

AGENTES CAUSAIS DE DOENÇAS DE PLANTAS: VÍRUS

1. RELAÇÃO DOS VÍRUS COM A CÉLULA E TECIDOS DA HOSPEDEIRA.
2. PROCESSO DE INFECÇÃO REPLICAÇÃO E MOVIMENTAÇÃO NA PLANTA.
3. SINTOMATOLOGIA E RECONHECIMENTO DE FITOVIROSES.

INFECÇÃO E REPLICAÇÃO DE VÍRUS DE PLANTAS

1. INTRODUÇÃO

2. PROCESSO INICIAL DE INFECÇÃO

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.

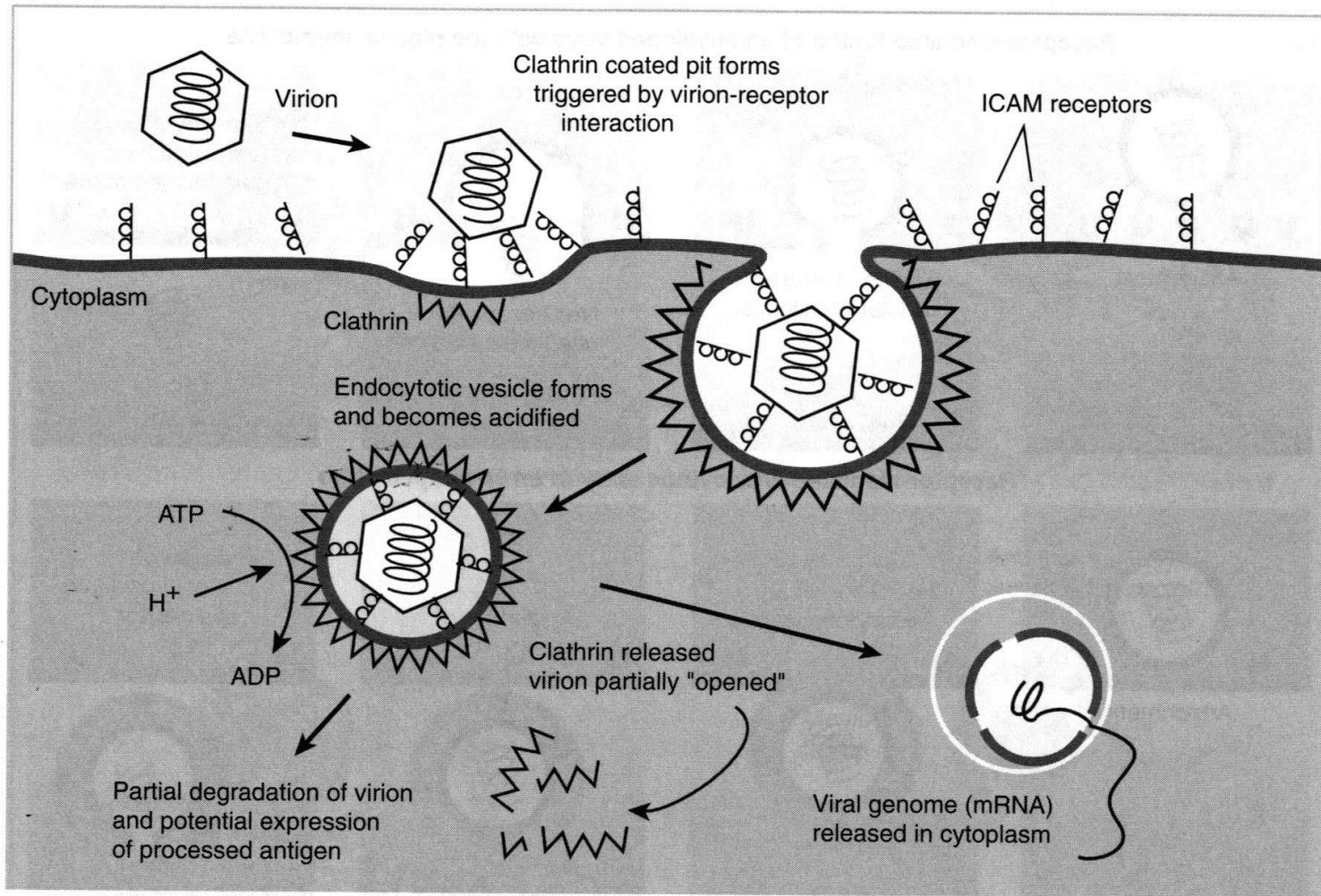


Fig. 6.2 Schematic of receptor-mediated endocytosis utilized by poliovirus for entry into the host cell. The endocytotic vesicle forms as a consequence of close association between the poliovirus-receptor complex and the plasma membrane.

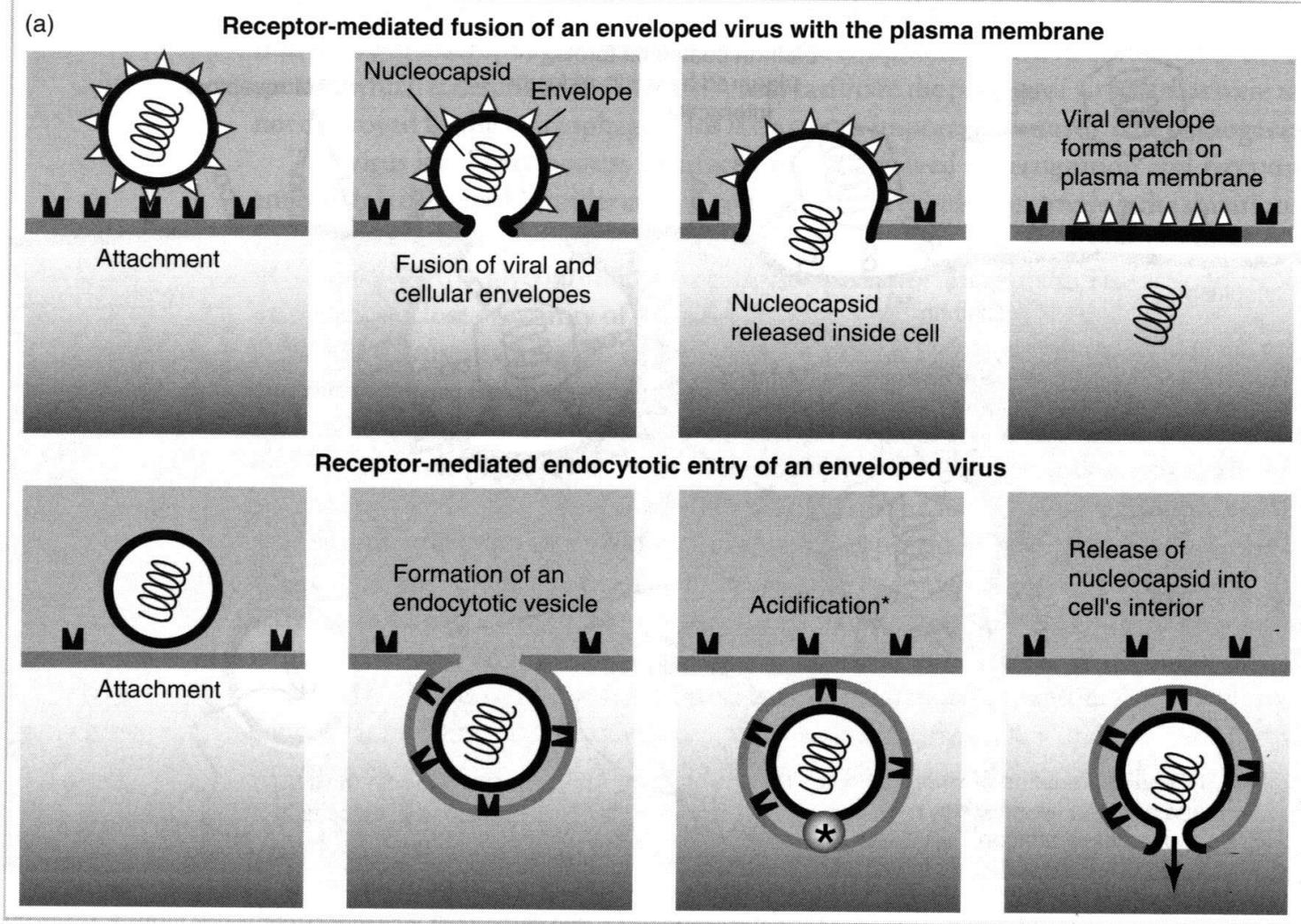


Fig. 6.3 *a.* The two basic modes of entry of an enveloped animal virus into the host cell. Membrane-associated viral glycoproteins either can interact with cellular receptors to initiate a fusion between the viral membrane and the cell plasma membrane, or can induce endocytosis. The fate of the input 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.)

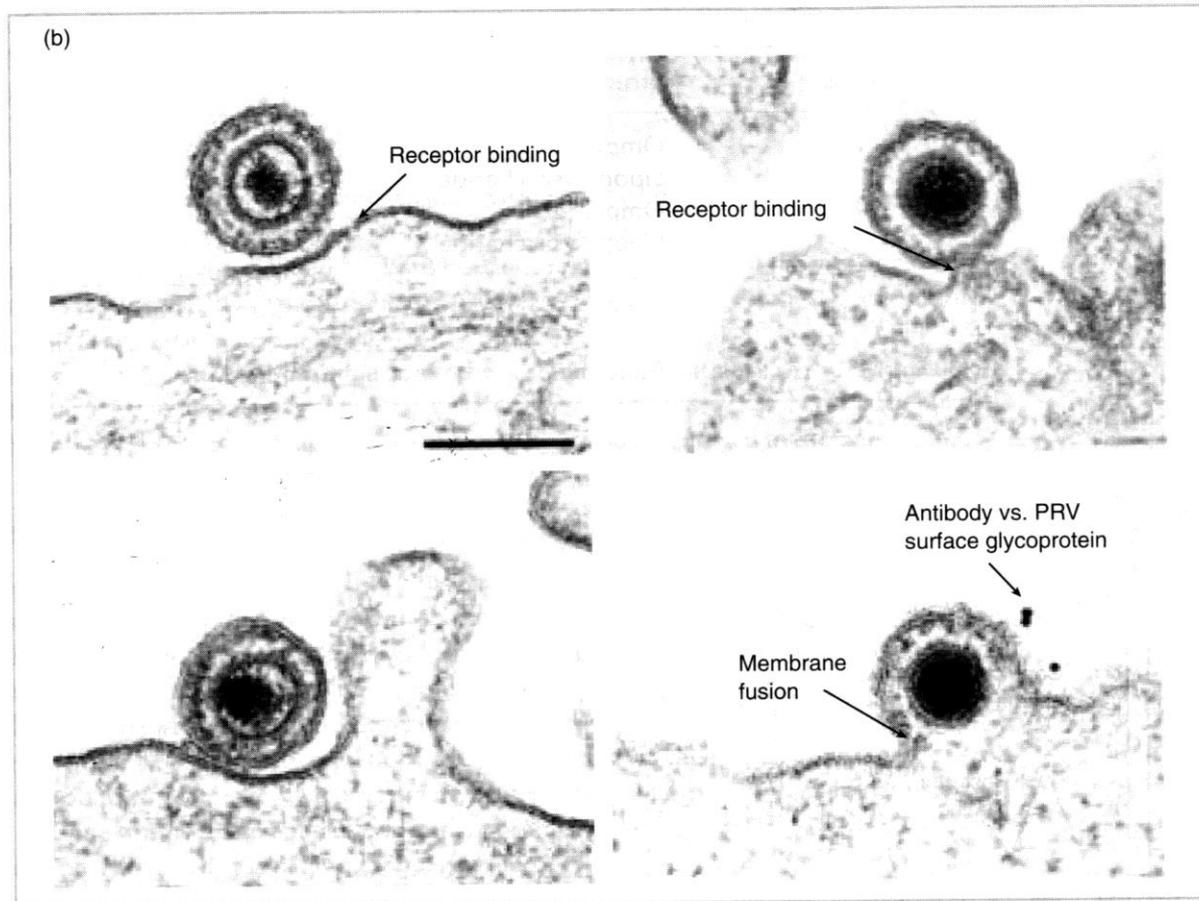


Fig. 6.3 *Continued*

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.)

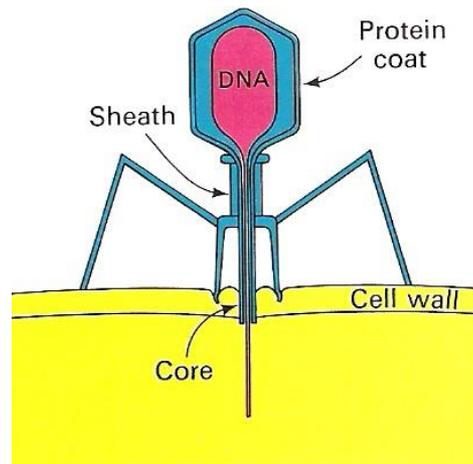
B. Vírus de bactéria (bacteriófagos)

a) Adsorção

Fibras longas: reconhecimento e aderência na célula.
Glicoproteínas, lipopolissacarídeos,
“sex pilus”: receptores na célula.

b) Penetração

c) Retirada da capa protéica



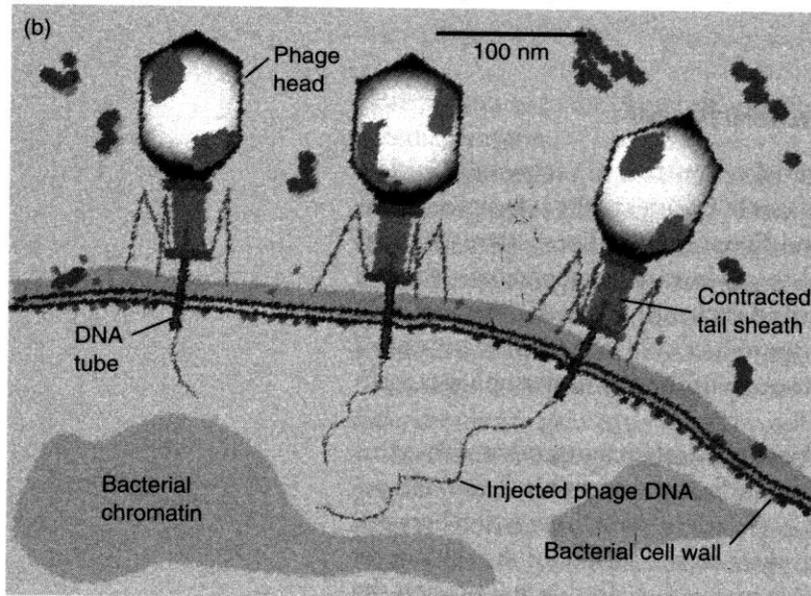
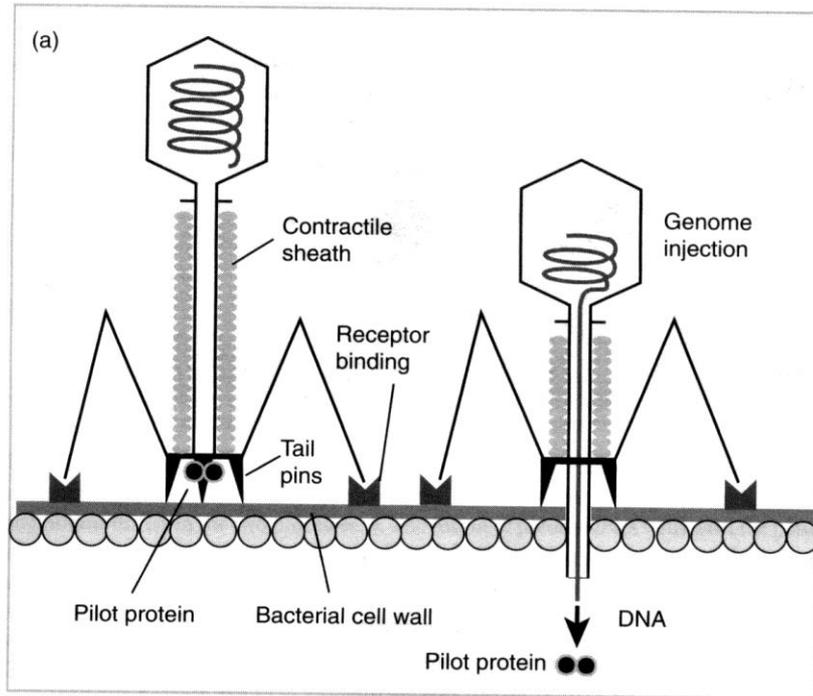


Fig. 6.4 Entry of T4 bacteriophage DNA into an *E. coli* cell. Initial attachment is between the fibers to the ompC lipopolysaccharide receptor on the bacterial cell wall (a). The binding of protein pins on the base plate to the cell wall leads to contraction of the tail fibers and sheath proteins, leading 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 em capa protéica de brome mosaic virus. Ambos Bromovirus. Infecção em caupi.

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

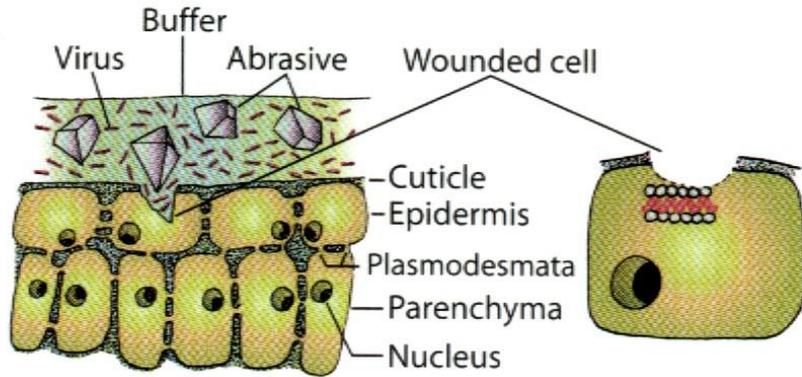
b) Penetração

- Dano na parede celular: entrada direta.

c) Retirada da capa protéica

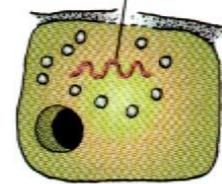
- Ácido nucleico é retirado da capa em alguns minutos.

INOCULAÇÃO POR FERIMENTO

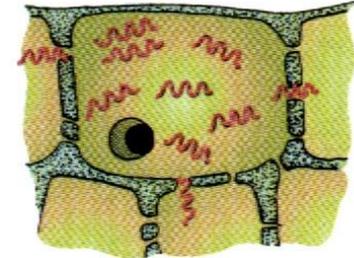


Virus taken in by wounded cell

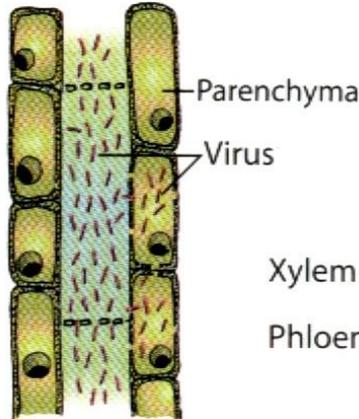
Viral nucleic acid



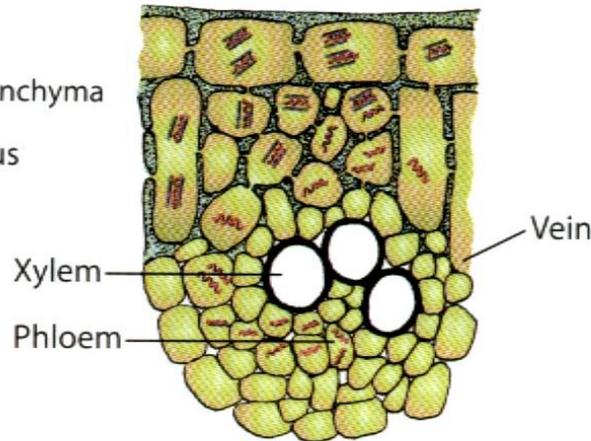
Viral nucleic acid freed from coat protein



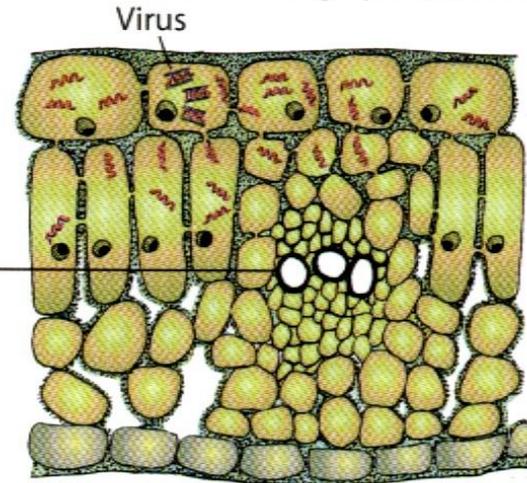
Viral nucleic acid replicates in cell. Some move to adjacent cells through plasmodesmata



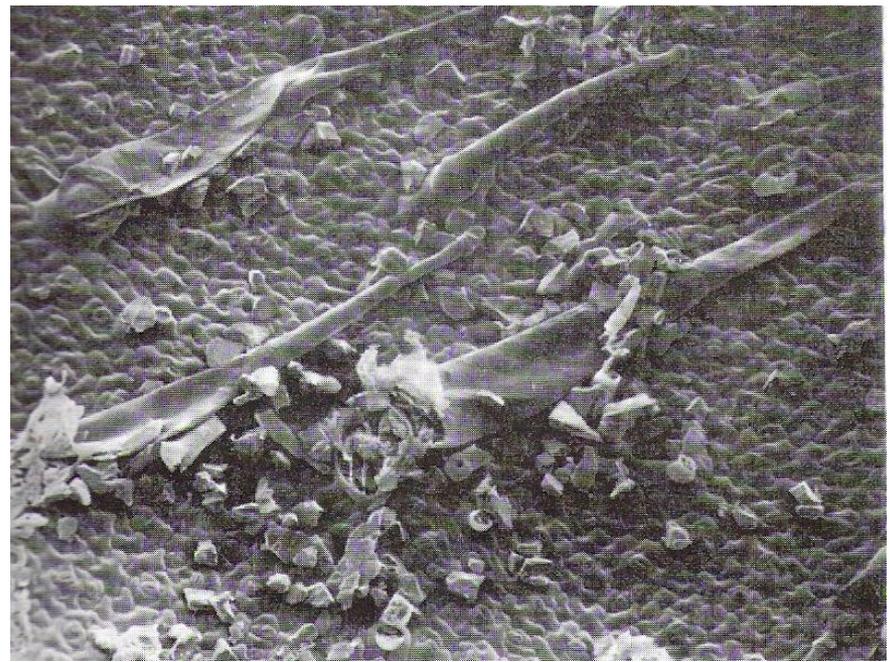
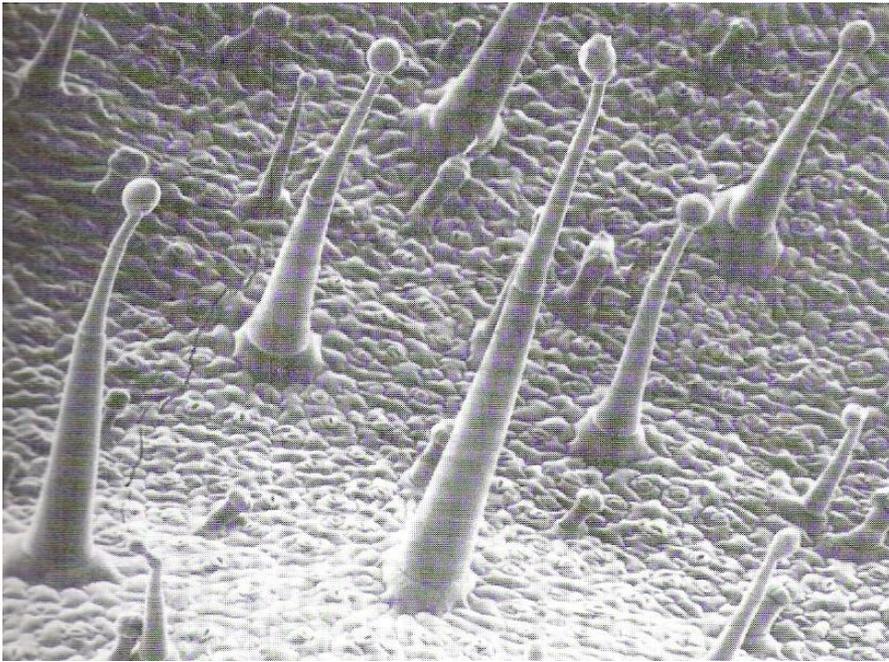
In phloem, viral nucleic acid or virus is carried with the photosynthate throughout the plant



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



INEFICIÊNCIA DA INOCULAÇÃO MECÂNICA:

- Pequeno n° de células da epiderme com ferimentos viáveis.
- Vida curta dos ferimentos viáveis.
- Distribuição irregular do vírus.
- Vírus adsorvido não infecta.
- Vírus que não se estabelece.

GÊNEROS DE VÍRUS DE PLANTAS DE ACORDO COM O TIPO DE ÁCIDO NUCLÉICO

RNA, fita simples (+)	62 (68,8)
RNA, fita simples (-)	07 (7,8%)
RNA, fita dupla	06 (6,8%)
DNA, fita simples	06 (6,8%)
DNA, fita dupla	09 (10%)

ROTAS PARA A EXPRESSÃO DE GENOMAS VIRAIS

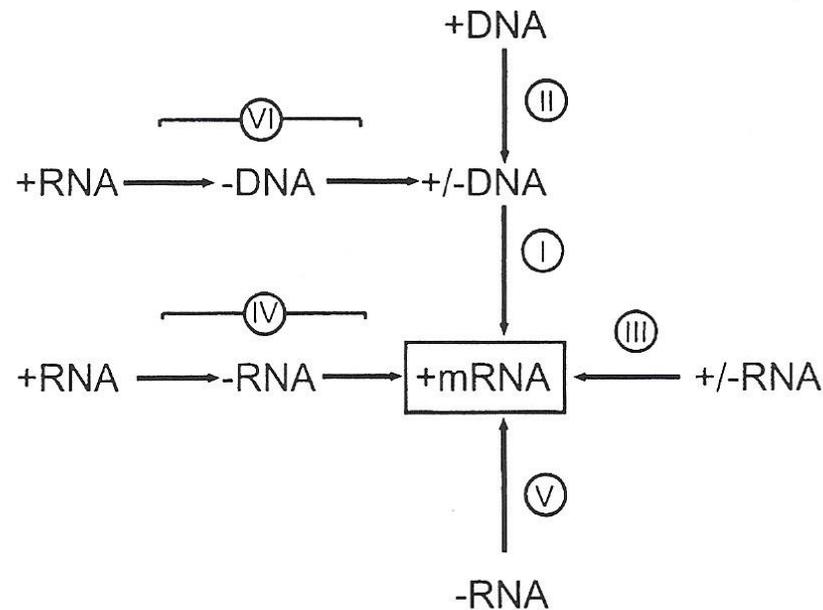
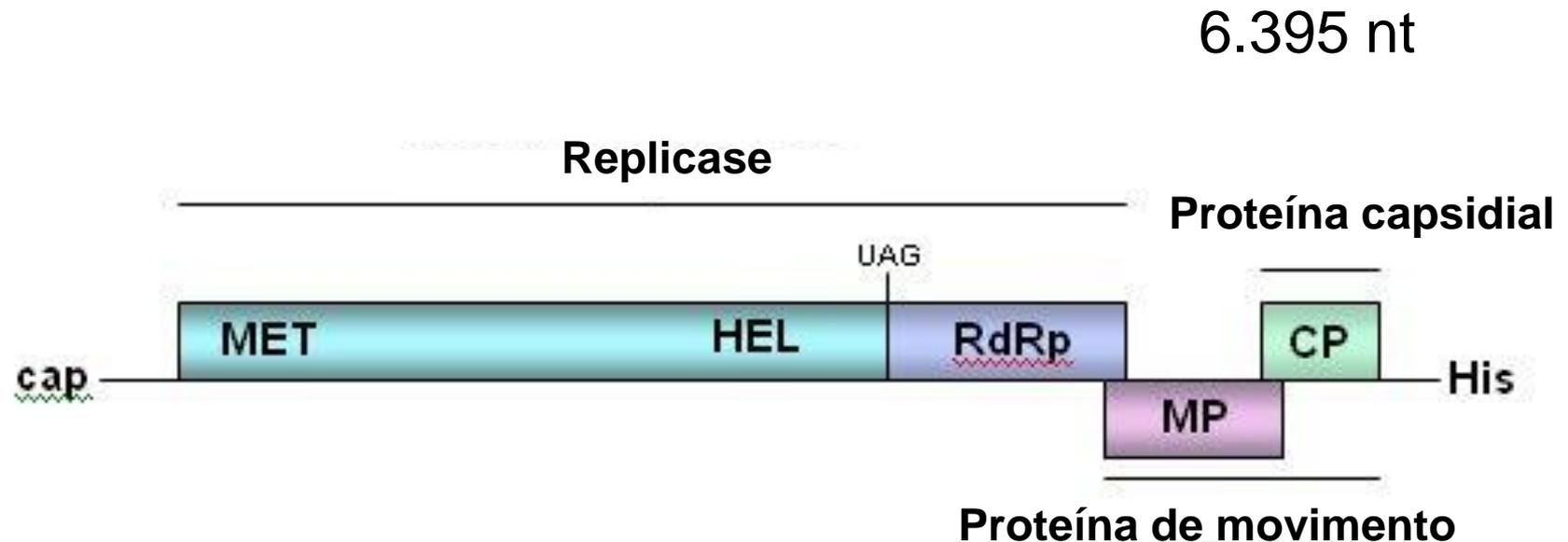


Fig. 7.1 Routing of viral genome expression through mRNA. Route I is transcription of dsDNA usually by host DNA-dependent RNA polymerase. Route II is the transcription of ssDNA to give the dsDNA template for I (e.g. geminiviruses). Route III is transcription of dsRNA, usually by virus-coded RdRp (e.g. 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.

REPLICAÇÃO DE VÍRUS DE PLANTAS: ss+RNA

Organização do genoma viral

Modelo: *Tobacco mosaic virus* (TMV)



MET: methyltransferase

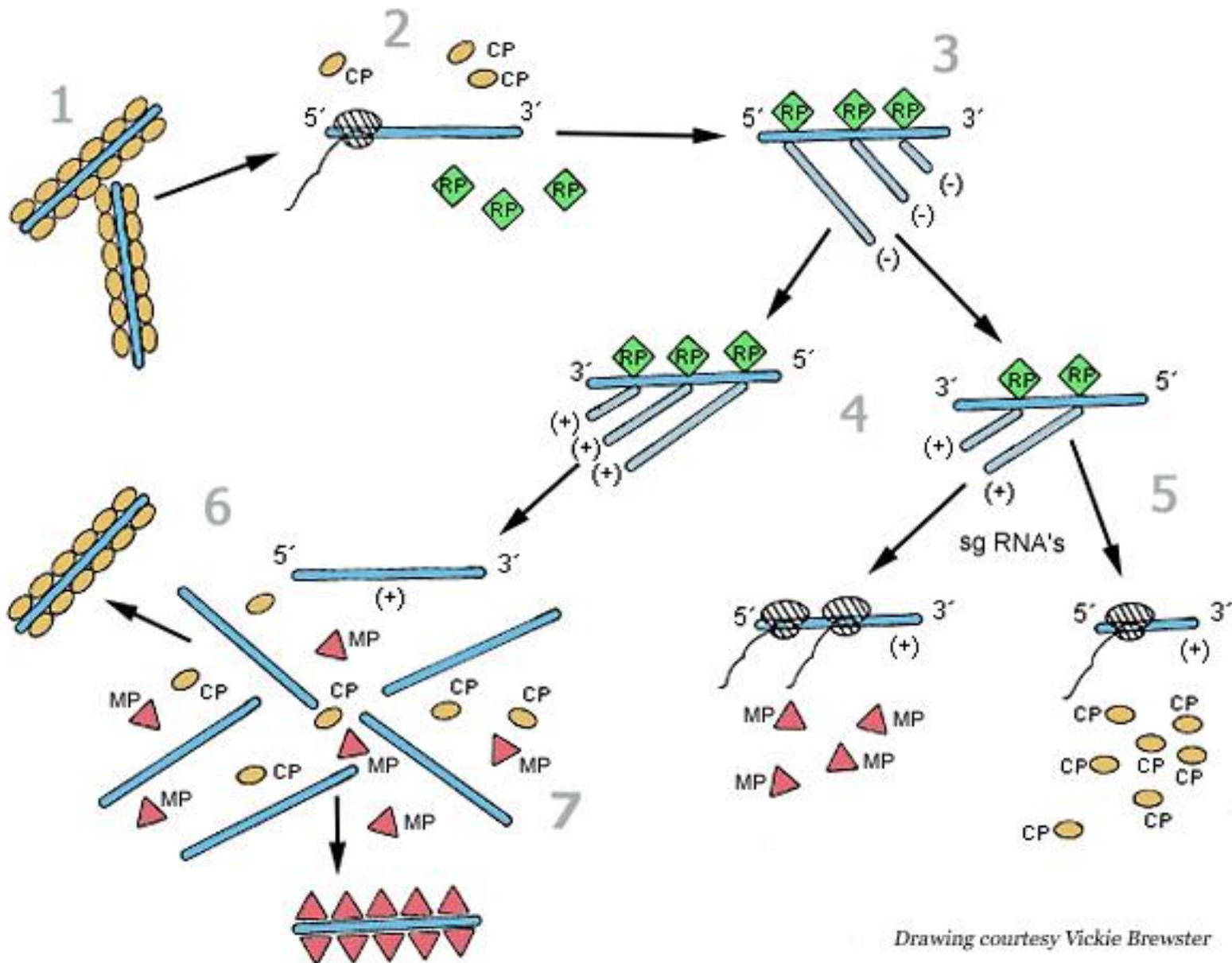
HEL: helicase

RdRp: RNA dependent RNA polymerase

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 da replicase (RP);
3. As replicases transcrevem o RNA viral (+ss) produzindo cópias complementares (-ssRNA);
4. O -ssRNA serve de molde para gerar RNA viral (+ss);
5. Ao mesmo tempo o -ssRNA 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 (+ss) 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



Drawing courtesy Vickie Brewster

REPLICAÇÃO DO TMV

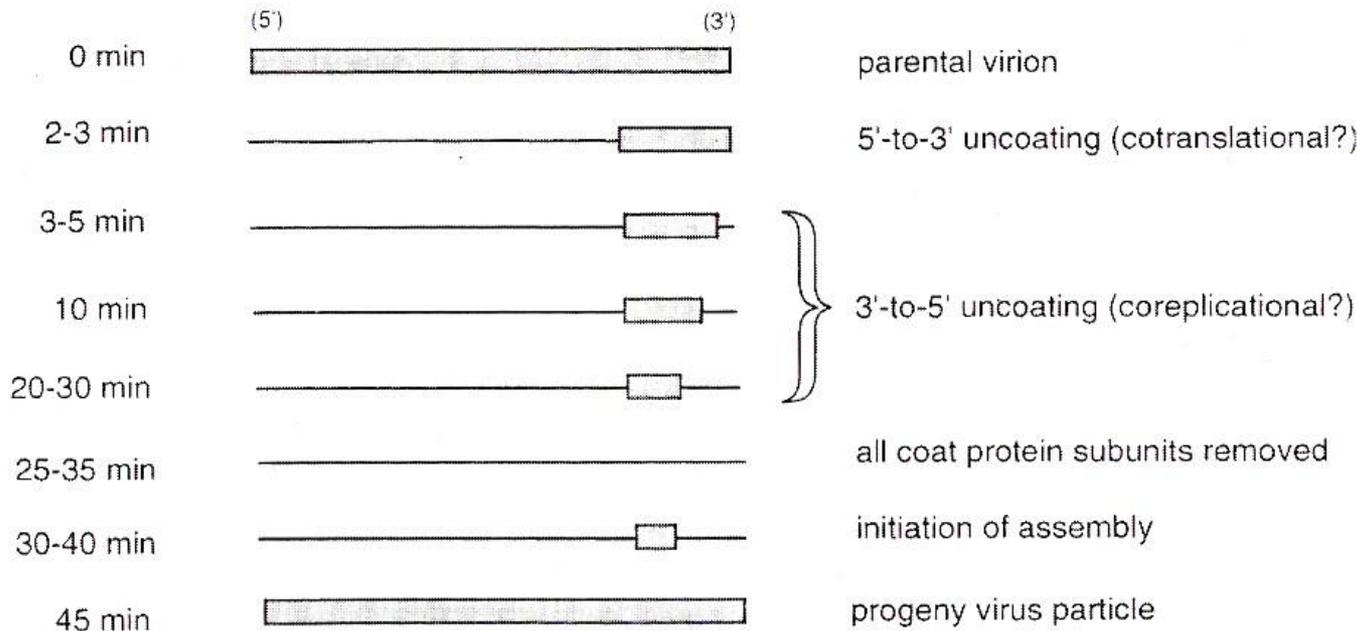


Fig. 7.6 Bidirectional disassembly of TMV particles *in vivo*. Coat protein subunits are removed in a 5' → 3' direction from *c.* 75% of the viral RNA in the first 2-3 minutes after inoculation of protoplasts. Uncoating the 3'-end of the RNA begins shortly thereafter and is completed by removal of subunits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

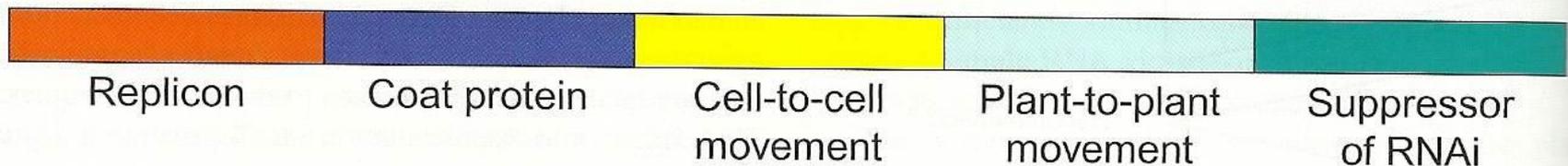
FUNÇÕES DAS PROTEÍNAS CODIFICADAS PELO VÍRUS

- Estrutural: proteína capsidial e proteínas do nucleocapsídeo, replicases em virus de –ssRNA e dsRNA.
- Enzimas: proteases, síntese de ácido nucléico (polymerase).
- Movimento e transmissão.
- Reconhecimento da célula hospedeira: não existe para vírus de plantas.

BOX 8.1 Virus Modules

Modules are defined as interchangeable genetic elements, each of which carries out a particular biological function.

A basic modular structure for a plant virus is shown in the figure.



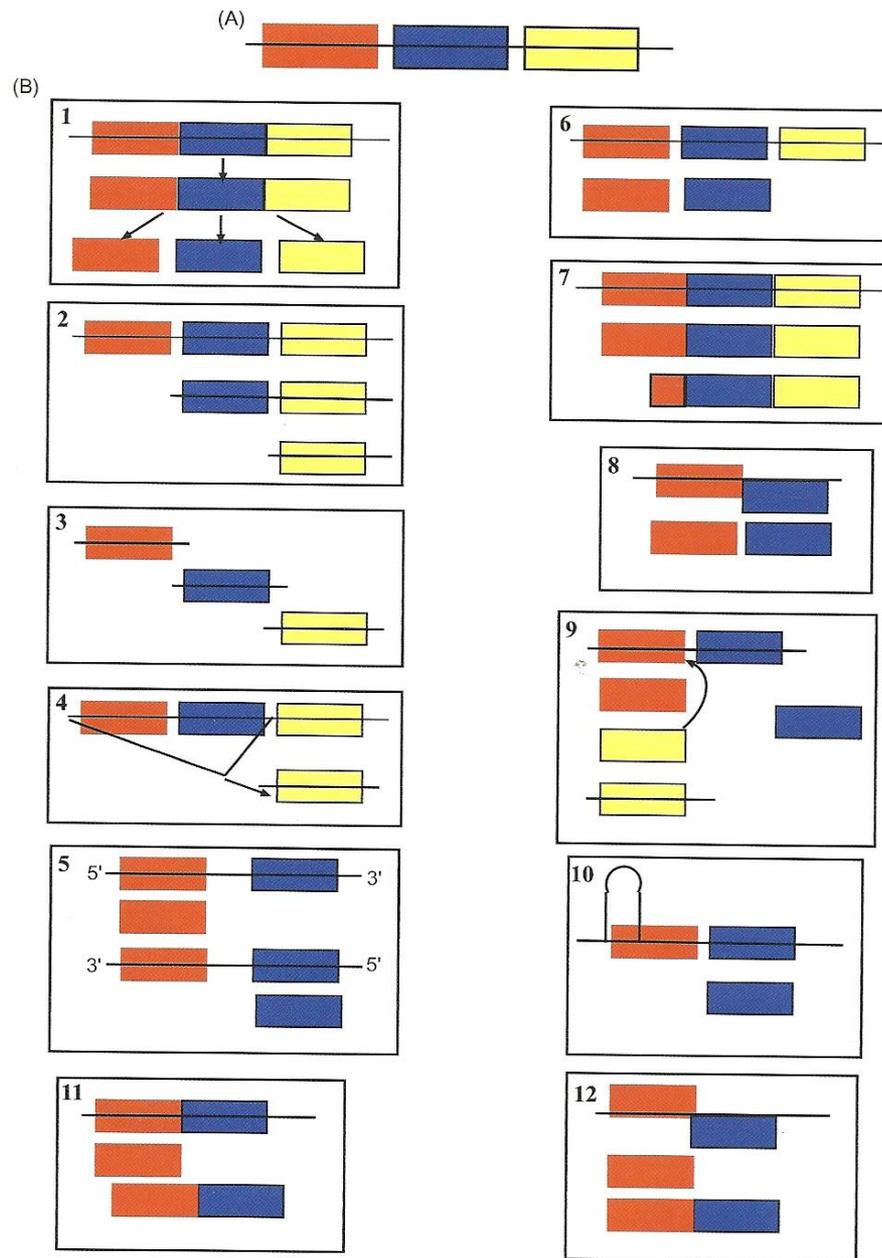
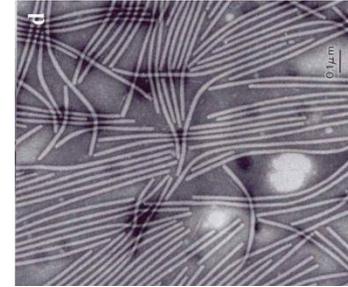
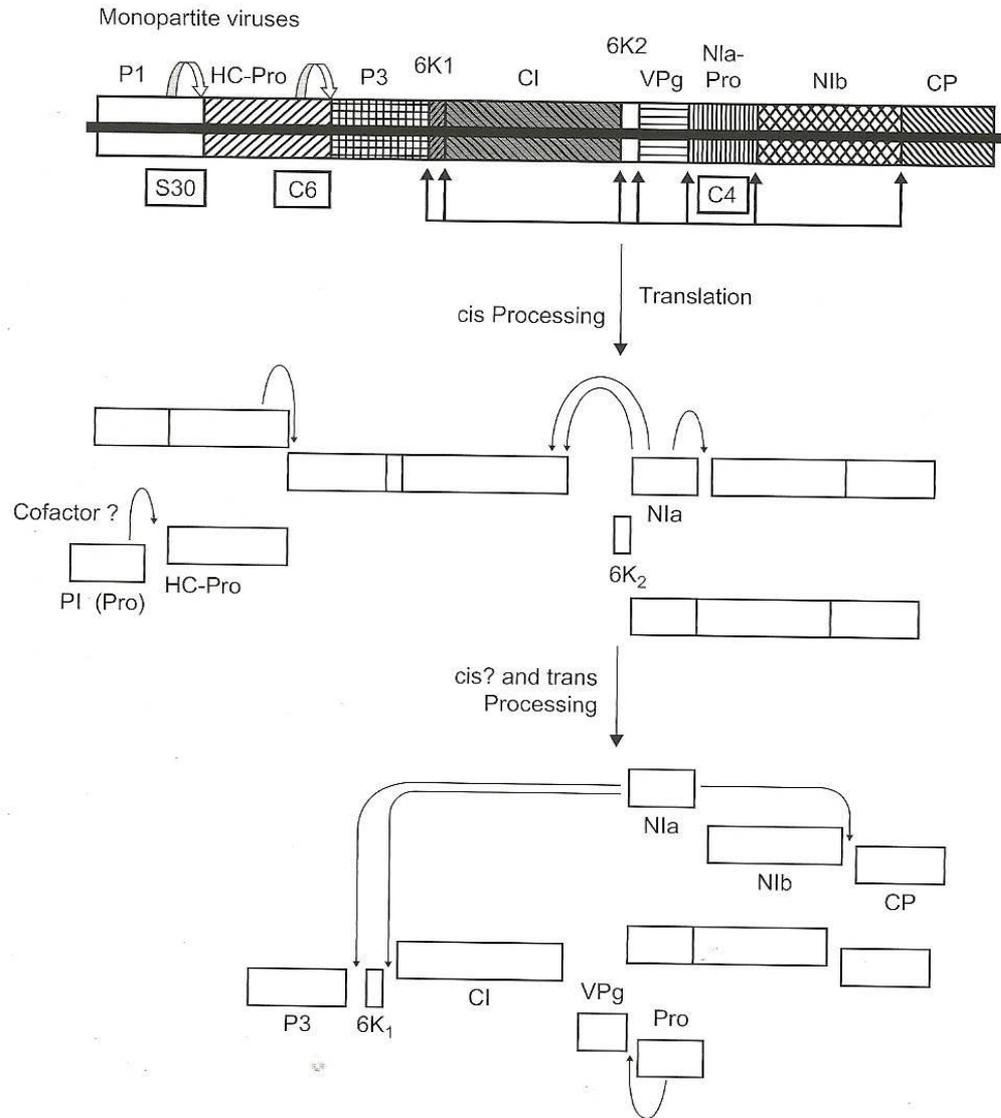


FIGURE 6.17 Diagram illustrating the 12 strategies that viruses have for overcoming the constraints of the eukaryotic translation of a polygenic viral genome with three ORFs. (B) The 12 strategies described in the text. *From Hull (2009) with permission of the publisher.*

“ESTRATÉGIAS” DO GENOMA PARA A SÍNTESE DE PROTEÍNAS

POLIPROTEÍNA (Potyvirus)



Poli-proteína: 320 kDa

CP: 20 kDa

2000 capsômeros
para uma CP

Table 7.1

Summary of Genome Strategies Adopted by 18 Single-Stranded Positive Sense RNA Plant Virus Groups

Number	Strategy (see Fig. 6.2)	Virus group	Number of ORFs	Number of proteins coded	
I	One strategy	Polyprotein	<i>Potyvirus</i>	1	8
II	One strategy	Subgenomic RNA	<i>Potexvirus</i>	5	4-5
			<i>Tombusvirus</i>	5	5
III	Two strategies	Subgenomic RNA plus read-through or frameshift protein	<i>Tobamovirus</i>	5	4-5
			<i>Luteovirus</i>	6	6-7
			<i>Carmovirus</i>	5	5-7
IV	Two strategies	Subgenomic RNAs and polyprotein	<i>Tymovirus</i>	3	3-5
			<i>Sobemovirus</i>	4	4-5
V	Two strategies	Multipartite genome and polyprotein	<i>Comovirus</i>	2	≈9
			<i>Nepovirus</i>	2	≈6
VI	Two strategies	Subgenomic RNAs and multipartite genome	<i>Bromovirus</i>	4	4
			<i>Cucumovirus</i>	4	4
			Alfalfa mosaic virus	4	4
			<i>Ilarvirus</i>	4	4
			<i>Hordeivirus</i>	7	7
VII	Three strategies	Subgenomic RNAs, multipartite genome, and read-through protein (or frameshift)	<i>Tobravirus</i>	5	5
			<i>Furovirus</i>	9	6-9
			<i>Dianthovirus</i>	4	4

MOVIMENTAÇÃO DOS VÍRUS DE PLANTAS

1. INTRODUÇÃO

Infecção

Replicação

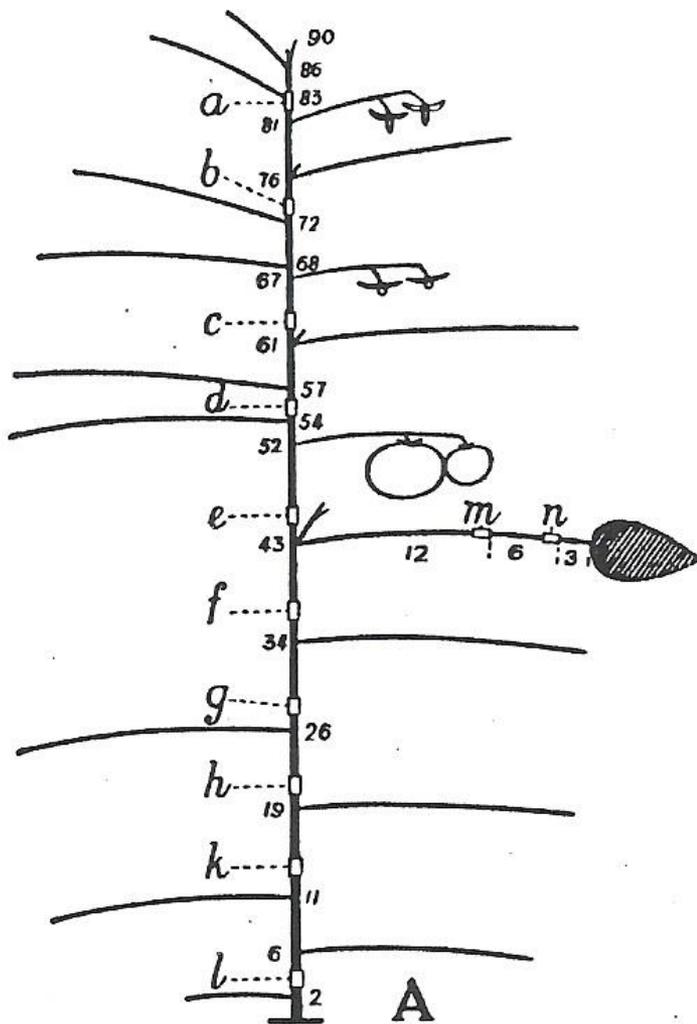
Invasão sistêmica

- a) movimento a partir da primeira célula infectada
- b) movimento da célula do parênquima para o tecido vascular
- c) movimento do tecido vascular de volta para células do parênquima

MOVIMENTO DO VÍRUS NA PLANTA

A. VISÃO GERAL

Samuel, G. 1934



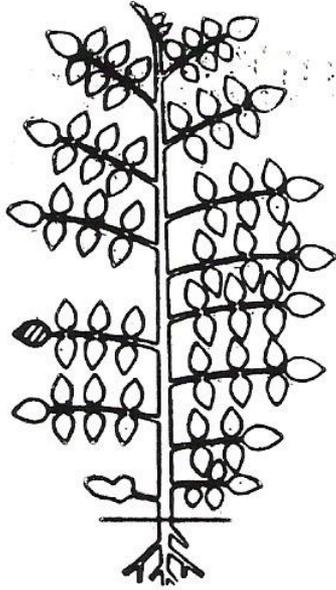
Inoc. *N. glutinosa* lesão local

Inoc. *N. tabacum* sistêmico

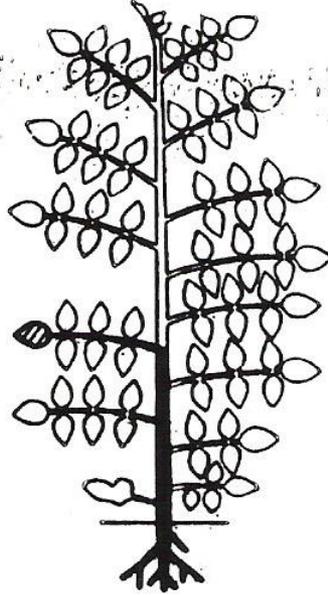
Enraizamento

Tubo de ensaio 1 semana inoc.
N. glutinosa lesão local

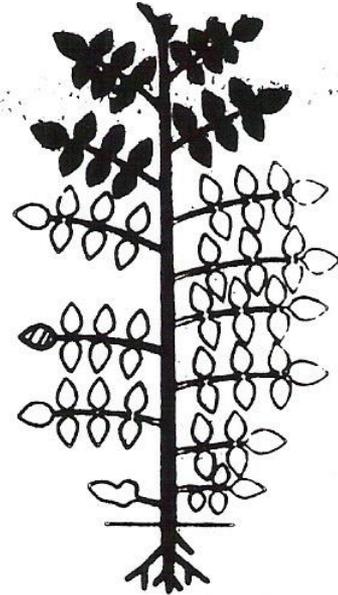
3 Days



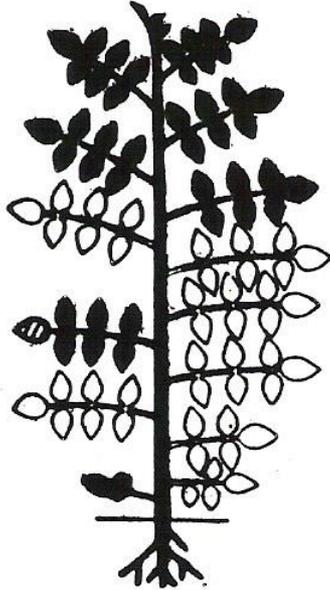
4 Days



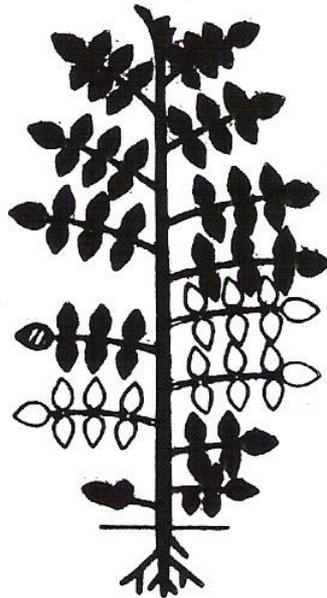
5 Days



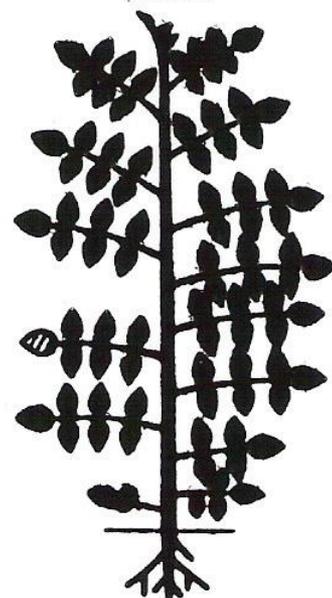
10 Days



18 Days



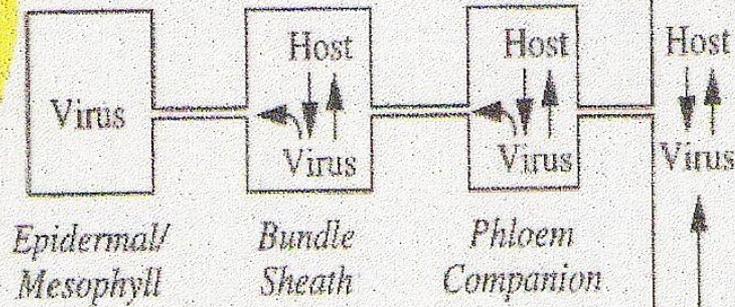
25 Days



4

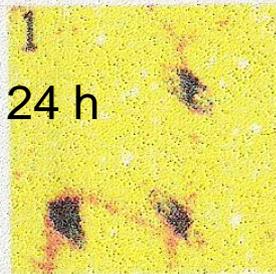
uidA = β -Glucoronidase (GUS)
Subst = 4-methylumbelliferyl glucoronade

Cell-to-Cell Movement — Systemically Infected Leaf

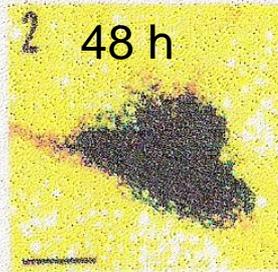


Tobacco etch virus

Long-Distance Movement



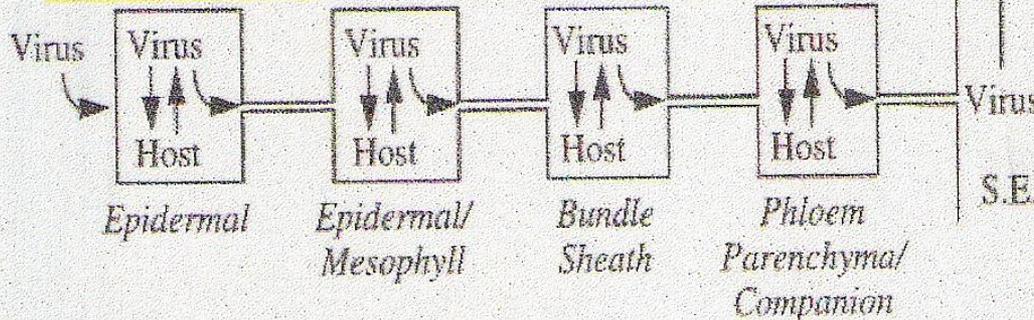
1
24 h



2
48 h



3
96 h

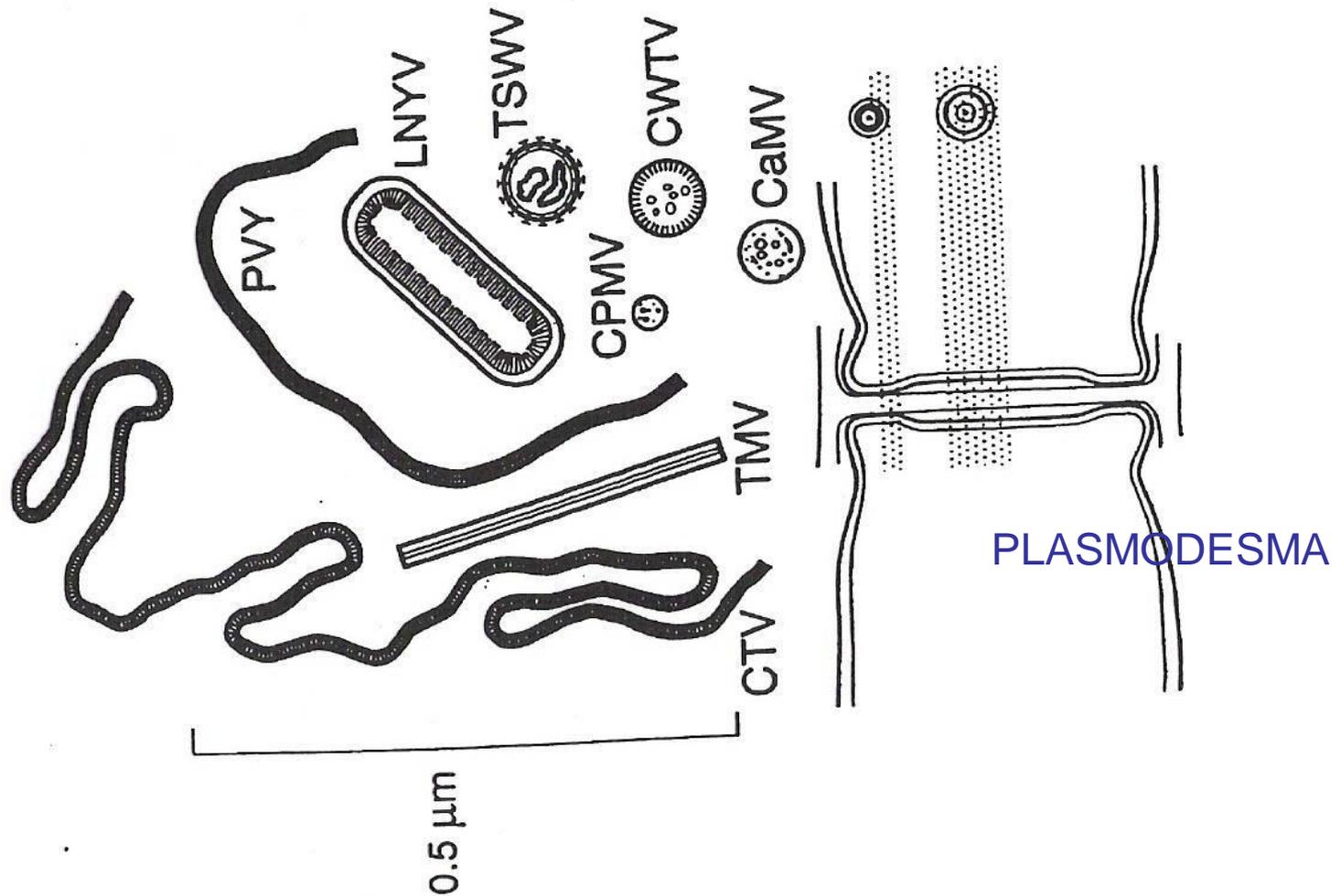


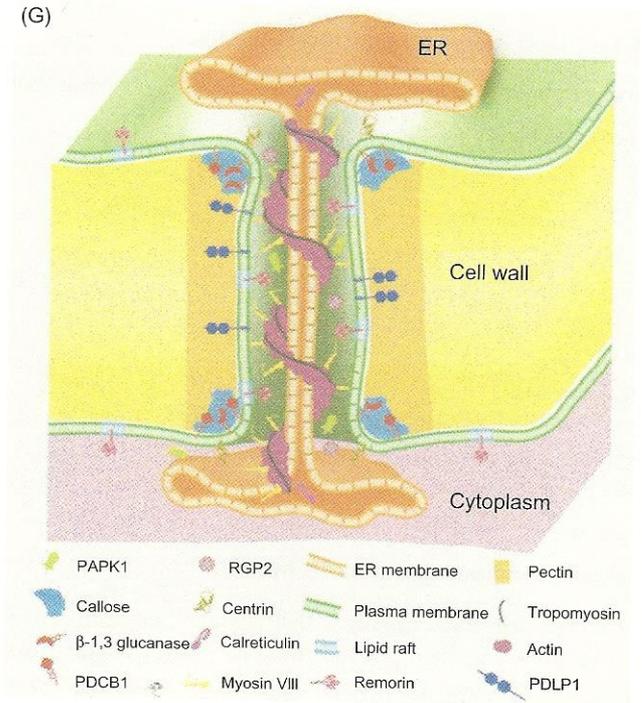
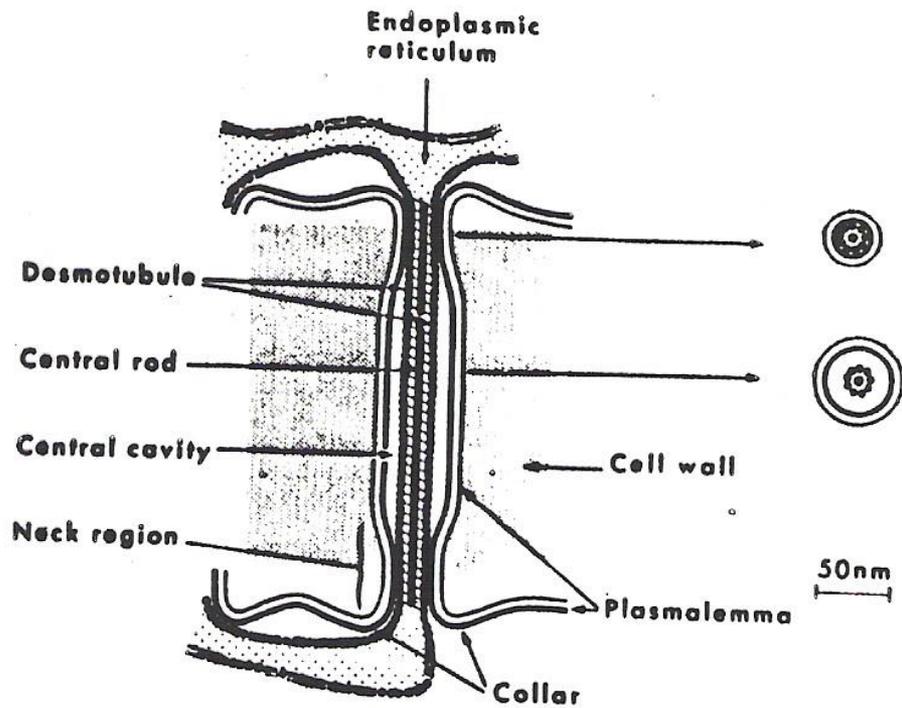
Cell-to-Cell Movement — Inoculated Leaf

Carrington et al., 1996

B. MOVIMENTO DE CÉLULA PARA CÉLULA

1. TRANSPORTE INTRACELULAR E INTERCELULAR





DIMENSIONS OF PLASMODESMATA (nm)

	Outer diameter of plasmalemma	Inner diameter of plasmalemma	Outer diameter of desmotubule	Inner diameter of desmotubule	Central rod
<i>Azolla</i> , young root cortical cells	35	25	16	7	3
<i>Hordeum</i> , young root (4 mm from tip) endodermal cells	46	33	20	9	3
<i>Hordeum</i> , older root (120 mm from tip) endodermal cells	60	44	20	10	4
<i>Abutilon</i> , distal cross-wall of stalk cell of nectary hair	44	29	16	10	3

From: Robards, A. W., Plasmodesmata in higher plants, in *Intercellular Communication in Plants: Studies on Plasmodesmata*, Gunning, B. E. S. and Robards, A. W., Eds., Springer-Verlag, Berlin, 1976, 15. With permission.

2. ESTRATÉGIAS DE MOVIMENTO:

a) TMV (super família proteína 30K)

b) Tubular

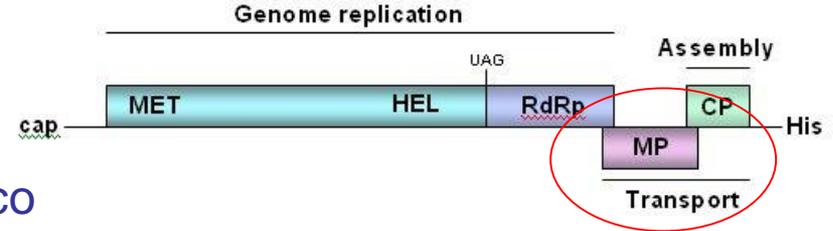
c) “Triple gene block”

d) Potyviruses

e) Geminiviruses

ESTRATÉGIA DO TMV

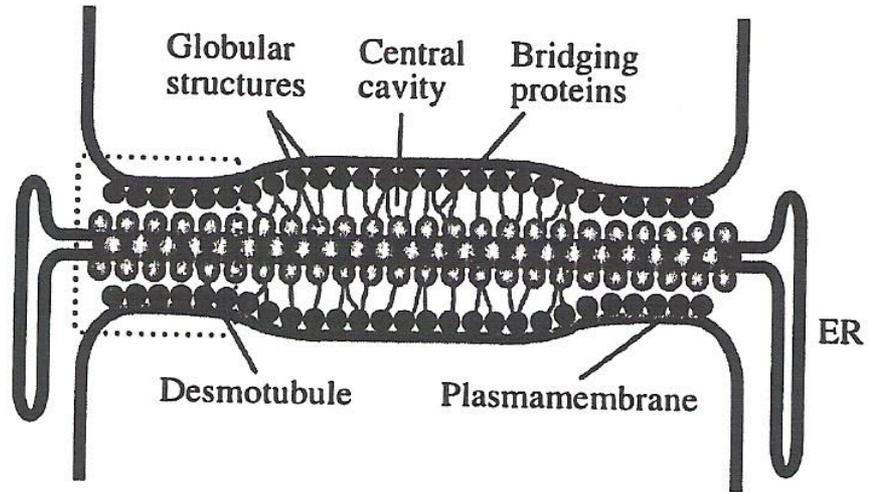
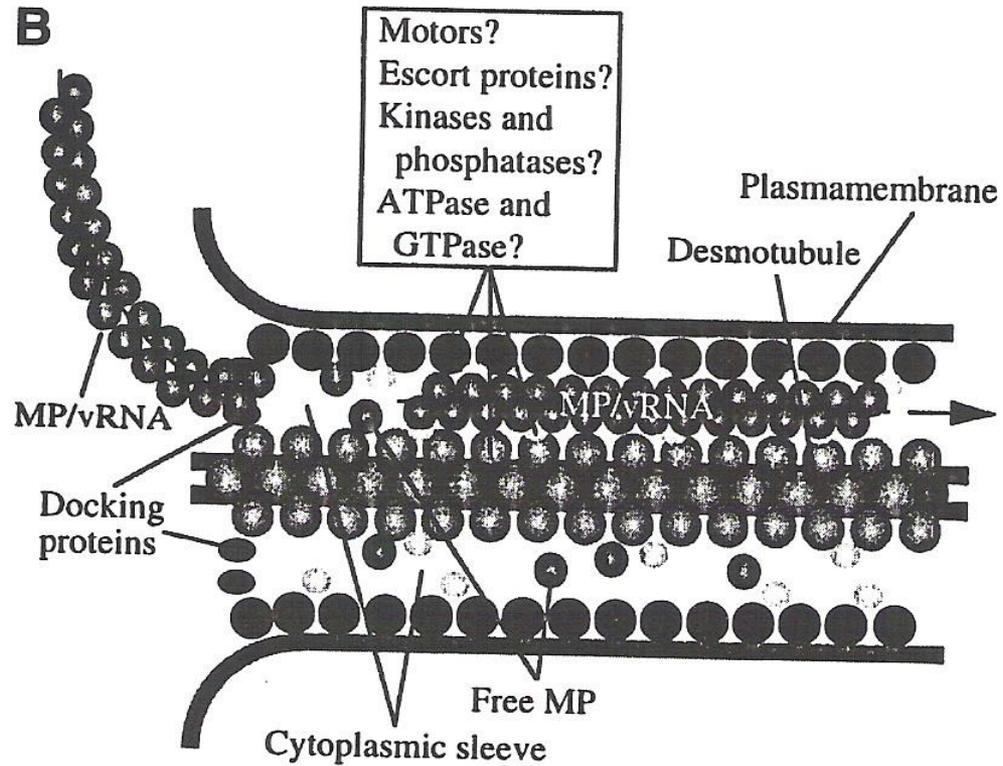
TMV - proteína 30 K.



- Associa-se ao ácido nucléico
- Aumenta diâmetro de plasmodesma, localiza-se e acumula-se neste
- Facilita o movimento do RNA para a célula vizinha
- planta transgênica

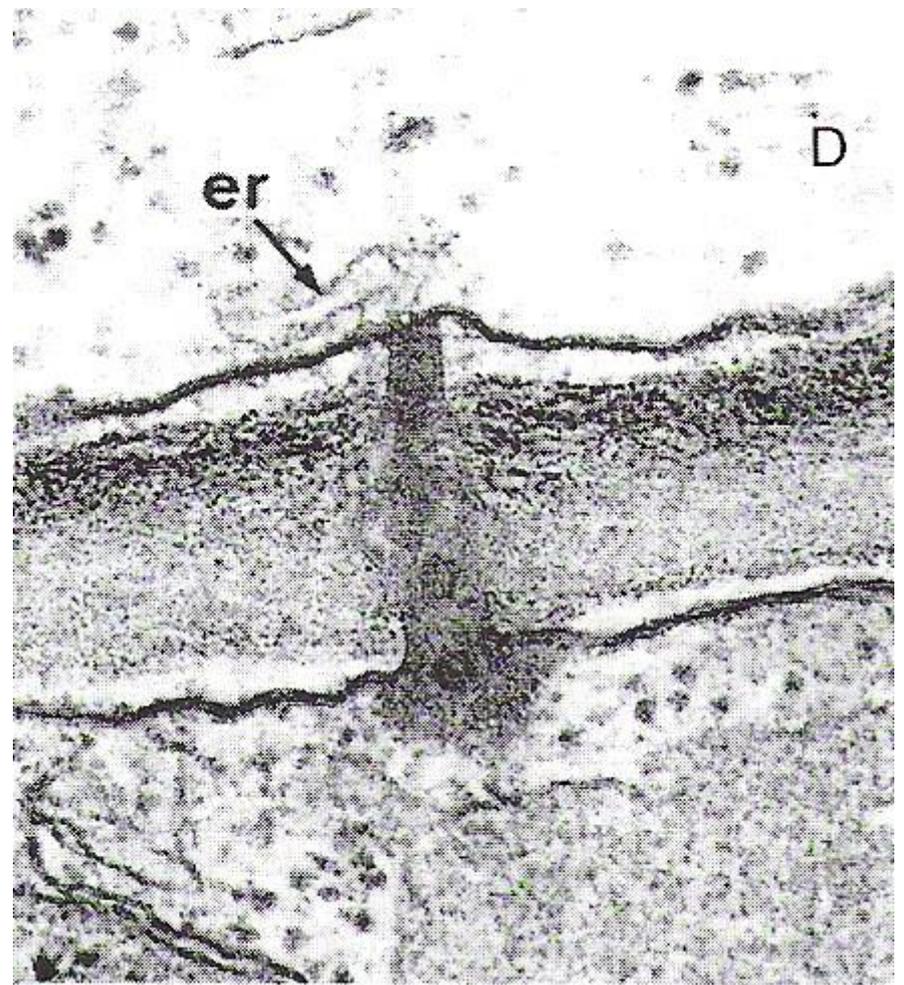
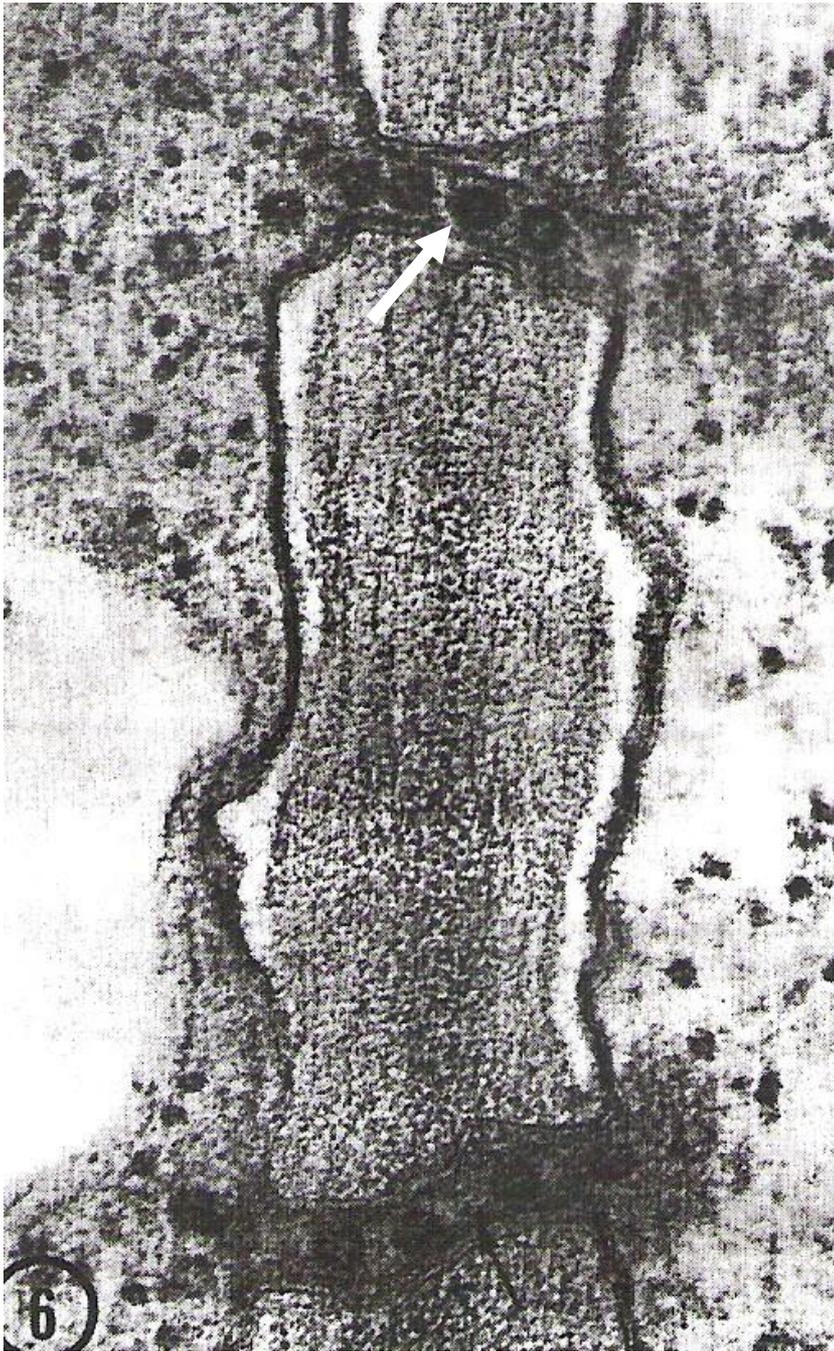
Isolado LS1 não é sistêmico a 32° C.

Em plantas transgênicas que produzem a proteína 30 K, LS1 invade sistemicamente.

A**B**

Estratégia Tubular

Vírus do mosaico da dália (Caulimovirus)
Kitajima & Lauritis, 1969

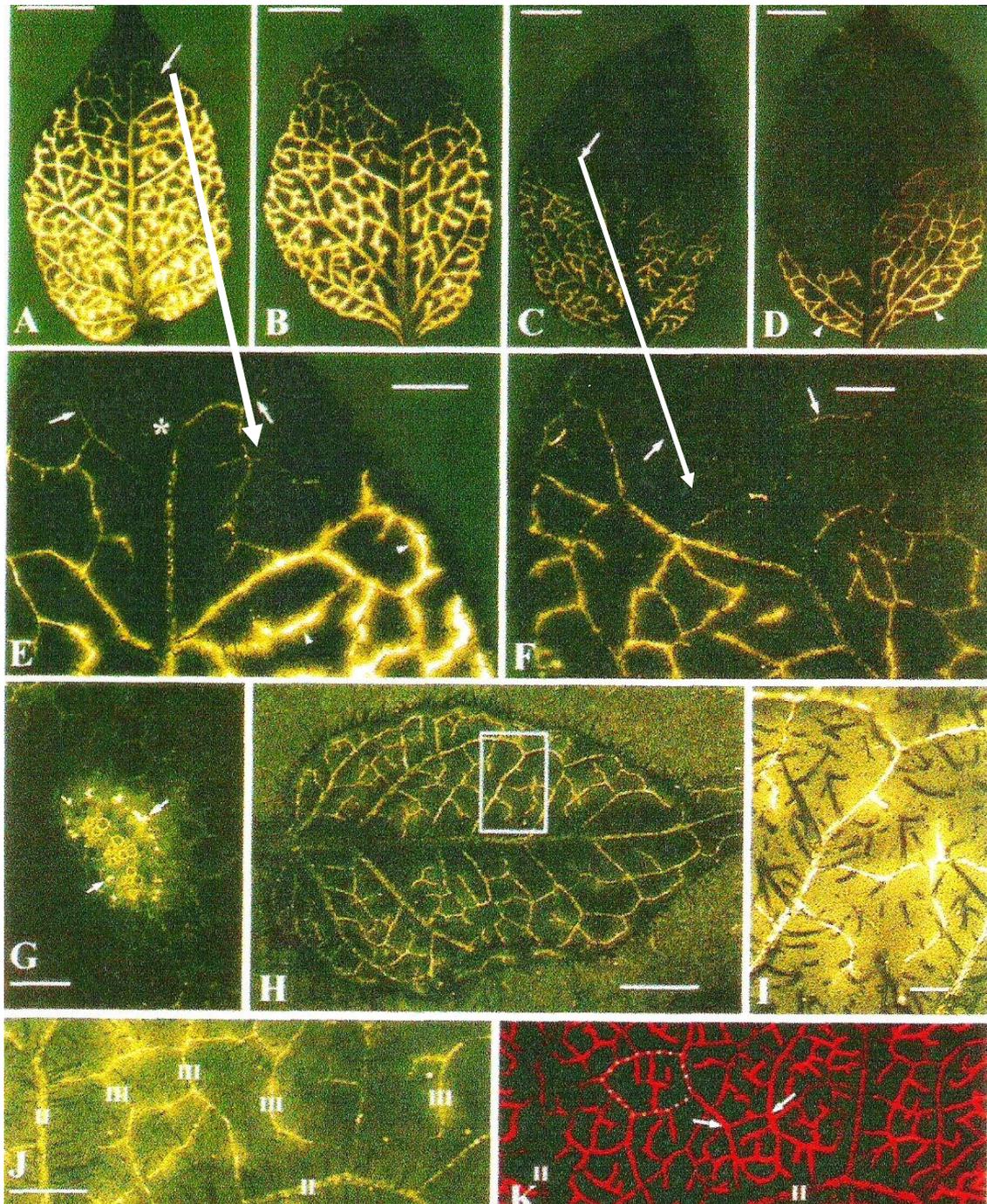


C. MOVIMENTO A LONGA DISTÂNCIA

- RÁPIDO
- Vírus sai do parênquima e vai para o floema.
- Movimento com produtos da fotossíntese.
- Envolvimento de proteína de transporte
- EX:

TMV: deleções ou adições no genoma que codifica a capa protéica (17,5 K) alterou o movimento sistêmico.

Transição
dreno-fonte



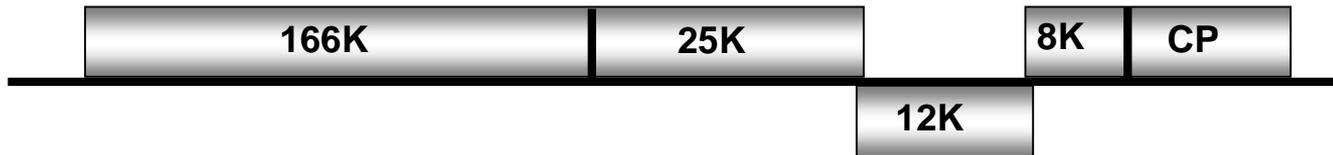
Corte transversal
do pecíolo: floema
marcado

Carboxyfluorescein

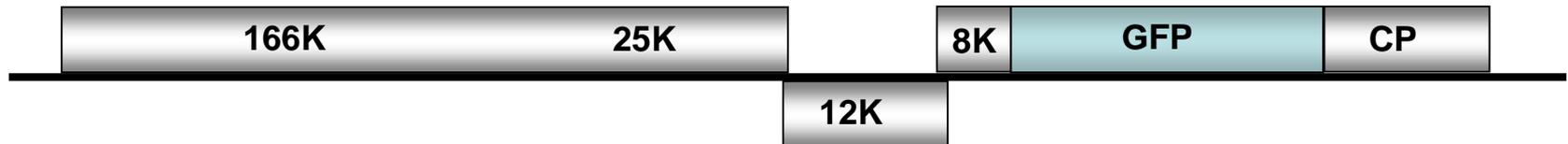
Texas Red dextran

Potato virus X (PVX)

PVX

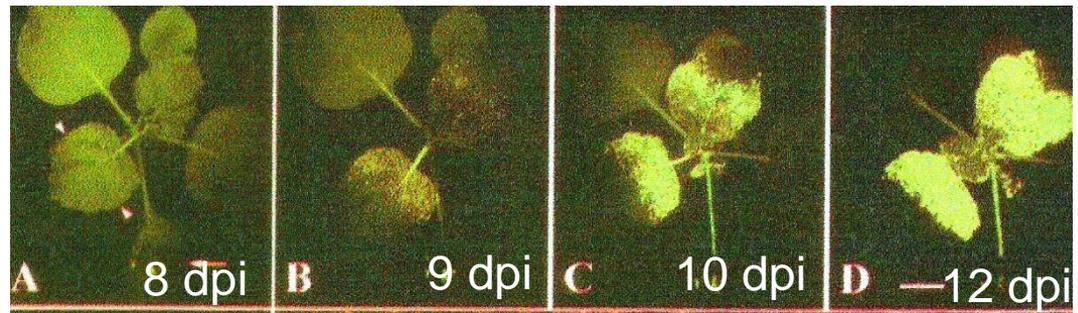


PVX.GFP

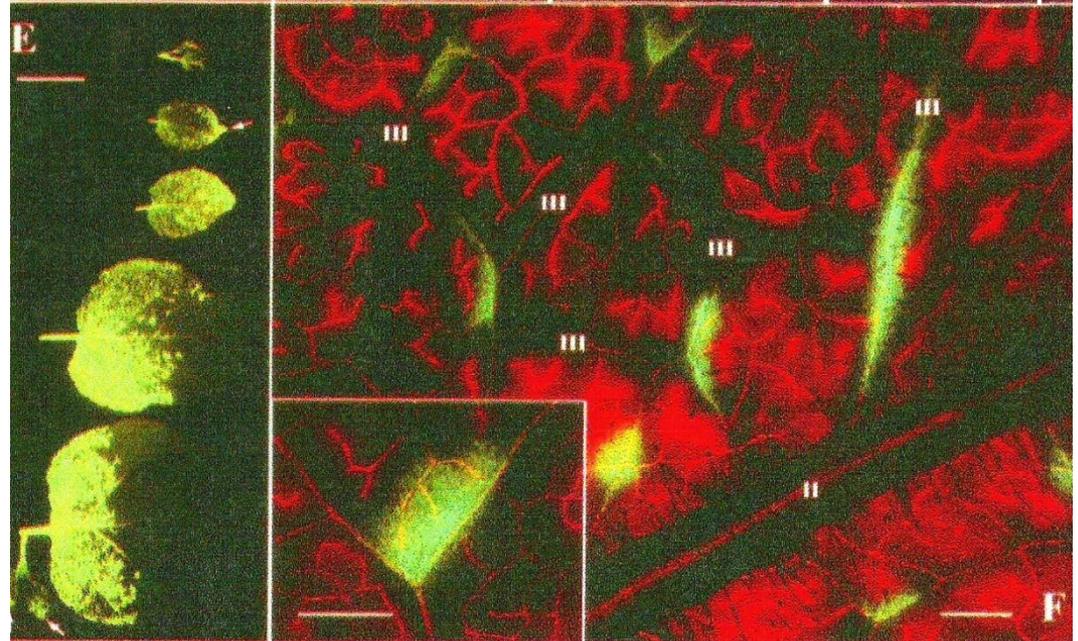


“green fluorescent protein”

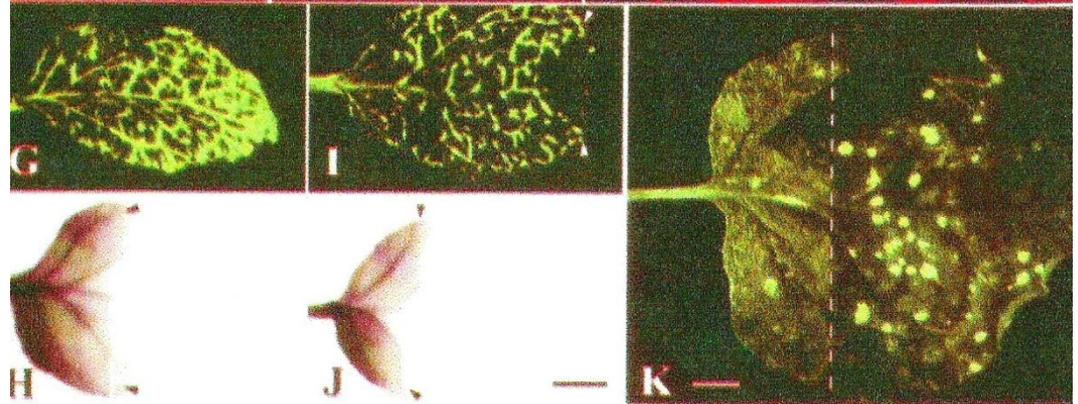
Folha inoculada: seta

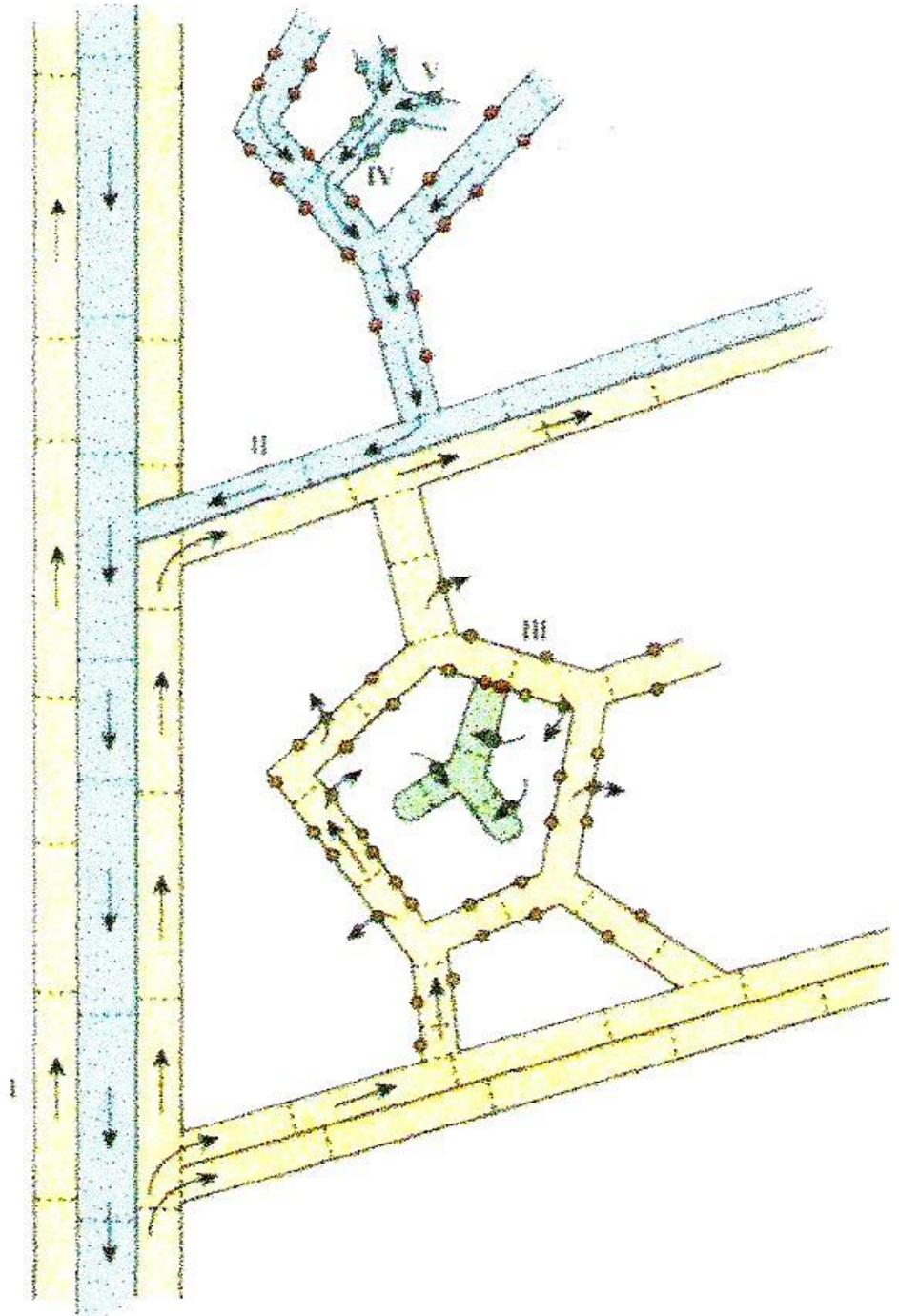
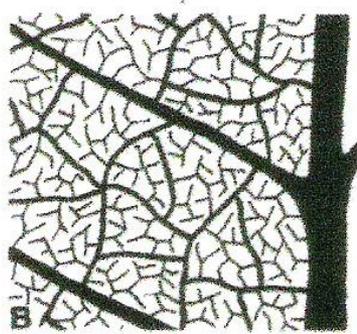
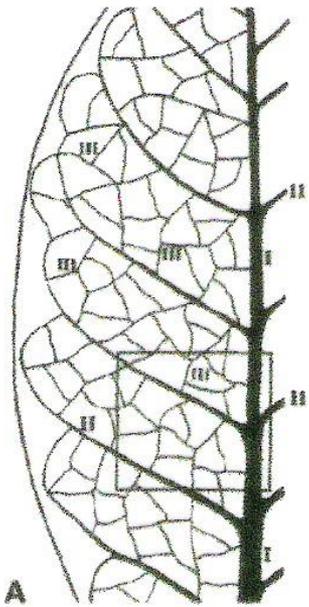


Folhas destacadas do ápice



PVX + GFP



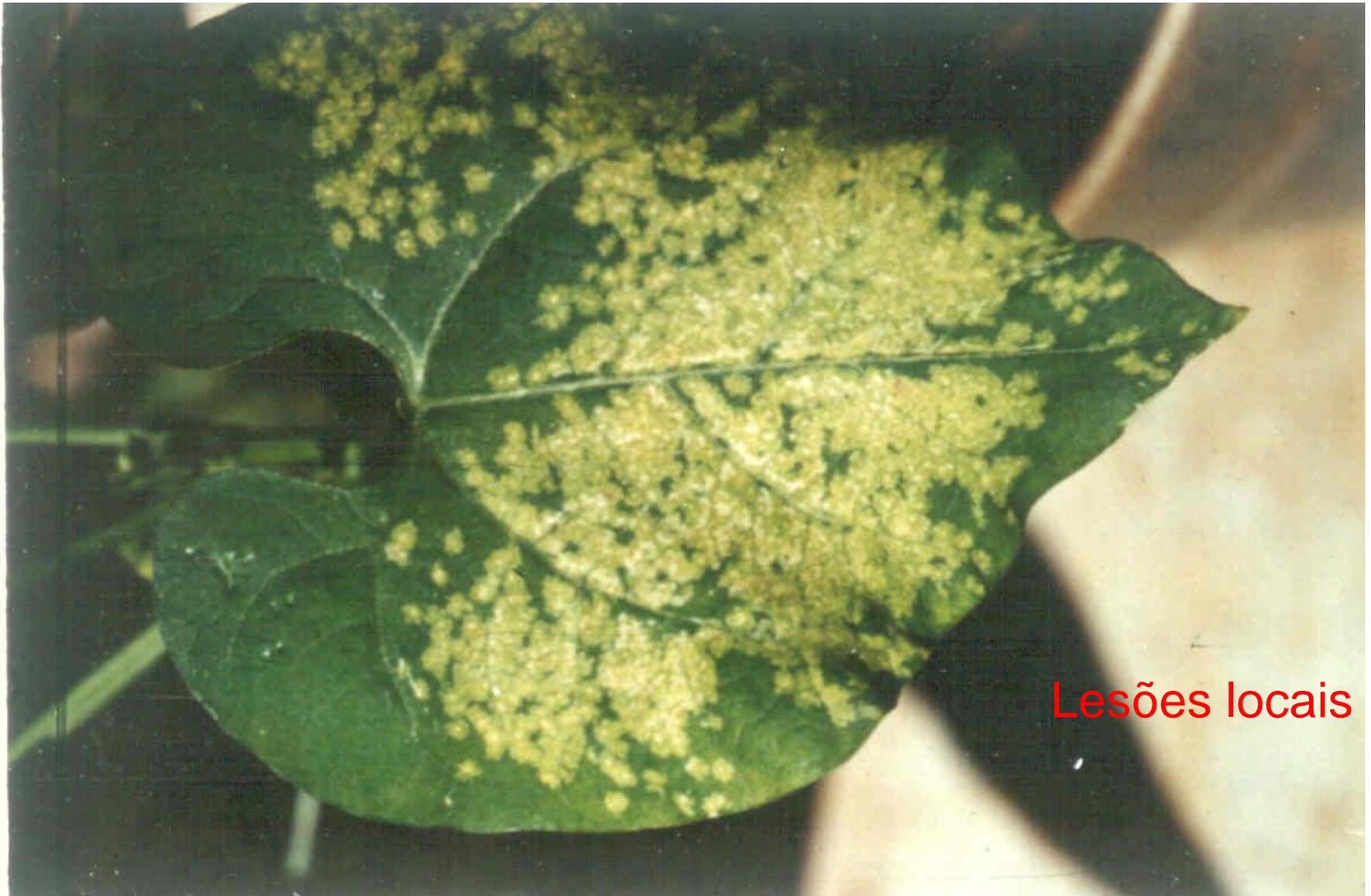


Fonte = azul (exporta)

Dreno = amarelo

SINTOMATOLOGIA E RECONHECIMENTO DE FITOVIROSES

1. SINTOMAS DE DOENÇAS CAUSADAS POR VÍRUS

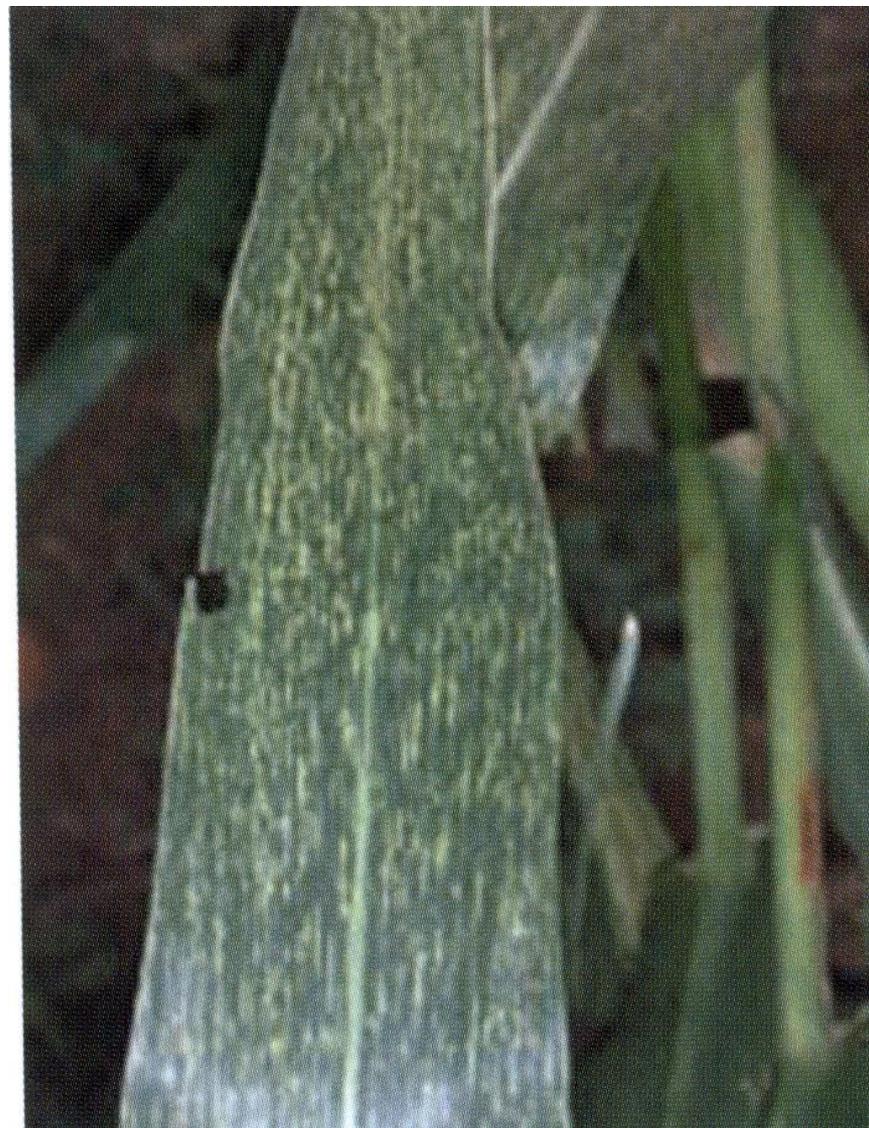


Lesões locais

Tomato chlorotic spot virus



Mosaico do fumo - TMV



Maize streak virus



Mosaico amarelo da abobrinha - ZYMV



Mosaico dourado em *Malvaviscos* sp



Cordão de sapato – PRSV-P em mamoeiro



Amarelo letal do mamoeiro Solo



Odontoglossum ringspot virus



Pinta verde do maracujazeiro



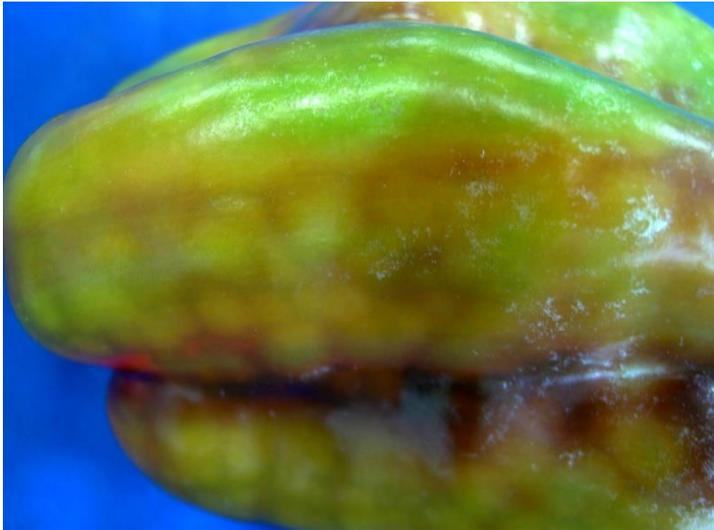
Endurecimento dos frutos do maracujazeiro - PWV

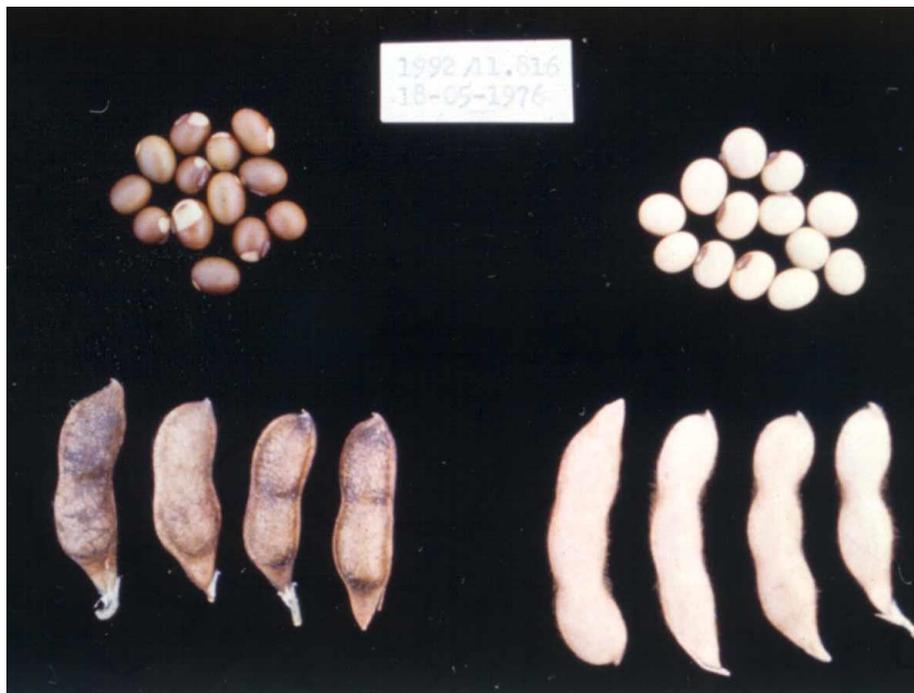


**Mosaico comum e amarelo da abobrinha
PRSV-W + ZYMV**



Vira cabeça: *Tomato spotted wilt virus*





Vírus do mosaico comum da soja



Rizomania da beterraba

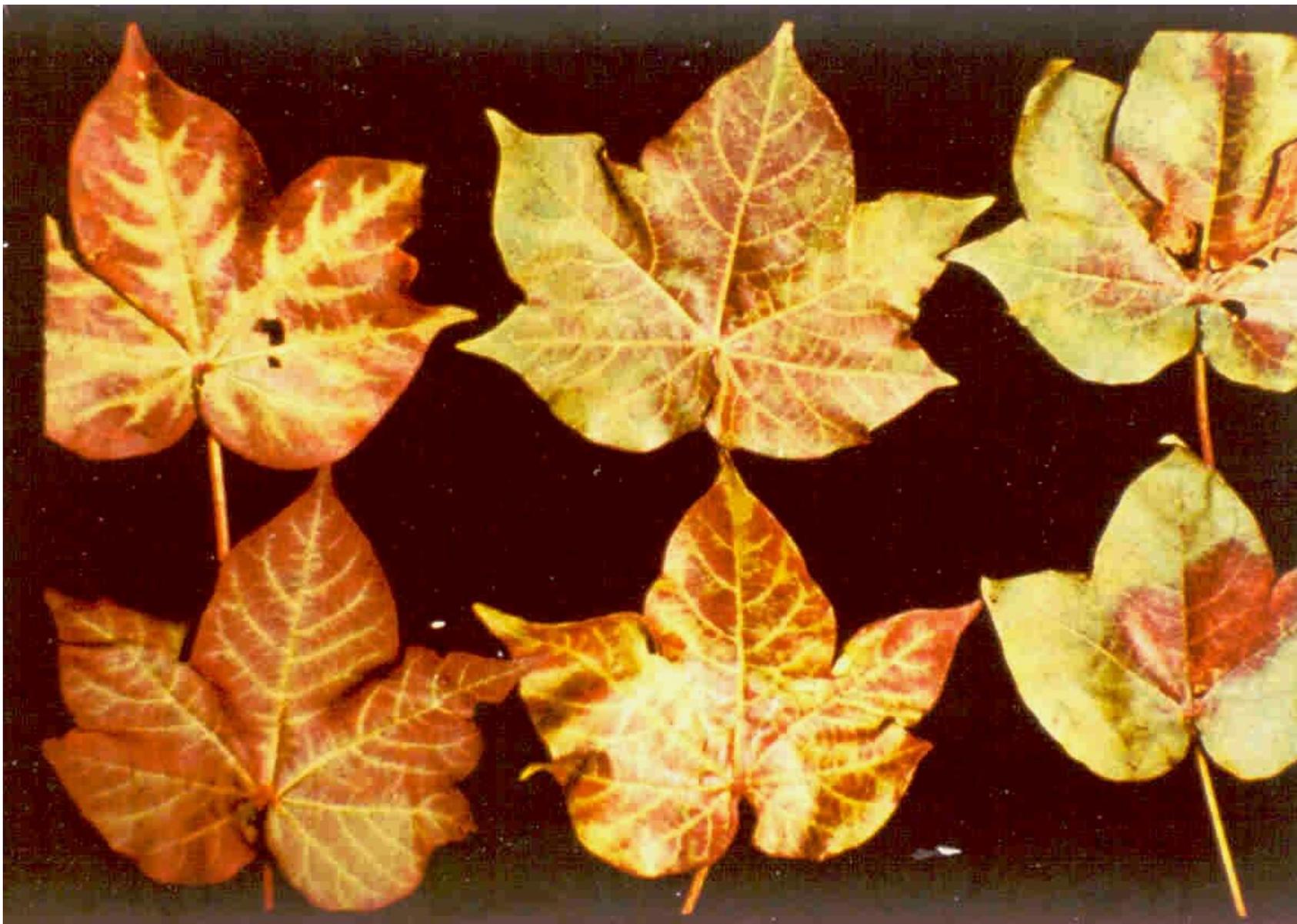


Sadias (esquerda e centro) e infectada (direita)

1. SINTOMAS SEMELHANTES AOS DAS FITOVIROSES



Variação genética em violeta Africana



Deficiência de Mg

Vermelhão (vírus)

Ácaro



**Ácaro branco em
em mamoeiro**



**Mosaico vs ácaro branco
em mamoeiro**



**Prateado da folha e
fruto branco de pepino =
toxemia**

**Prateado da folha =
genético**



Bemisia tabaci





Diuron em mamoeiro



INJÚRIAS



**Choque térmico
em violeta Africana**

**Baixa temperatura
em orquídea**

