

Stress and Plants

- biotic stress -

Responses to Plant Pathogens

- Plants must continuously defend themselves against attack from:
 - bacteria
 - viruses
 - fungi
 - invertebrates (+ some vertebrates)
 - other plants
- Because their immobility precludes escape, each plant possesses both a **preformed** and an **inducible** defense capacity
- In **wild plant populations**, most plants are **healthy most of the time**; if disease does occur, it is usually **restricted** to only **a few plants** and affects only a small amount of tissue
- Disease, the outcome of a successful infection, **rarely kills** a plant **in natural growth conditions**

Why do we study Plant Pathogens?

There are three main reasons:

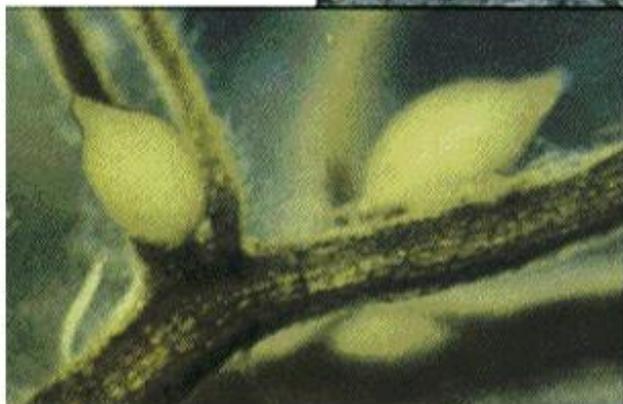
- (1) A detailed study of plant-microbe interactions should provide **sustainable practical solutions** for the control of plant disease in agricultural crops. Indeed, growing **monocultures** of **genetically uniform** crop species can lead to severe **outbreaks** of disease (**epidemics**)

(A) *Sugar beet nematode interaction*



*The central rows show severe damage from **Heterodera schachtii***

(B)



Mature female nematode bodies filled with eggs, attached to the sugar beet roots

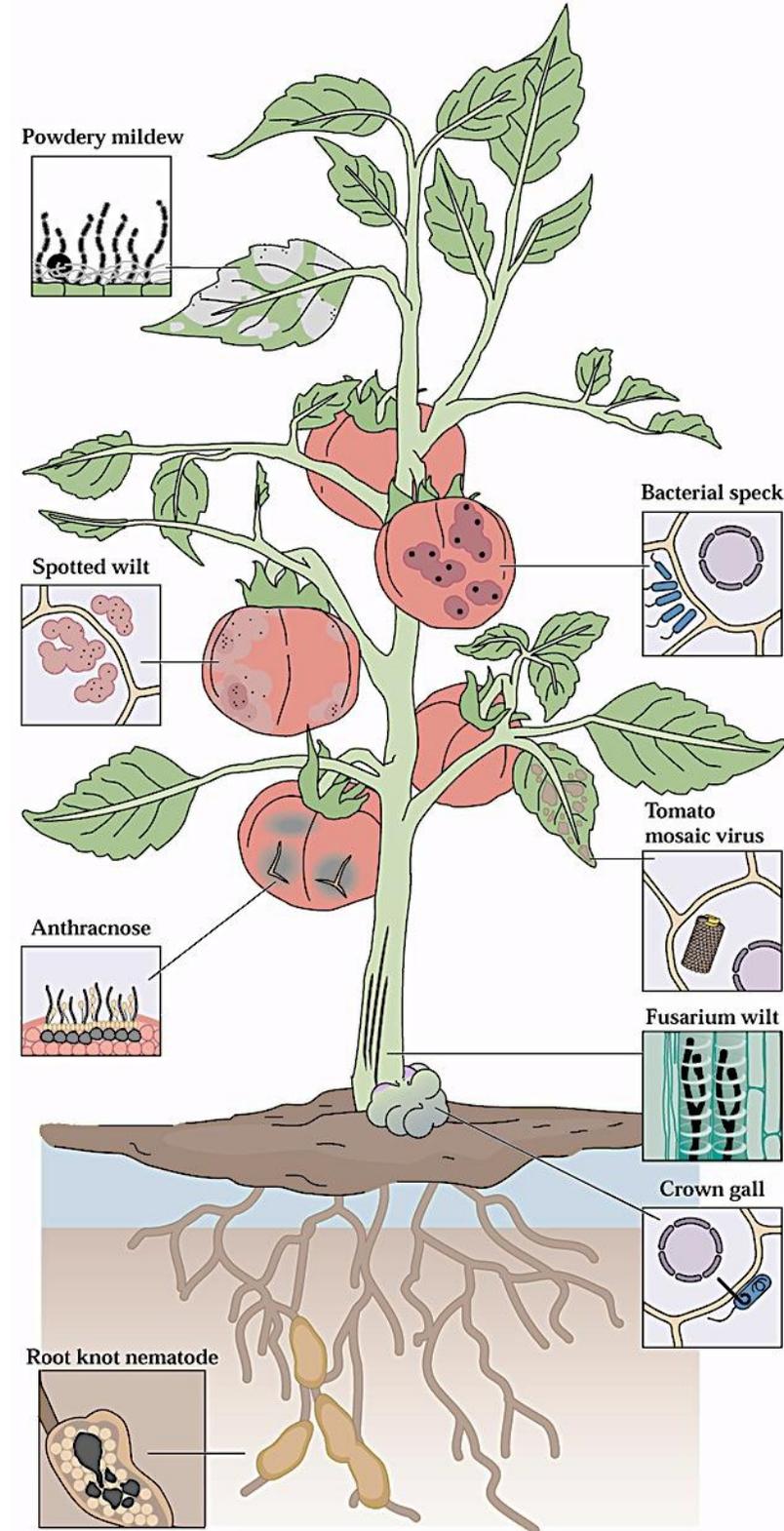
- (2) such studies could help **elucidate** the **signaling** mechanisms by which plant cells **cope** with a stress situation (different answers/ signaling pathways for different stresses?)
- (3) study of plant-pathogen interactions can lead us to understand how organisms from **different kingdoms** communicate with one another

Cladosporium fulvum (leaf mold fungus) sporulating on tomato leaves



Plant Pathogens

- A plant pathogen is defined as an organism that, to complete a part or all of its life cycle, **grows inside the plant** and, in doing so, has a **detrimental effect** on the plant
- **Roots** and **shoots** of all plants come into contact with plant pathogens. Each pathogen has evolved a specific way to invade plants:
 - **mechanical** pressure surface layers
 - **enzymatic** attack
 - natural **openings** (stomata, lenticells)
 - use of previously **wounded** tissue
 - ...
- Most microbes attack only **a specific part** of the plant and produce characteristic disease symptoms, such as a **mosaic**, **necrosis**, **spotting**, **wilting**, or **enlarged** roots.
- Tomato plants, for instance, are attacked by more than **100** different pathogenic microorganisms



Pathogen attack strategies

- Once inside the plant, one of three main attack strategies is deployed to utilize the host plant as a substrate:
 - **necrotrophy**, in which the plant cells are killed
 - **biotrophy**, in which the plant cells remain alive;
 - **hemibiotrophy**, in which the pathogen initially keeps cells alive but kills them at later stages of the infection.
- The success of certain widespread plant pathogens can be attributed to several main factors:
 - rapid and **high** rate of **reproduction** during the main **growing season** for plants
 - efficient **dispersal** mechanism by wind, water or **vector** organisms such as insects
 - **different** types of reproduction (often sexual) toward the end of each plant growing season to produce a second type of structure (spore, propagule) allowing long-term **survival**
 - high capacity to generate **genetic diversity**
 - **monoculture** of crop plants *VERSUS* **well-adapted** pathogen **genotypes**

Fungal Plant Pathogens use a wide Range of Pathogenesis Strategies

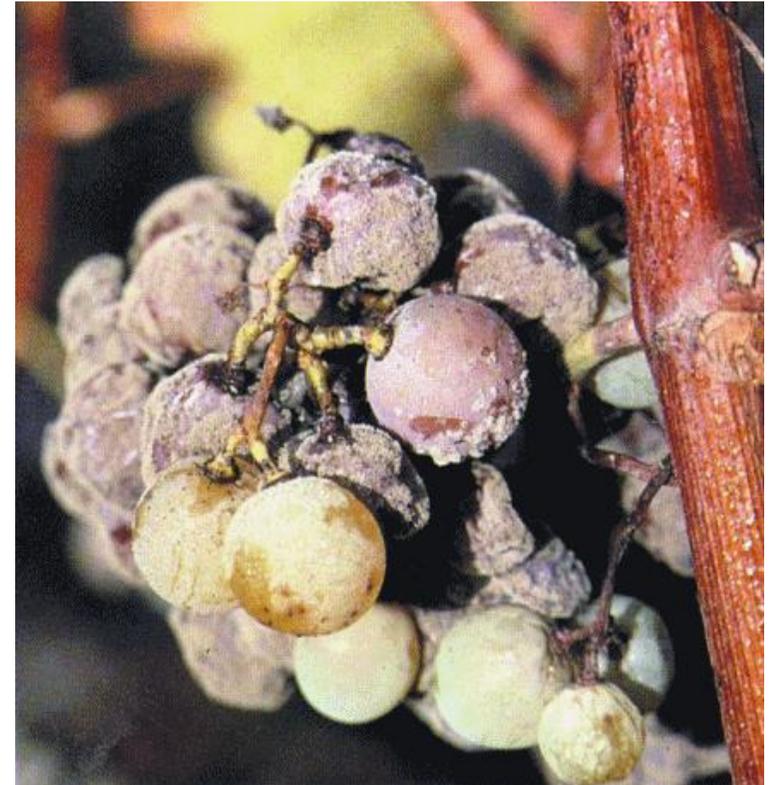
- Less than 2% of the approximately 100.000 known fungal species are able to colonise plants and cause disease
- necrotrophic species that produce cell wall-degrading enzymes tend to attack a broad range of plant species

Botrytis cinerea, the gray mold fungus, sporulating on grapes. This necrotroph secretes large numbers of cell wall-degrading enzymes and thereby destroys plant tissue in advance of the colonizing hyphae.

Plants respond to the degradation of cell wall by mounting defense responses that include enzymes that, in turn degrade, fungal cell wall

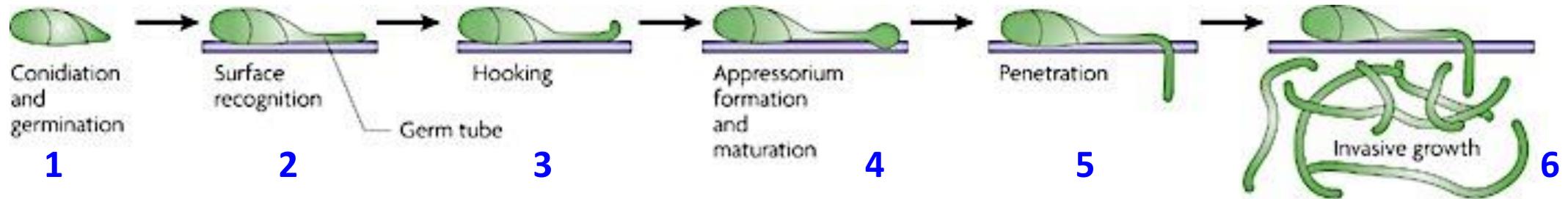


This process, if controlled, can be used to produce sweeter wines (« Pourriture Noble »)



- **biotrophic** fungi keep host cells alive and usually exhibit a **high degree of specialisation** for individual plant species

***Magnaporthe grisea*, agent of the rice blast disease**



Nature Reviews | Microbiology

1. In nature, *M. grisea* conidia (also called **spores**) are dispersed by **wind** or **rain** and deposited on the leaves of susceptible plants

2. When the deposited conidia are in an environment with a **ready supply of water** (for example, a dew drop), they germinate to produce **elongated cells named germ tubes**, that are the **precursors to hyphae**

3. If the germ tube senses contact with an appropriate '**inductive**' surface, it **ceases growth** and hooks itself

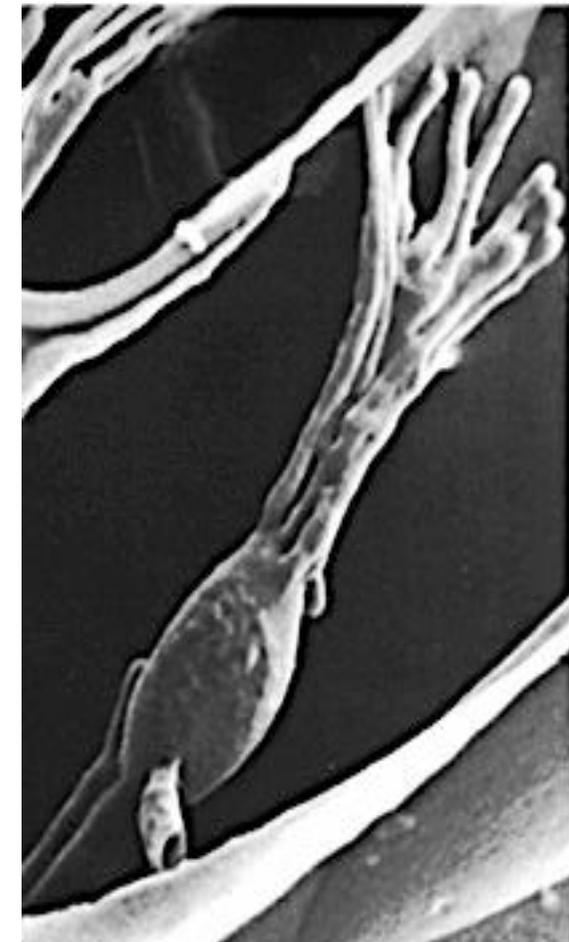
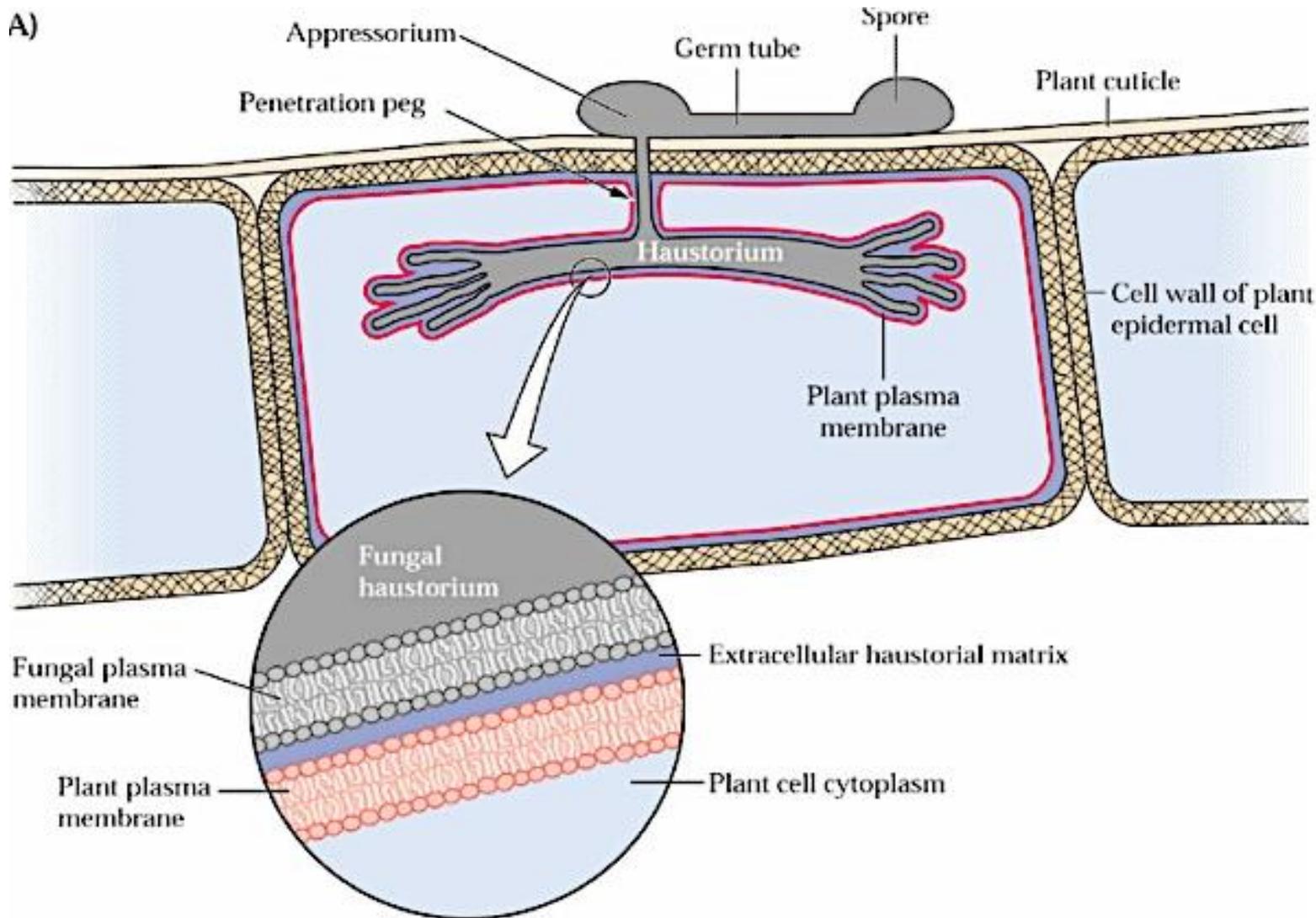
4. A specialised structure, the **appressorium**, acquires water from the dew drop by accumulating **glycerol** and other compatible solutes. Eventually, the appressorial glycerol concentration exceeds **3 M** and, as a result, **extremely high turgor pressure** is generated

5. Using this high turgor pressure, the penetration plug and secondary germ tube produced by the appressorium exert sufficient force to **breach the cuticle** of the plant or, in vitro, to push through inert non-biological materials such as **Teflon**.

6. Once within the epidermal cells of the plant, '**infection hyphae**' grow intracellularly and spread from cell to cell, producing the characteristic **lesions** of rice blast.



- To utilize the living plant cells as a food substrate, biotrophic fungi, after penetration of the rigid cell wall form an **haustorium**, which causes **invagination** of the **plasma membrane**
- This specialised feeding structure **increases** the **surface contact** between the two organisms, thus **maximizing nutrient and water flow** to favour fungal growth



- **hemibiotrophic** fungi sequentially deploy a **biotrophic** and then a **necrotrophic** mode of nutrition
- the switch is usually triggered by **increasing nutritional demands** as fungal biomass increases



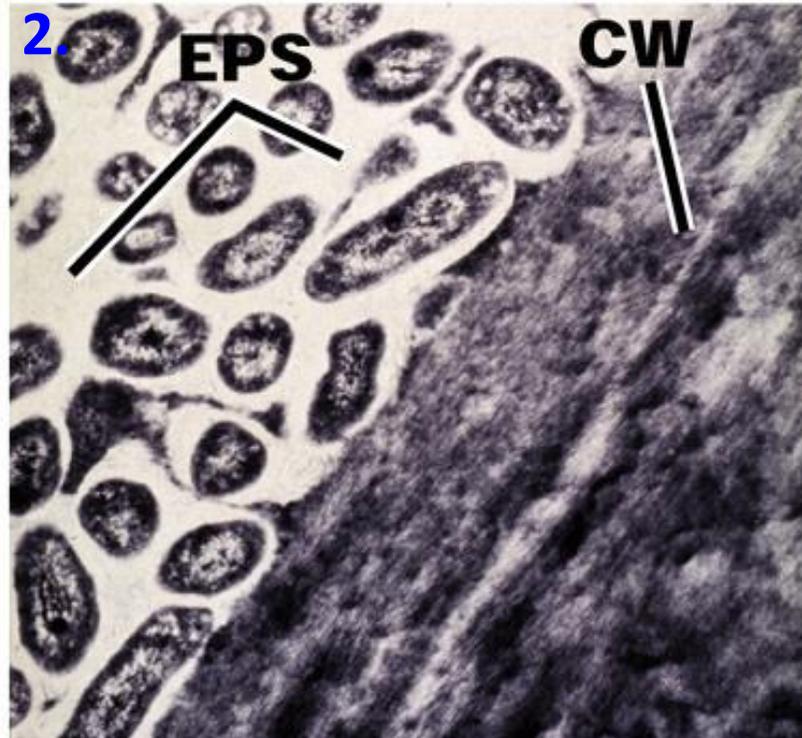
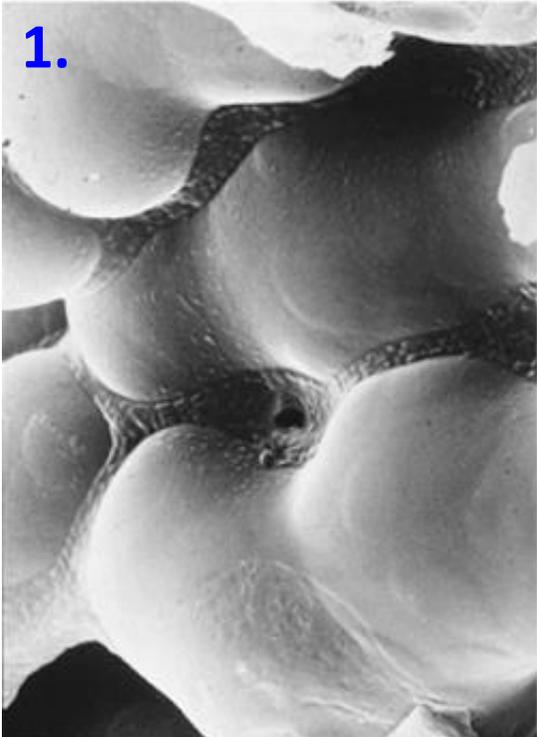
*For example, **Phytophthora infestans**, which causes late blight disease of potato, was responsible for the devastating blight disease epidemic in Ireland in 1846 and 1847, resulting in the Irish famine and emigration of more than one million people to the United States and other countries. Today this fungus still causes large losses in annual yields*

- The hemibiotrophic lifestyle of this pathogen facilitates its progress from leaf **infection** to **sporulation** in only **three days**.
- If **moist, cool conditions** prevail, the entire foliage of a potato field can be destroyed **within two weeks**

Bacterial Pathogens of Plants (phytobacteria)

- Phytopathogenic bacteria specialise in colonising the **apoplast** to cause spots, vascular wilts, and blights
- most are **Gram-negative** rod shaped bacteria from the genera *Pseudomonas*, *Xanthomonas*, and *Erwinia*
- Two features characterise bacteria-plant relationships:

First, during their parasitic life, most bacteria reside within the intercellular spaces of the various plant organs or in the **xylem**



1. *Xanthomonas campestris* bacteria colonising in the intercellular air spaces of a Brassica leaf

2. Ordinarily, the bacteria are surrounded by an extracellular polysaccharide material (EPS) and proliferate in close contact with the plant cell walls (CW)

Second, many cause considerable plant tissue damage by secreting either **toxins**, extracellular polysaccharides (**EPSs**), or **cell wall-degrading enzymes** at some stage during pathogenesis

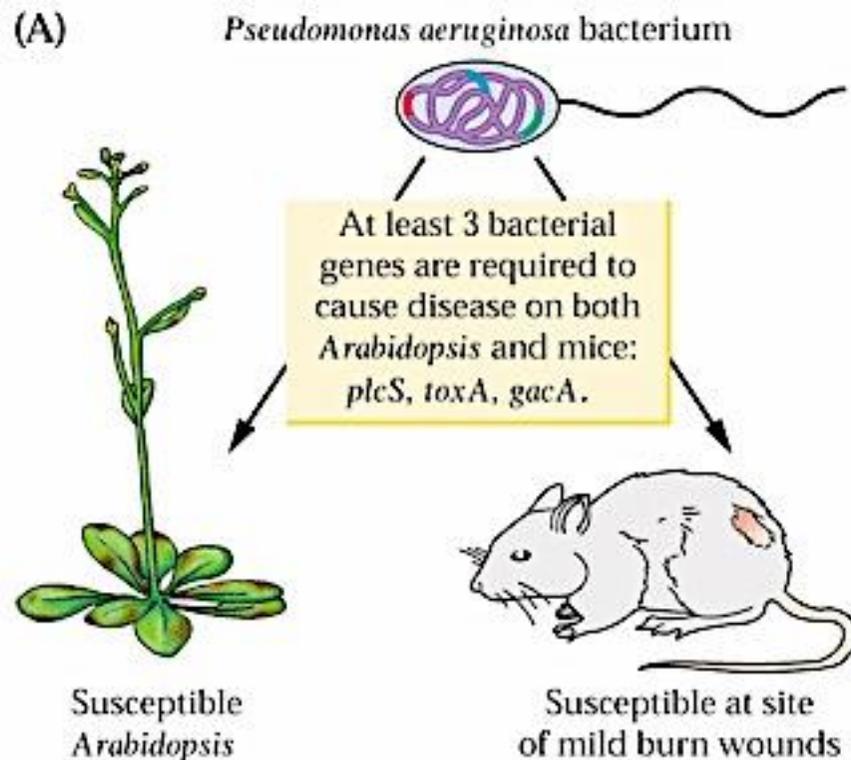
- the secreted EPSs, which entirely surround the growing bacterial colony, may aid bacterial virulence – for example, by saturating intercellular spaces with water or by blocking the xylem, producing **wilt symptoms**

- a common sign of the disease can be observed when cut stem sections are placed in **clear water**. It consists of a viscous white spontaneous **slime** streaming from the cut end of the stem. This stream represents the bacterial **ooze** exuding from the cut ends of **colonized vascular bundles**



- bacteria that deploy **pectic enzymes**, such as *Erwinia*, cleave plant cell wall polymers either by hydrolysis (polygalacturonases) or through beta-eliminations (pectate or pectin lyases)

- Several bacterial genes in the **hypersensitive response and pathogenicity cluster** (*hrp*), are **absolutely** required for bacterial pathogenesis
- many *hrp* gene sequences from plant bacteria are very similar to the genes required for pathogenesis in bacteria that infect animals.
- One known strain of *Pseudomonas aeruginosa* is capable of causing disease in both *Arabidopsis* and mice :



(B)

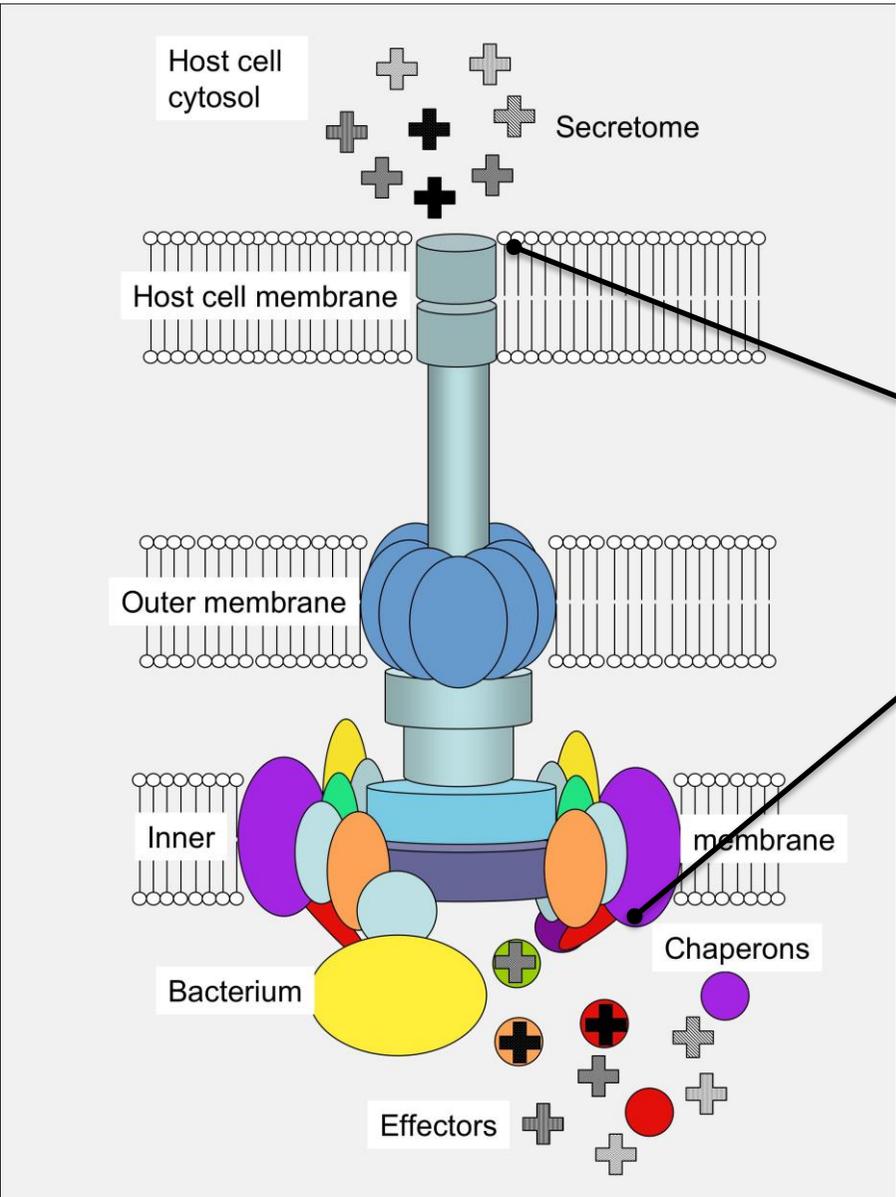
<i>P. aeruginosa</i> genotype	<i>Arabidopsis</i> (bacterial titer/cm ²)	Mouse (% lethality)
Wild type	1 × 10 ⁷ (full disease)	77
● <i>plcS</i> mutant	1 × 10 ⁵ (less severe)	40
● <i>toxA</i> mutant	1 × 10 ⁶ (less severe)	40
● <i>gacA</i> mutant	5 × 10 ³ (attenuated symptoms)	0

plcS encodes a **phospholipase S** that degrades phospholipids of eukaryotic, but not prokaryotic cells

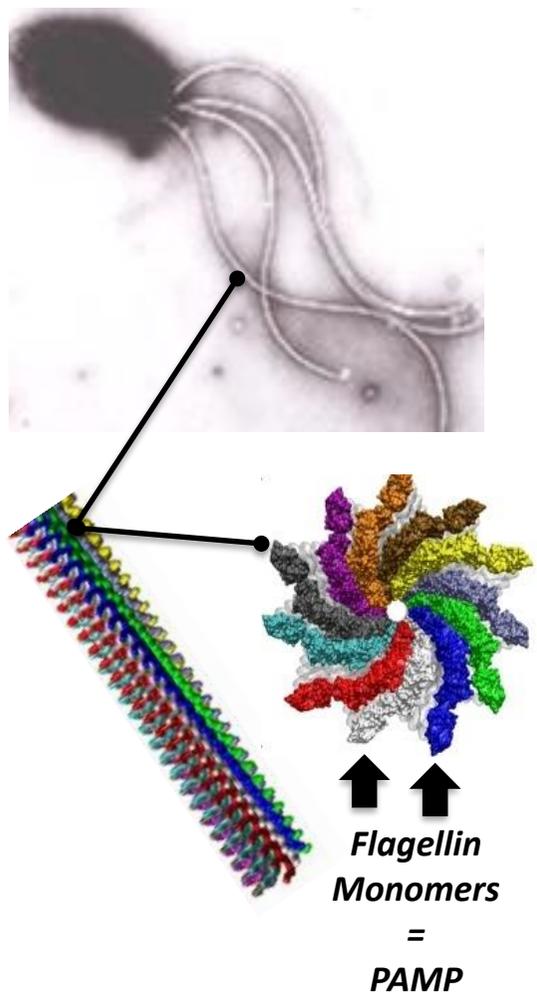
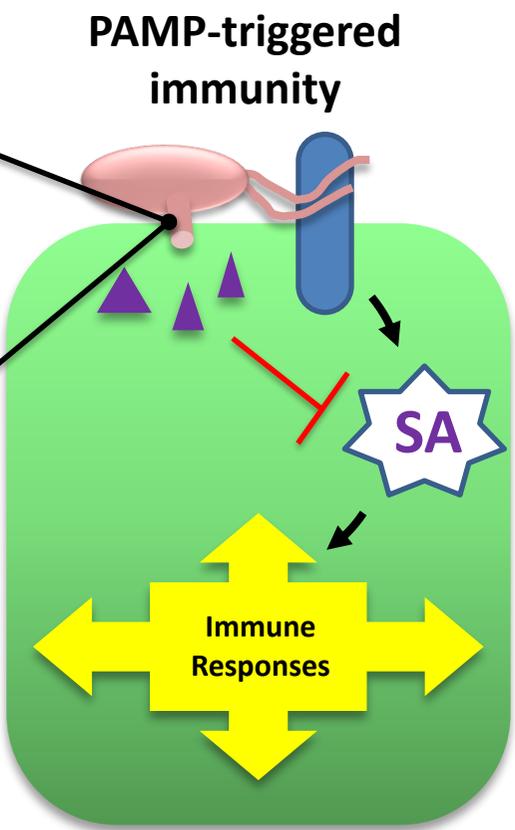
toxA encodes a **exotoxin A** that inhibits protein synthesis by **ribosylating** eukaryotic **Elongation Factor 2**

gacA encodes a **transcriptional regulator** of several *hrp* genes

- Some bacteria (e.g. *Pseudomonas*) use a **type-III secretion system** to deliver virulence factors into host cells



- The delivered material is called 'secretome' and is primarily aimed at suppressing **PAMP triggered immunity**



The particular case of Agrobacterium tumefaciens

- ethiological agent of the “**crown gall**” disease, characterized by the development of **tumors** on roots and lower part of the stems (the crown)

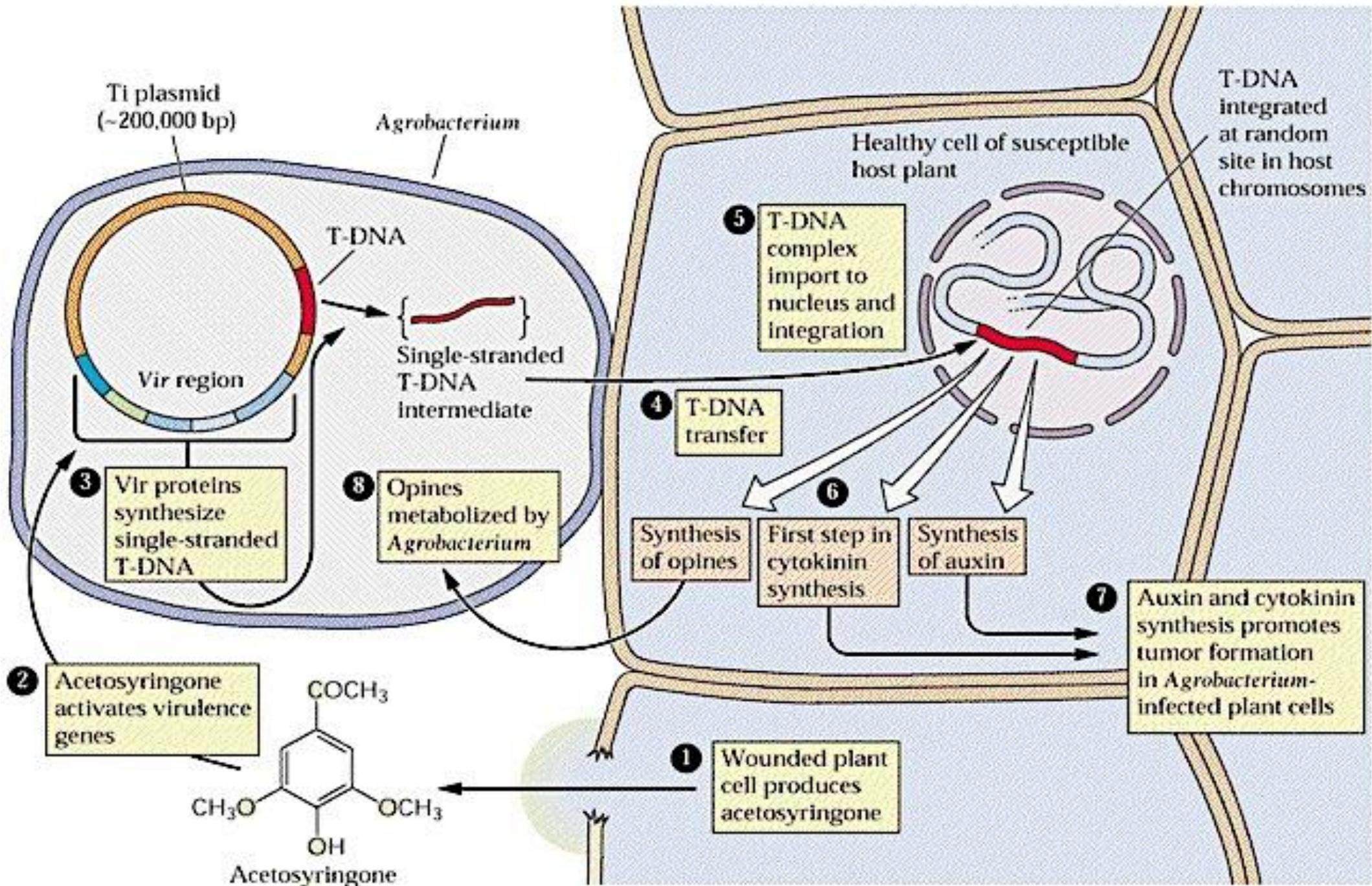


Agrobacterium tumefaciens
as they begin to infect a
carrot cell



Agrobacterium tumefaciens gall at the root of *Carya illinoensis*

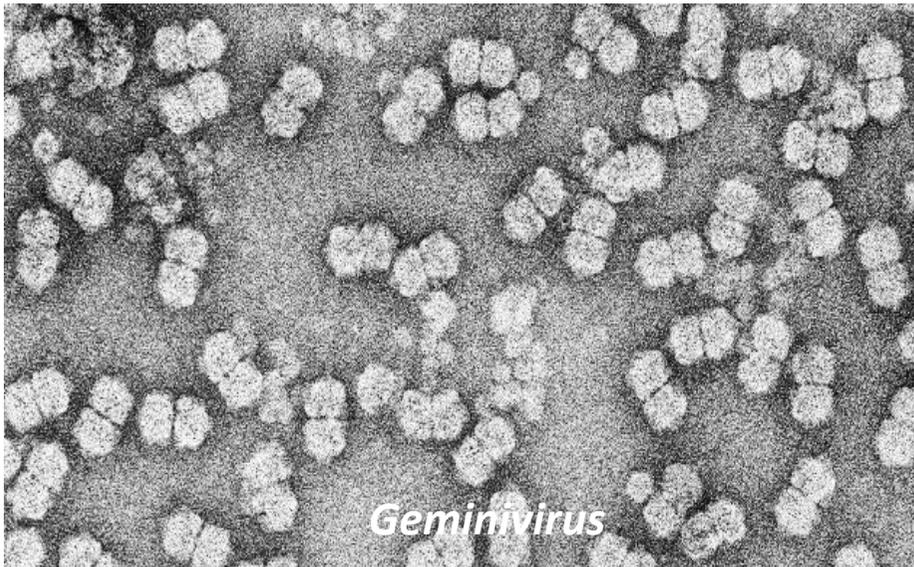
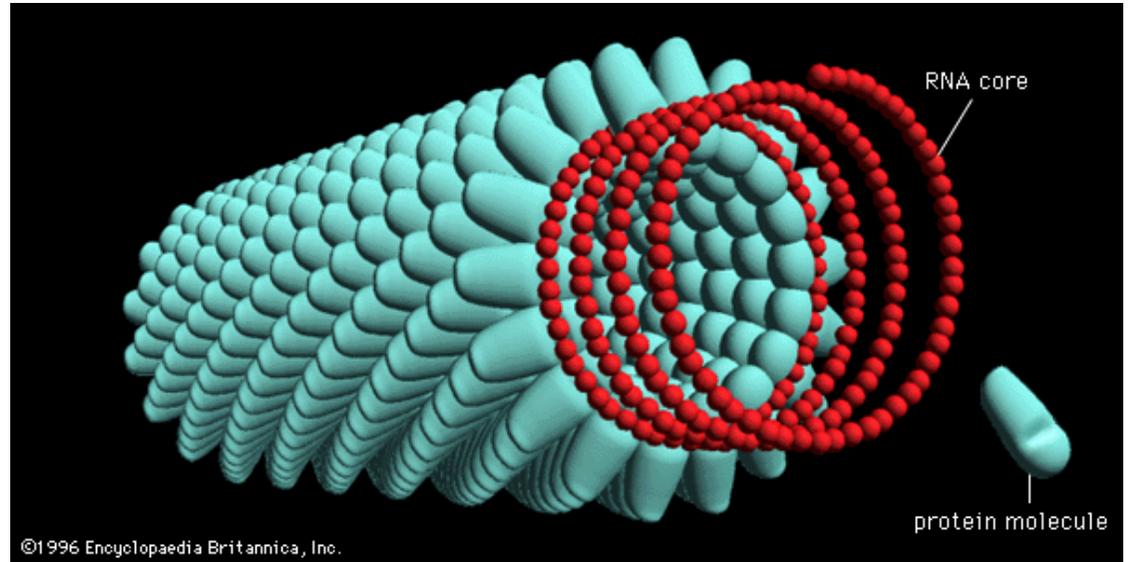
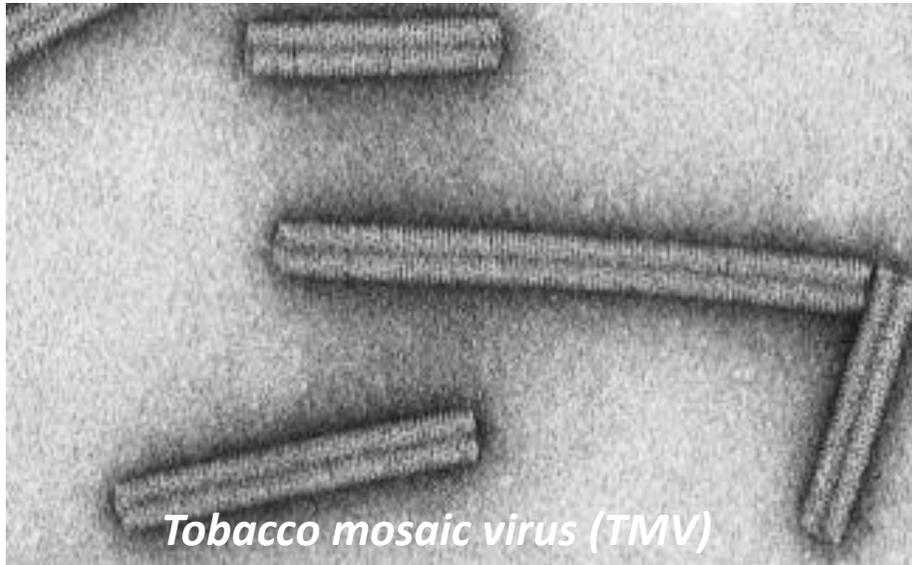
Agrobacterium T-DNA transfer as a natural case of genetic engineering



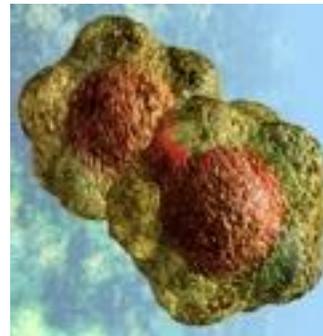
- ‘disarmed’ T-DNA (without Ti-genes) are widely used to produce transgenic plants

Plant Viruses (phytoviruses)

- More than 40 families of DNA and RNA plant viruses exist, most are single-stranded (ss) positive-sense RNA viruses



- Far fewer plant viruses have DNA genomes but they are among the most economically important, such as Geminiviruses, with circular, single-stranded DNA genome packaged into twin particles, hence their name

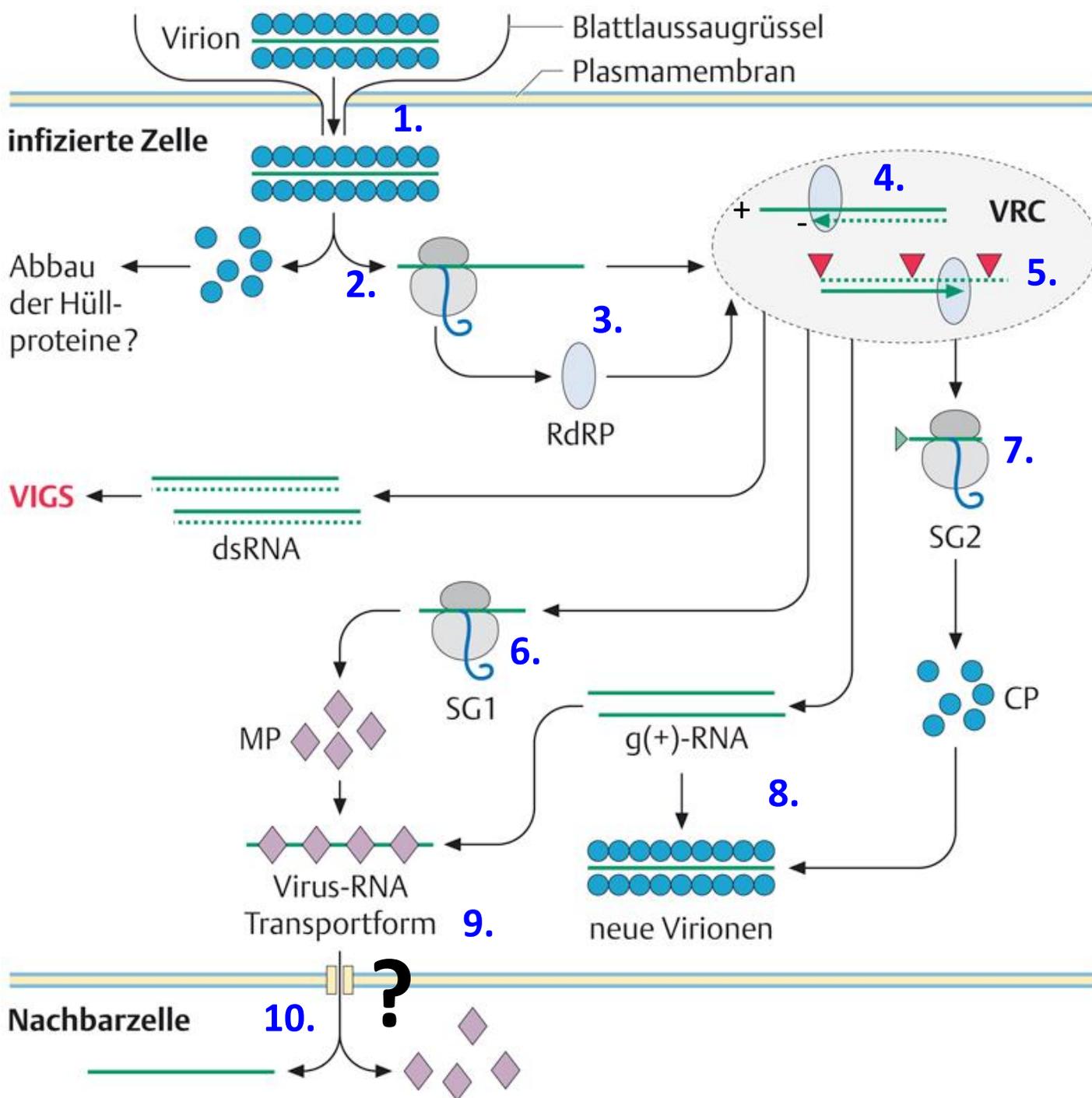


- Symptoms of viral infection include tissue yellowing (**chlorosis**) or browning (**necrosis**), **mosaic** pattern, and plant **stunting**



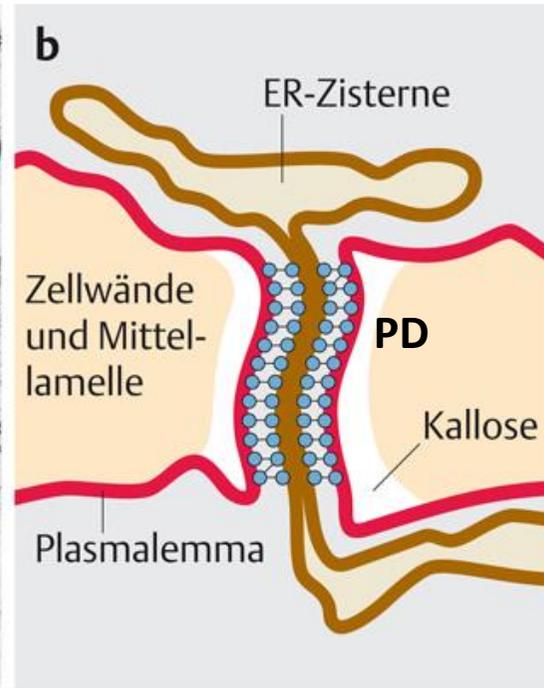
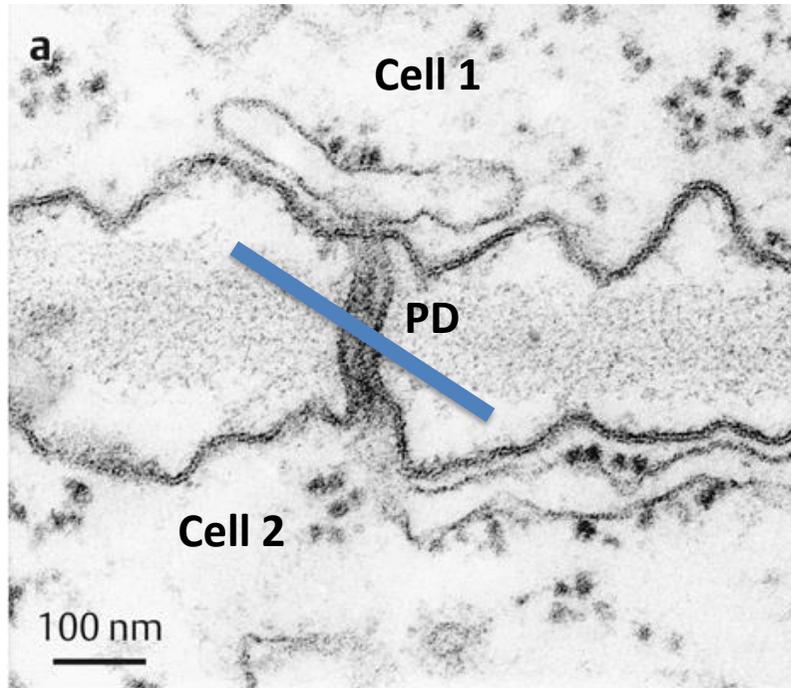
- Plant viruses are **biotrophs** and face 3 major **challenges**
 - how to **replicate** in the cell initially infected
 - how to **move into adjacent cells** and the **vascular** system
 - how to **supress** host defense systems
 - how to get **transmitted** to another plant
- Genome replication for **positive-strand RNA** viruses occurs in the **cytoplasm**
- Genome amplification of **ssDNA** geminiviruses, and some **negative-strand ssRNA** viruses, occurs in the **nucleus**
- Subsequent **transport** of the virus particle occurs through **plasmodesmata**: in contrast to animal viruses, plant viruses **never cross the plasma membrane** of the infected cell

Tobacco-Mosaic-Virus replication



1. Virus entry via cell damage or insect feeding
2. Uncoating of the viral genomic (G) RNA
3. Translation of the (G)RNA yields the viral replicase (RdRP)
4. The (+) (G)RNA strand is copied into a complementary (-) strand in the Viral replication Complex (RLC)
5. The 2 subgenomic (SG)RNAs are initiated internally on the (-) strand
6. (SG)RNA1 is translated into movement protein (MP)
7. (SG)RNA2 is translated into coat protein (CP)
8. More (G)RNA is generated and coated by CP, generating an RNP complex
9. RNP associates with the MP to create a virus transport form
10. The virus moves through plasmodesmata; the cycle is reiterated

Ultrastructure and composition of plasmodesmata (PD)



- Plasmodesmata are **pores** connecting all plant cells together, thereby creating of a **cytoplasmic continuum** known as **symplasm**

- Plasmodesmata are constituted of **ridge proteins** linked to an endoplasmic reticulum (ER) continuum between cells

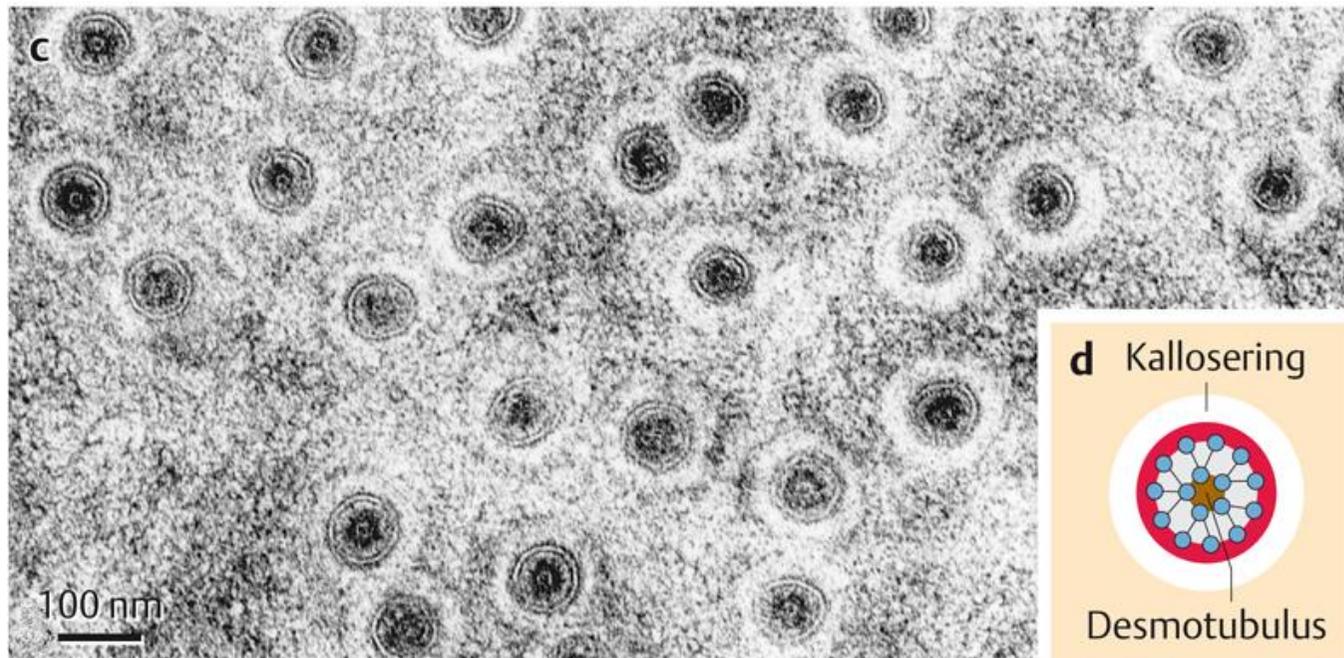
- The **neck** of plasmodesmata is formed by **callose** deposition surrounded by **plasma membrane**

- Many plasmodesmata connect plant cells to each others and forms channels known as **desmotubules**

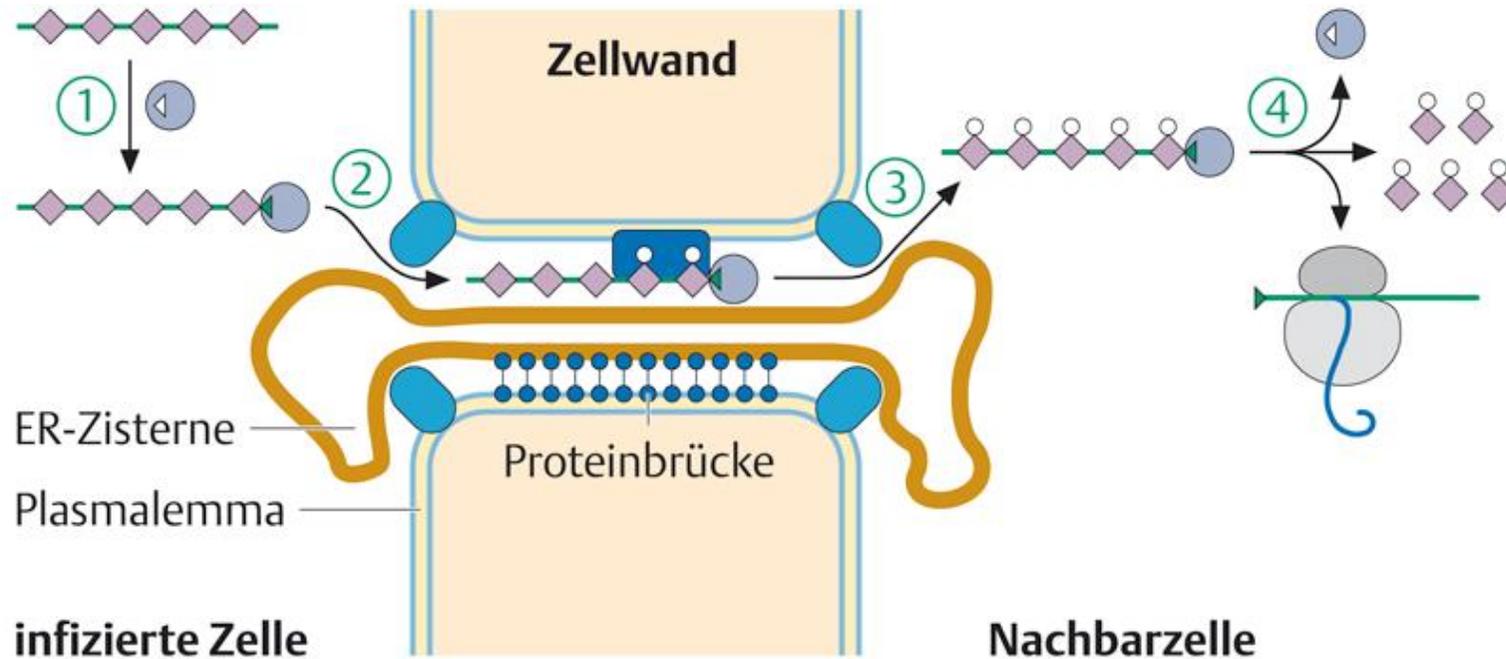
- **Desmotubules** can accomodate **passively** the movement of proteins of **up to 50 kDa ...**

- ...but viral RNP are **> 1000 kDa!**

- Hence the need for **Movement Proteins** for viruses to **actively** enlarge plasmodesmata **aperture**



Systemic Spread of Plant Viruses



1. MP binds chaperone proteins

2. RNP-chaperone complex binds to an elusive PD-bound receptor

2-bis. The MP becomes phosphorylated by an unknown protein kinase

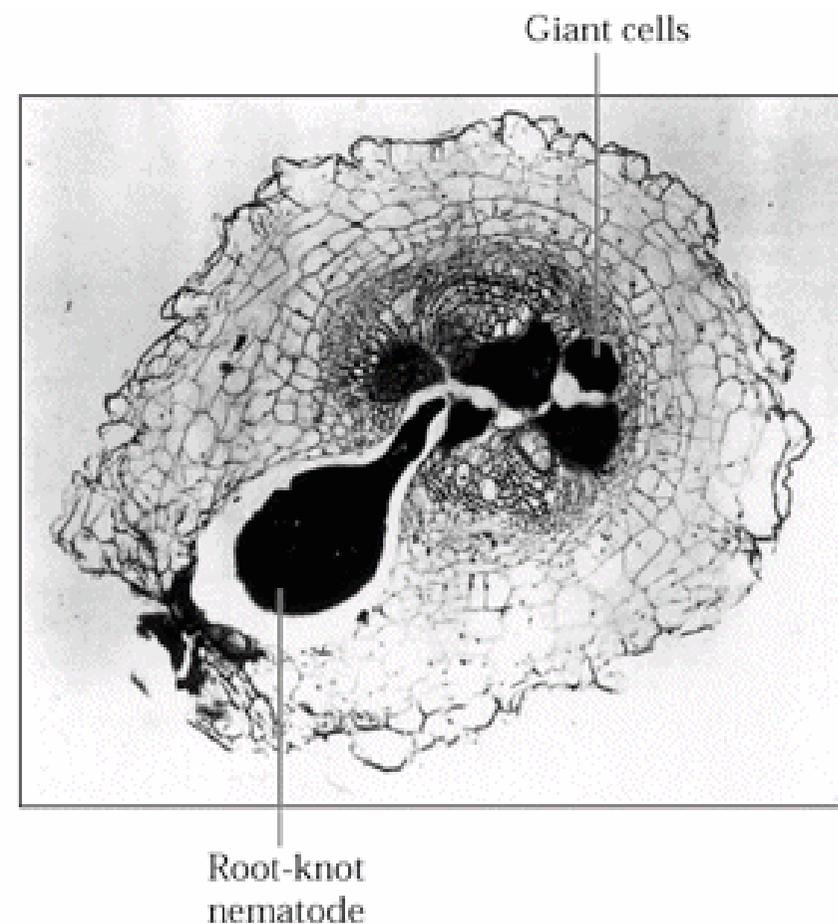
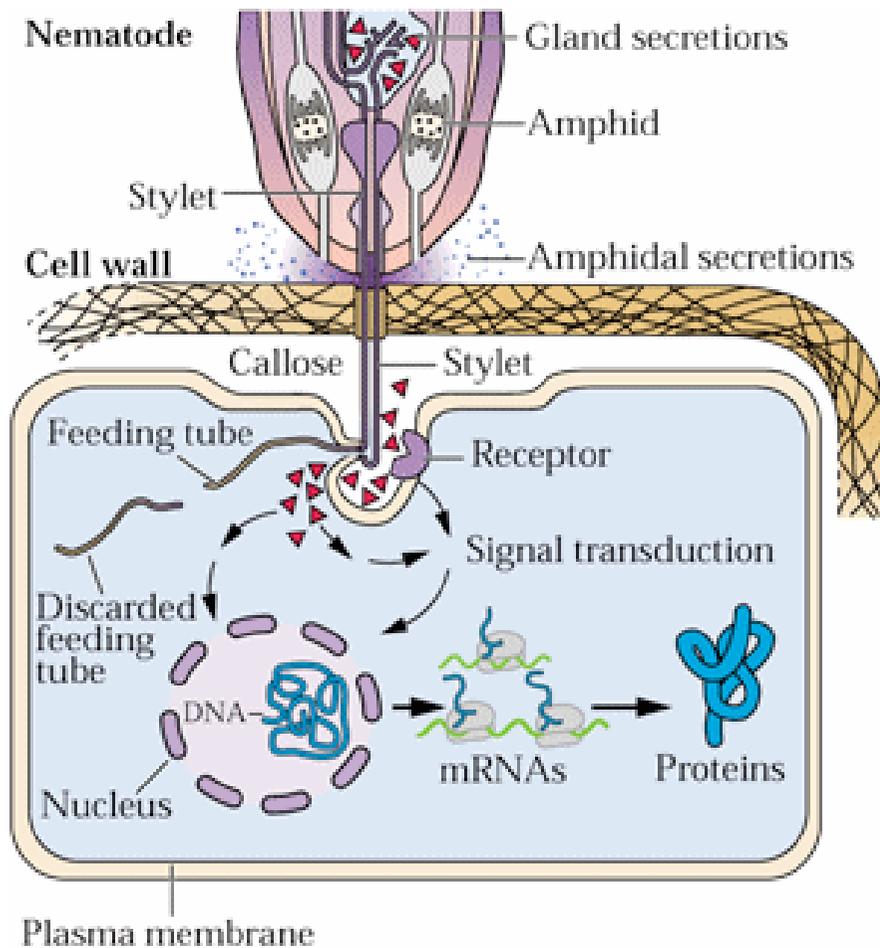
3. The activated RNP-MP complex then uses the ER membrane to move

4. MP, chaperone and RNP are disassembled and the viral RNA initiates a new replication cycle



Plant Pathogenic Nematodes

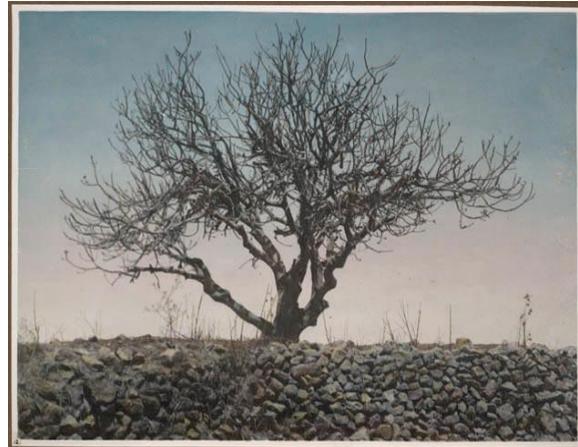
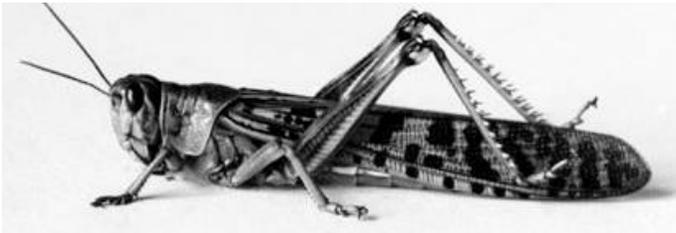
- More than **20 genera** of plant nematodes cause plant diseases. Infections by these round worms (ca. 1 mm long) are nearly always **confined** to the **plant root system**
- Some use their **amphidal secretions** to digest the plant cell wall and penetrate the host cell with their **stylet**
- Effector proteins delivered into host cells induce **cell division and gigantism**, transforming dividing cells into a **feeding factory**



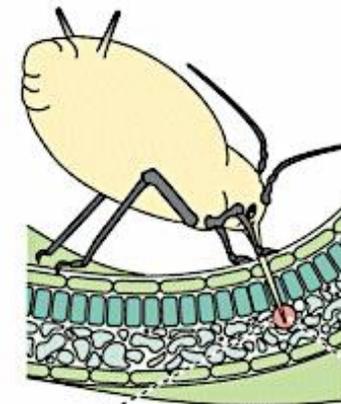
Feeding Arthropods not only damage Plants directly but also facilitate colonisation by Viral, Bacterial, and Fungal Pathogens

- myriads of insect species feed, reproduce, and shelter on plants. Two broad categories of herbivorous insects are recognised: a) **chewing** and b) **sap sucking**

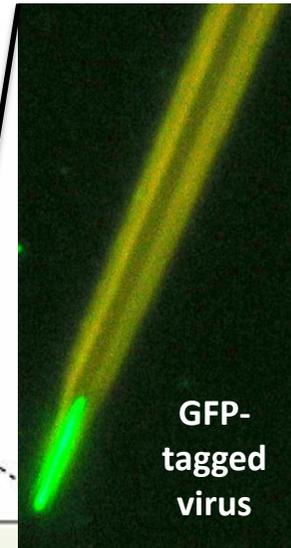
The 1915 locust plague (March to October), was a plague of locusts that stripped areas in and around Palestine of almost all vegetation



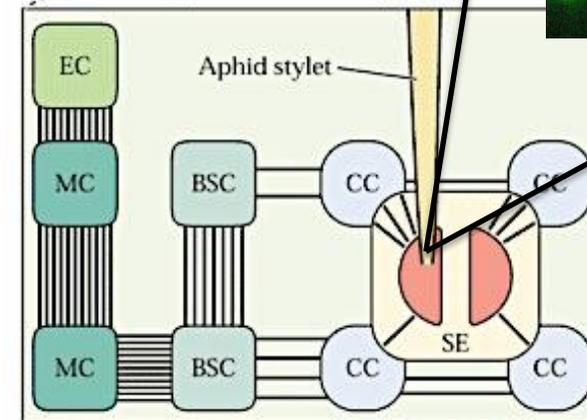
*Colorado potato beetle
(Leptinotarsa decemlineata)*



Vascular bundle penetrated by the insect feeding stylet



GFP-tagged virus



SE: phloem sieve elements

CC: companion cells

BSC: bundle sheath cell

MC: mesophyll cell

EC: epidermal cell

Plant Defense Systems

- Only a **very small proportion** of pathogen infections are likely to result in a **diseased plant**. Four main reasons account for most failures of pathogens to infect plants successfully:
 - the plant species attacked is **unable** to support the life-strategy requirements of the particular pathogen and thus is considered a **nonhost**
 - the plant possesses **preformed structural barriers** or **toxic** compounds that confine successful infection to specialised pathogen species (**nonhost resistance**)
 - on recognition of the attacking pathogen, **defense mechanisms** are activated such that the invasion remains **localised**; many of these mechanisms involve hormones (*cf.* previous lectures)
 - **environmental** conditions change and the pathogen **dies** before the infection process has reached the point at which it is no longer influenced by adverse external stresses