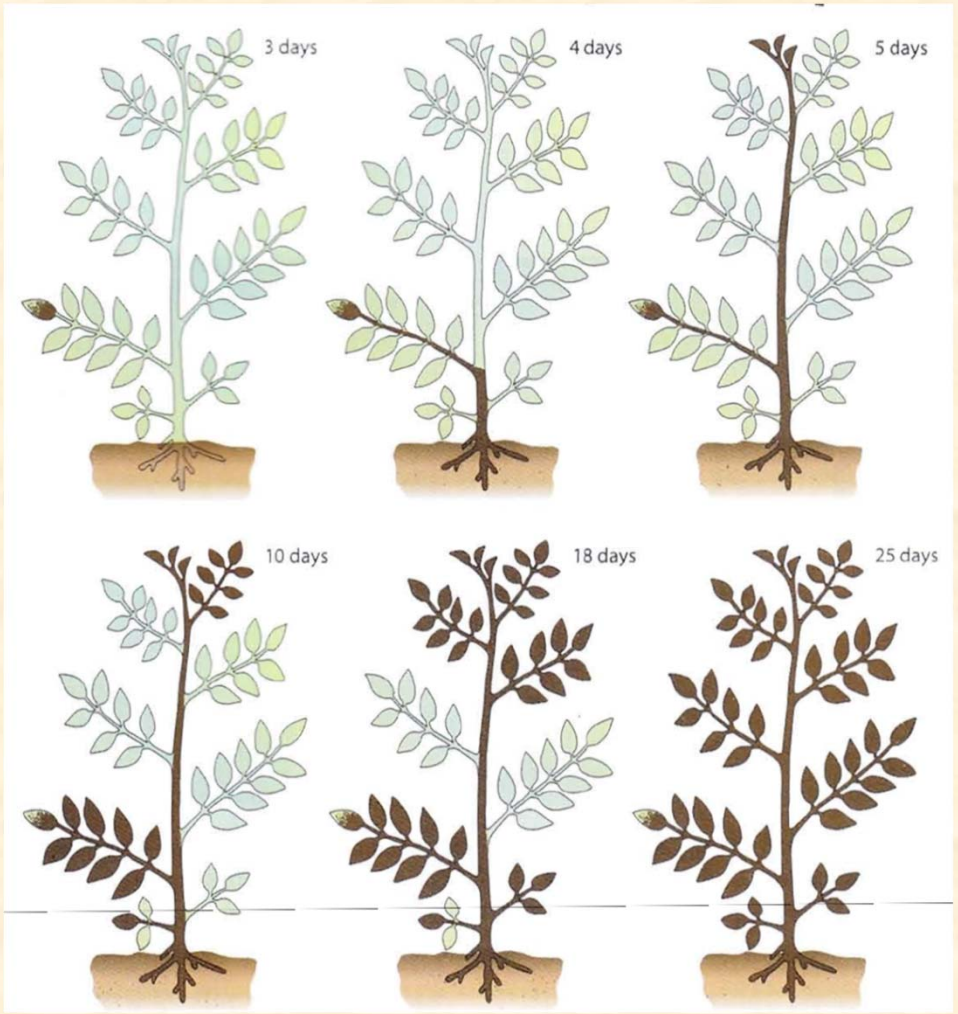
Plant Viruses: Movement and Distribution within Plants



Outline:

1. Cell-to-cell

2. Systemic

3. Virus movement and distribution of viruses within a plant

4. Virus movement and distribution of viruses within a leaf

5. Tissue tropism

“Susceptible” Infection:

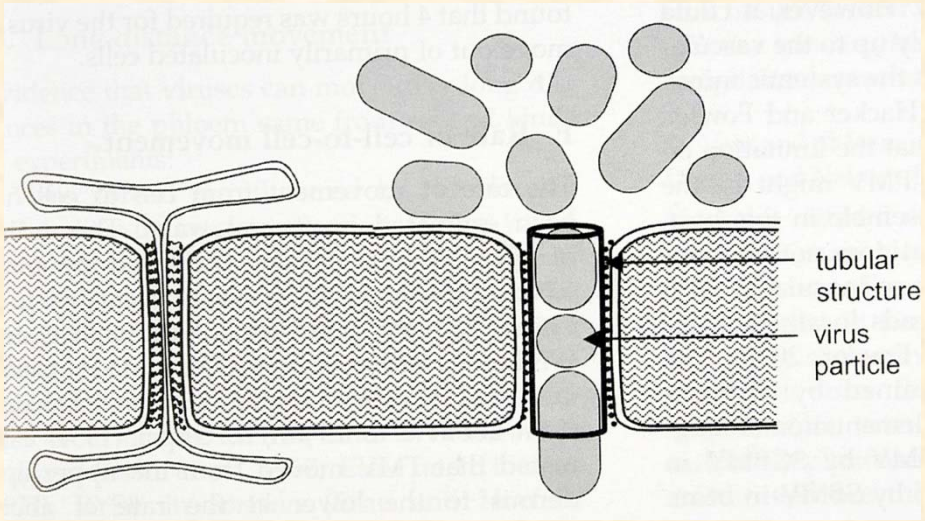
- Virus enters a plant cell,
- Replicates in the cell,
- Moves to neighboring cells,
- Moves into the phloem, then to sinks (root and shoot apices), and then spreads to neighboring cells
- Virus replication in apical cells may cause developmental abnormalities
- Cellular abnormalities give rise to foliar symptoms

- Symptoms develop in leaves days/ weeks after infection

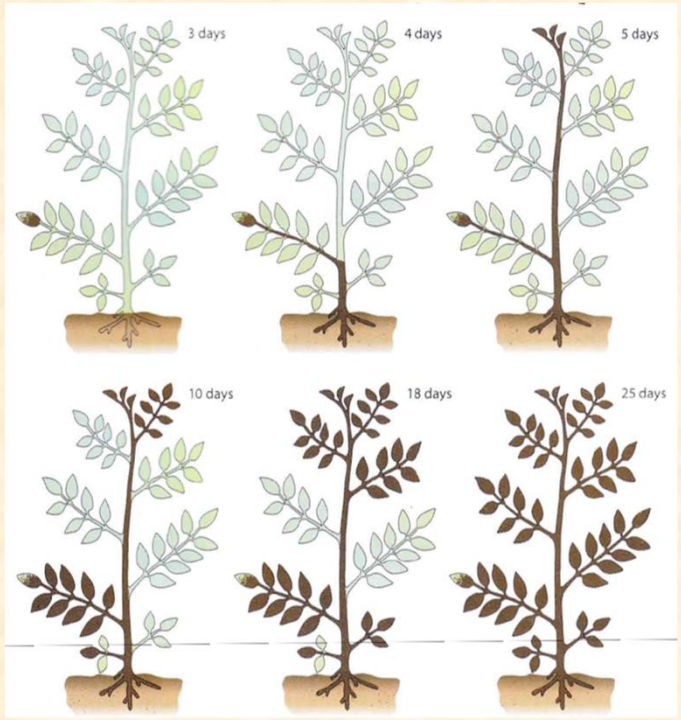
- Symptoms develop in leaves distant from site of inoculation

When virus infects a plants – there are two kinds of movement:

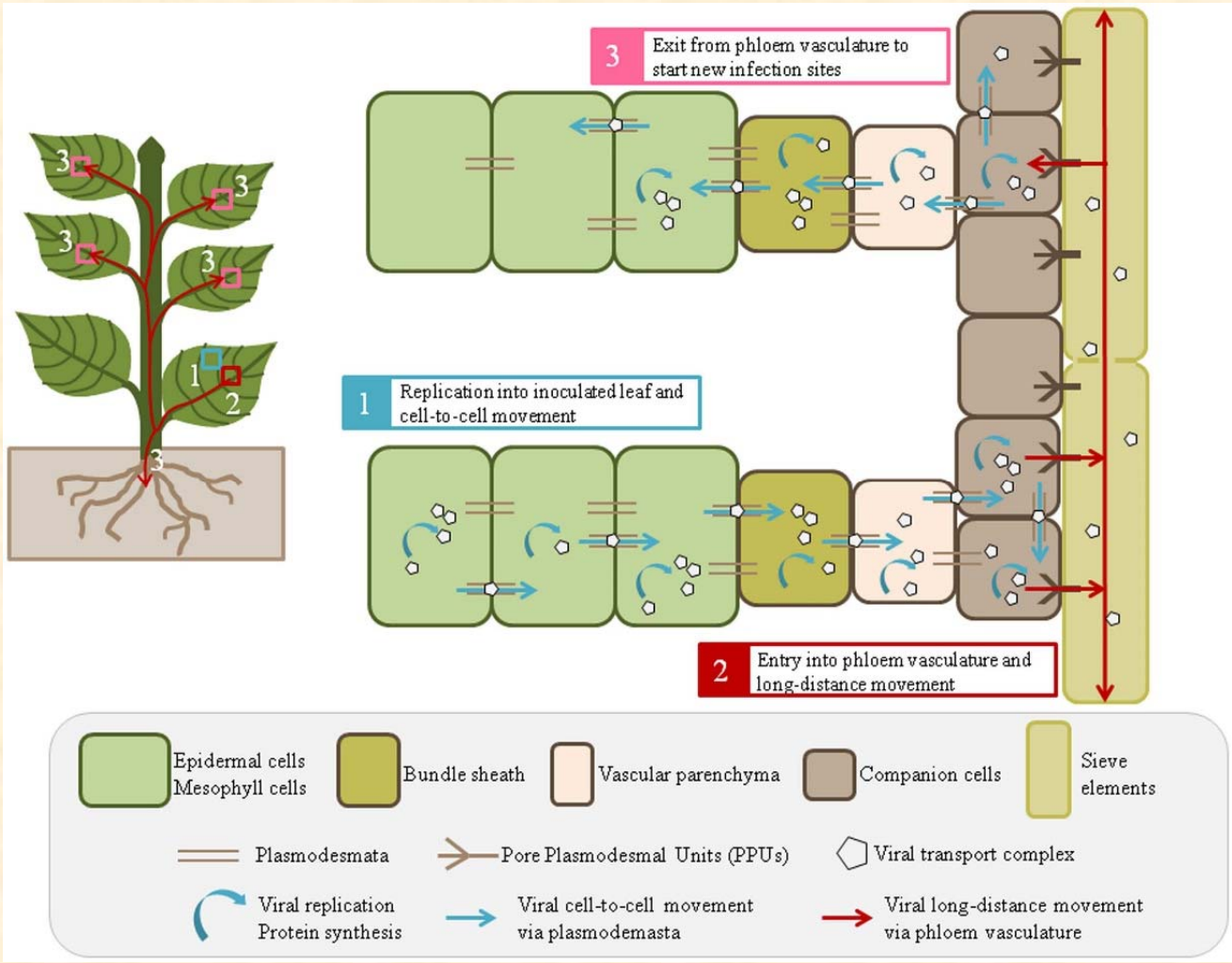
Cell to Cell



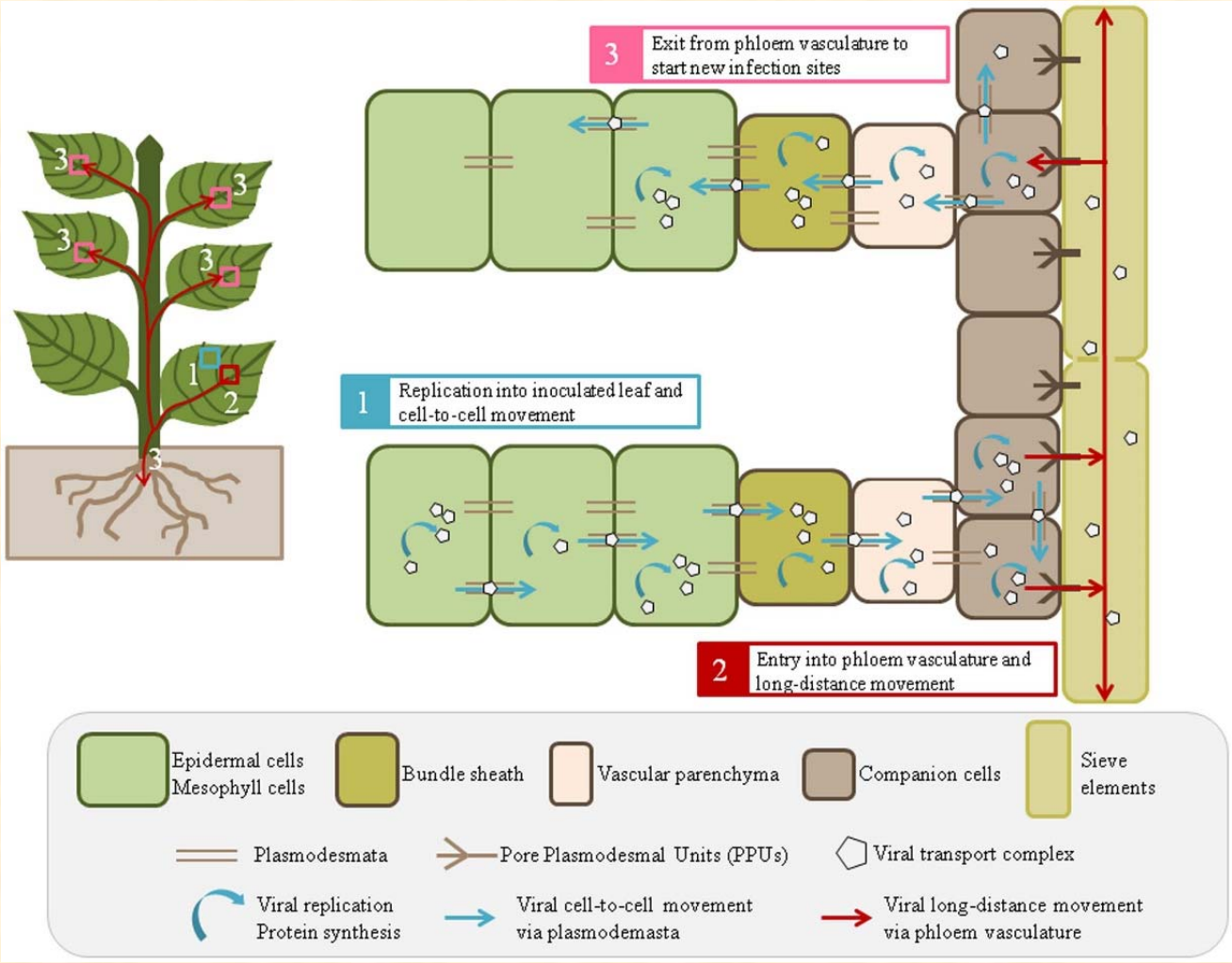
Systemic



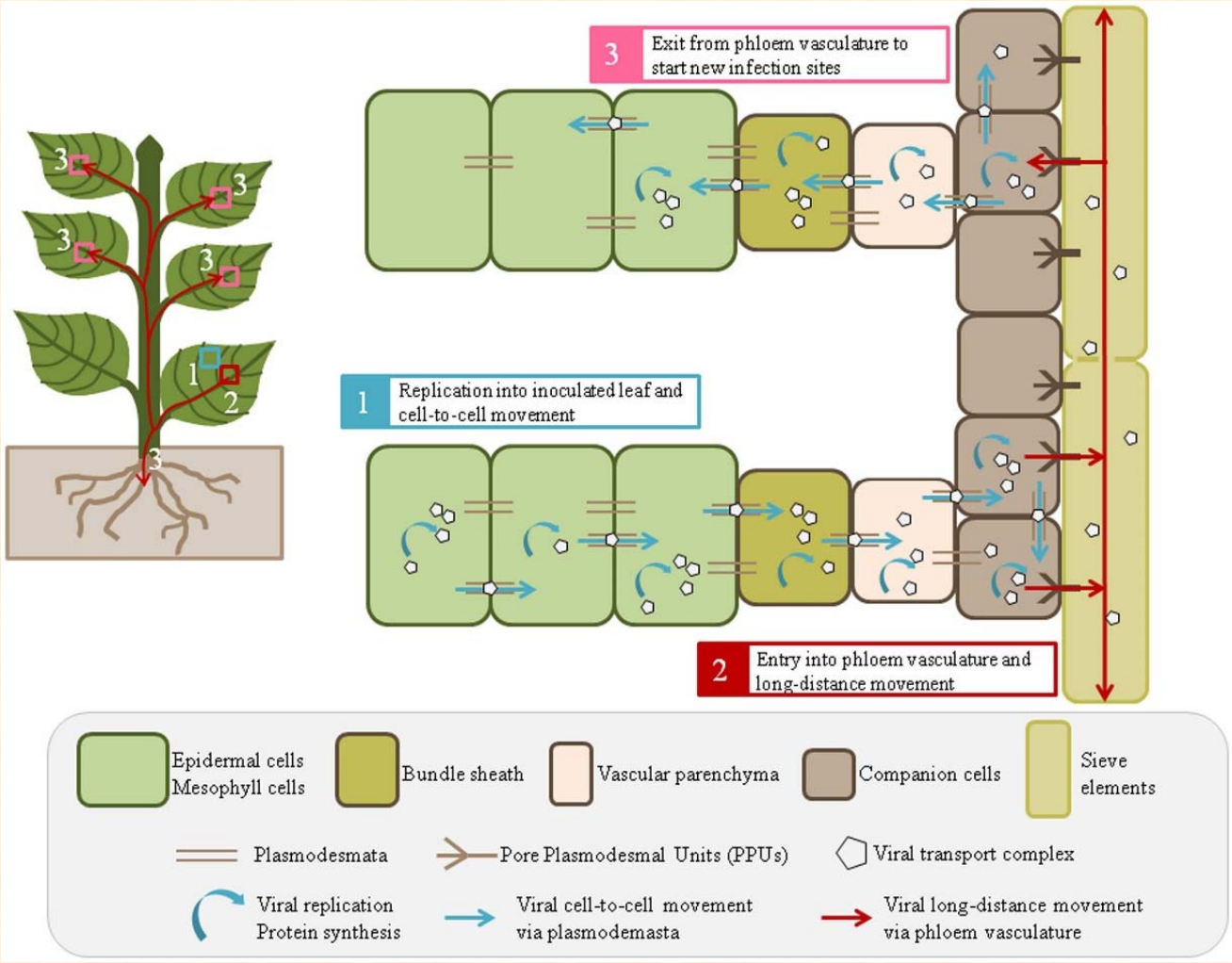
1) When a virus is inoculated into a leaf epidermal cell, the virus must move cell-to-cell if the infection is to spread.



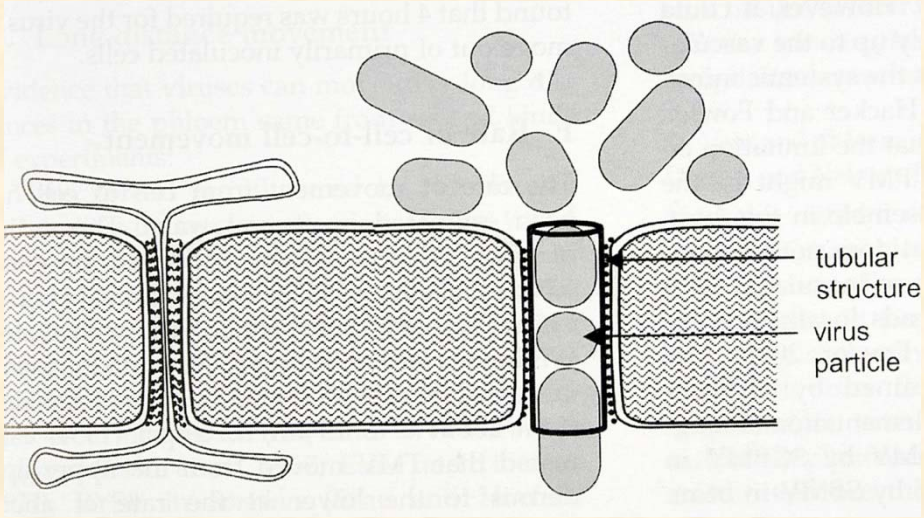
2) In order for the virus to spread systemically, it must invade the mesophyll cells below the epidermis and pass through bundle sheath, parenchyma, and companion cells before finally entering the sieve element.

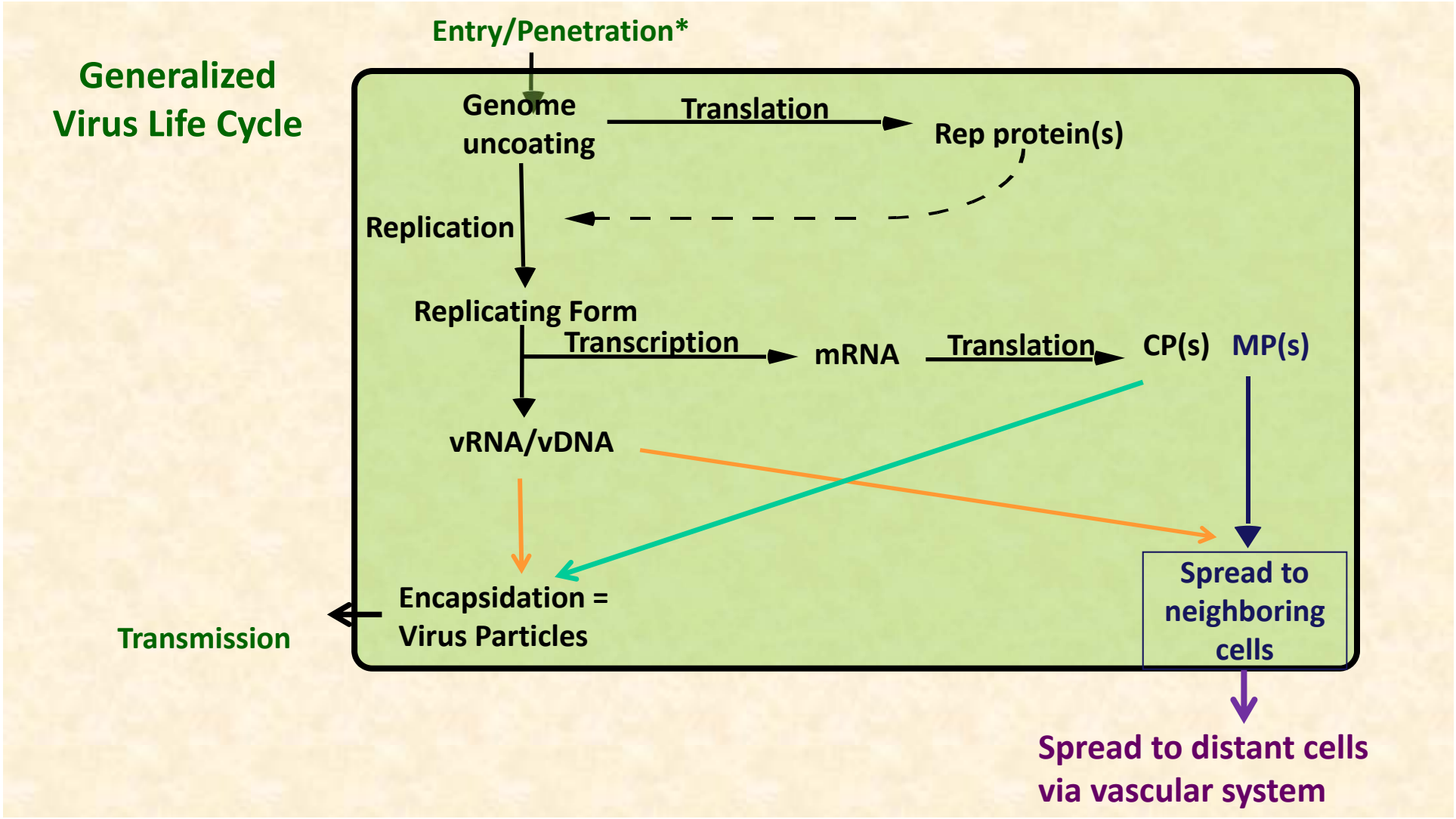


3) Once the virus has gained access to the phloem, it may be transported to other leaves (sinks) both up and down where the process of cell-to-cell movement may begin anew.



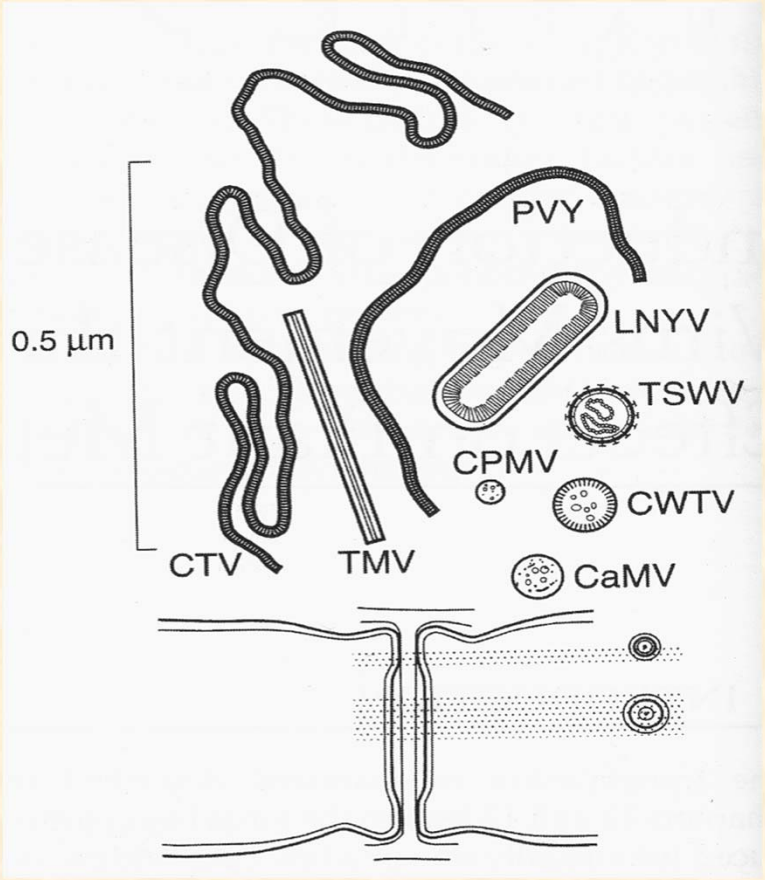
1. Cell to Cell Movement




Generalized
Virus Life Cycle
Entry/Penetration*
Genome
uncoating
Translation
Rep protein(s)
Replication
Replicating Form
Transcription
mRNA
Translation
CP(s)
MP(s)
vRNA/vDNA
Encapsidation =
Virus Particles
Transmission
Spread to
neighboring
cells
Spread to distant cells
via vascular system

Cell to Cell:
Relative sizes of some plant viruses compared with the size of plasmodesma

CTV, 2µm X 10 nm
TMV, 300 nm X 18 nm
PVY, 750 nm X 11 nm
LNYV, 220 nm X 80 nm
TSWV, 80 nm
CWTV, 70 nm
CaMV, 50 nm
CPMV, 28 nm



Encapsidated plant viruses are too large to move through plasmodesmata

Cell to Cell:

At least 3 mechanisms of cell-cell movement

a. Mechanism used by Tobamoviruses (TMV, *Tobacco mosaic virus*)

b. Mechanism used by Comoviruses, Nepoviruses, Caulimoviruses, Tospoviruses

c. Mechanism used by Begomoviruses

In all cases viruses produce 1 or more proteins that allows cell-to-cell movement (Movement protein - MP)

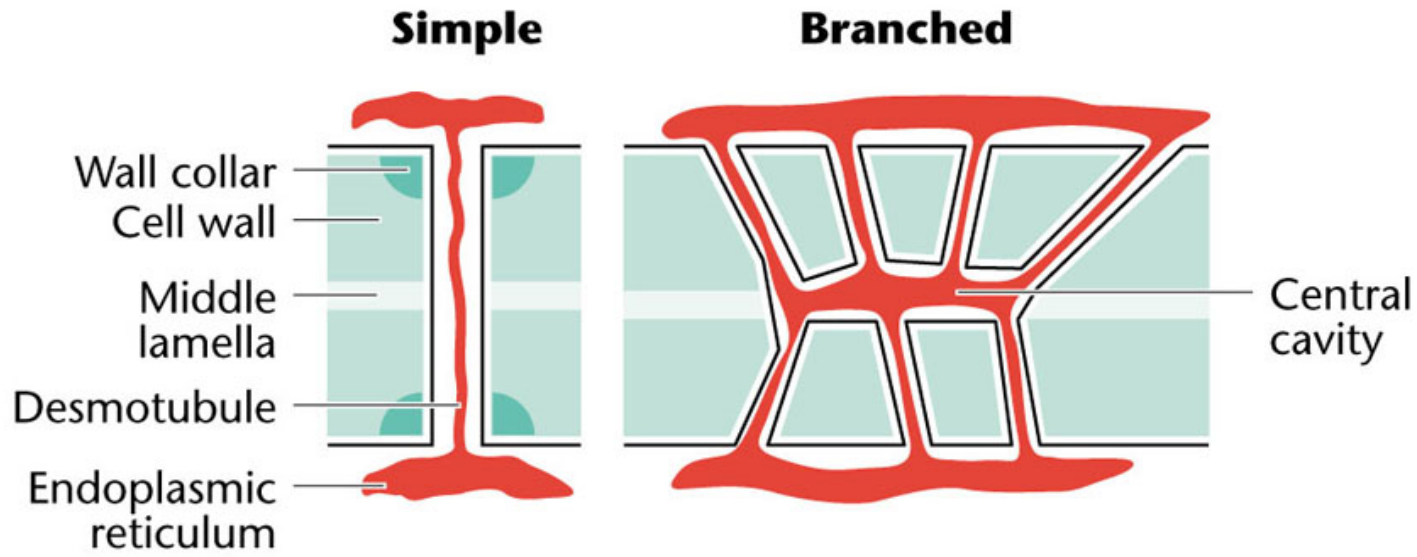


Figure 3 Comparison of simple and branched forms of plasmodesmata. In branched plasmodesmata, several adjacent plasmodesmal canals converge to form an enlarged central cavity.

a. Mechanism used by tobamoviruses

- TMV MP binds and elongates single-stranded nucleic acids (RNA, DNA).

- TMV MP increases plasmodesmatal size exclusion limit from 0.7 kDa to approx. 20 kDa.

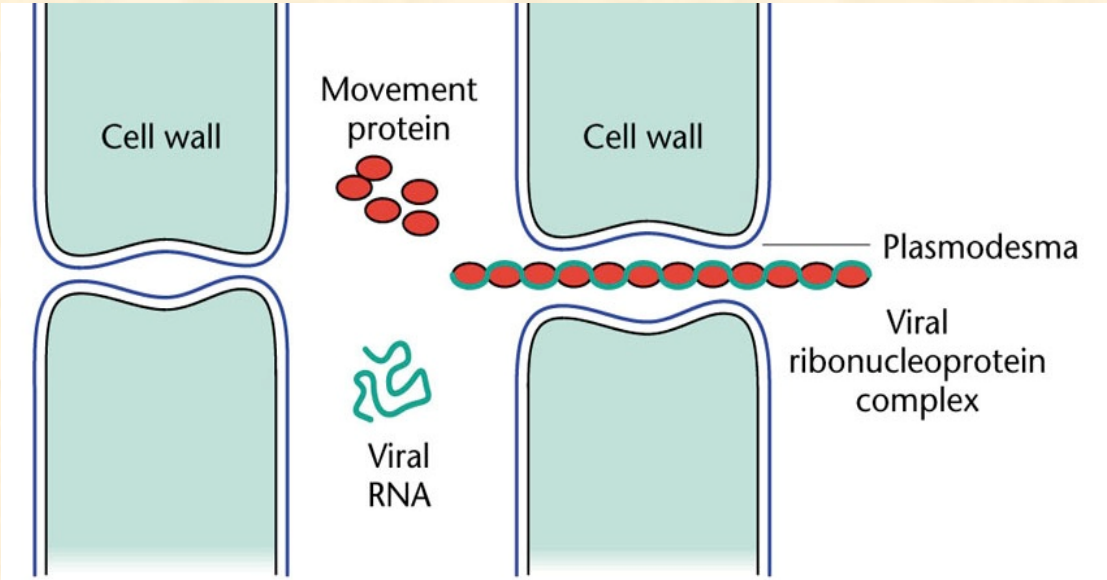
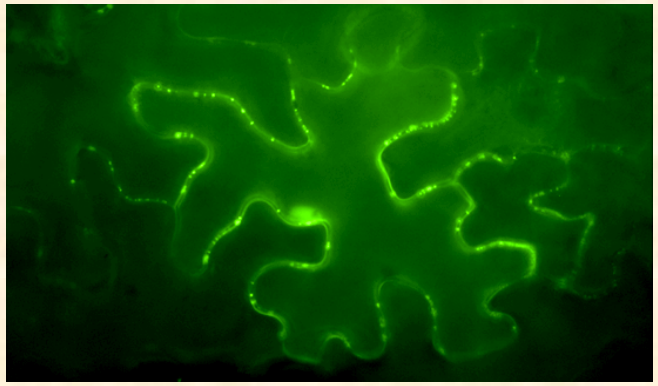


Figure 4 Diagrammatic representation of the movement of the *tobacco mosaic virus* (TMV) genome through plasmodesmata. The viral RNA is trafficked through the 'gated' plasmodesmal pore, together with the viral movement protein, as a linear ribonucleoprotein complex.

TMV movement protein:

- In TMV infected plants, and in 30k transgenic plants, the MP was localized to the plasmodesmata.

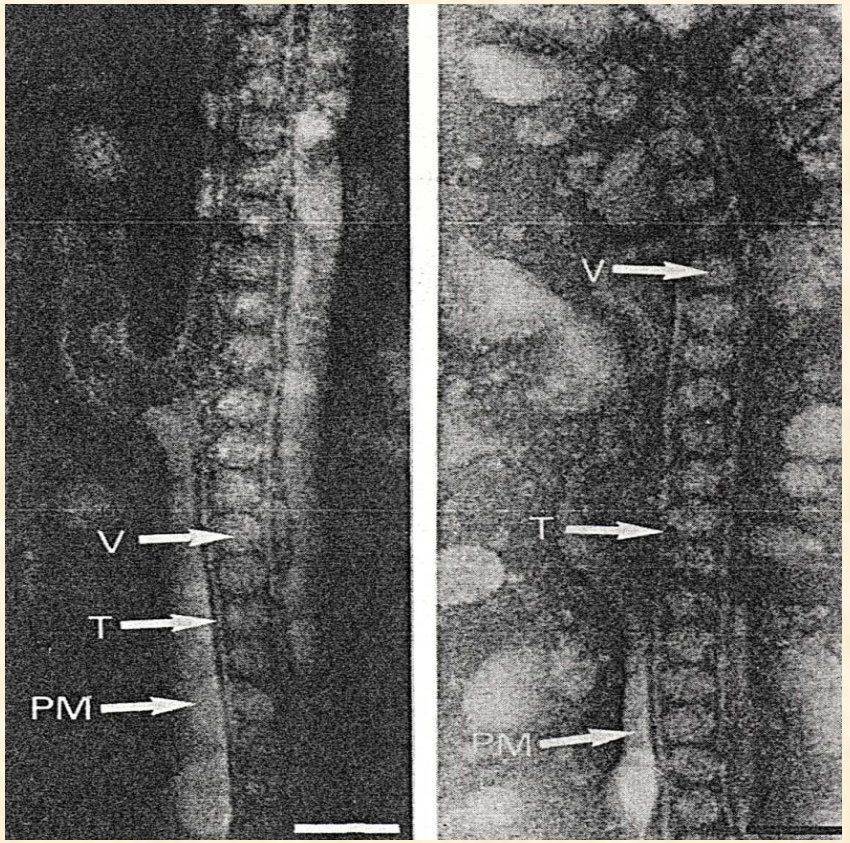


Transient expression of TMV MP:GFP after 24 h

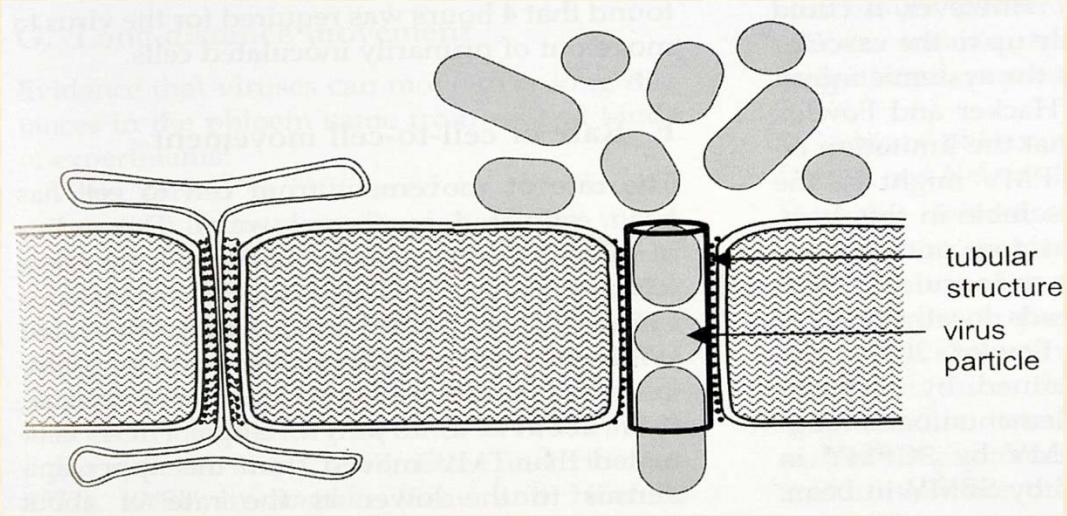
- The MP is phosphorylated by a cell-wall associated protein kinase. Regulation of plasmodesmatal transport by the MP is dependent on phosphorylation.
- The MP associates with cytoskeletal structures and with cortical ER.
- The coat protein is not required for cell-to-cell movement of TMV

b. Mechanism used by comoviruses CPMV (*Cowpea mosaic virus*)

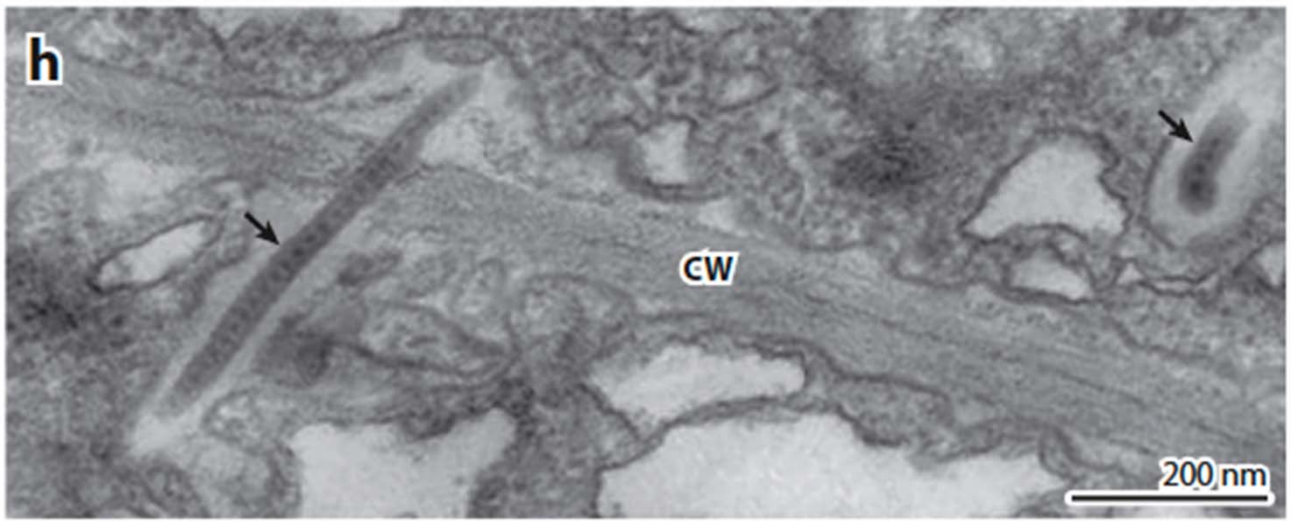
- CPMV MP does not bind nucleic acids
- The viral CP is essential for cell-to-cell movement



- A short MP tubular structure forms in the plasmodesmata pore allowing CPMV virions to move from cell-to-cell.



2. Mechanism used by nepoviruses TRSV (*Tobacco ringspot virus*)

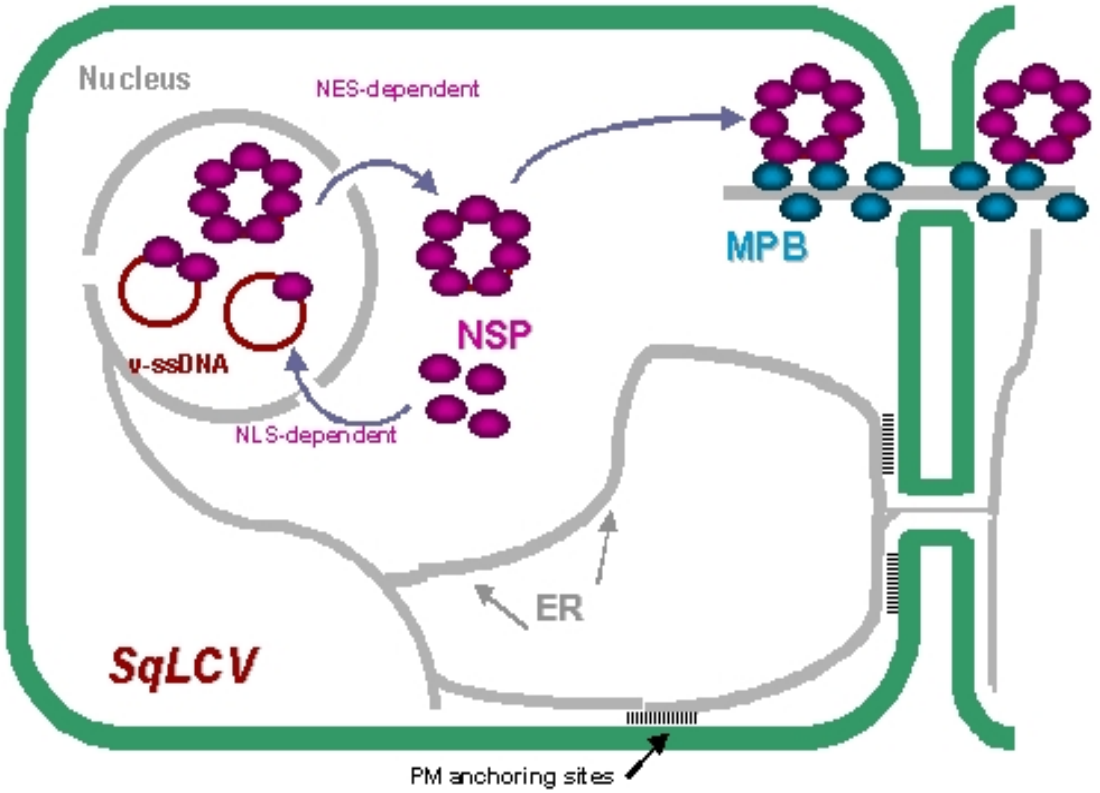


Laliberte et al., 2010. Annu. Rev. Phytopathol. 48:69-91.

- Tubular structures containing virus-like particles in *Tomato ringspot virus* (ToRSV)-infected *Nicotiana clevelandii* plant.
- Tubular structures are composed of TRSV movement protein
- Tubular structures will form in the absence of TRSV coat protein

c. Mechanism used by begomoviruses

- Nuclear shuttle protein (NSP) and MP are both required for cell-to-cell movement.
- Both NSP and MP bind single-stranded nucleic acids.
- Coat protein is not required



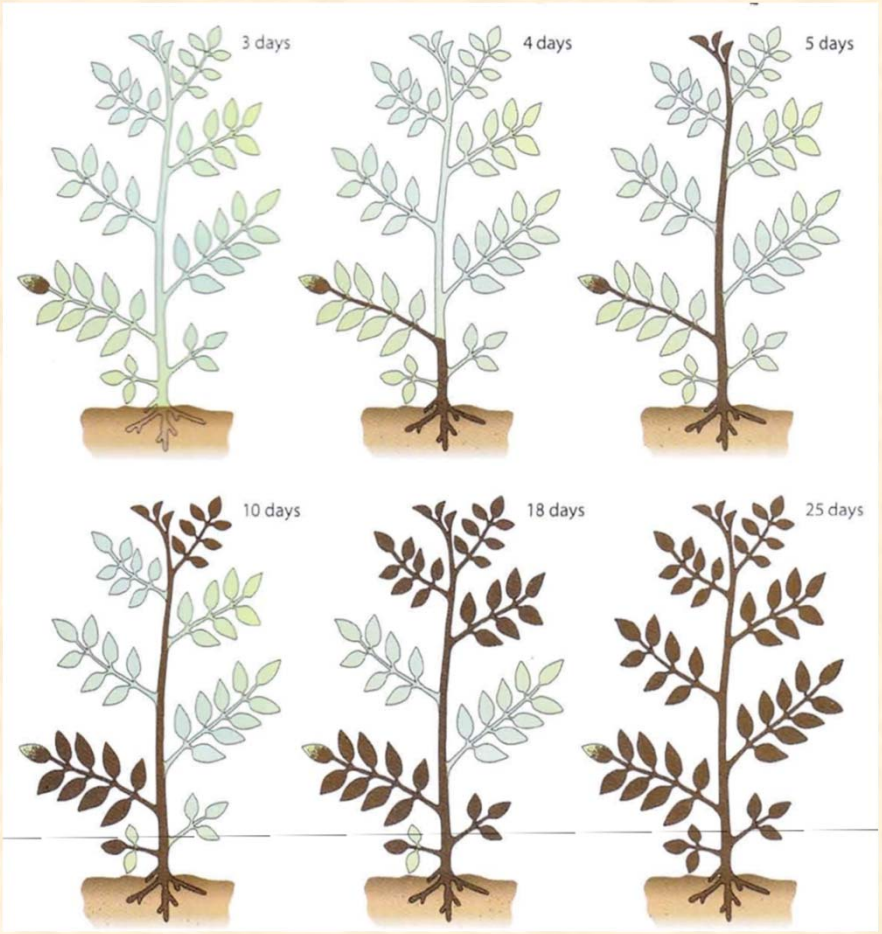
From Lazarowitz and Beachy Plant Cell 11:535 (1999)

Cell to cell movement:

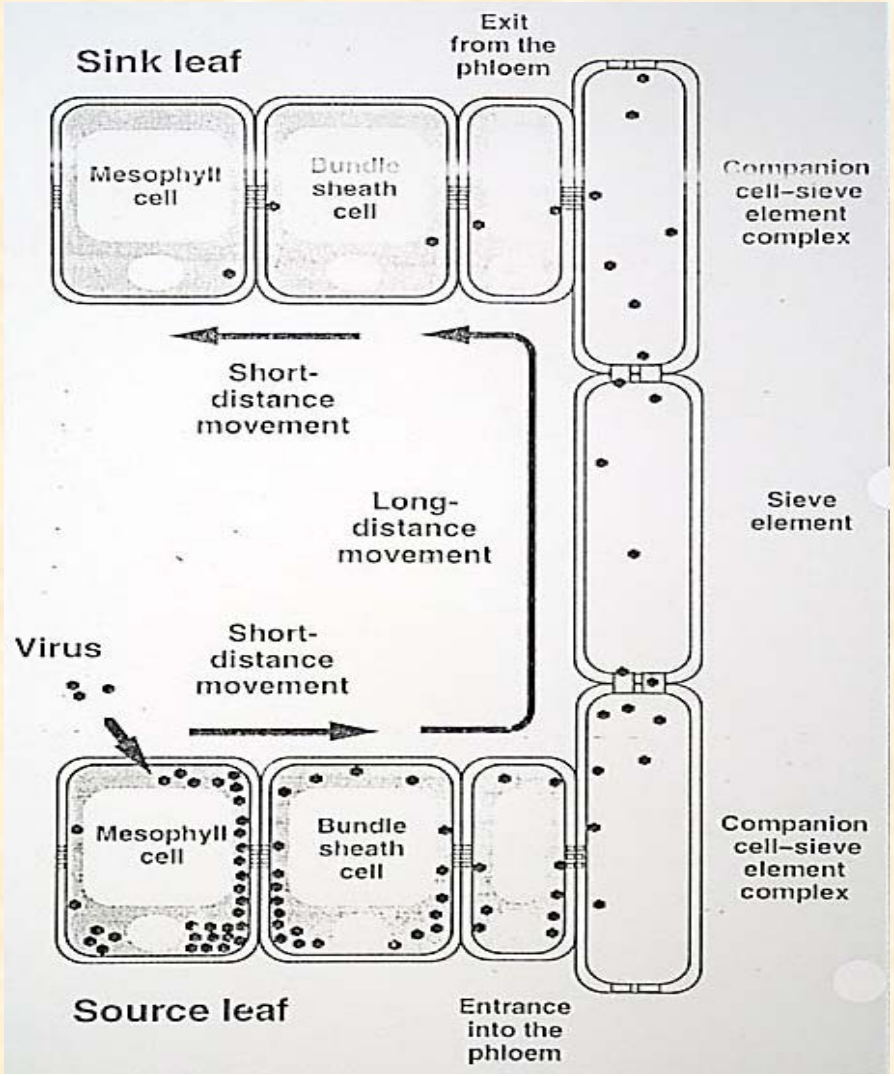
- **Cell-to-cell movement often occurs in local lesions**

- **Cell-to-cell movement in local lesions can be followed by systemic invasion**

2. Systemic (Long distance) Movement



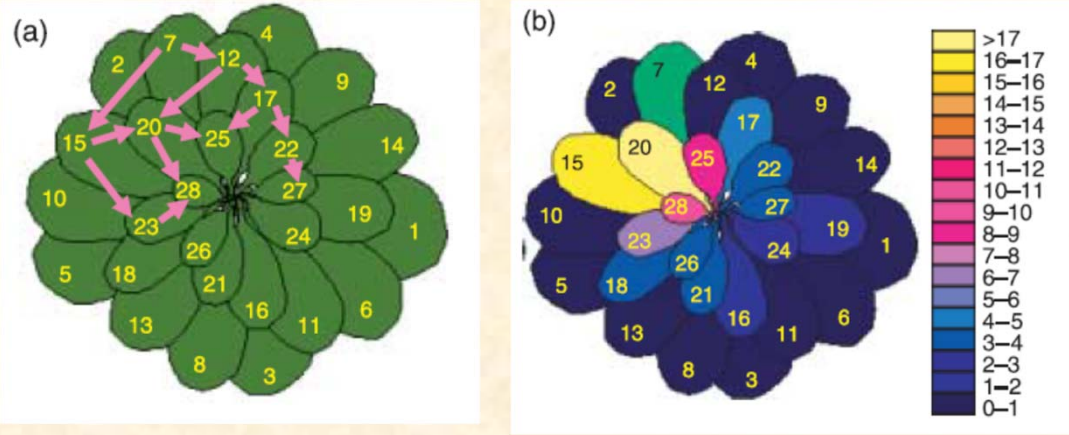
- Viral long distance movement is from source to sink leaves
- The process is very fast once it reaches the phloem
- Viruses can move as virions or as complexes of proteins and viral genomes (RNA or DNA)



An important characteristic of long-distance translocation is that not all sinks are equally supplied by source leaves and that source leaves preferentially serve sinks with a direct vascular connection – referred to as an **orthostichy** (a vertical row of leaves arranged one directly above another).

If you know the orthostichy of a plant, then the movement of virus within the plant is relatively predictable.

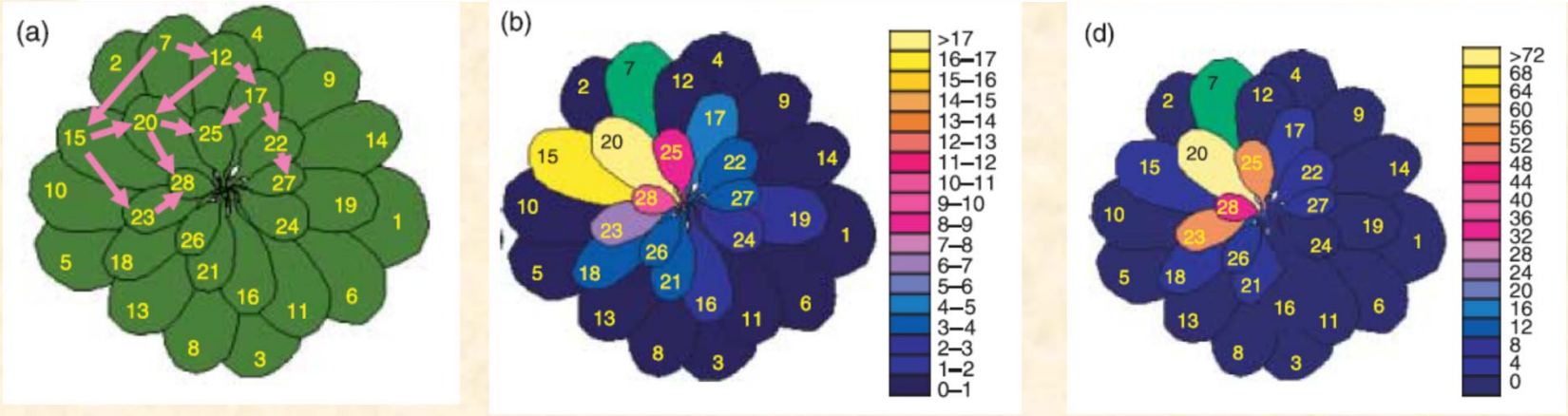
Patterns of movement of photo-assimilates and *Cauliflower mosaic virus* (CaMV) in *Arabidopsis* rosettes



(a) Diagram of a 28-leaf *Arabidopsis* rosette, with leaves numbered in the order in which they emerged. Arrows indicate direct vascular connections.

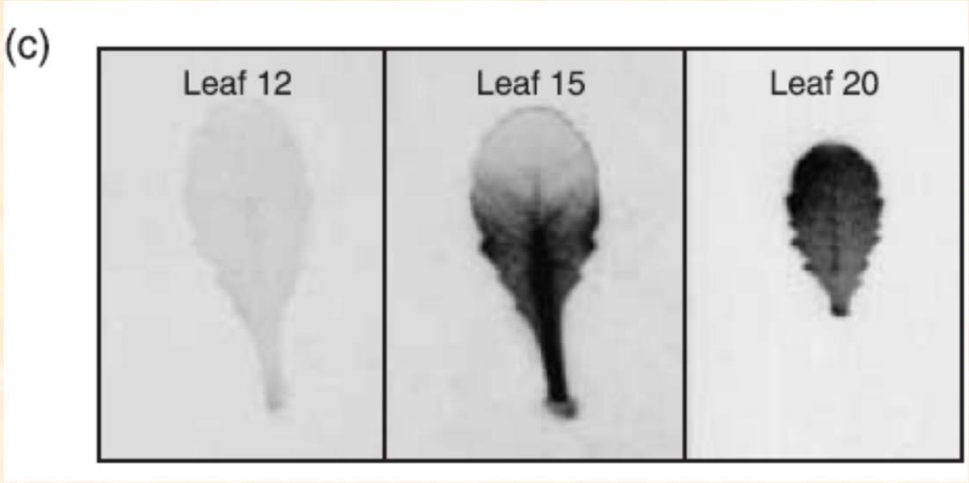
(b) Patterns of sugar translocation, determined by measuring the accumulation of 14C 5 d after applying 14C-labelled sucrose to leaf 7. Values for individual leaves are color-coded (see color bar on right) according to the percentage of the total 14C signal. Leaf no. 7 has been colored green to highlight the initial site of application.

Patterns of movement of photo-assimilates and *Cauliflower mosaic virus* (CaMV) in *Arabidopsis* rosettes

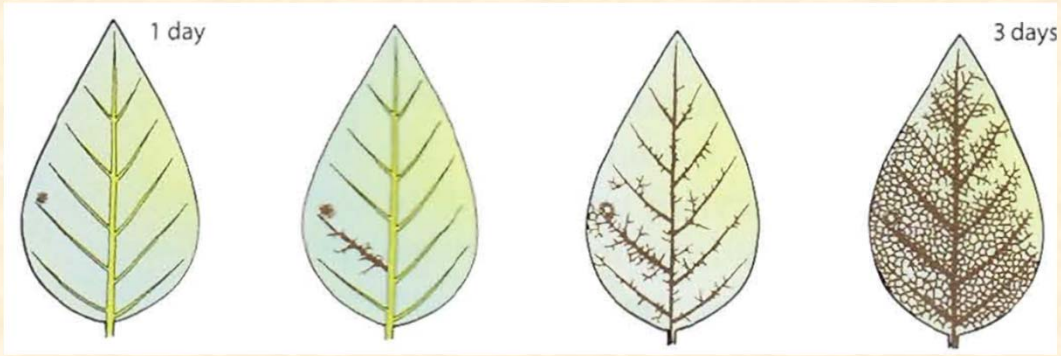


(d) Patterns of symptom appearance of CaMV at 31 dpi. Leaves were given a weighted score depending on the order in which symptoms appeared, with the first leaf in which symptoms (vein chlorosis) appeared scoring 4, the second scoring 3, the third scoring 2 and the fourth scoring 1. The diagram shows the aggregate score for each leaf from 27 plants that developed symptoms. Leaves have been color-coded depending on their aggregate score according to the color bar on the right. The inoculated leaf (no. 7) always developed local lesions and is colored green.

Roberts et al *New Phytologist* (2007) **175**: 707–717



(c) Representative autoradiograph images of leaves 12, 15 and 20, showing the radioactivity in each leaf, derived from the uptake of [14C]-sucrose from I-7. Leaves were harvested 5 d after application.



Movement of TMV within a leaf from point of inoculation, shows similar pattern of spread as uptake of sucrose, suggesting similar routes of movement

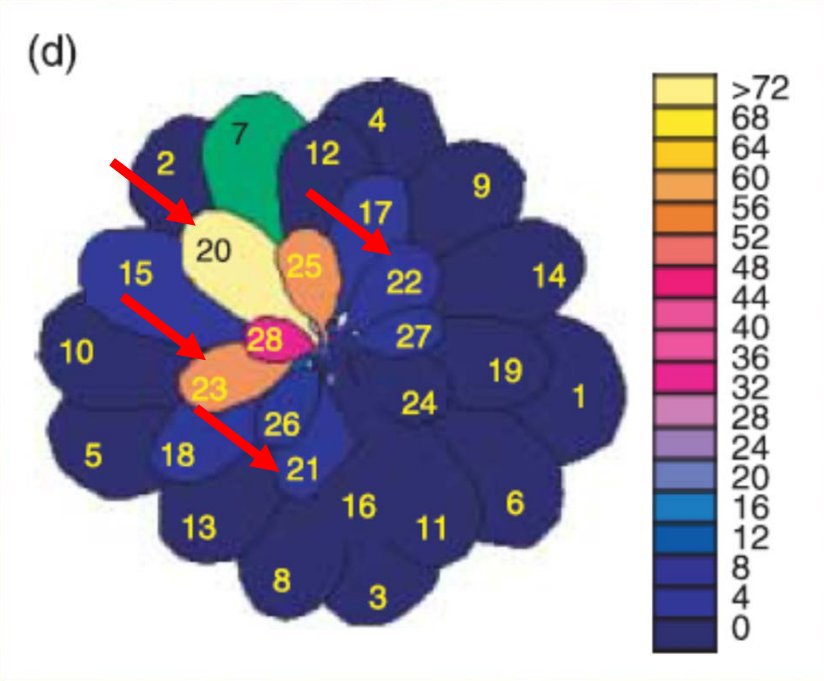
Host factors known to be involved in long distance movement of viruses:

- Pectin methylesterase (PME) purified from tobacco leaves interacts with the MP of TMV and that interaction is required for TMV cell-to-cell movement. Suppression of PME expression in tobacco led to delayed systemic movement of TMV.

- Elicitor responsive protein (IP-L protein). Repression of IP-expression led to a delay in virus accumulation in non-inoculated leaves which suggests that a high expression level of IP-L is important for efficient systemic infection of *Tomato mosaic virus*

- ORF3 protein of *Groundnut rosette virus* (GRV, *Tombusviridae, Umbravirus*), interacts with fibrillarin to form a ringlike structure of ORF3-fibrillarin complex seen both in vitro and in GRV infected plants which is also infectious. it is postulated that binding with ORF3 alters the structure of fibrillarin to facilitate viral movement.

3. Virus movement and its relationship to distribution of viruses within a plant



Amount of virus in a leaf can vary greatly from leaf to leaf

Compare:

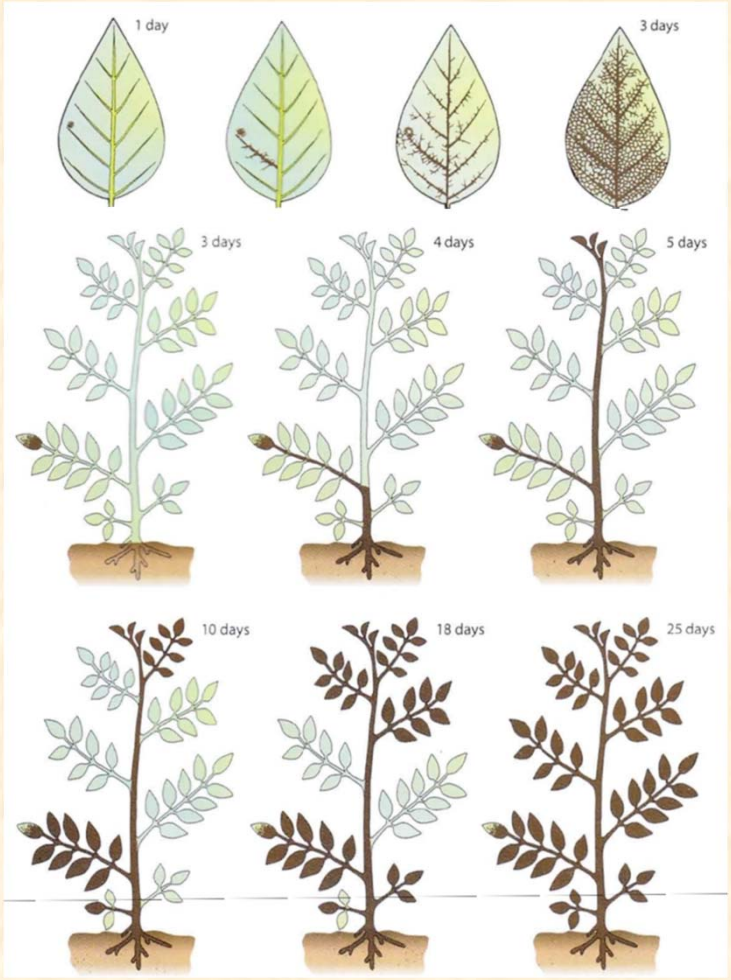
leaf 20 with 21 then 22 then 23

Illustration showing the spread of TMV in a tomato plant

Spread within a leaf from the site of inoculation

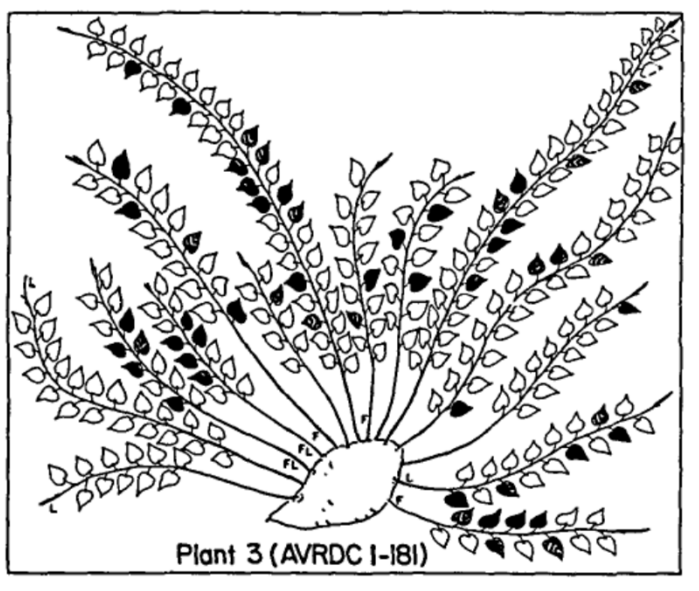
Spread from the inoculated leaf to the rest of the plant

*Virus can only be found in leaves that form after infection. Those formed before inoculation will rarely have virus-infected cells or detectable virus protein or nucleic acid.



From: Agrios, G. N. Plant Pathology, 4e, Academic Press

Example of very uneven distribution of two potyviruses (*Feathery mottle virus, Sweet potato latent virus*) in sweet potato plants (based on detection of the coat protein by ELISA)



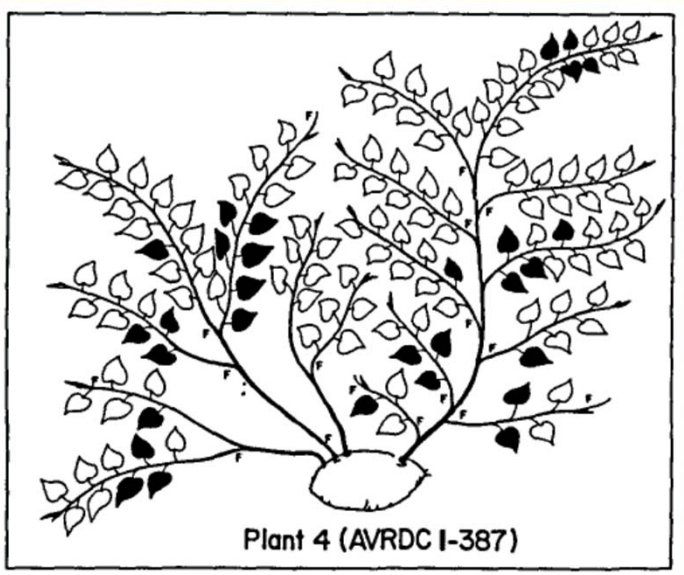


Figure 2. Diagrammatic presentation of distribution of FMV and SPLV in leaves, stems and branches of plant 3 of a sweet potato plant of line AVRDC I-181 and distribution of FMV in plant 4 of a sweet potato line AVRDC I-387.

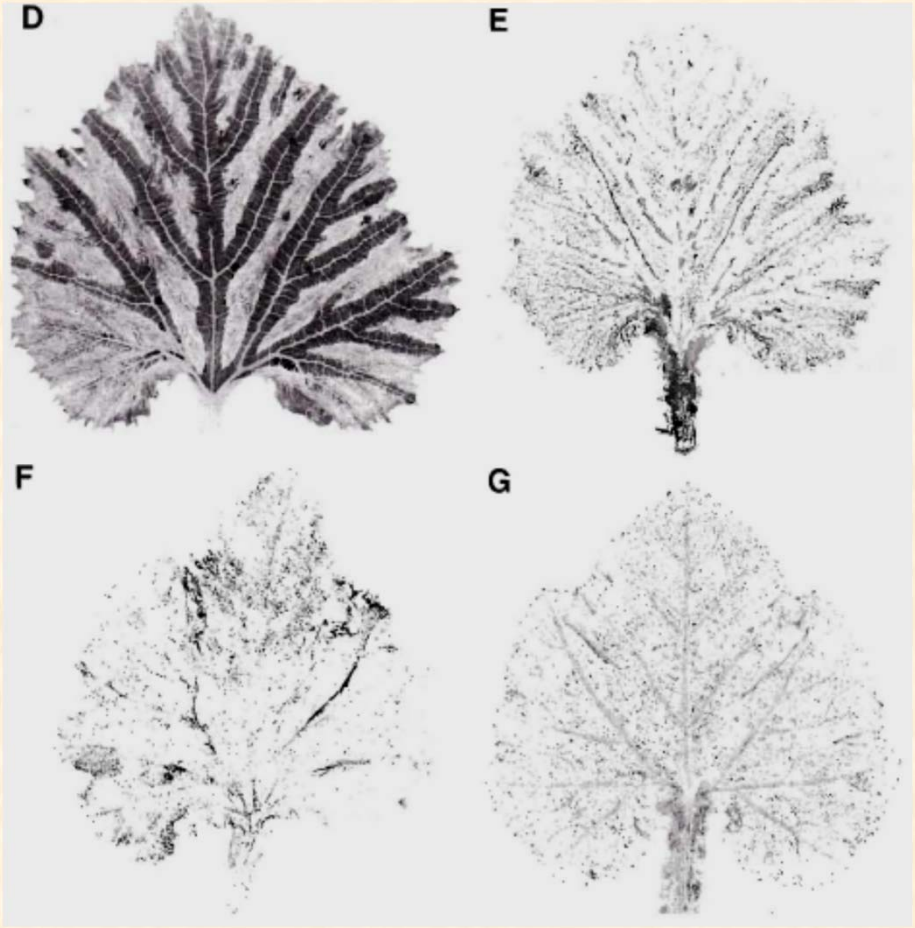
Virus spread and distribution within a plant:

- Varies among virus/host combinations
- Dynamic – distribution changes over time
- Impacts success of detection
- Important for transmission (mechanical, vector, seed)

4. Virus movement and its relationship to distribution of viruses within a leaf

Different viruses are distributed differently in leaves of different plants of the same host species/cultivar

Squash leaf infected with ZYMV



Immunoblot of leaf to the left, dark spots indicate presence of ZYMV coat protein

Immunoblot of leaf infected with CMV

Immunoblot of leaf infected with SqMV

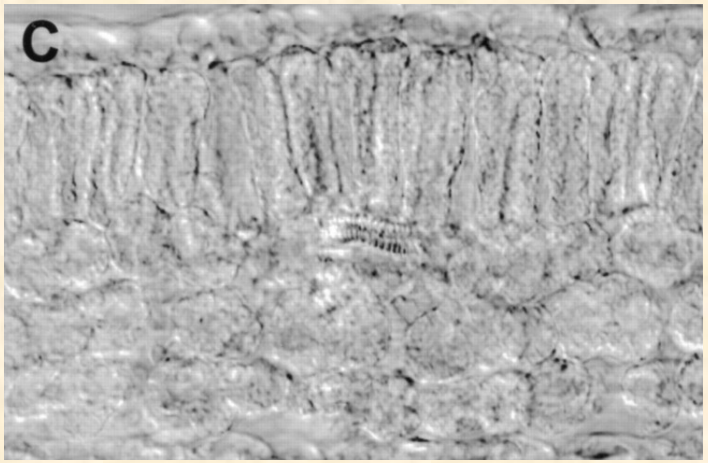
Virus spread and distribution within a leaf:

- Varies among virus/host combinations
- Dynamic – distribution changes over time
- Impacts success of detection
- Important for transmission (mechanical, vector, seed)

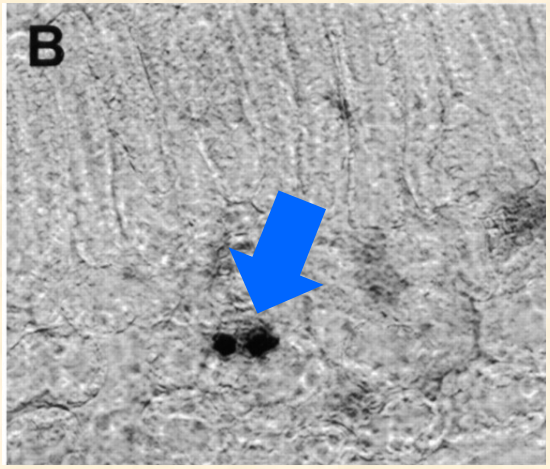
5. **Tissue tropism -** preference of viruses to replicate in tissues

- Viruses do not replicate in all plant tissues, most viruses replicate in specific tissues
- Viruses do not replicate in all the cells in a particular tissue type, and many uninfected cells can be found nearby infected ones
- So the distribution of virus-infected cells is almost never uniform
- Most plant viruses replicate in the cytoplasm of cells, some replicate in the nucleus, a few replicate in both locations

Some viruses replicate only in parenchyma cells that are in contact with the phloem (phloem-associated parenchyma)

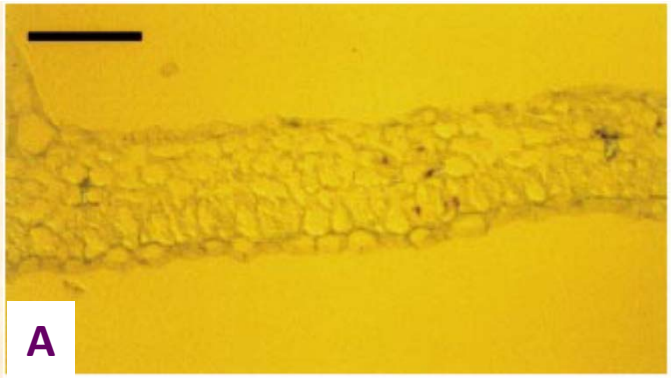


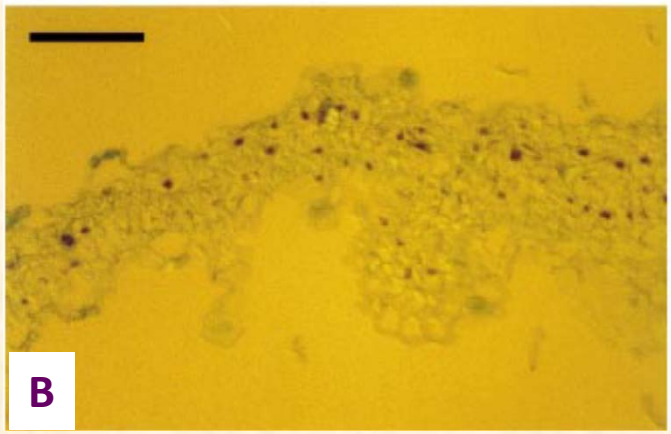
Healthy leaf



Leaf infected with a species of *Begomovirus* showing 2 virus infected nuclei in phloem and phloem-associated parenchyma

Marc R. Morra, and Ian T. D. Petty Plant Cell 2000;12:2259-2270



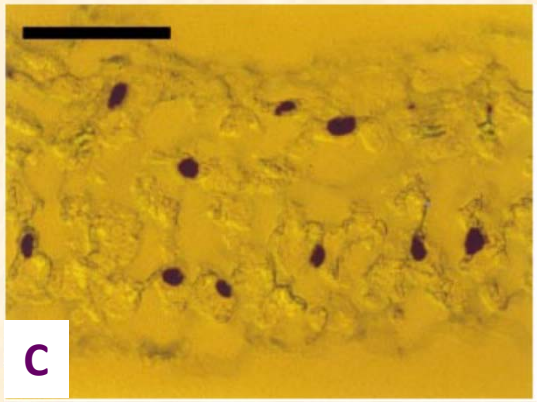


Distribution of infected cells changes over time

A. Early in infection, TGMV DNA is predominantly associated with phloem cells and, to a lesser extent, adjacent palisade parenchyma cells.

B. TGMV DNA is detectable in the nuclei of spongy and palisade parenchyma cells at a later stage of infection when the leaves exhibit severe leaf curl symptoms.

C. Close up of **B** showing infected nuclei

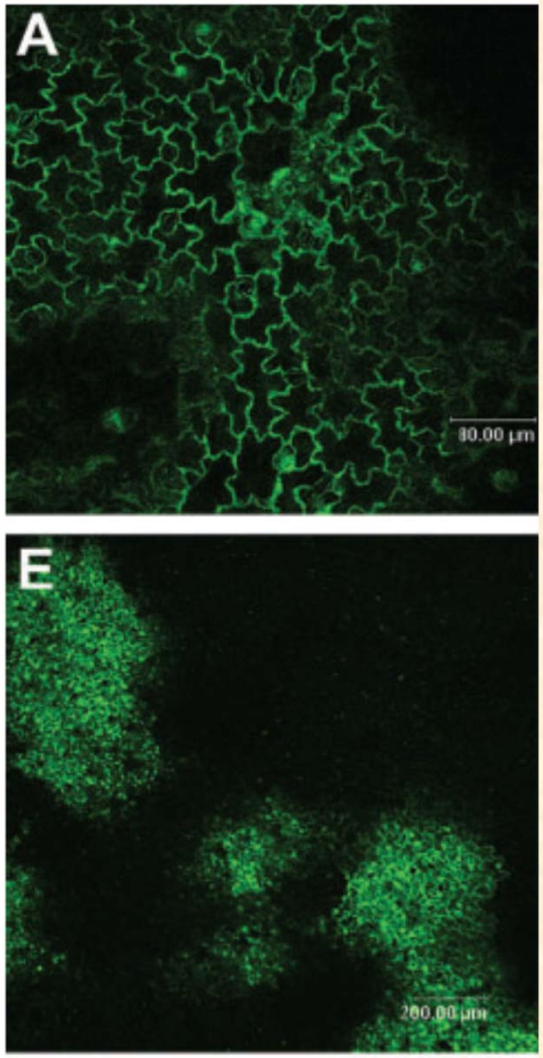


Reporters – allow us to see where viruses are located
1) Used as labels for viral protein IgG (immunodetection)

A. Leaf infected with a potyvirus showing infection of many of the epidermal cells of tobacco. (infected cells are outlined in green due to IgG labeled with **GFP**, dark areas are areas with uninfected cells)

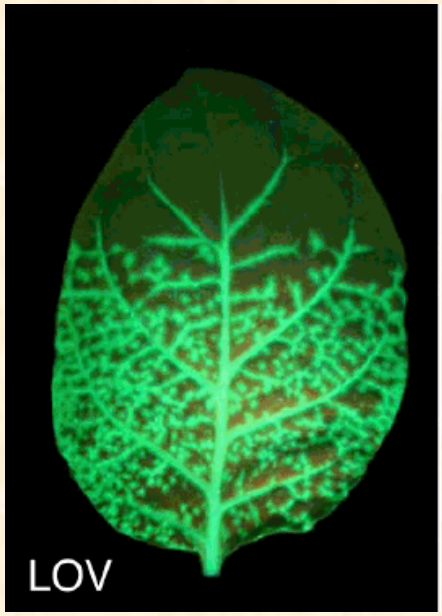
E. Leaf infected with a potyvirus showing infection of many of the mesophyll cells. Green indicates an infected cell - notice that infected cells occur in clusters and that many cells are not infected (dark areas)

GFP – green fluorescing protein from the jellyfish, *Aequorea victoria*

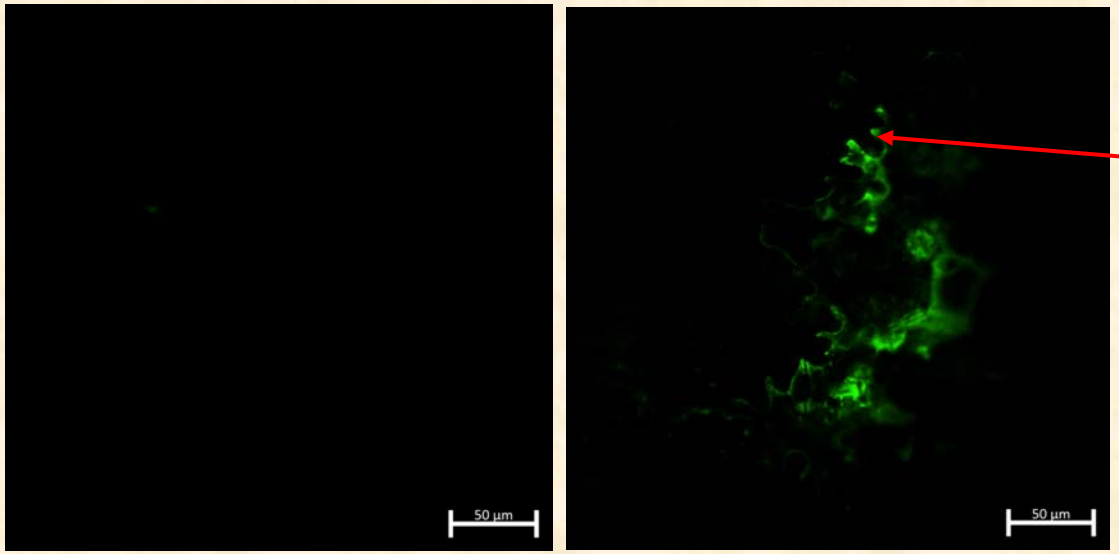


Reporters – allow us to see where viruses are located

2) OR fused to viral proteins which are expressed in plants with viral or other promoters



Tobacco mosaic virus (TMV) modified to express a CP-LOV hybrid protein



Left: healthy tomato leaf (control)
Right: ToMoV A CP-/GFP+ construct expressing GFP transmitted in tomato by a whitefly (viewed in a Confocal laser scanning microscope)

LOV – fluorescent reporter derived from a plant phototropin

Common fluorescent reporters:

LOV – fluorescent reporter derived from a plant phototropin
GFP – green fluorescing protein from the jellyfish, *Aequorea victoria*

N. benthamiana leaf infected with a begomovirus, PepGMV A CP-/eGFP+ construct showing expression of eGFP in epidermal cells – in nuclei and plasmalemma
(Viewed using a confocal laser scanning microscope)

Virus is present in the epidermis due to the method of inoculation (agro-infiltration)