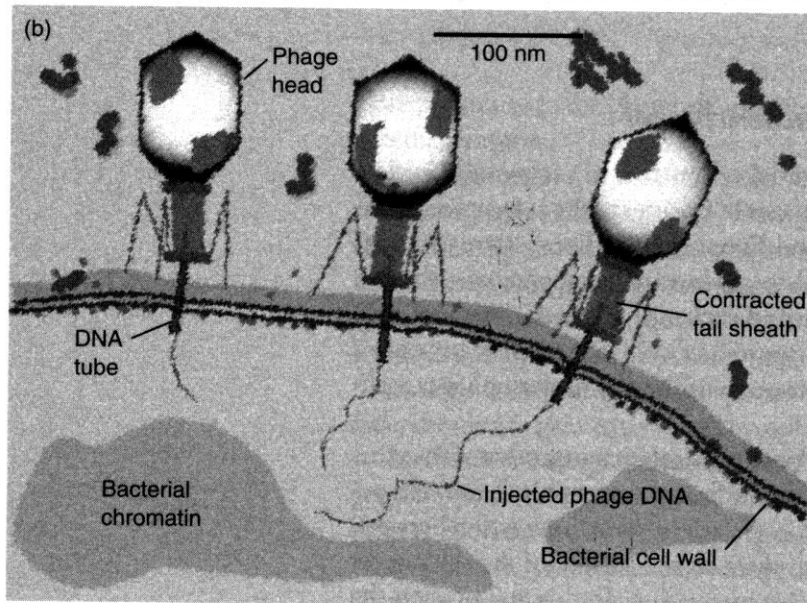
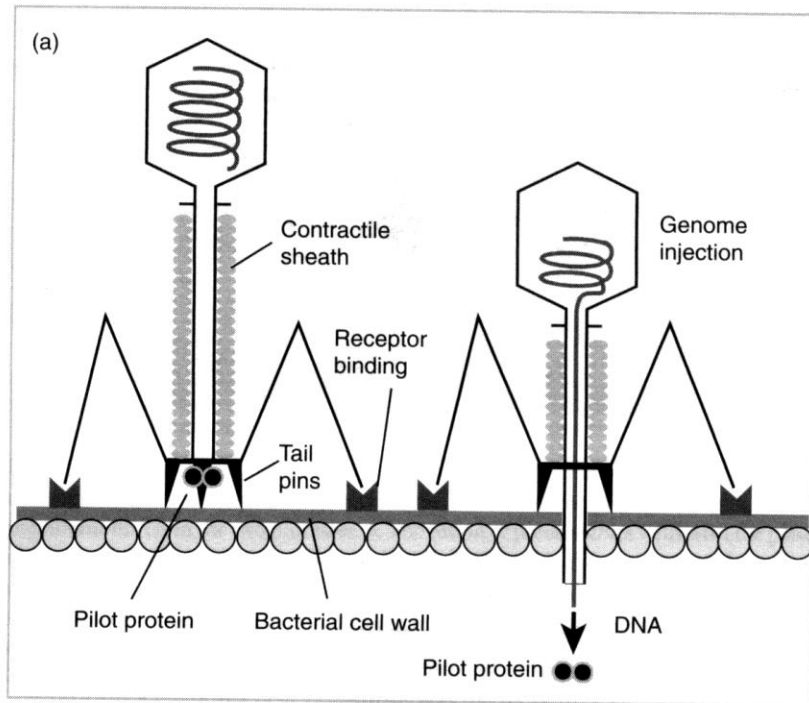


Fig. 6.4 Entry of T4 bacteriophage DNA into an *E. coli* cell. Initial attachment is between the fibers to the ompC lipopolysaccharide receptor on the bacterial cell wall (a). The binding of protein pins on the base plate to the cell wall leads to contraction of the tail fibers and sheath proteins, leading to insertion of the tail tube through the cell wall. As shown in the electron micrograph (b), phage pilot protein (arrow) allows the highly charged viral DNA genome to penetrate the bacterial plasma membrane and enter the cell. Phage DNA can be seen as shadowy lines emanating from the tail tube. (From Dimmock, N.J., and Primrose, S.B. *Introduction to Modern Virology*, 4th edn. Boston: Blackwell Science, 1994.)



## C. Vírus de plantas : inoculação por ferimento

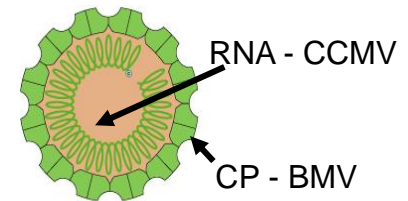
### a) Adsorção

- Não há evidências de que a capa protéica tem função de reconhecimento ou adsorção.

EX:

RNA *Cowpea chlorotic mottle virus* (CCMV) em capa protéica de *Brome mosaic virus* (BMV). Ambos gênero *Bromovirus*. Infecção em caupi.

- Não há evidências de que há receptores de vírus nas células da planta.



### b) Penetração

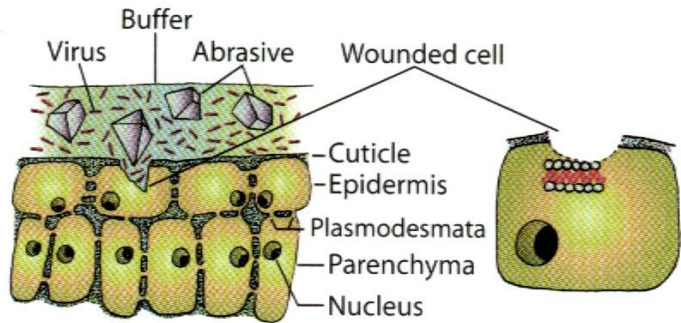
- Dano na parede celular: entrada direta.

### c) Retirada da capa protéica

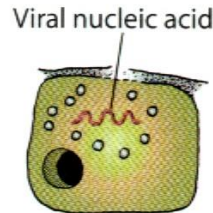
- Ácido nucleico é retirado da capa em alguns minutos.

# INOCULAÇÃO POR FERIMENTO

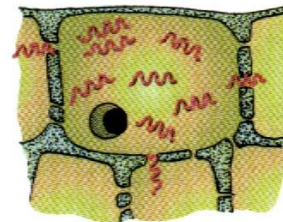
## Transmissão mecânica experimental



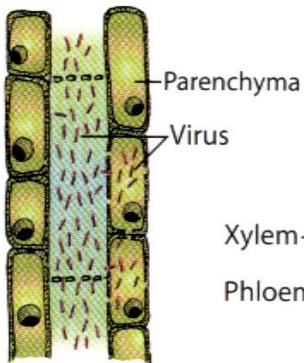
Virus taken in by wounded cell



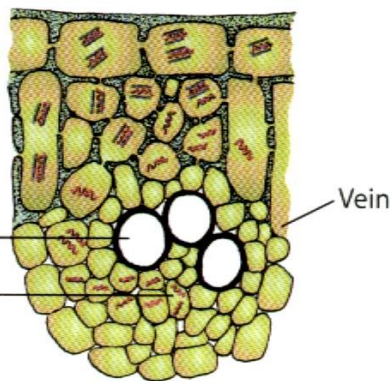
Viral nucleic acid freed from coat protein



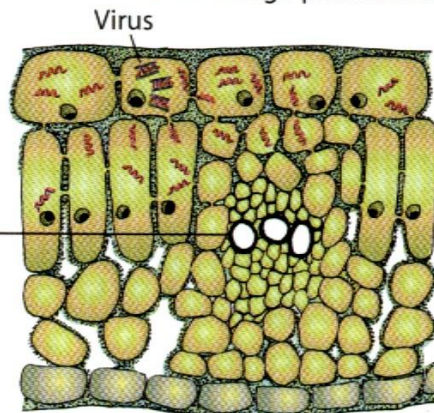
Viral nucleic acid replicates in cell. Some move to adjacent cells through plasmodesmata



In phloem, viral nucleic acid or virus is carried with the photosynthate throughout the plant



Viral nucleic acid or virus reaches phloem vessel through plasmodesmata of parenchyma cells

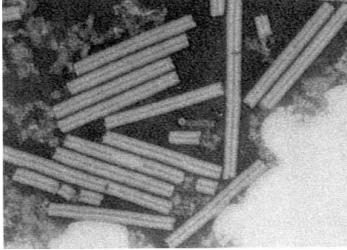


Viral nucleic acid multiplies in new cells and spreads to adjacent cells. Some of the early formed nucleic acid is coated with protein and forms virus

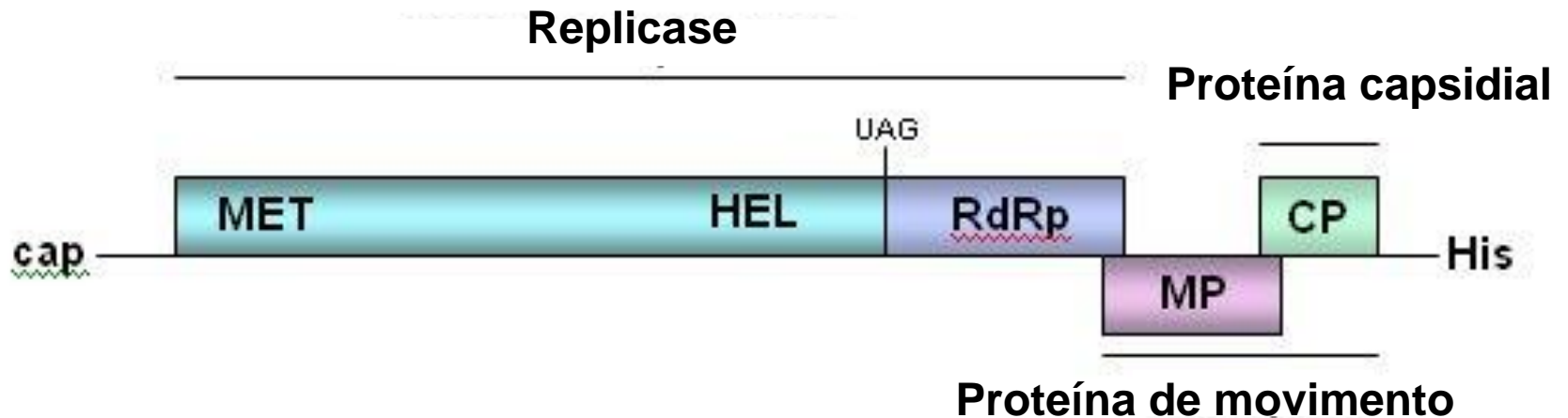
## Vetor



# Ciclo de uma partícula viral RNA fs(+)

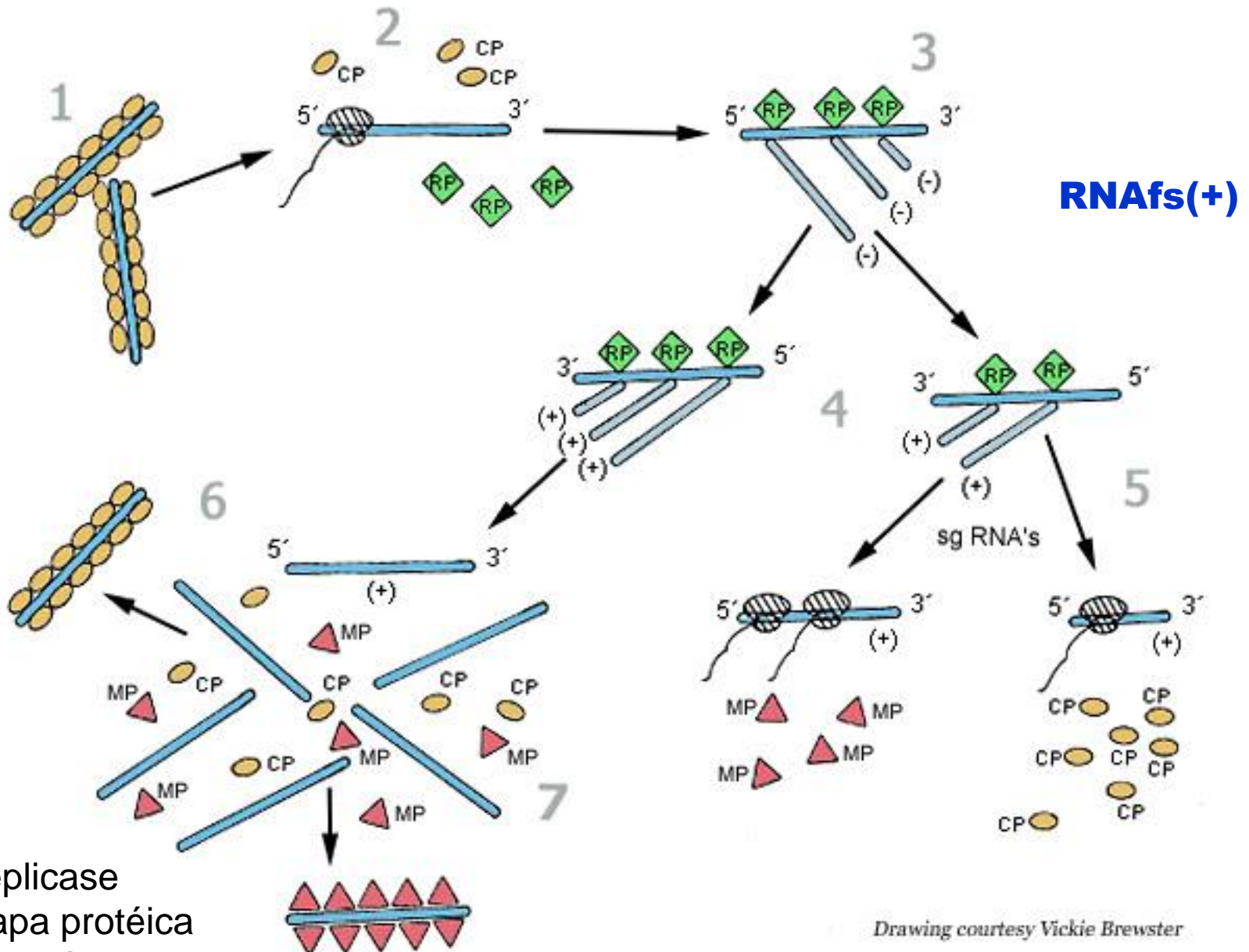


## Organização do genoma viral Modelo: *Tobacco mosaic virus* (TMV)



6.395 nt

# Tobacco mosaic virus - TMV



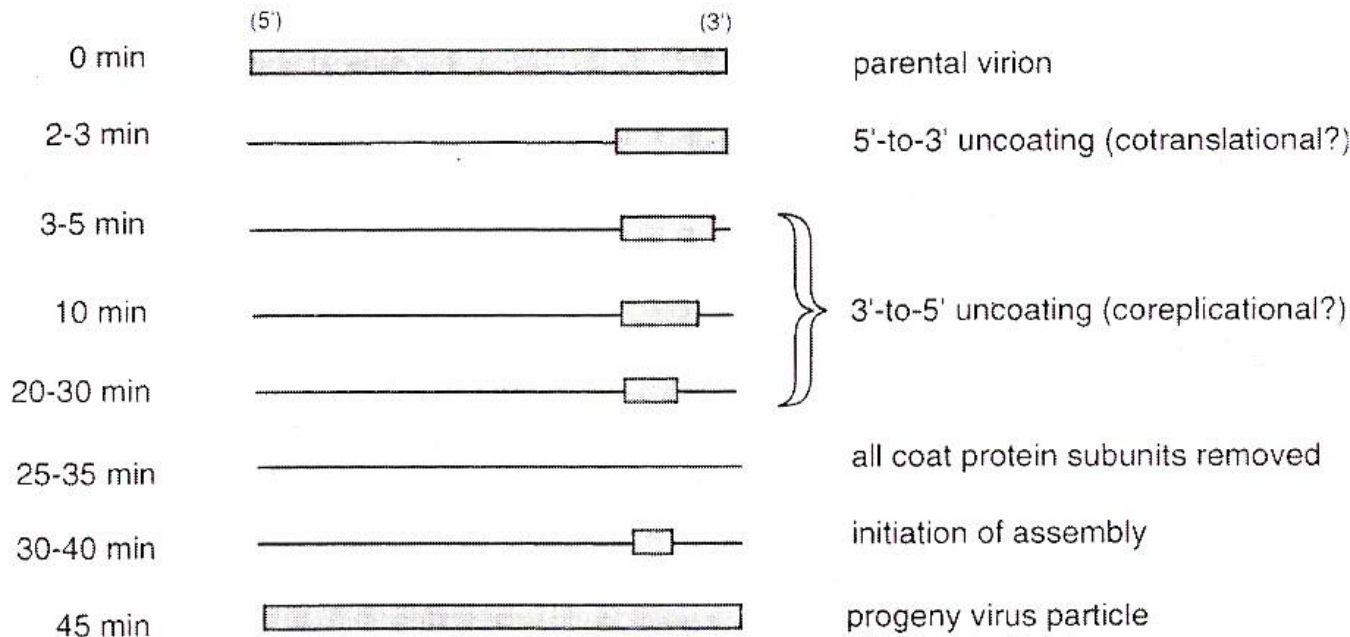
Drawing courtesy Vickie Brewster

# ETAPAS DO CICLO DE REPLICAÇÃO DO TMV

1. O TMV entra na célula por ferimentos;
2. A capa protéica é retirada e ao mesmo tempo os ribossomos traduzem o RNA para síntese das replicases (RP);
3. As replicases transcrevem o RNA viral (fs+) produzindo cópias complementares (RNAfs-);
4. O RNAfs- serve de molde para gerar RNA viral (fs+);
5. Ao mesmo tempo o RNAfs- serve de molde para gerar RNAs sub-genômicos, que serão usados para sintetizar as proteínas de movimento (MP) e capsidial (CP);
6. Parte do RNA viral (fs+) é encapsulado pelas proteínas capsidias;
7. Parte do RNA viral é “protegido” pela proteína de movimento para mover-se para a célula vizinha e novo ciclo de replicação.

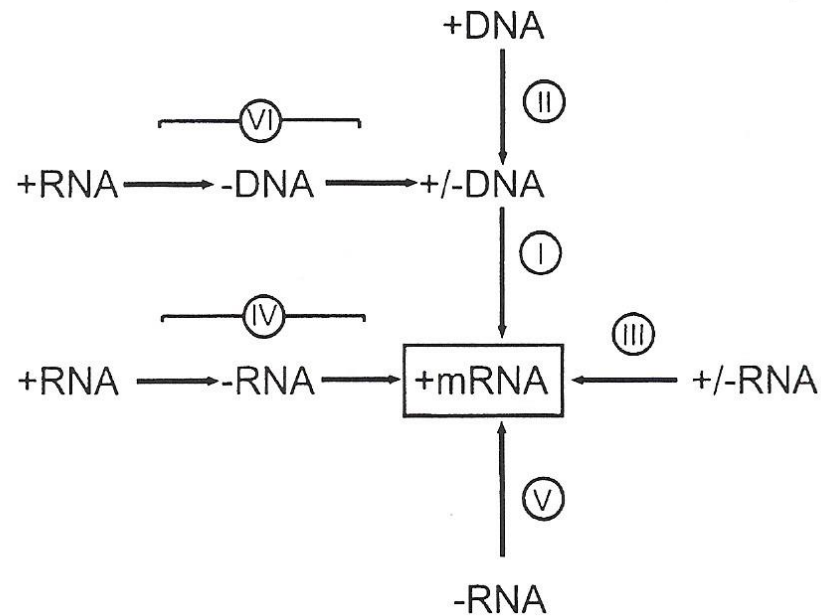


# REPLICAÇÃO DO TMV



**Fig. 7.6** Bidirectional disassembly of TMV particles *in vivo*. Coat protein subunits are removed in a 5' → 3' direction from *c.* 75% of the viral RNA in the first 2-3 minutes after inoculation of protoplasts. Uncoating the 3'-end of the RNA begins shortly thereafter and is completed by removal of subunits in the 3' → 5' direction. From Wu *et al.* (1994), with kind permission of the copyright holder, © The National Academy of Sciences, USA.

# ROTAS PARA A EXPRESSÃO DE GENOMAS VIRAIS



**Fig. 7.1** Routing of viral genome expression through mRNA. Route I is transcription of dsDNA usually by host DNA-dependent RNA polymerase. Route II is the transcription of ssDNA to give the dsDNA template for I (e.g. geminiviruses). Route III is transcription of dsRNA, usually by virus-coded RdRp (e.g. reoviruses). Route IV is replication of (+)-strand RNA via a (-)-strand template by virus-coded RdRp—the viral (+) strand is often the template for early translation (the (+)-strand RNA viruses). Route V is transcription of (-)-strand virus genome by virus-coded RdRp (e.g. tospoviruses). Route VI is reverse transcription of RNA stage of retro- and pararetro-viruses leading to the dsDNA template for mRNA transcription. From Baltimore (1971), with permission.

# Vírus - disseminação

## A. VÍRUS DE PLANTAS



**Afídeos (pulgões)**



**Mosca branca**



**Tripes**

**Outros vetores: Ácaros; Cigarrinhas; Besouros; Cochonilhas, Fungos; Nematoides; Protozoários**

- Sementes: 1/5 dos vírus, taxa variável
- Pólen
- Propagação vegetativa e enxertia
- Mecânica: operações culturais

# Vírus - disseminação

## B. VÍRUS DO HOMEM

<b>VÍRUS</b>	<b>VETOR</b>	<b>HOSPEDEIRO</b>
Poliovirus	Fezes humana (água/alimento)	Homem
HIV	Injeção direta no sangue	Homem
Catapora	Aerossol	Homem
Febre amarela	Mosquito	Macaco tropical
Dengue, Zica	Mosquito	Homem, mosquito, primata
Hepatite A	Fezes humana (água/alimento)	Homem
Hepatite B e C	Injeção direta no sangue	Homem
Raiva	Mordida animal infectado	Vertebrado
Rhinovirus/Coronavirus	Aerossol	Homem

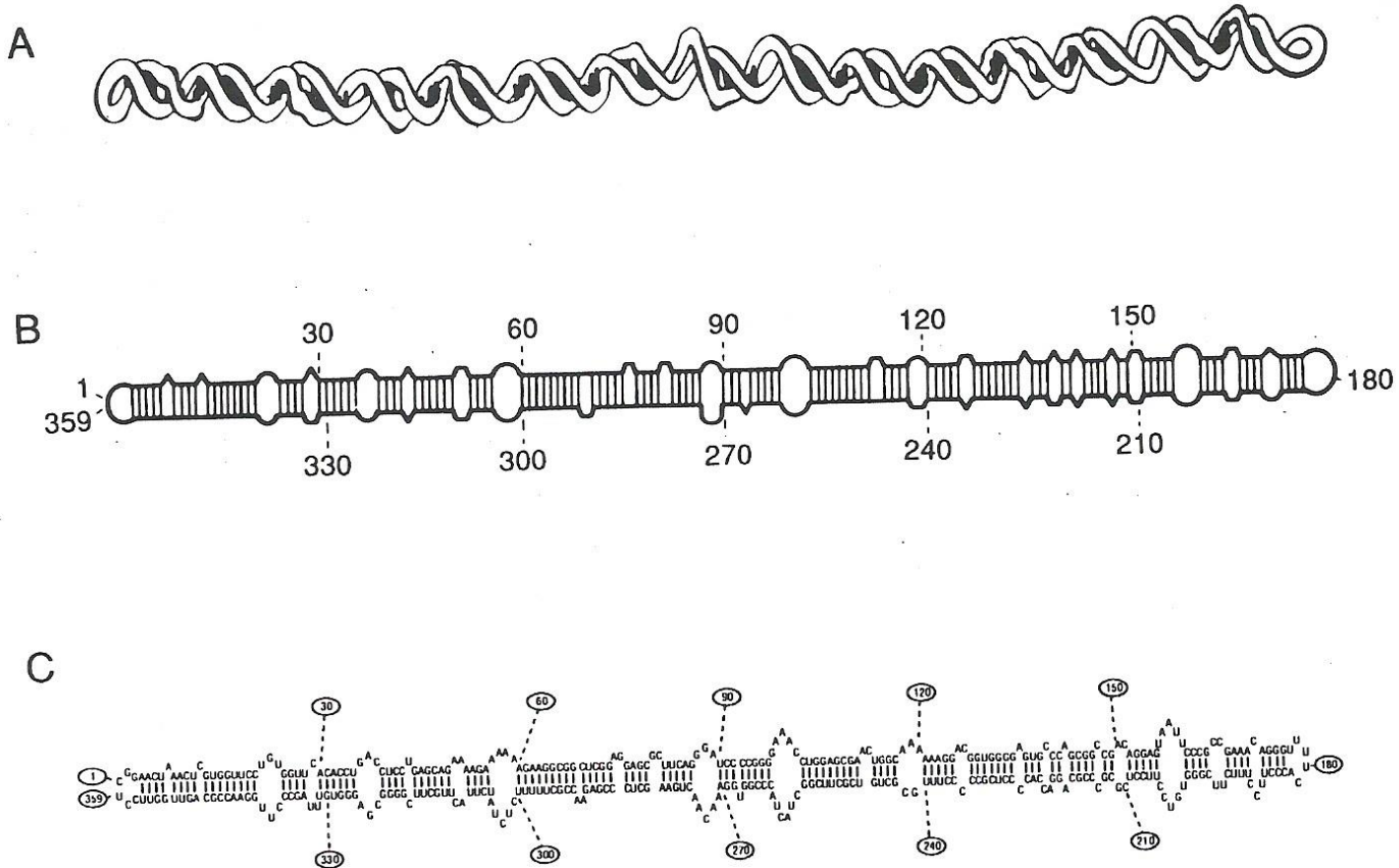
# Viroïdes



# Viroides

- Infectam somente plantas
- Partículas de RNA circular menores que vírus (apenas 200-400 pb)
- Desprovidas de capa protéica.
- Genoma aparentemente não contém genes!!!

# VIROIDES



***Potato spindle tuber viroid*** (PSTVd): A = representação tridimensional da molécula; B = estrutura secundária proposta e C = seqüência de nucleotídeos (Matthews, 1991)



Mosaico latente do pessegueiro

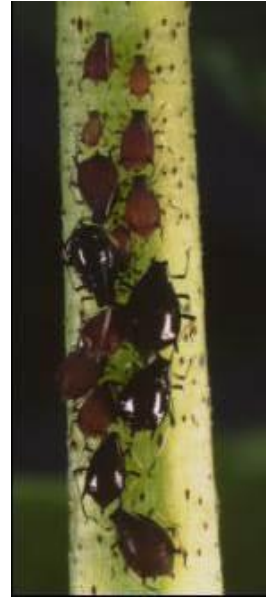


Afilamento do tubérculo da batata

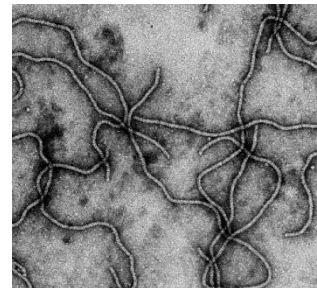
# **IMPORTÂNCIA DOS VÍRUS NA AGRICULTURA**

# TRISTEZA DOS CITROS (*Citrus tristeza virus* - CTV)

- Brasil, 1937  
11 milhões de árvores;  
9 milhões mortas
- Américas, Espanha e Israel:  
100 milhões de árvores  
mortas ou improdutivas



Pulgão preto  
*Toxoptera citricida*





# Controle maioria de laranjas doce: tolerância



**Baía/Azeda**

**Baía/Cravo**

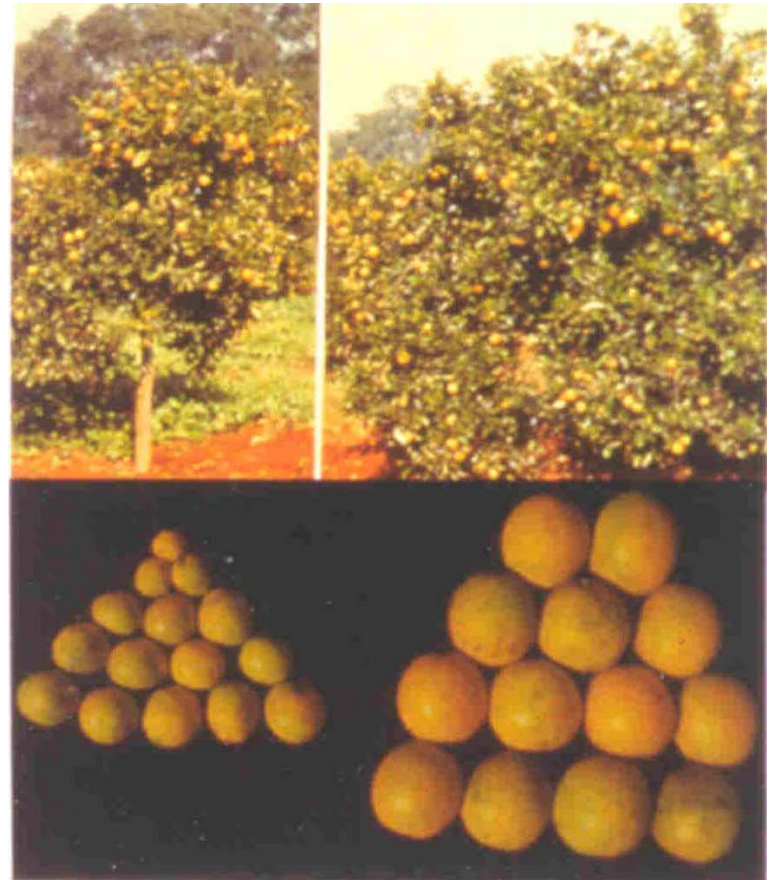
# Controle laranja Pêra: premunização (“vacinação”)

**Brasil, Austrália,  
África do Sul**

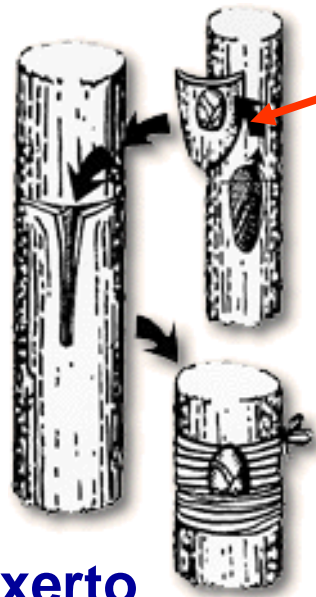
**Pêra (Brasil):**

**1977 = 7 milhões**  
**1980 = 8 milhões**  
**1987 = 50 milhões**  
**1992 = 80 milhões**  
**2008 = ~70 milhões**

**África do Sul: Pomelos**  
**1992 = 22,5 milhões**



## Premunização de mudas de laranja Pêra



Borbulha de  
'Pêra' premunizada

Porta enxerto  
livre de vírus

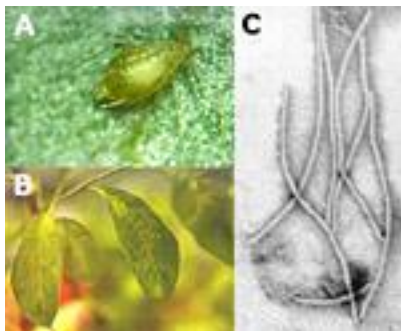


Mudas premunizadas

# **VÍRUS EXÓTICOS QUE PODEM PREJUDICAR O AGRONEGÓCIO**



# PLUM POX VIRUS - PPV



**Potyvirus**

**Afídeos, ñ persistente**



# ***PLUM POX VIRUS - PPV***

- **Infecta: pessegueiro, ameixeira, nectarina, etc.**

- **Na América do Sul, presente no Chile**

**(Rosales et al., Act Hortic. 1998)**

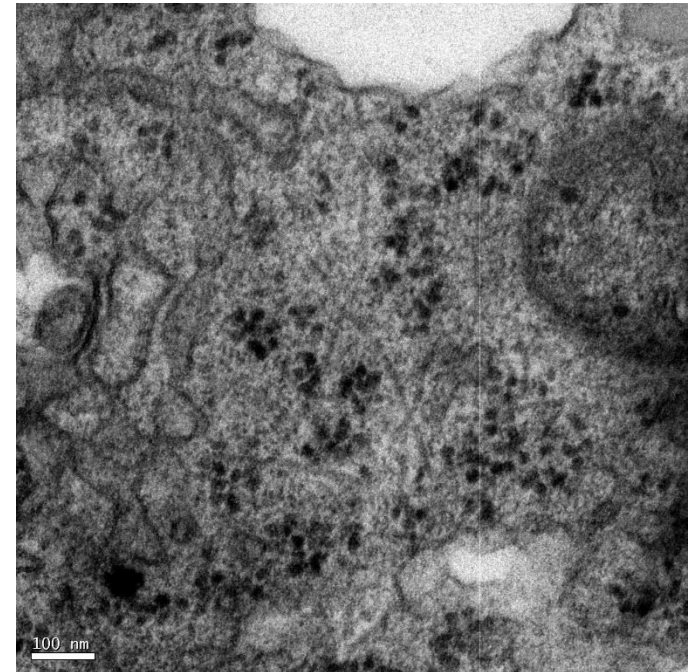
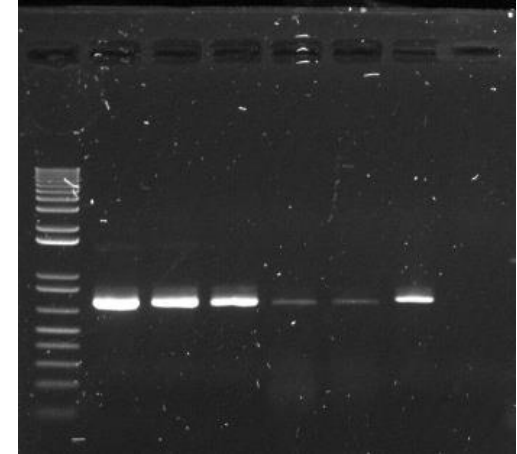
**(Detectado na Argentina)**

- **Vetores: 20 espécies de afídeos**

***Aphis gossypii*, não coloniza *Prunus***

***Myzus persicae*, coloniza *Prunus***

# PPV EM FEIRA EM PIRACICABA, SP, 2014



QUALITY PRODUCE OF CHILE

GOIII Fresh & Safety Produce

**GEORUIT**

DURAZNOS / SWEET SEPTEMBER

**PEACHES/YELLOW FLESH**

PLASTICO 9 KN / VOLUME FILL CONVENTIONAL

GTIN: 07800121144013    Size: 0052    CAT-1    Pack Date: Mar07

LOT: 00G50641

41

Identification (0032)

41    GDN: 404922948272    Packing Identification    Size: 0052

90388    CSP: 90388    Package: PBCG9

REPORTER: GEORUIT LTDA (CSE 87763) - PRODUCT OF CHILE

(56-2) 726 0461 - E-mail: georuite@georuit.com

# Plum Pox in North America: Identification of Aphid Vectors and a Potential Role for Fruit in Virus Spread

Frederick Gildow, Vern Damsteegt, Andrew Stone, William Schneider, Douglas Luster, and Laurene Levy  
Phytopathology 2004

TABLE 4. Transmission of *Plum pox virus* (PPV)-PENN-7 from infected peach fruit to healthy peach seedlings by four aphid species during 3-day unrestricted probing tests<sup>a</sup>

Aphid species	Test 1	Test 2	Total	Percentage (%)
<i>Myzus persicae</i>	5/10	5/10	10/20	50
<i>Aphis fabae</i>	0/10	0/10	0/20	0
<i>A. spiraecola</i>	5/10	2/10	7/10	35
<i>Brachycaudus persicae</i>	0/10	0/10	0/20	0

<sup>a</sup> Data expressed as number of healthy seedlings infected over number of seedlings infested with 50 to 100 aphids per seedling during a 3-day exposure to PPV-infected fruit collected from PPV-infected trees in York County, PA in 2002. Positive transmissions initially were verified by enzyme-linked immunosorbent assays of each infested peach seedling  $\approx$ 90 days post-inoculation. Systemic infection of seedlings then was verified by real-time polymerase chain reaction following a 90-day vernalization at 2°C and regrowth of foliage.

Gildow et al., 2004

# Mudança no procedimento para importação de hospedeiros do *Plum pox virus*

Defesa Vegetal 24/01/2018

Anteriormente, era exigido que os produtos estivessem livres da praga *Plum pox virus* (PPV).

Agora, além desta declaração adicional (DA), também é exigida a certificação de que os produtos não apresentam risco quarentenário com respeito ao PPV (DA14).

Países e produtos para os quais a DA14 passa a ser exigida: Argentina, Chile, Espanha, Estados Unidos, Irã, Israel, Itália, Portugal e Turquia e os produtos são ameixa, cereja, cereja ácida, damasco, nectarina e pêsego.

Os estudos de análise de risco de pragas, bem como as medidas determinadas a partir destes, fortalecem a proteção da agricultura brasileira contribuindo para o desenvolvimento e evitando prejuízos decorrentes do desequilíbrio causado pela possível introdução de pragas.

