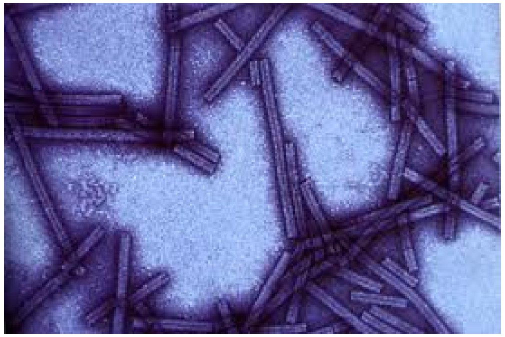
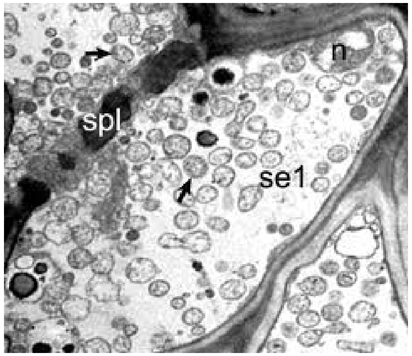
Molecular plant pathology



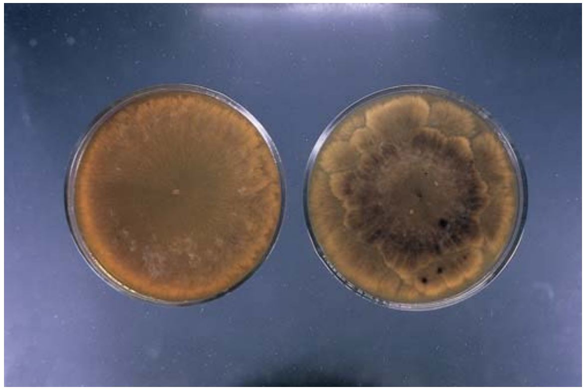


Assist. Prof. Martina Šeruga Musić

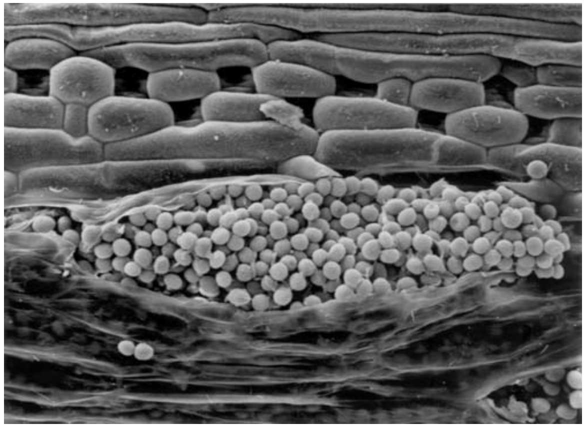
acad. year 2016/17

FUNGI

- Kingdom Fungi – Eumycota
- about 15 000 phytoppathogenic fungi – the most numerous plant pathogens
- fungi - parasites, saprophytes (saprotrophs), symbionts (mutualism)
- taxonomy of fungi
- important divisions and orders:



Ascomycota order *Erysiphales* (powdery mildew)
(Ascomycetes) order *Helotiales* (rot)



Basidiomycota order *Ustilaginales* (smuts)
(Basidiomycetes) order *Pucciniales* (*Uredinales*) (rusts)

Erysiphales – powdery mildew

Microsphaera alphitoides
(Erysiphae alphitoides)

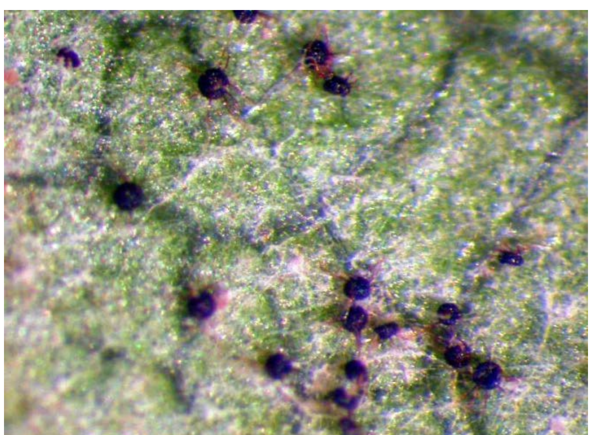
- powdery mildew of oak

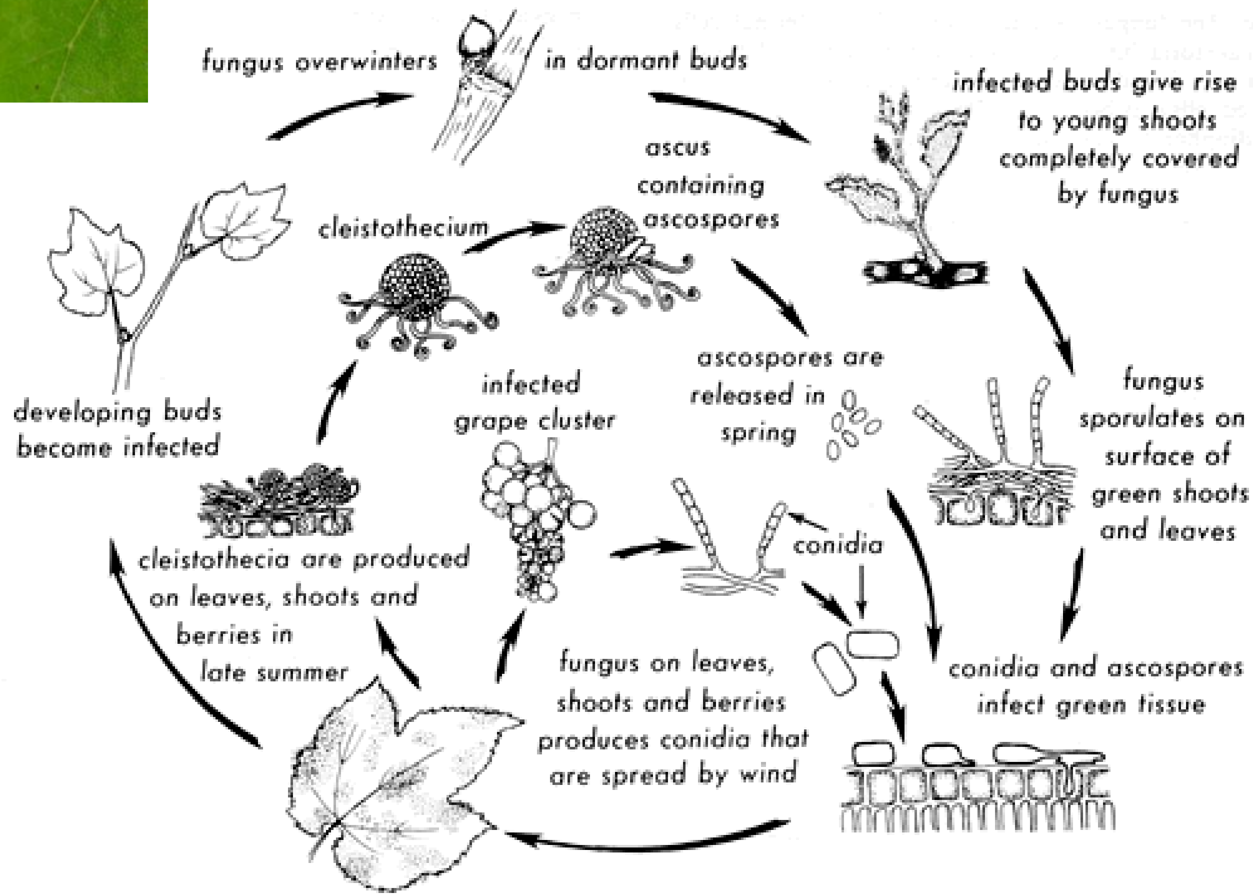
- powdery mildew of wheat

Blumeria graminis
(Erysiphe graminis)

- grapevine powdery mildew – polycyclic disease

Uncinula necator (Erysiphe necator; Oidium tuckeri)



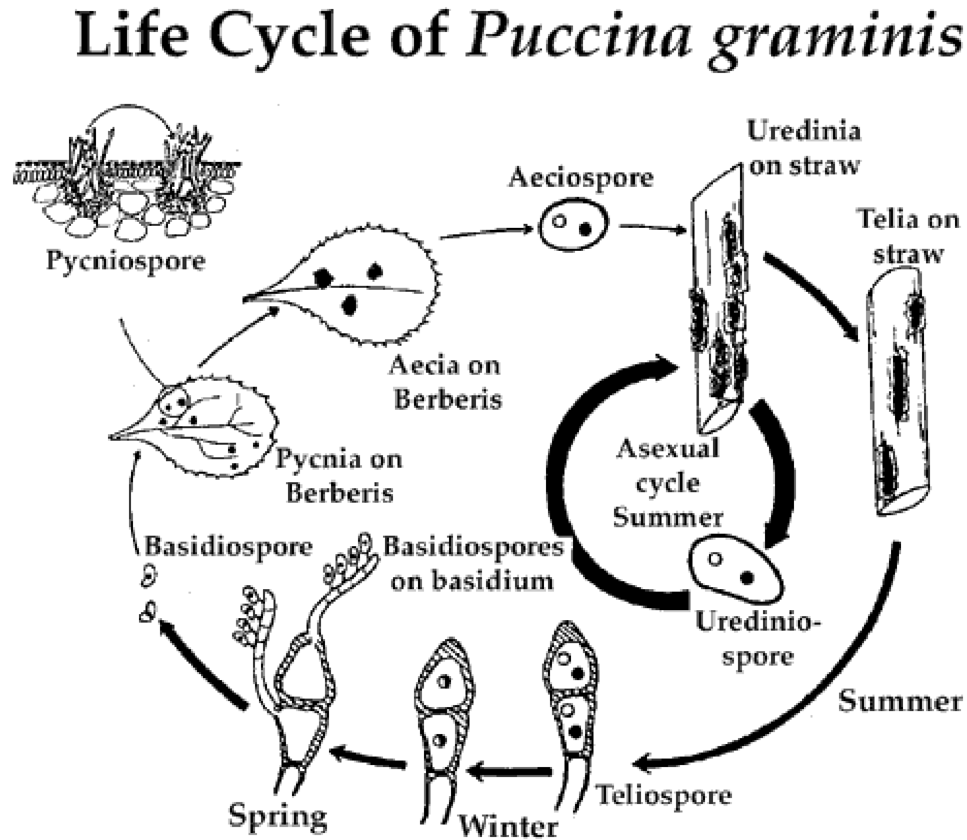


Genomes of fungi from order *Erysiphales*

- large genomes – around 180 Mbp
- genome of *Erysiphe graminis* - powdery mildew of wheat – in 2013 about 82 Mbp was sequenced which is almost half od the genome estimated to 180 Mbp - 6540 genes were annotated
- it was discovered that this genome is one of the most repetitive ones – 90 % of this genome is consisted of repetitive sequences, mostly transposable elements
- genetic flexibility, varibility and pathogen variation potential
- genome mosaicism – mosaic of old haplogroups that existed prior to domestication of wheat – adaptation to hexaploid wheat genome
- Genome of *Erysiphe necator* – around 50 MBp were sequenced
- Highly repetitive genome – adaptation by genomic structural variations
- resistance to fungicides

Pucciniales (Uredinales) - rusts

Puccinia graminis – stem wheat rust



- macrocyclic disease - up to 5 types of spores
- haplospores (haploid gametes) – basidiospores and pycniospores; dikaryotic spores – urediniospores, teliospores and aeciospores
- alternativne host – common barberry (*Berberis vulgaris* L.)

Hemileia vastatrix – coffee rust

BRANDON KEIM SCIENCE 06.11.13 9:30 AM

DISEASE OUTBREAK THREATENS THE FUTURE OF GOOD COFFEE

A rust-blighted leaf on a farm in Colombia. *Image: International Center for Tropical Agriculture/Flickr*

TECHNOLOGY INNOVATION, THE INTERNET, GADGETS, AND MORE. JUNE 6 2014 10:31 AM

Not Your Cup of Tea

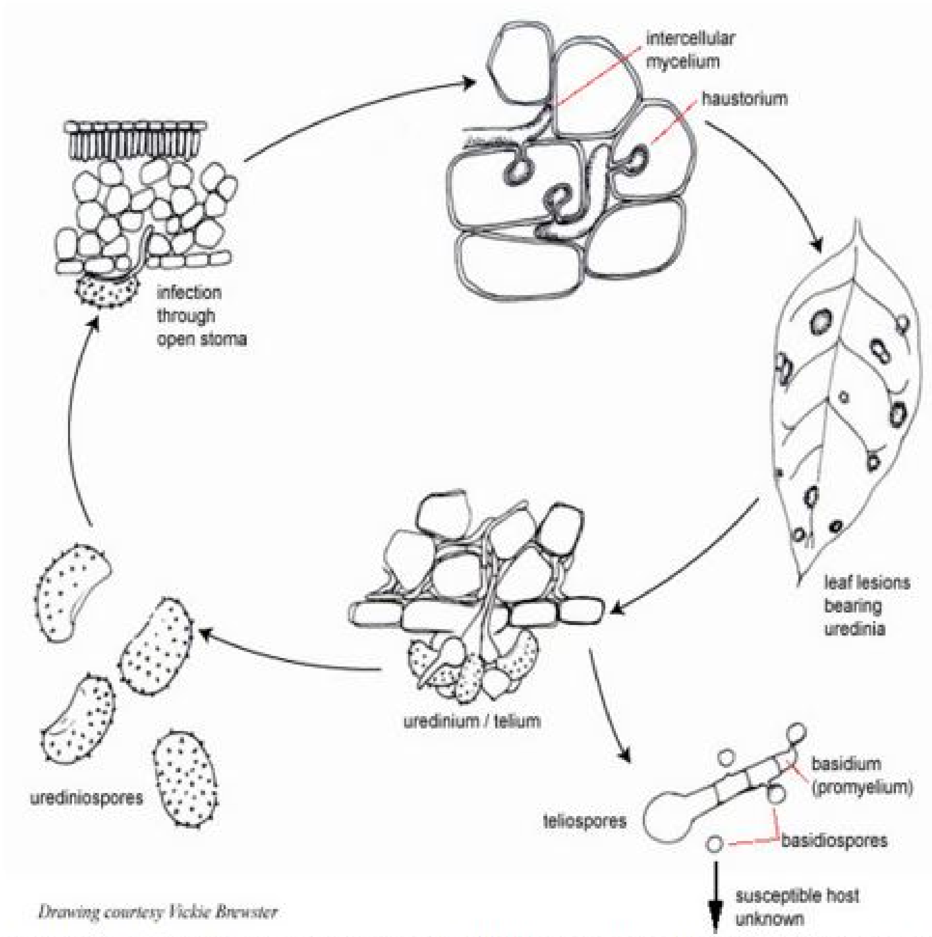
Hemileia vastatrix turned the Brits off coffee. Could it do the same to the rest of the world?

By Alison Griswold

Coffee leaf rust afflicts coffee leaves at a plantation in Pérez Zeledón, Costa Rica, on July 12, 2013.

Photo by Juan Carlos Ulate/Reuters

Disease Cycle and Epidemiology



The disease cycle is a simple one. Urediniospores initiate infections that develop into lesions that produce more urediniospores (Figure 6)

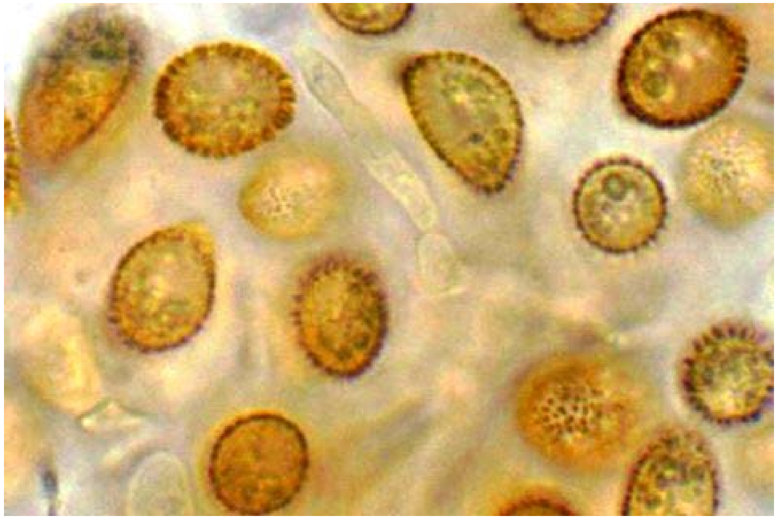
- hyperparasitic fungus *Verticillium hemileiae* – colonize coffee rust lesions and may reduce the viability of urediniospores of the coffee rust pathogen – biological control

Ustilaginales - smuts

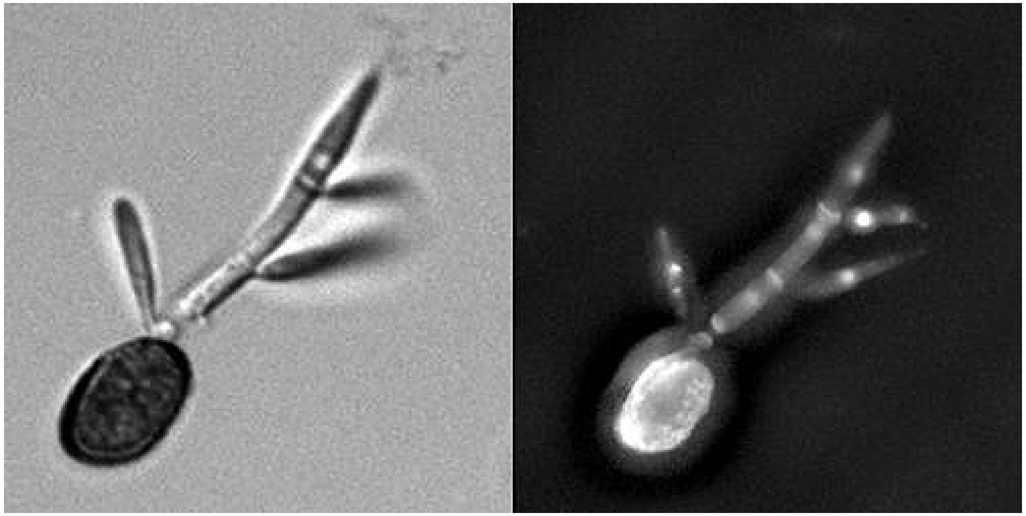
Ustilago maydis – corn smut

- *huitlacoche* – delicacy in Mexico
- tumors or "galls” - made up of much-enlarged cells of the infected plant, fungal threads and blue-black spores
- ustilagine – alkaloid with similar effect to ergotamine

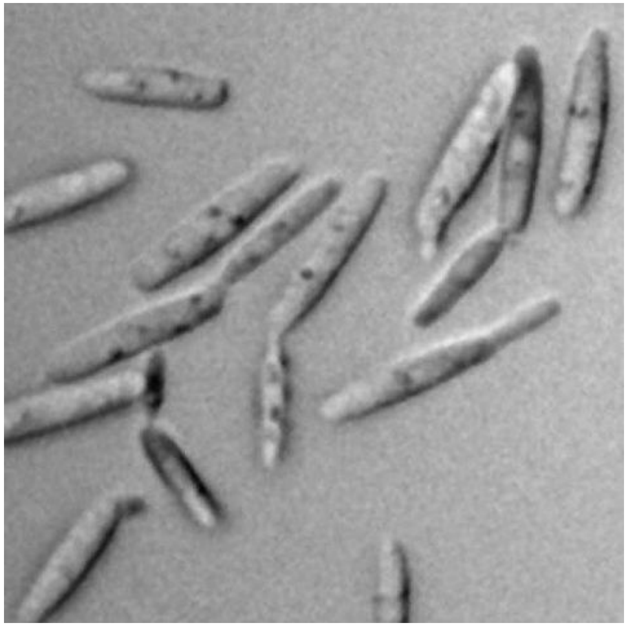
Life cycle

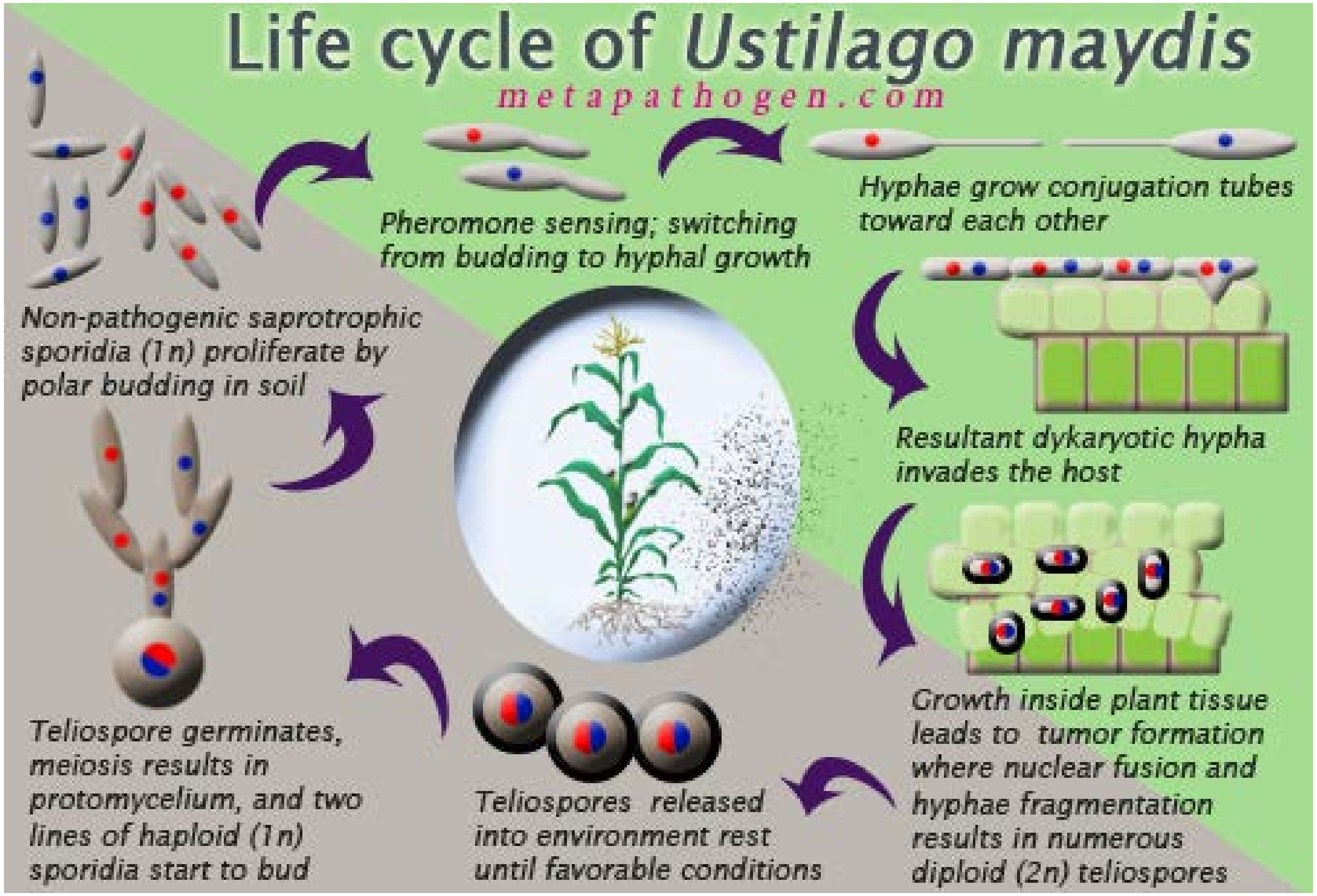


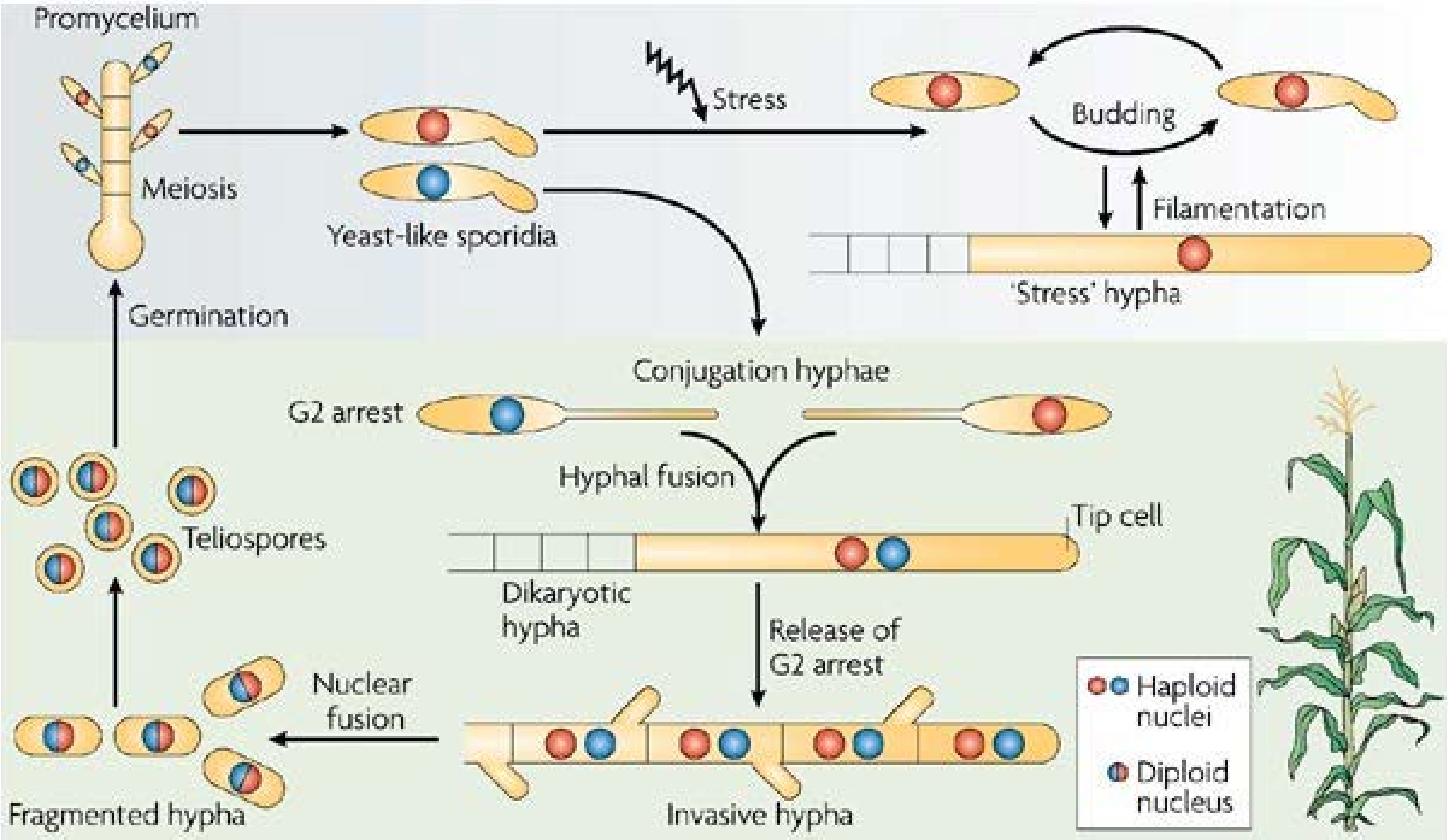
dikaryotic teliospores



haploid sporidia

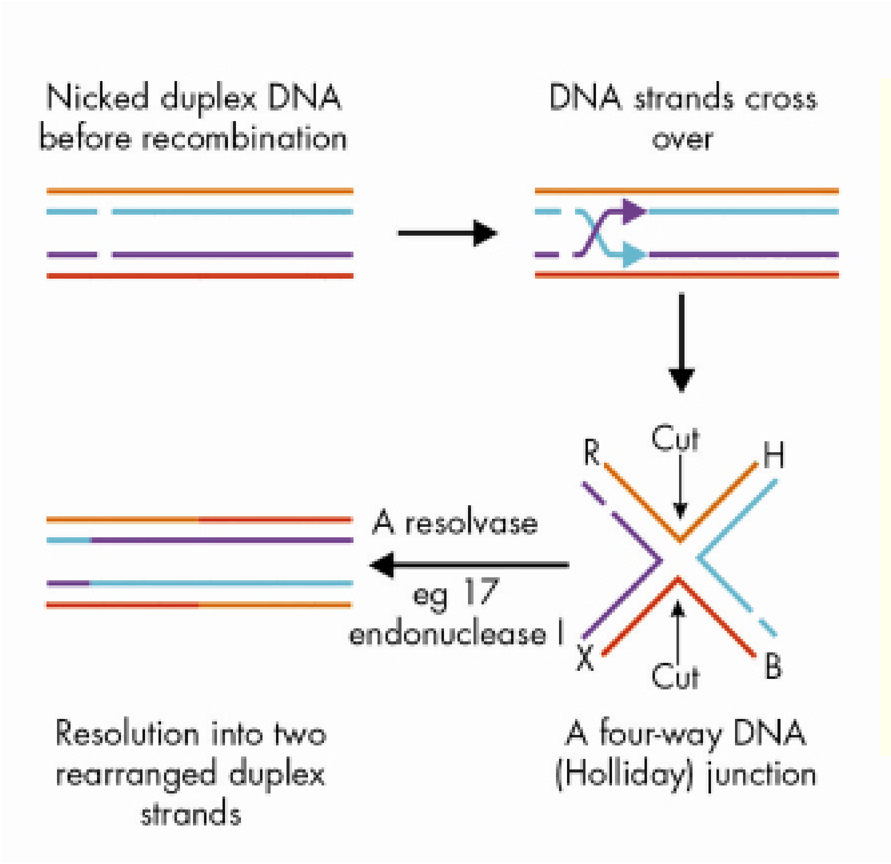



Life cycle of Ustilago maydis
metapathogen.com
Pheromone sensing; switching from budding to hyphal growth
Hyphae grow conjugation tubes toward each other
Non-pathogenic saprotrophic sporidia (1n) proliferate by polar budding in soil
Resultant dykaryotic hypha invades the host
Growth inside plant tissue leads to tumor formation where nuclear fusion and hyphae fragmentation results in numerous diploid (2n) teliospores
Teliospore germinates, meiosis results in protomycelium, and two lines of haploid (1n) sporidia start to bud
Teliospores released into environment rest until favorable conditions



Nature Reviews | Microbiology

- very good model organism – complete genome of 20 Mbp is sequenced in 2006
- studies of various biological phenomena - types of mating in fungi , plant - pathogen interactions, the mechanism of homologous recombination and repair
- Robin Holliday - 1964 - Holliday recombination model



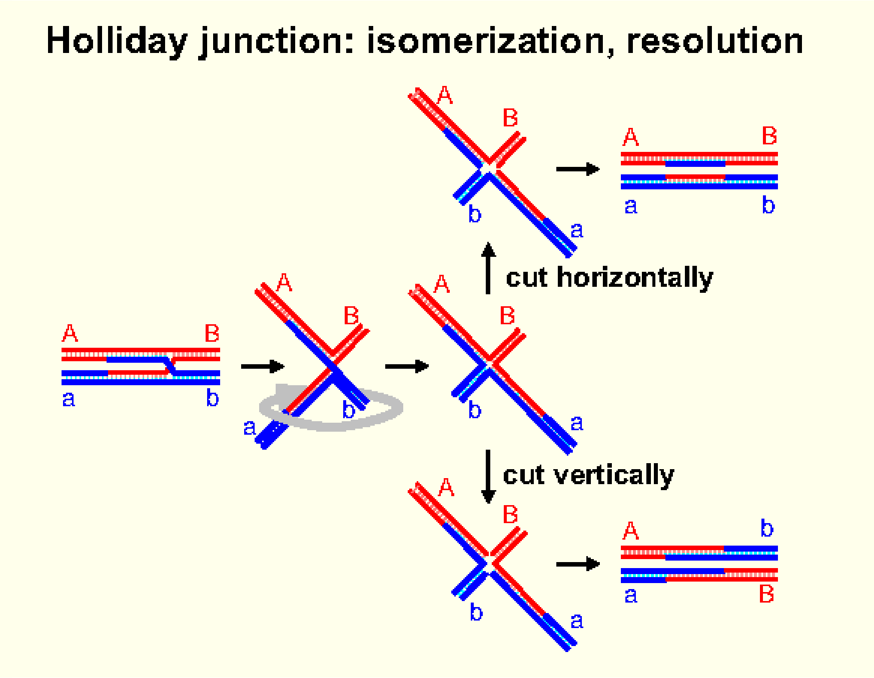
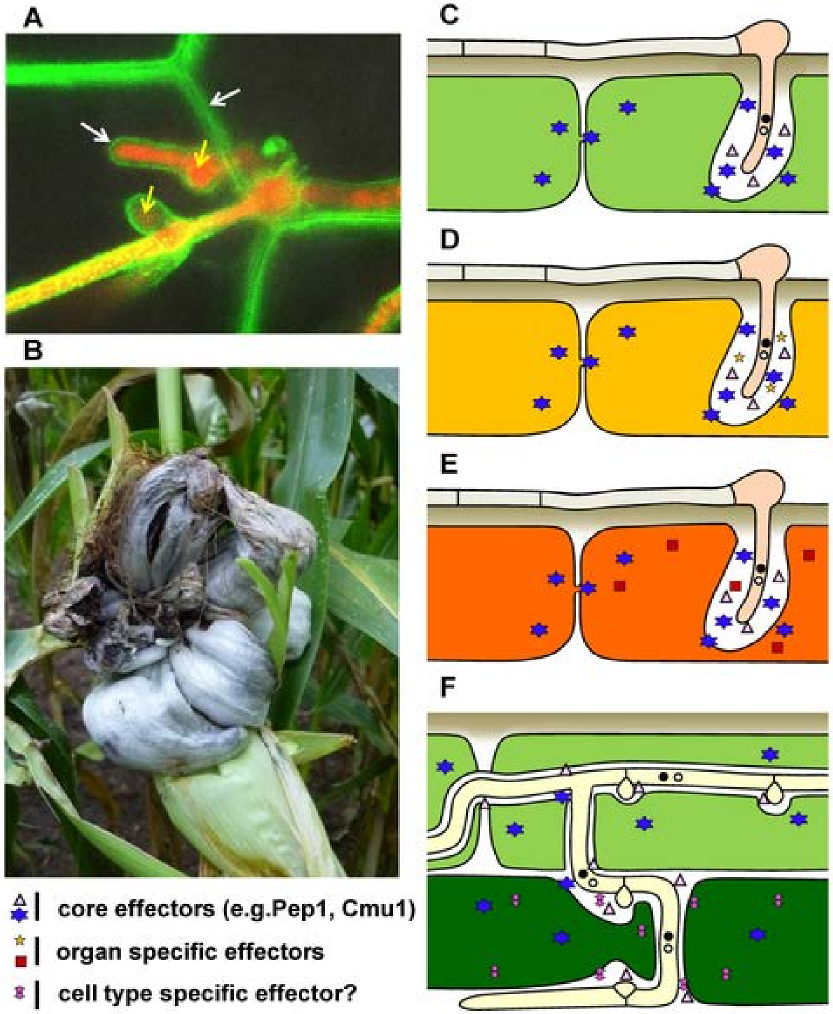


Figure 1. Disease symptoms and schematic presentation of effector cocktail use in different maize organs and tissues infected by U. maydis.

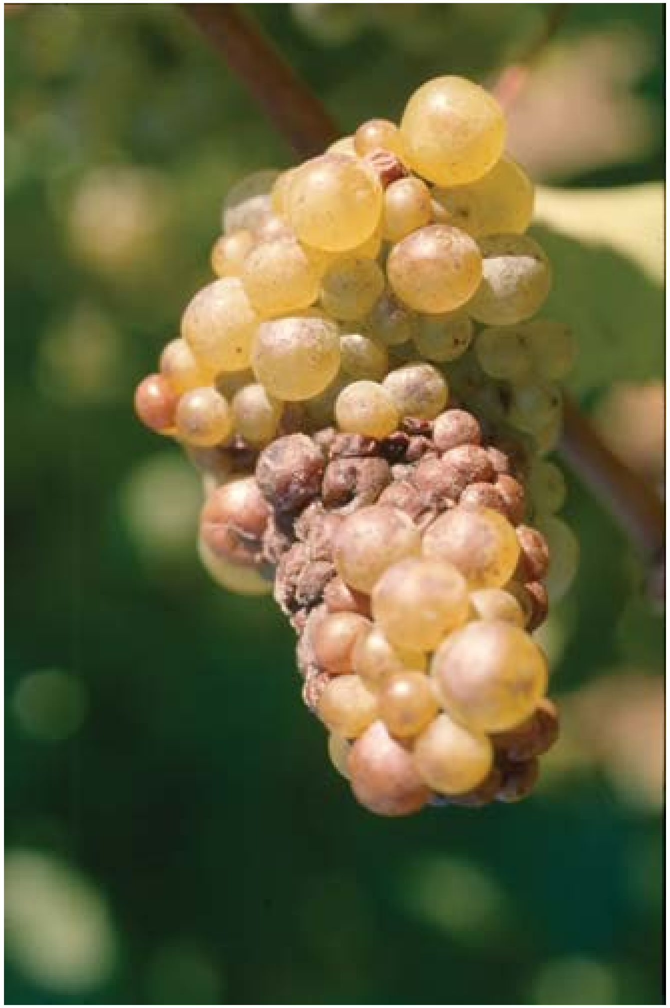


Djamei A, Kahmann R (2012) Ustilago maydis: Dissecting the Molecular Interface between Pathogen and Plant. PLoS Pathog 8(11): e1002955. doi:10.1371/journal.ppat.1002955
http://www.plospathogens.org/article/info:doi/10.1371/journal.ppat.1002955

PLOS | PATHOGENS

Sclerotiniales - rots

Botrytis cinerea – grey mold rot



OOMYCETES

• fungus-like microorganisms

• originally classified among fungi

• kingdom Chromalveolata (Chromista), phylum Heterokonta ?

• disputed taxonomy

• class *Oomycetes* – "egg fungi"; water molds

genus *Pythium*

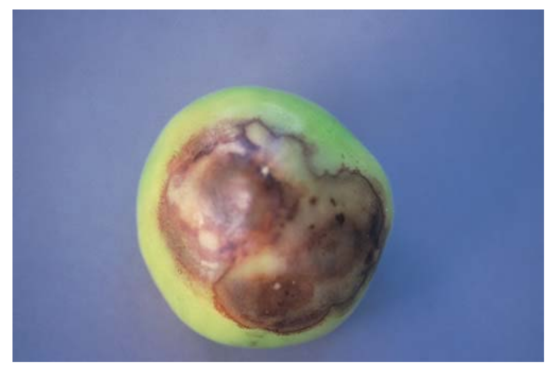
Phytophthora

Plasmopara

Peronospora

genus *Pythium*

Phytophtora infestans



Phytophtora capsici

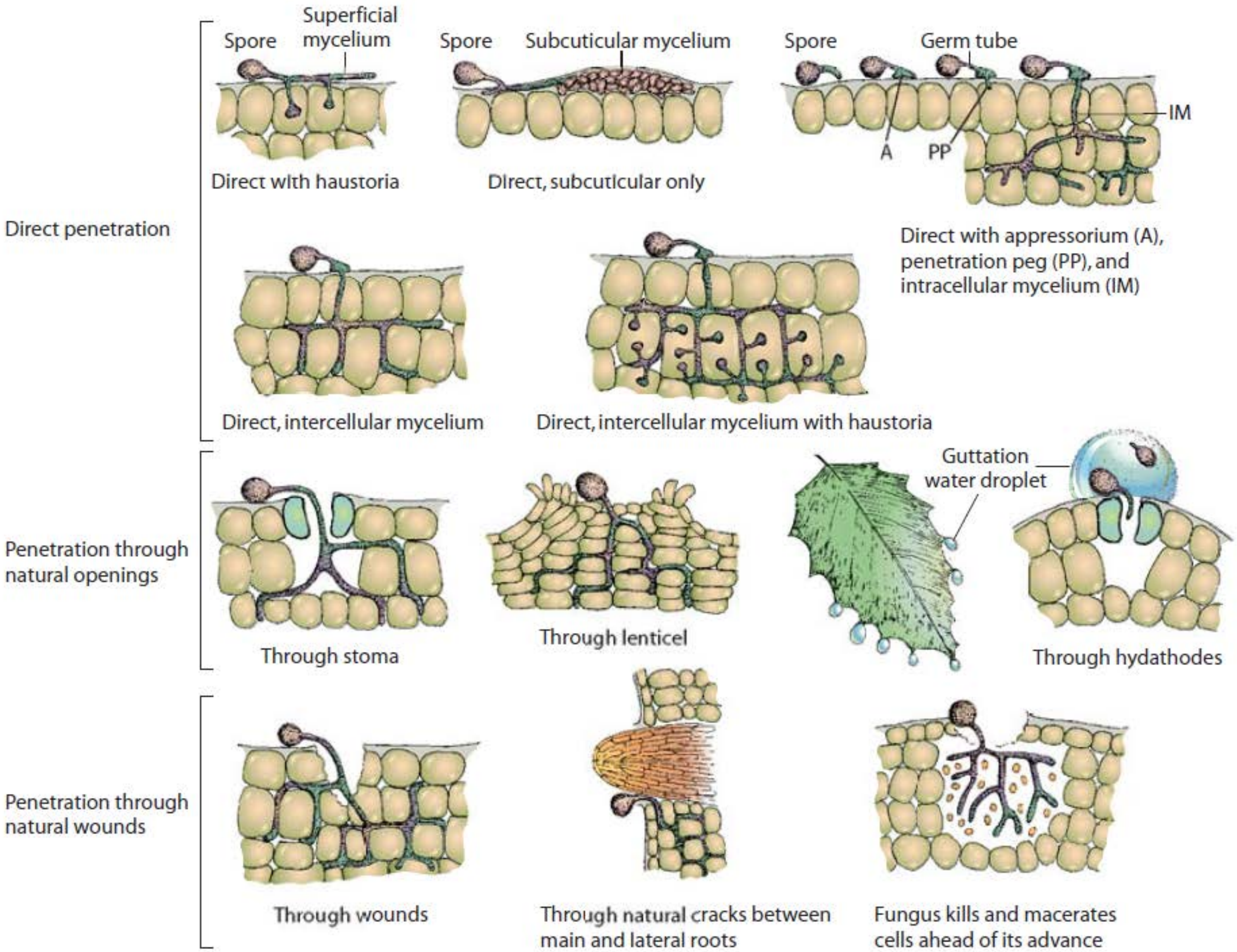
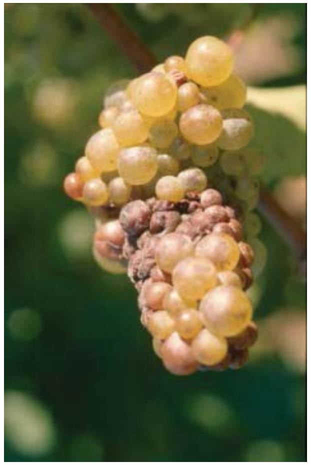


FIGURE 2-5 Methods of penetration and invasion by fungi.

MECHANISM OF PATHOGENICITY IN FUNGI AND OOMYCETES

- each pathogen is using a unique strategy for obtaining nutrients and survival
- necrotrophy vs. biotrophy
- necrotrophic parasites - kill and destroy the host cell, then use the released nutrients from the dead matter
- biotrophic parasites - colonize plant cells and direct nutrients for their growth
- hemibiotrophic parasites - biotrophic initial phase and subsequent necrotrophic
- some fungi are not pathogenic *per se*, but are considered as pathogens since they contain mycotoxins and flavonoids harmful for human and animals - *Aspergillus flavus*, *Claviceps pupurea*



Botrytis cinerea – siva plijesanj

Uncinula necator - pepelnica

How pathogens attack the host cell?

- chemical weapons against chemical weapons pf hosts
- effector biology
- EFFECTORS - all proteins and other small molecules of pathogens that alter cell structure and function of the host

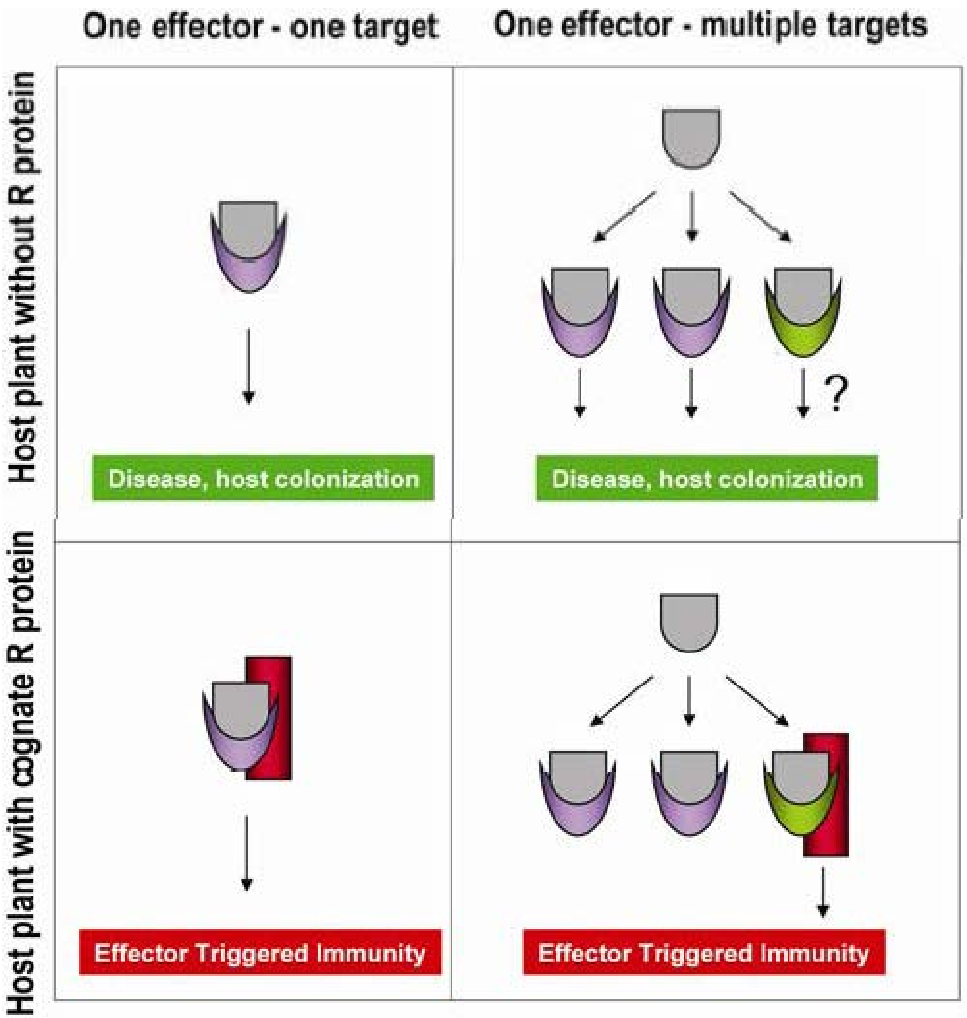
  -virulence factors and toxins (facilitate infection)

  - avirulence factors and elicitors (induce a defense response of the host)

  -in a broader sense and degradation enzymes and PAMPs (*pathogen -associated molecular patterns)*

- different pathogens have different mechanisms by which they are delivered to the host cell
- in biotrofic fungi and Oomycetes effectors are delivered into the host cells mostly through haustoria

One effector can have multiple target molecules



Gene-for-gene relationship

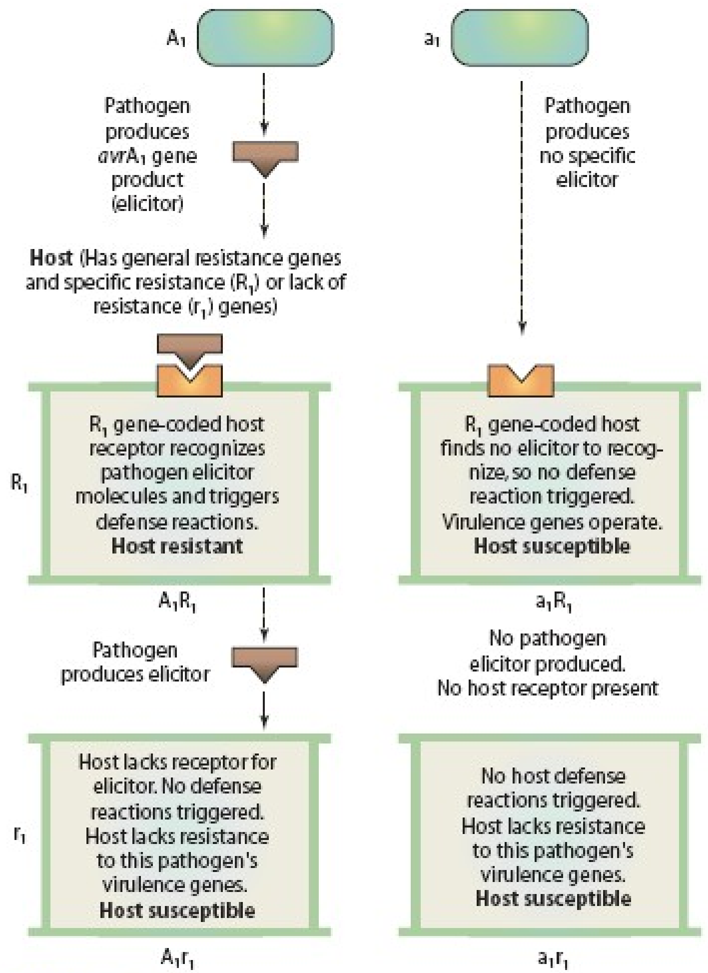
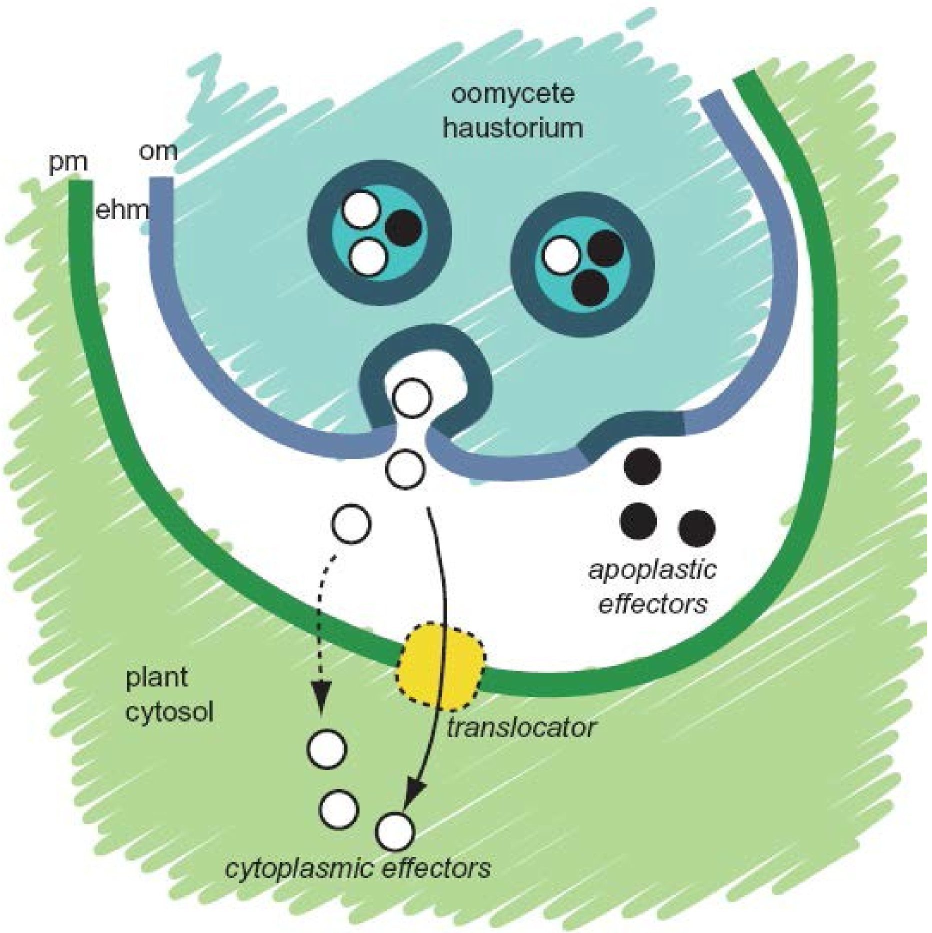
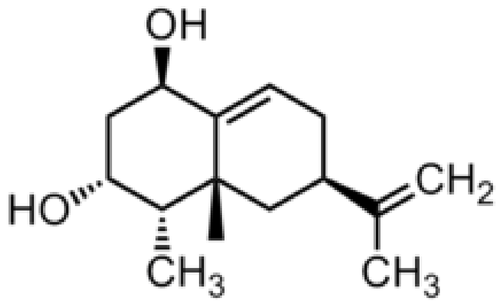


FIGURE 4-11 Basic interactions of pathogen avirulence (A)/virulence (a) genes with host resistance (R)/susceptibility (r) genes in a gene-for-gene relationship and final outcomes of the interactions.

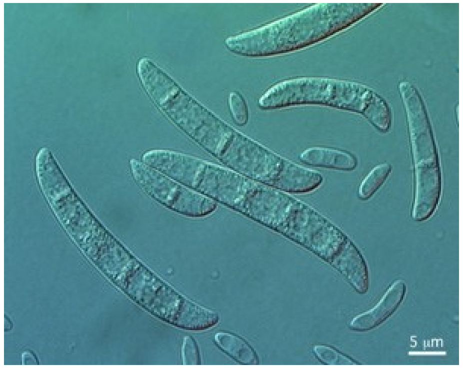
- some effectors act in the apoplast - extracellular space between the surfaces of the pathogen and the host
- *Cladosporium flavum* - effectors Avr2, Avr9, Avr4, ECP2 - inhibitors of cysteine proteases
- *Phytophthora infestans* – EPI1, EPI10, EPIC210 - inhibitors of plant hydrolases (glucanase), serine and cysteine proteases
- *Ustilago maydis* - PEP1 - inhibits peroxidase corn POX12
- in general - such effectors act by inhibition and protection from plant hydrolytic enzymes



- the cross of the barrier of a host cell - healthy plants have phytoanticipins -preformed antimicrobial compounds and inhibitors of enzymes that break down the cell wall – for example inhibitory protein of polygalacturonase (PGIPs)
- detoxification of phytoanticipins - *Geumannomyces graminis* - avenacinase - degrades avenacin - present in the rye
- detoxification of phytoalexins
- PHYTOALEXINS - toxic antimicrobial compounds produced in plants after the infection by pathogens or under stress conditions - mainly terpenoids and alkaloids - terpenes , flavonoids ...



- example : peas (*Pisum sativum*) has phytoalexin pisatin - most pathogenic fungi which infect beans can detoxipfy pisatin by the enzyme pisatin demethylase

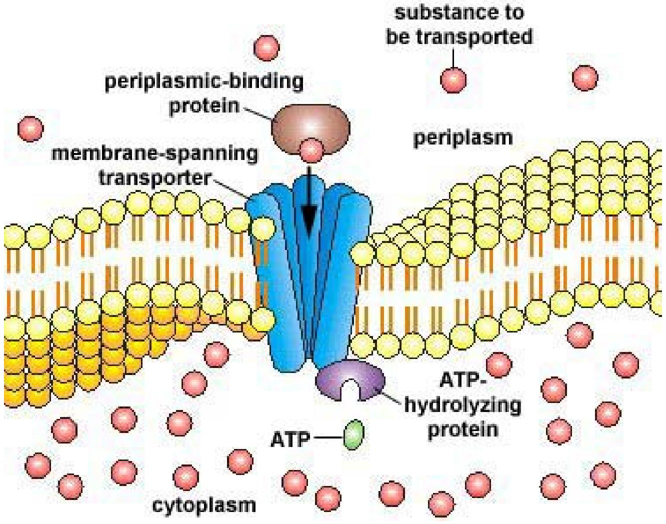


- In fungi *Nectria haematococca* (*Fusarium solani*) - genes *PDA1* and *Cyp57* involved in detoxification of pisatin

- the same fungus can detoxify medicarpin and maackiain through genes encoding mono – oxidases

- *Botrytis cinerea* - produces an enzyme laccase (stilbene oxidase) - detoxifies phytoalexin resveratrol

- alternative for detoxification of phytoalexins may be the possibility of their transport and elimination from the cell via transporter proteins - ATP - binding cassette (ABC) transporters



- example : phytoalexin sakuranetin from rice induces the expression of *ABC1* gene in the fungus *Magnaporthe grisea*

- fungi use the same transport system from the transport and elimination of fungicides and other toxic substances from the cells

• secretion of the enzymes tht degrade the cell wall (*CWDE; cell wall degrading enzymes*) :

- pectinases – endo and exo-polygalactorunases, pectate-lyases…
- celullases
- cutinases
- ligninases
- protease
- lipases
- amylases

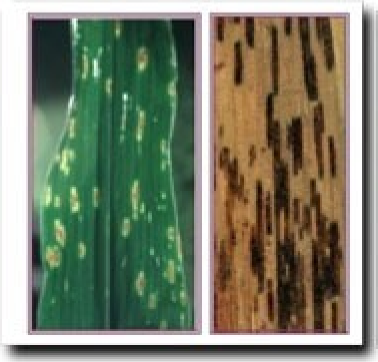
- the role of toxins
- fungi produce an array of toxins that could harm the plant tissue or cause the plant cell death or the change in the expression of plant genes – nectrotic symptoms or formation of galls

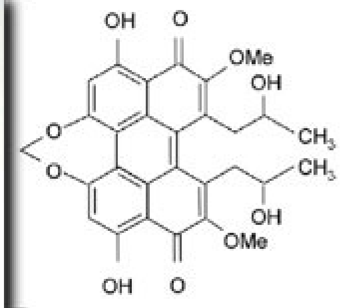
- specific (selective) toxins
    - genus *Cochliobolus* – victorin, T-toxin, HC-toxin, HS-toxin – infecting rye, corn, sugar cane
    - *Alternaria alternata* – AK-toxin, ACT-toxin, AF-toxin, AM-toxin, AAL-toxin – infecting pear, tangerines, strawberry, apple, tomato

• unspecific (nonselective) toxins

- •fusicoccin – *Fussicoccum amygdali (Phomopsis amygdali)* – almond
- cercosporin – *Cercospora* spp.
- tentoxin – *Alternaria* spp.
- naphtazarine – *Nectria haematococca* – peas
- dothistromin – *Dothistroma septospora* - pine

•not pathogenicity factors and effectors!





cercosporin

- mycotoxins – not toxic for the plant, but could be harmful for human and animals that consume infected plant material

  - aflatoxin – *Aspergillus flavus* and *Aspergillus parasiticus*

    - carcinogenic and teratogenic agent

  - fumonisin – *Fusarium moniliforme* – infecting corn
  - ergotamine – *Claviceps purpurea* – rye
  - trichothecin – *Fusarium* sp. - wheat

Effectors – avirulence factors - Avr proteinis

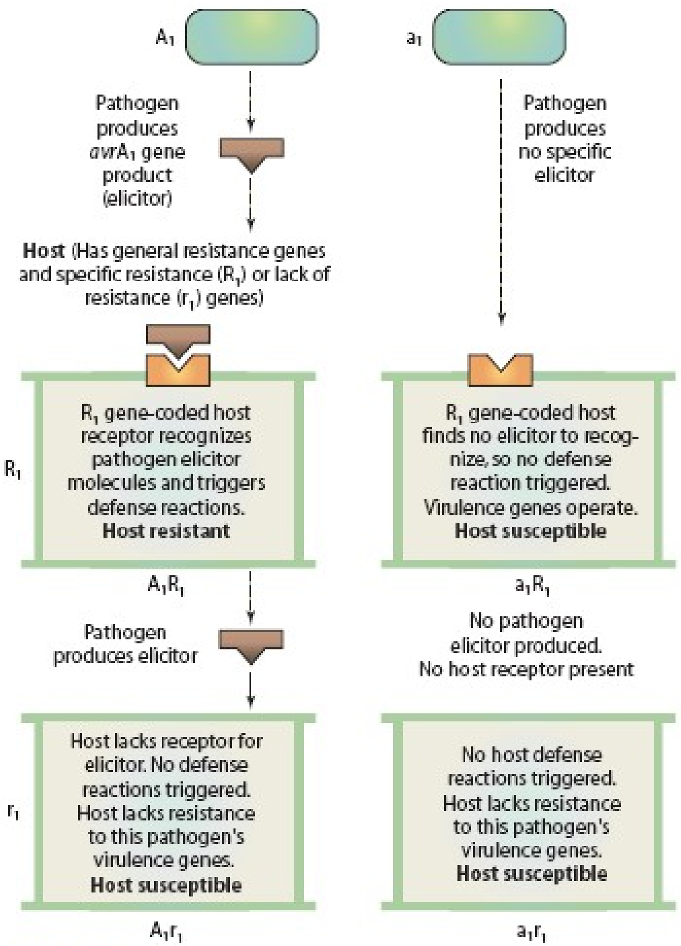


FIGURE 4-11 Basic interactions of pathogen avirulence (A)/virulence (a) genes with host resistance (R)/susceptibility (r) genes in a gene-for-gene relationship and final outcomes of the interactions.

- their role – not unambiguos – elicitors? – induce host defense response, patogenicity factors?, *house-keeping* genes?

- co-evolution of plant hosts and pathogens

  - *Cladosporium fulvum* - *Avr2*, *Avr4*, *Avr9*, *ECP2* genes

  - *Magnaporthe grisea – Avr-Pita, Pwl1, Pwl2*

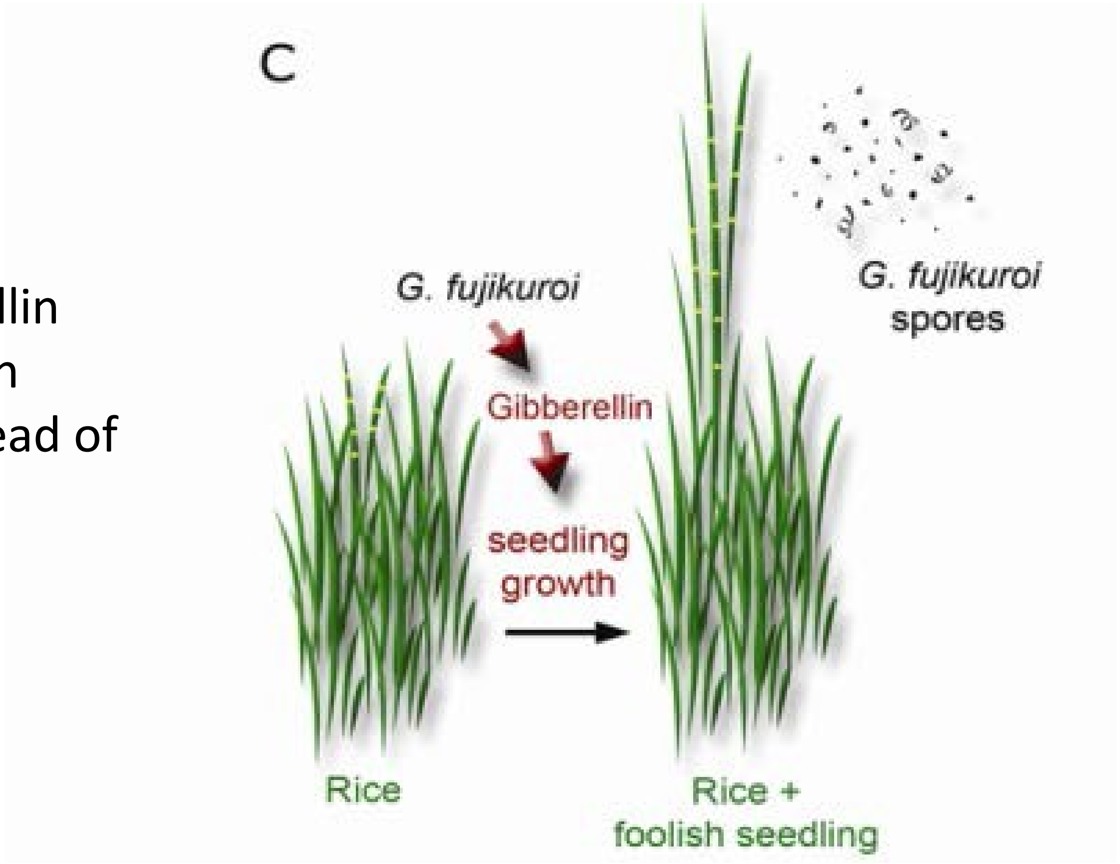
  - *Rhynchosporium secalis – Nip1*

  - *Phytopthora* sp. – *Avr1b, Avr3a, Avr3b, Avr3c……* *CRN*-effectors, *INF1* – other effectors

  - *Erysiphae graminis (Blumeria graminis) – Avra10* - HIGS concept (*host-induced gene silencing*)

Effectors – use the molecular mimicry approach and alter the plant development in that way

•example - *Giberella fujikuroi* – produces phytohormone giberellin and induces apical growth which benefits the fungi by easier spread of spores



Mechanisms of genetic variability in fungi and Oomycetes

- mating-type loci – heterothallic or homothalic system - regulation
- chromosome instability – loss of telomeric sequences
- horizontal gene transfer – gene clusters encoding secondary metabolites
- mobile genetic elements– transposons
- heterokaryosis
- the role of mycoviruses – dsRNA viruses – *Cryphonectria parasitica* – fungi – the agent of chestnut blight disease - hypovirulence - Hypovirus