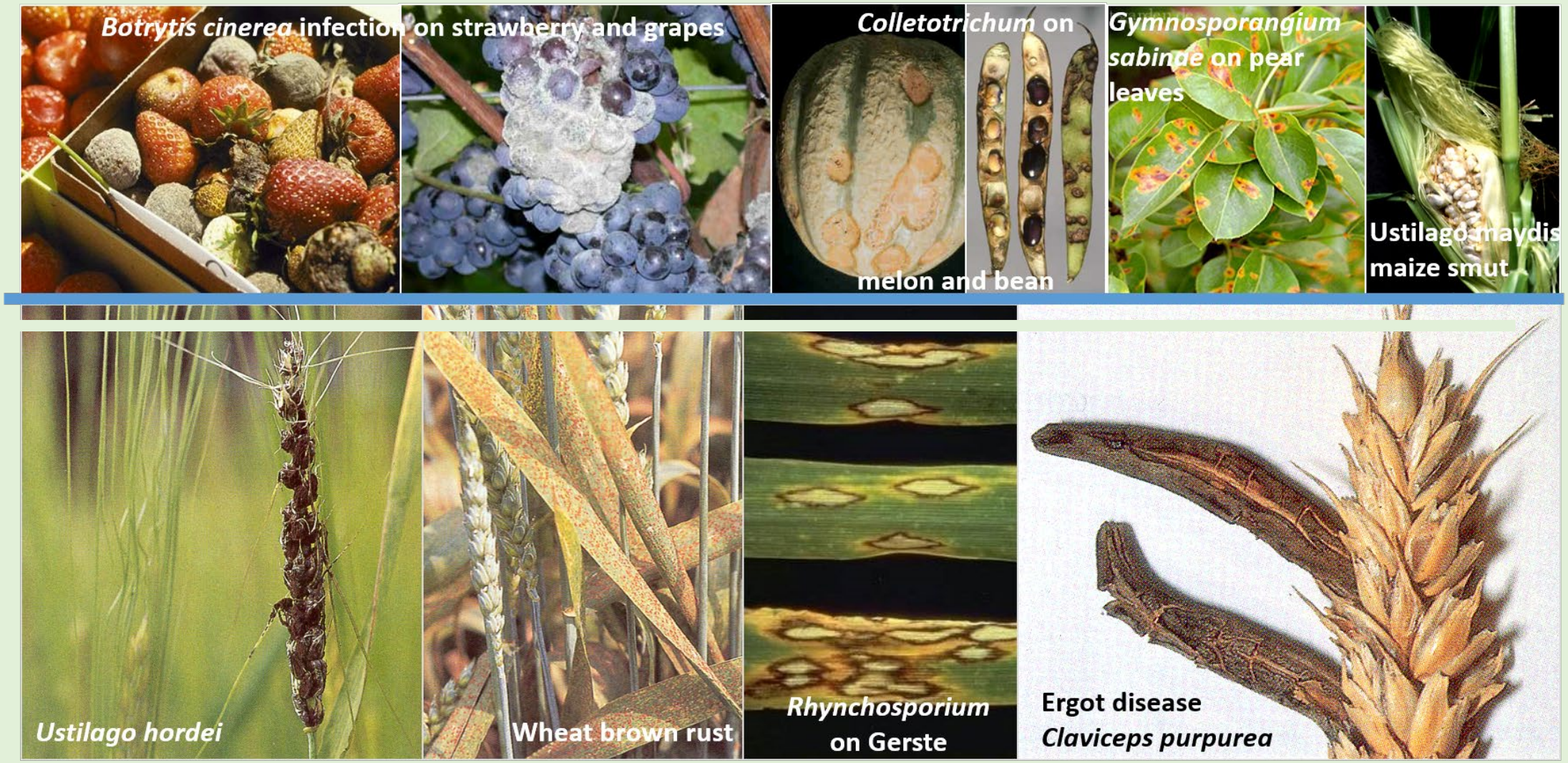
Conceitos introdutórios da genética de interações patógeno-planta

Profa. Dra. Chirlei Glienke
BIOGEMM-UFPR

Diferentes formas de interação de patógenos com seus hospedeiros



Diferentes formas de interação de patógenos com seus hospedeiros

A doença é a exceção, as plantas saudáveis são as mais comuns

- Como as plantas se defendem contra microrganismos patogênicos?
- Por que alguns microrganismos são capazes de causar doenças?
- Como contornam as respostas de defesa das plantas?
- Quais fatores de patogenicidade e virulência foram identificados?

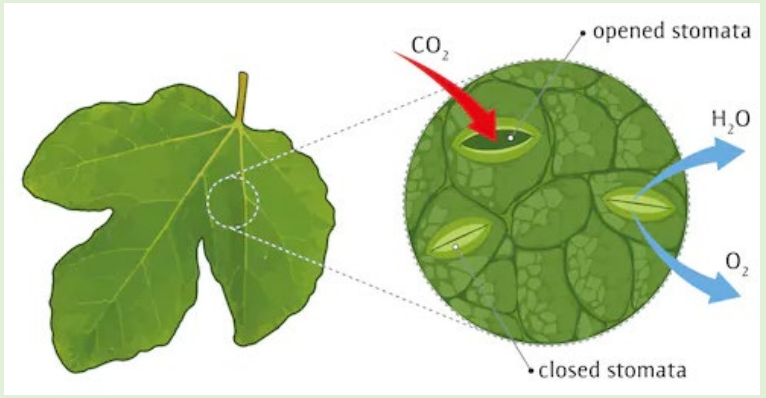
Infecção vegetal

Force or enzymatic lysis, different ways of host invasion

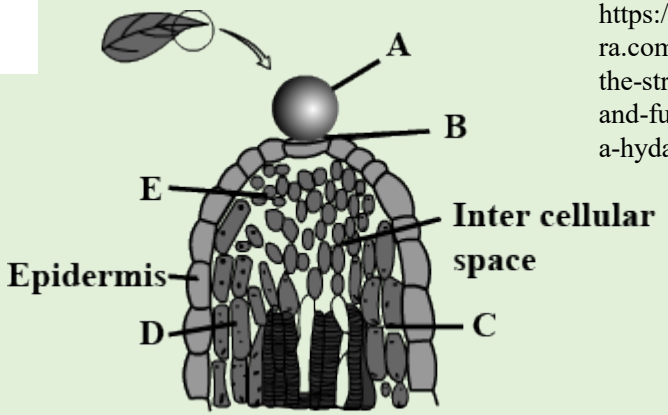
https://www.quora.com/What-is-the-structure-and-function-of-a-hydathode

natural openings

- stomatal pores
- hydrathodes



https://vivadifferences.com/difference-between-stomata-and-hydathodes/

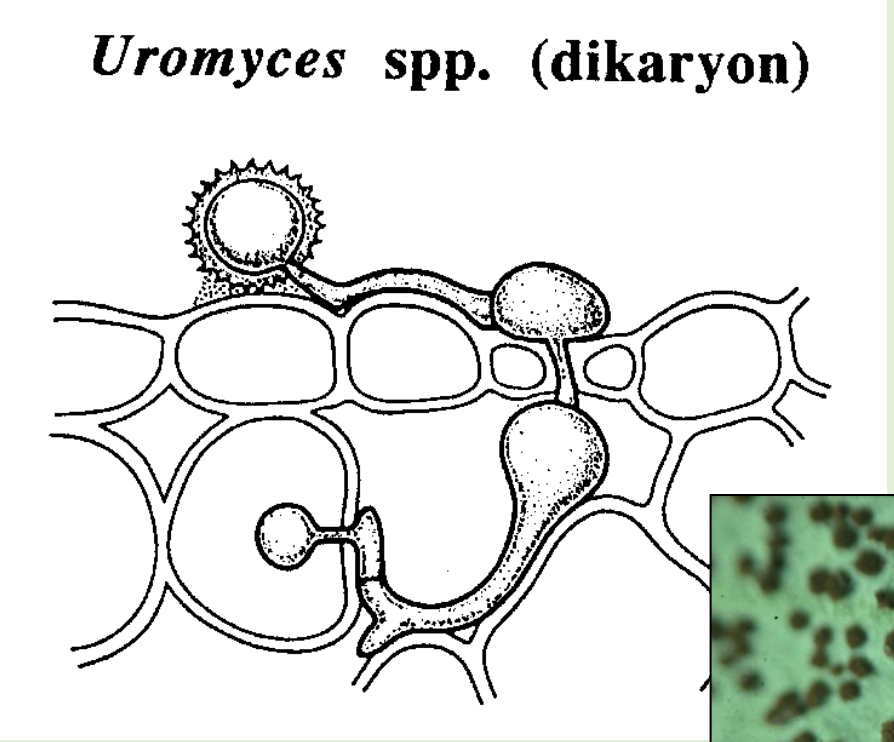


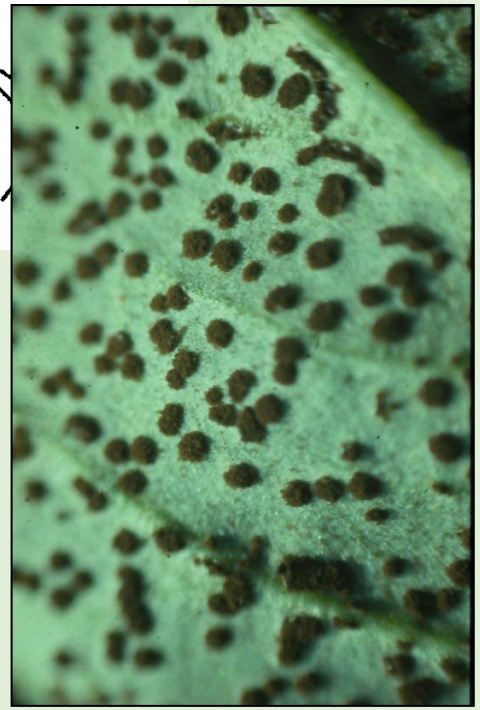
https://haygot.s3.amazonaws.com/questions/155402_a4be6bd8464d4ff888a86d546d4a89ab.png

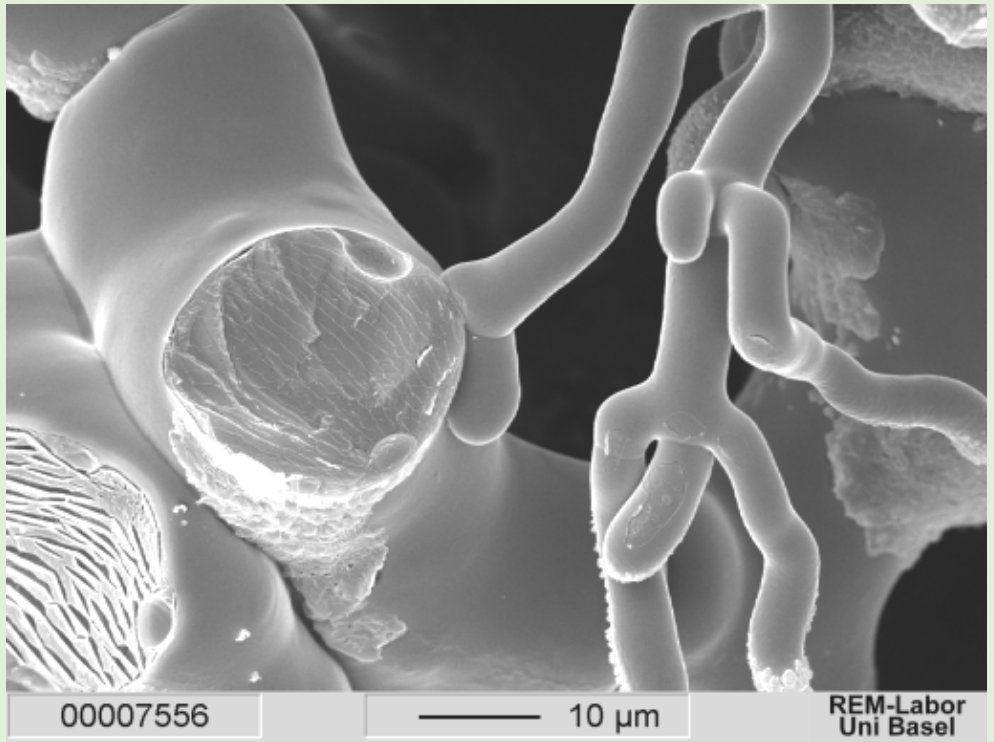
intact plant surface

- CWDEs (cell wall-degrading enzymes)
- force exertion

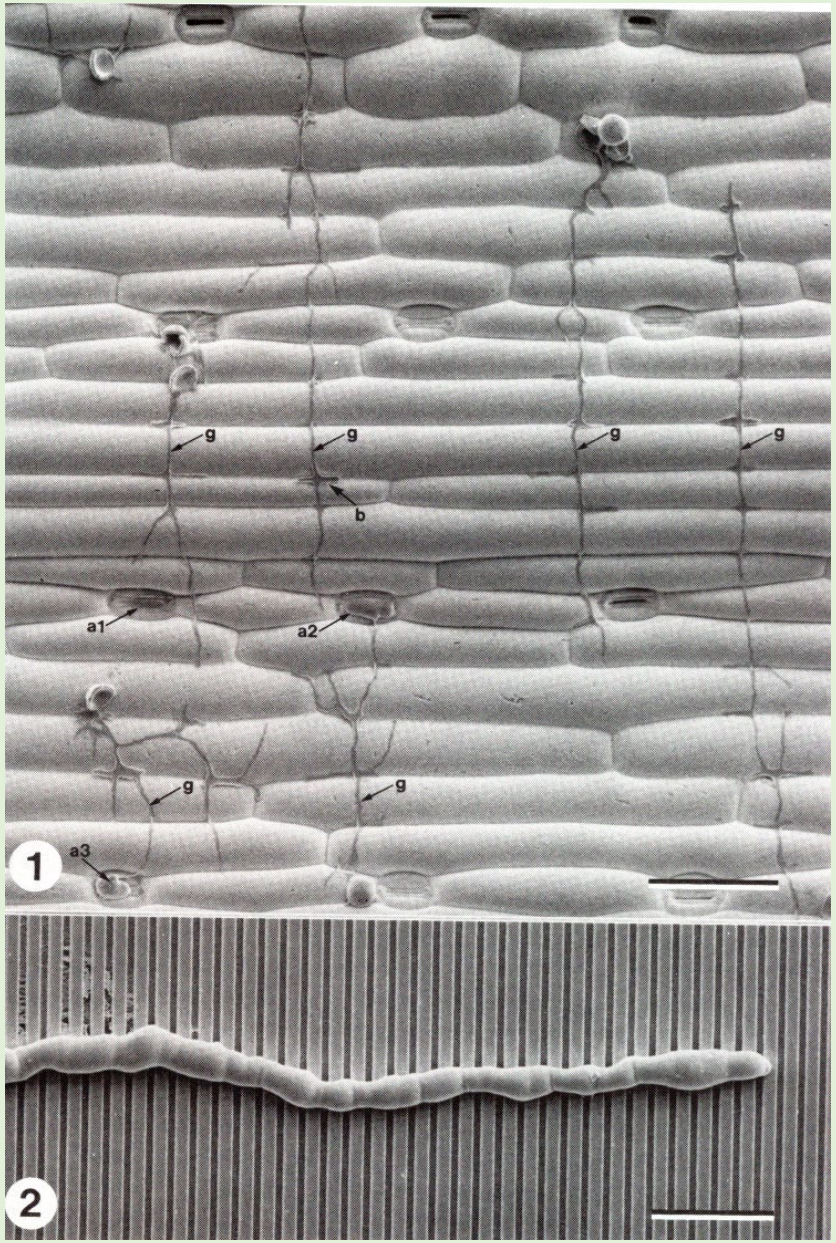
Encontrar e penetrar estômatos

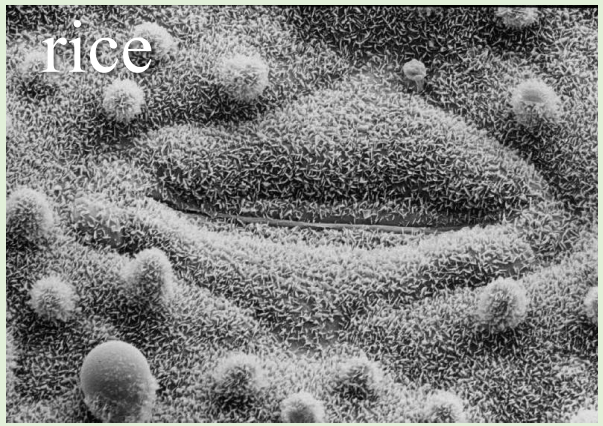


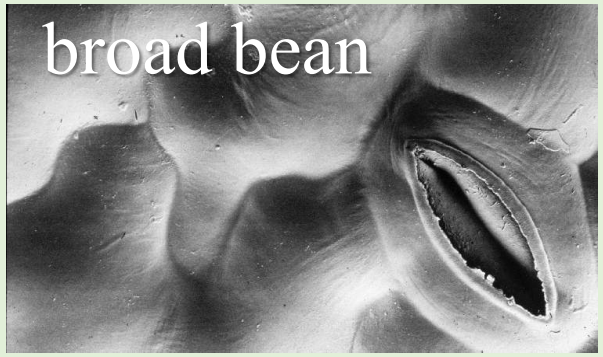


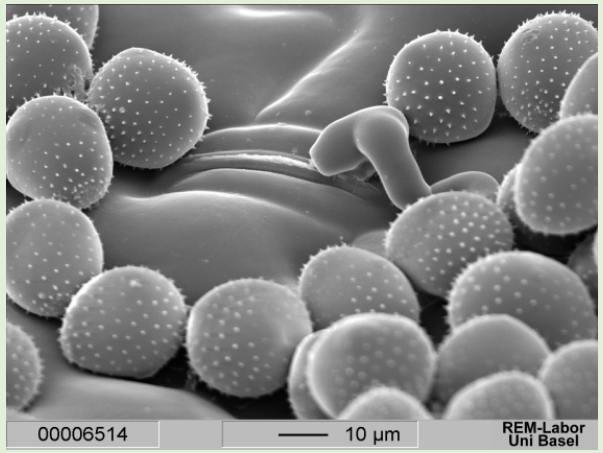


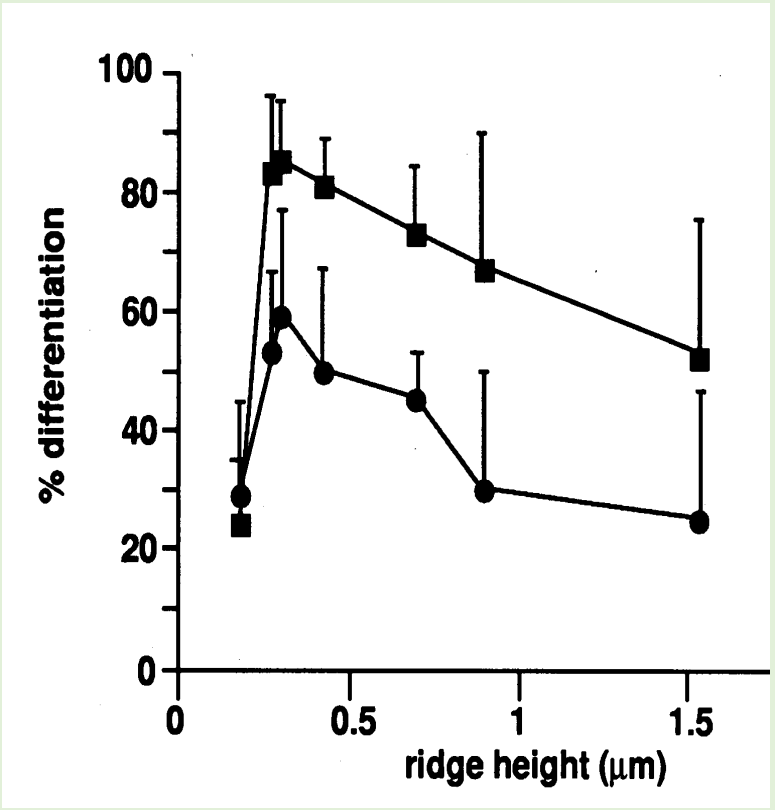
Encontrar e penetrar estômatos



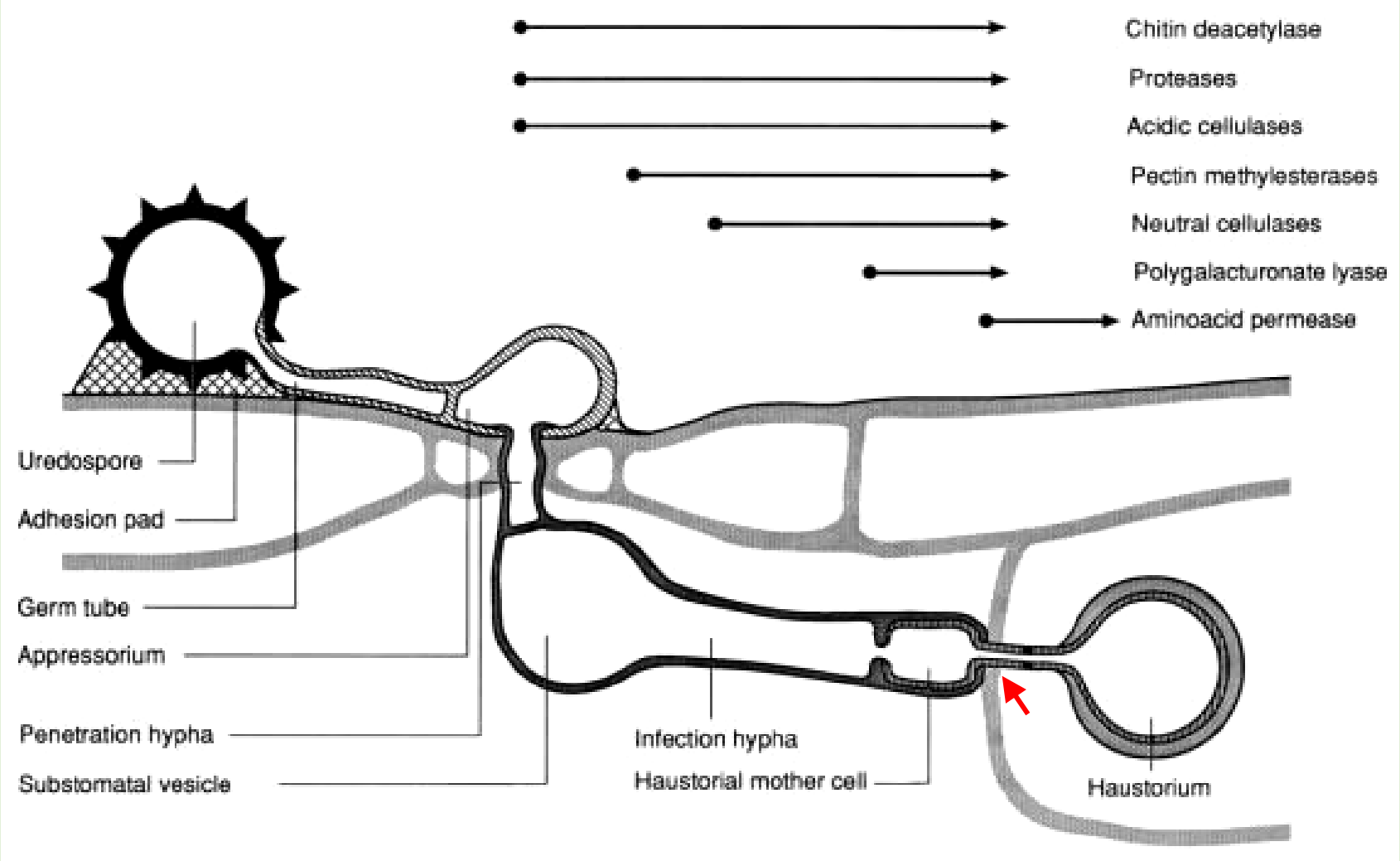








Encontrar e penetrar estômatos

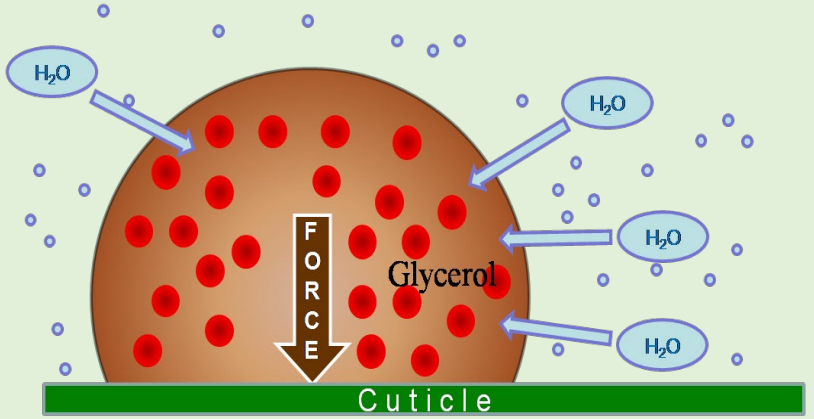


Infecção vegetal

Force or enzymatic lysis, different ways of host invasion

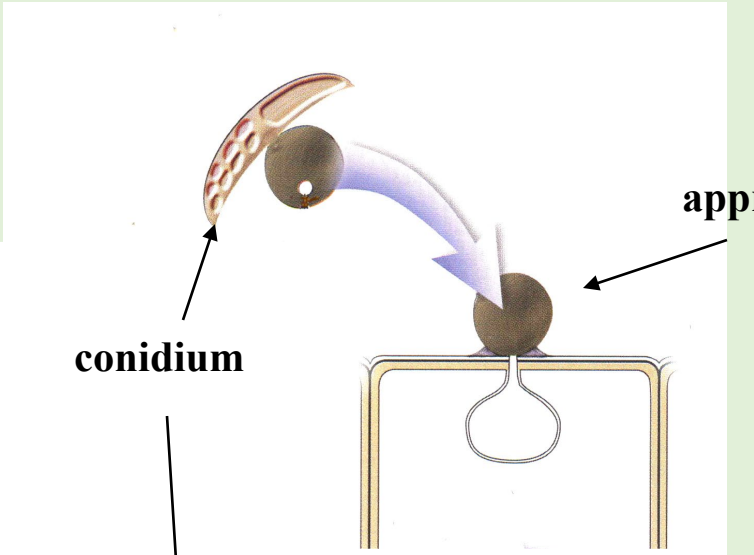
natural openings

- stomatal pores
- hydrathodes

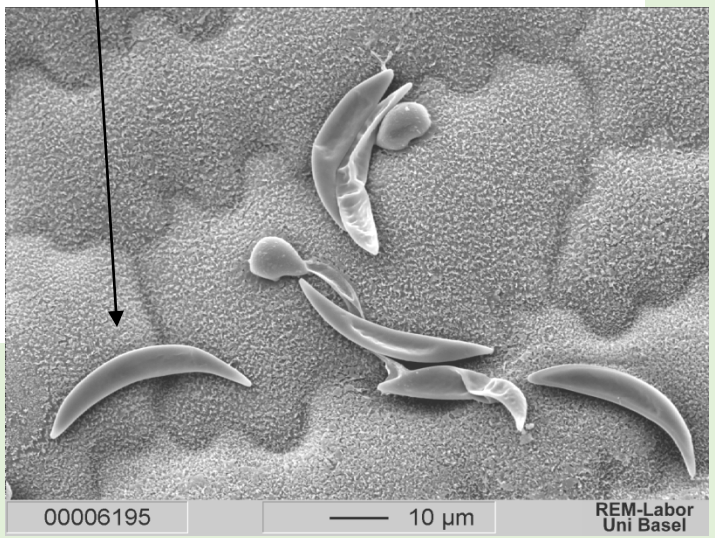


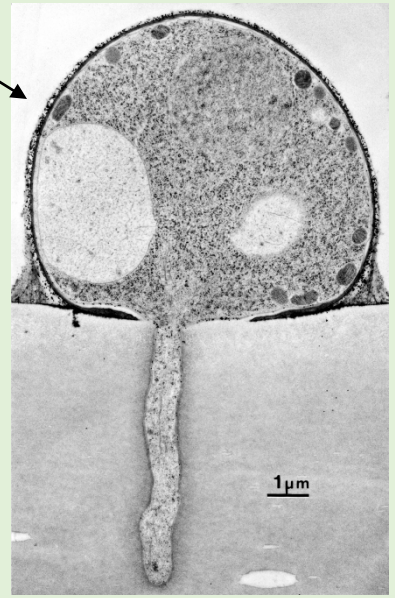
intact plant surface

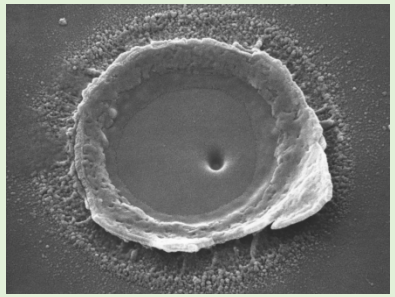
- CWDEs (cell wall-degrading enzymes)
- force exertion

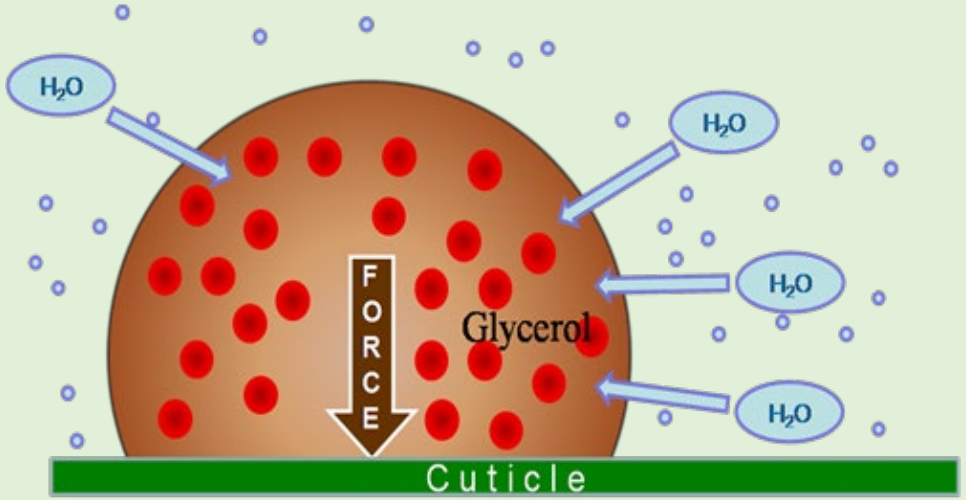


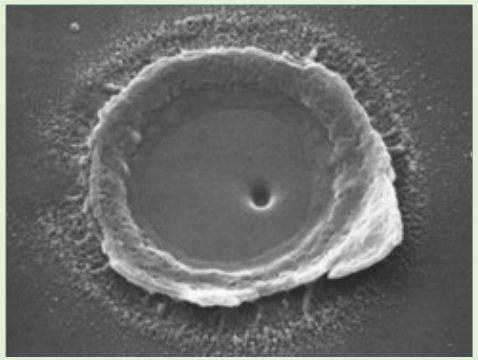
Examples: ***Colletotrichum graminicola***
Magnaporthe oryzae
***Phyllosticta* spp.**



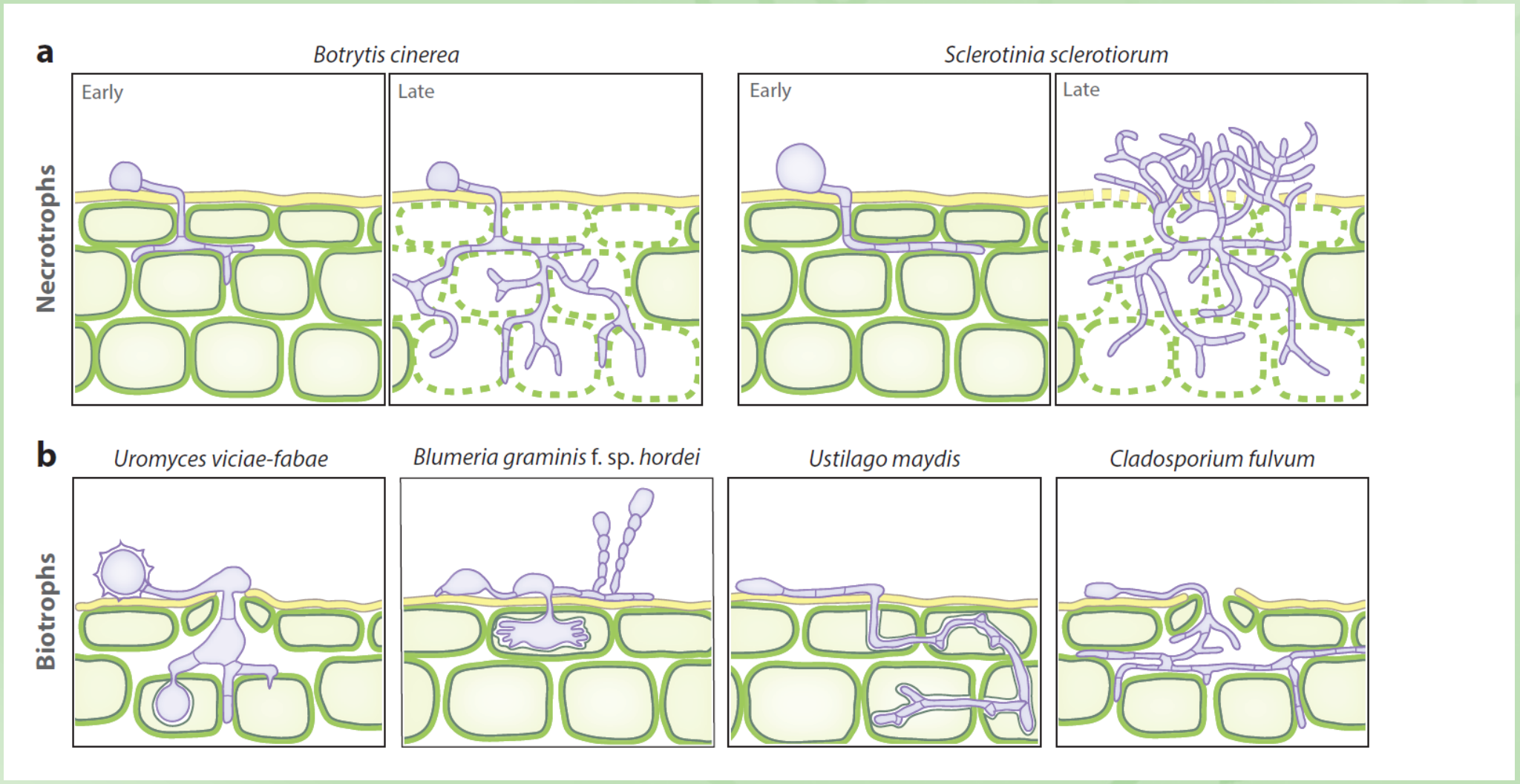




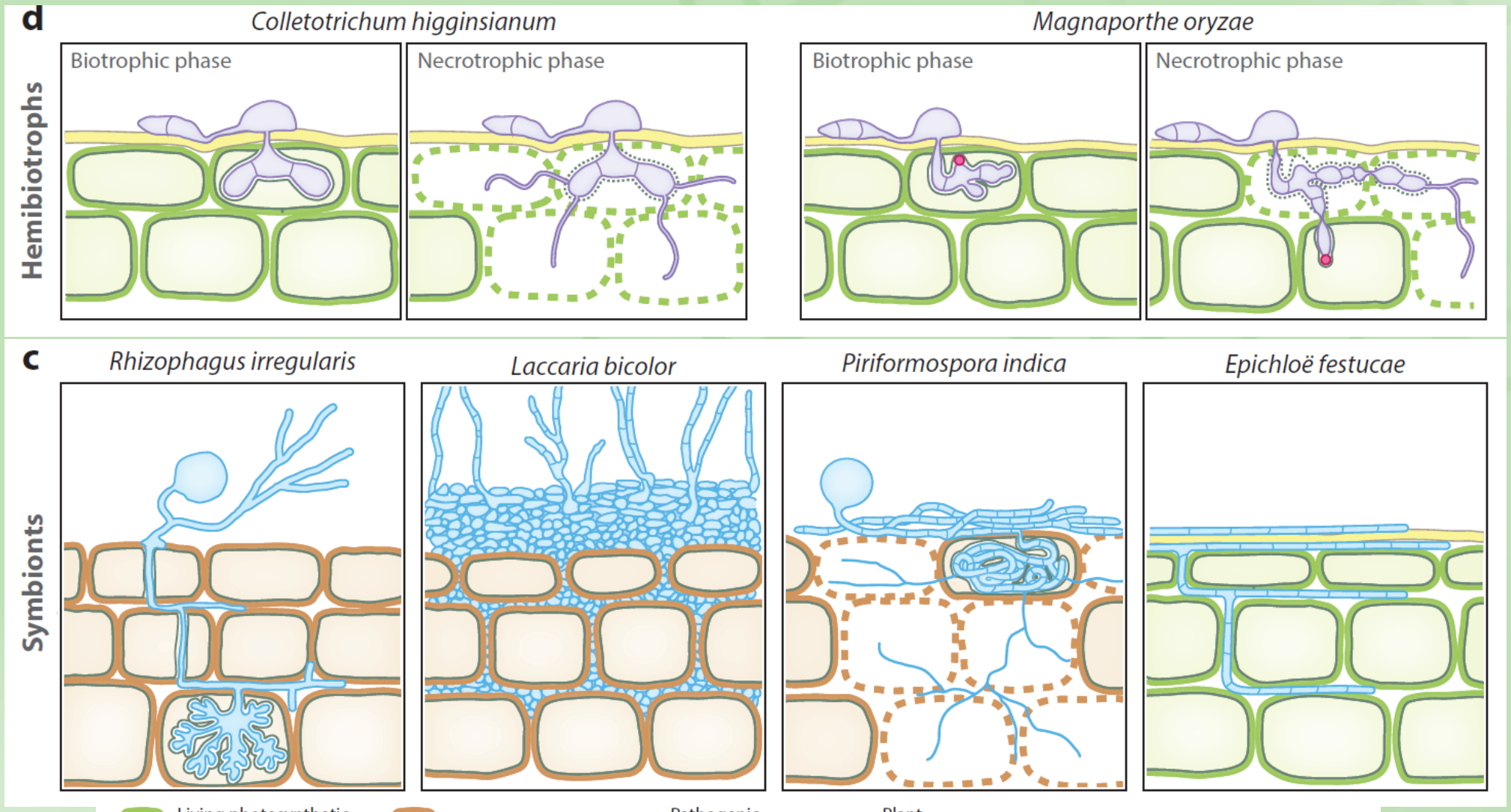




Estratégias de interação de diferentes patógenos com os hospedeiros – lifestyles



Estratégias de interação de diferentes patógenos com os hospedeiros – lifestyles



Fungal lifestyles

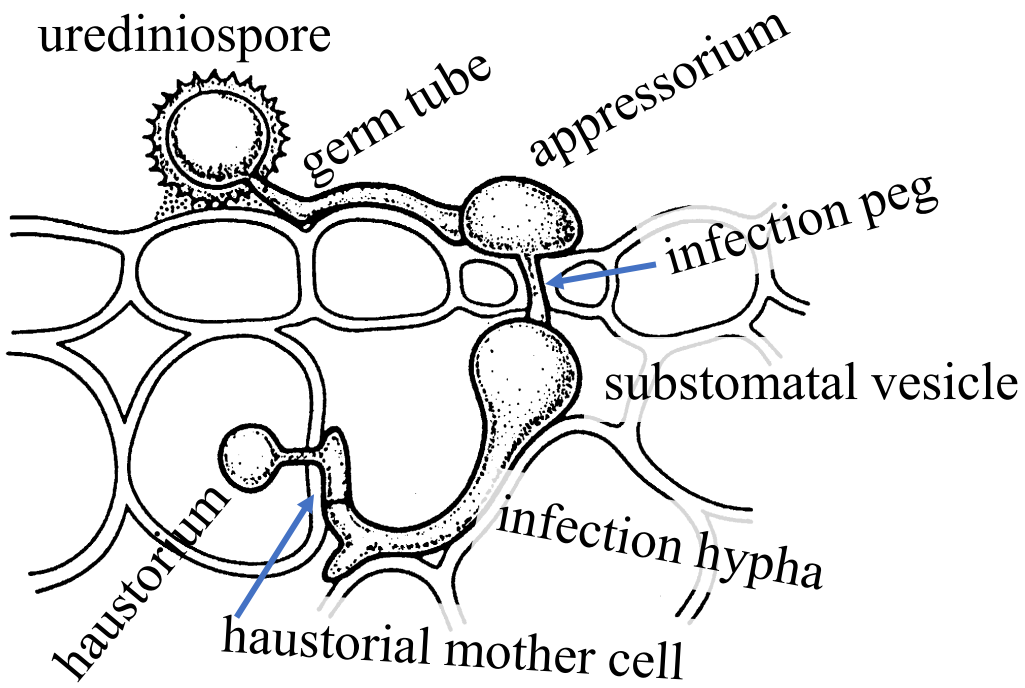
Fungos fitopatogênicos diferenciam estruturas de infecção mais ou menos complexas, muitas vezes refletindo seu estilo de vida

Biotrofia

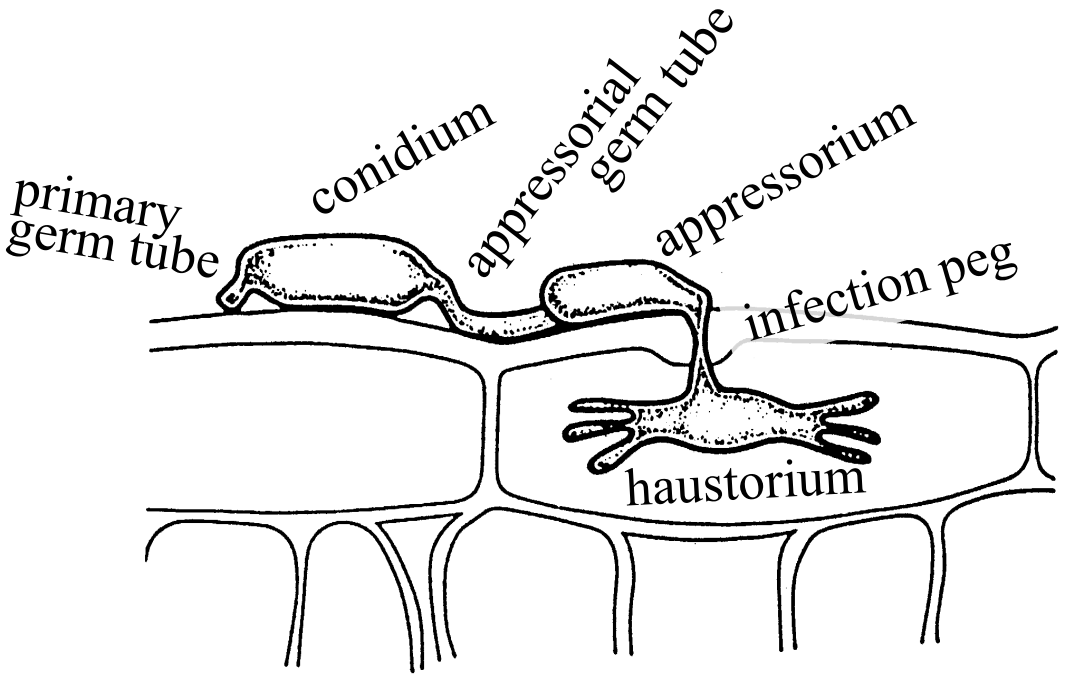
- Os fungos biotróficos requerem células hospedeiras vivas - Se a célula hospedeira morre, o patógeno também morre
- A maioria dos fungos biotróficos diferencia haustorio, mas alguns vivem como endófitos
- Exemplos: ferrugens, oídio, vários míldios e o protista Plasmodiophora brassicae, causador da doença da raiz do clube de plantas brassicaceae

Biotrofia – Estruturas de infecção

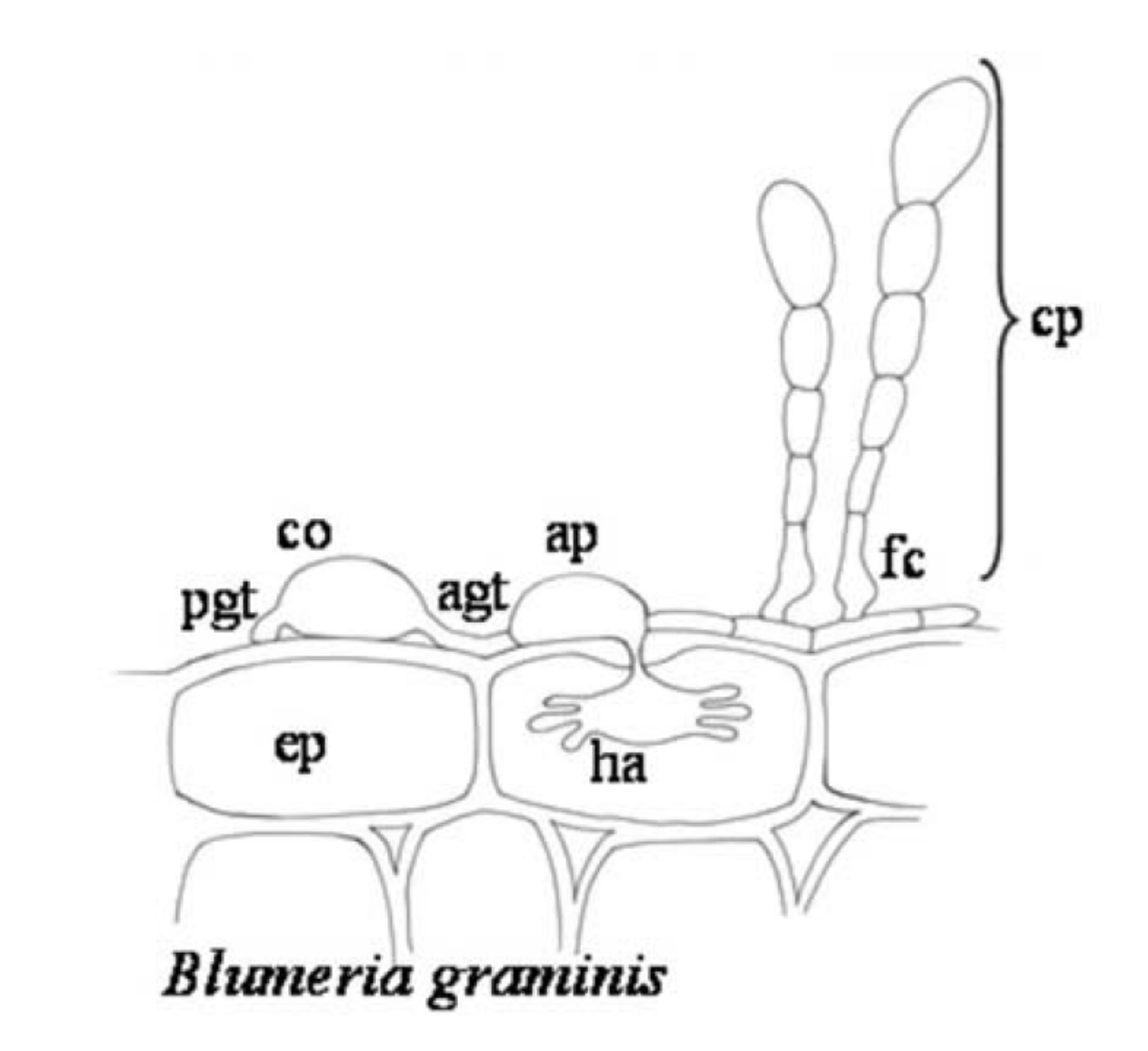
Uromyces viciae-fabae



Blumeria graminis f. sp. *hordei*

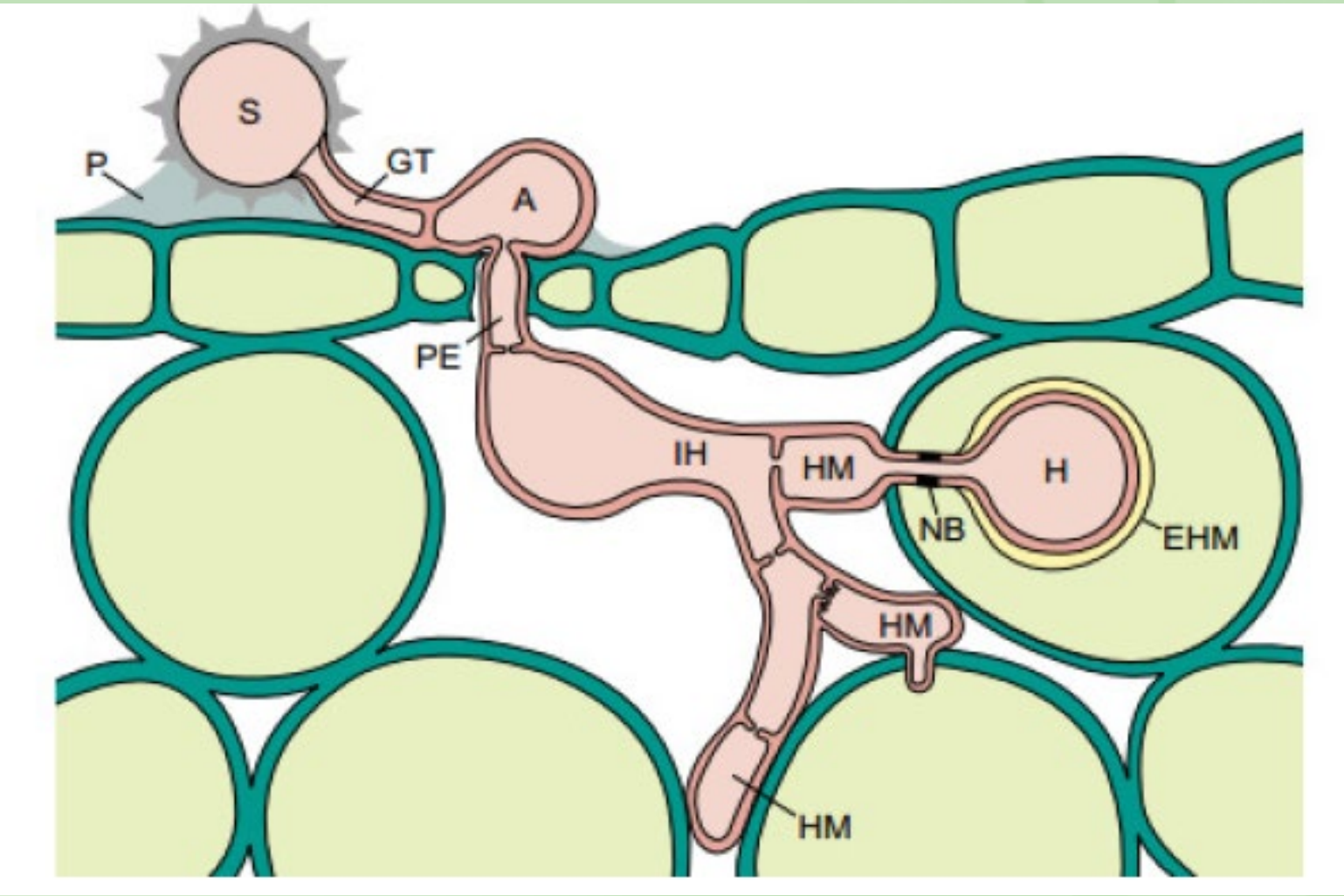


Biotrófico obrigatório *Blumeria graminis*



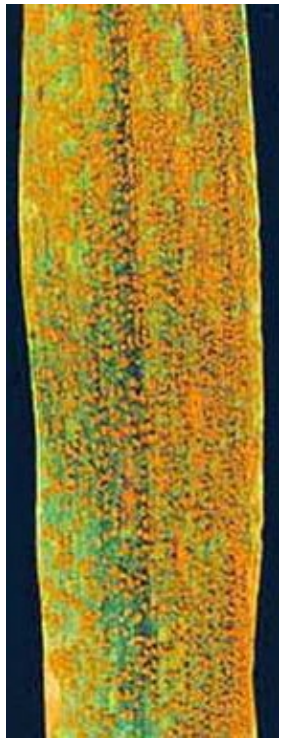
Primary and an appressorial germ tube (pgt; agt),
Appressorium (ap)
Epidermal cell of the host (ep)
Haustorium (ha)
Conidiophores (cp),
Foot cell (fc).

Infecção pelo fungo biotrófico *Uromyces fabae*

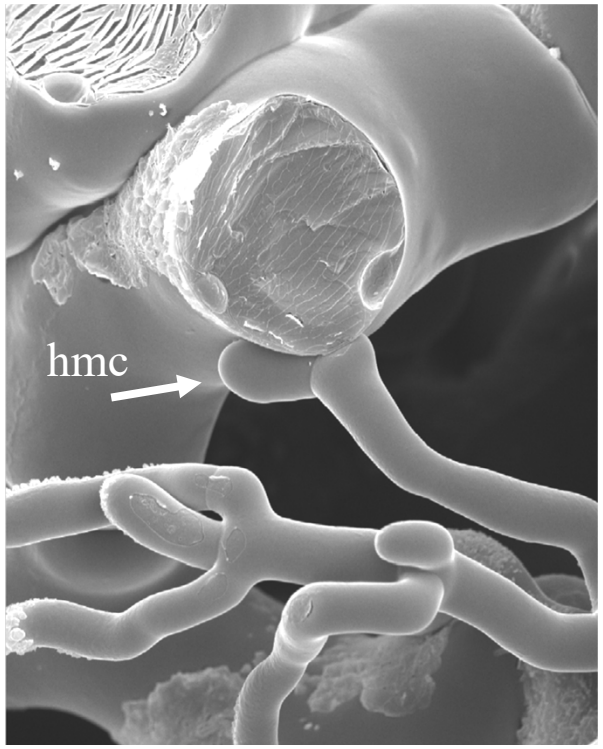


S: anurediospore
GT: Tubo germinativo
P: adhesion pad
A: appressorium (entra pelo estômato)
PE: Hifa de penetraçáo
IH: Hifa de penetração
HM: haustorial mother cell (HM)
H: haustorium
NB: dark-staining neck-band
EHM: extrahaustorial Membrane

Biotrofia



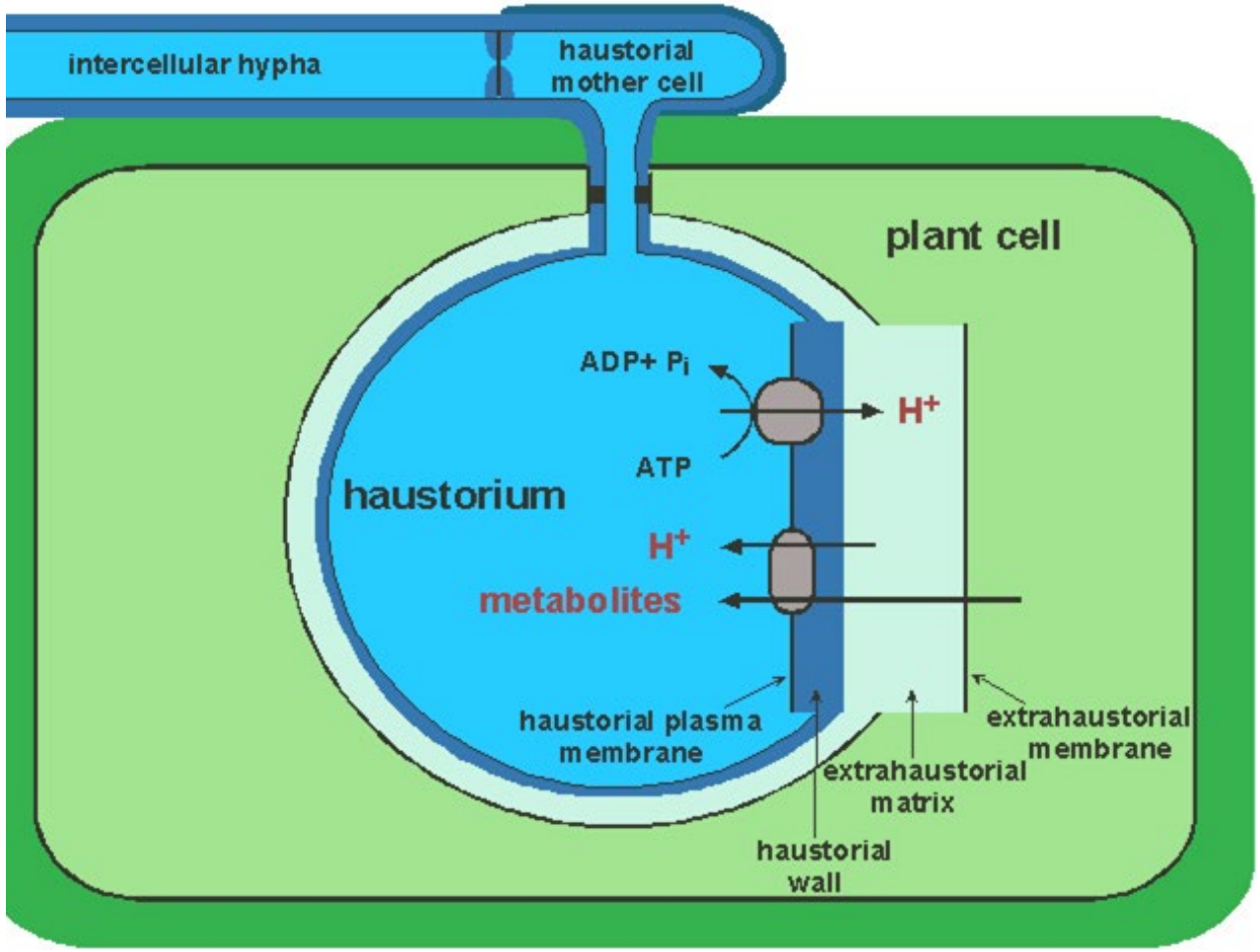
Leaf rust disease symptom



Fungal hyphae in the intercellular space of the host leaf

Função do haustorio

Hexose and amino acid uptake through haustorial H^+-symport.



Fungos biotróficos de importância econômica

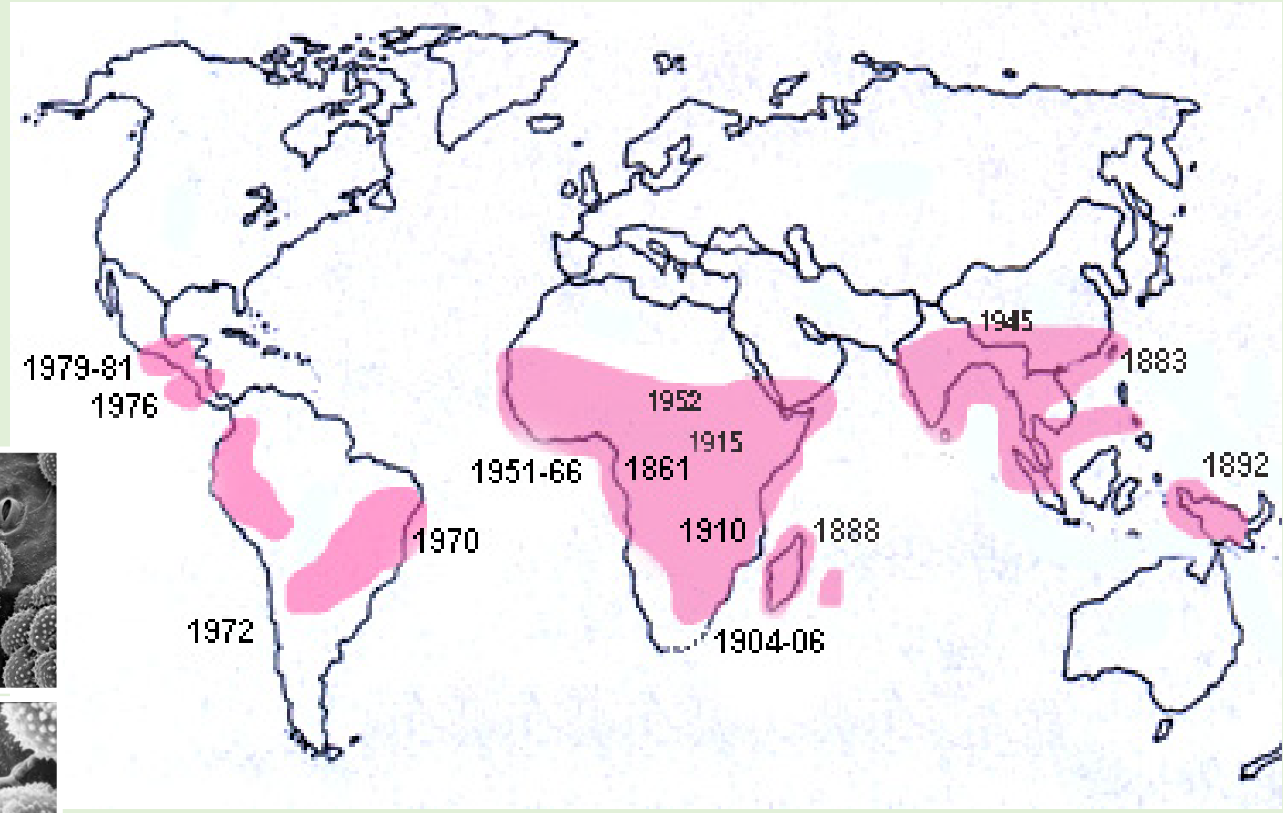
Species	Host(s)	Disease
Erysiphe graminis	Grasses	Powdery Mildew of Grasses
Erysiphe macrospora	Elm	Powdery Mildew of Elm
Erysiphe necator	Grape	Powdery Mildew of Grape
Leveillula taurica	Cotton	Powdery Mildew of Cotton And Many Other
Phyllactinia alnicola	Oak, Alder, Lilac	Powdery Mildew of Oak, Alder, Lilac, Currant
Ovariopsis	Hazel, Ash, Birch	Powdery Mildew of Hazel, Ash, Birch
Podosphaera leucotricha	Apple, Plum	Powdery Mildew of Apple, Plum
Podosphaera macularis	Hops	Powdery Mildew of Hops
Coleosporium ipomoeae	Sweet Potato, Pine Needle	Sweet Potato Rust, Pine Needle Rust
Cronartium flaccidum	Scotch Pine Blister	Scotch Pine Blister Rust
Melampsora epitea	Poplar-Conifer	Poplar-Conifer Rusts
Phakopsora pachyrhizi	Soybean	Soybean Rust
Kuehneola uredinis	Blackberry	Blackberry Stem and Leaf Rust
Phragmidium tuberculatum	Blackberry, Rose, Rosaceous Hosts	Rust of Blackberry, Rose, Rosaceous Hosts
Phragmidium violaceum	Blackberry	Blackberry Rust
Gymnosporangium sabinae	Pear	Incense Cedar-Pear Rust, Juniper Gall Rust, Pear Trellis Rust
Uromyces gladioli	Iridaceae	Rusts of Iridaceae
Tranzschelia discolor	Peach	Peach Rust
Hemileia vastatrix	Coffee	Coffee Rust

Biotrofia

Rust fungi - pathogens causing dramatic yield losses

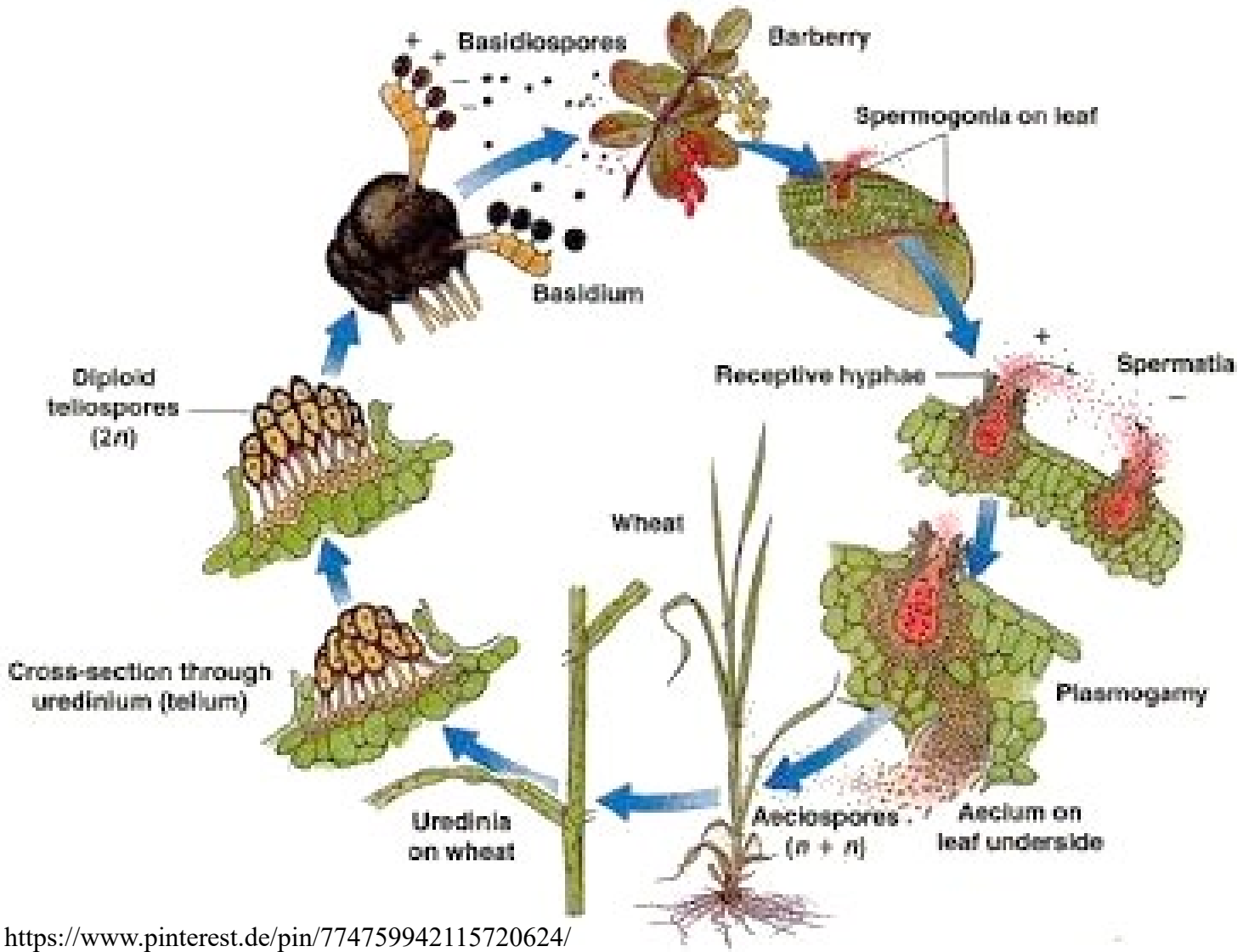
The coffee rust fungus *Hemileia vastatrix*

Worldwide coffee rust migration



Biotrofia

Rust fungi - pathogens with a complex disease cycle



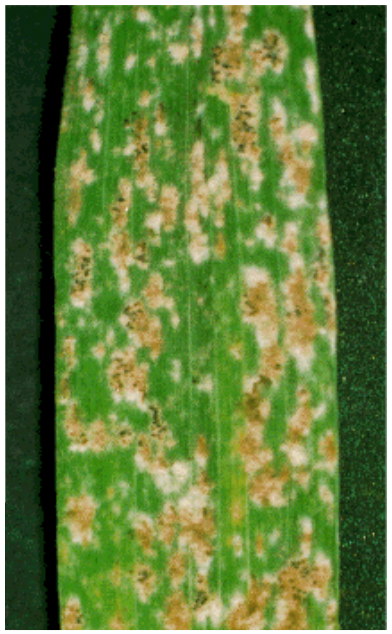
https://www.pinterest.de/pin/774759942115720624/

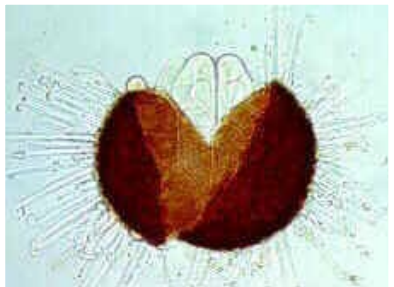
The wheat stem rust fungus
Puccinia graminis f. sp. *tritici*

Five spore types,
three of which are infectious:

teliospores	diploid	2n
basidiospores	haploid	n
spermatia	haploid	n
aeciospores	dikaryotic	n+n
urediniospores	dikaryotic	n+n

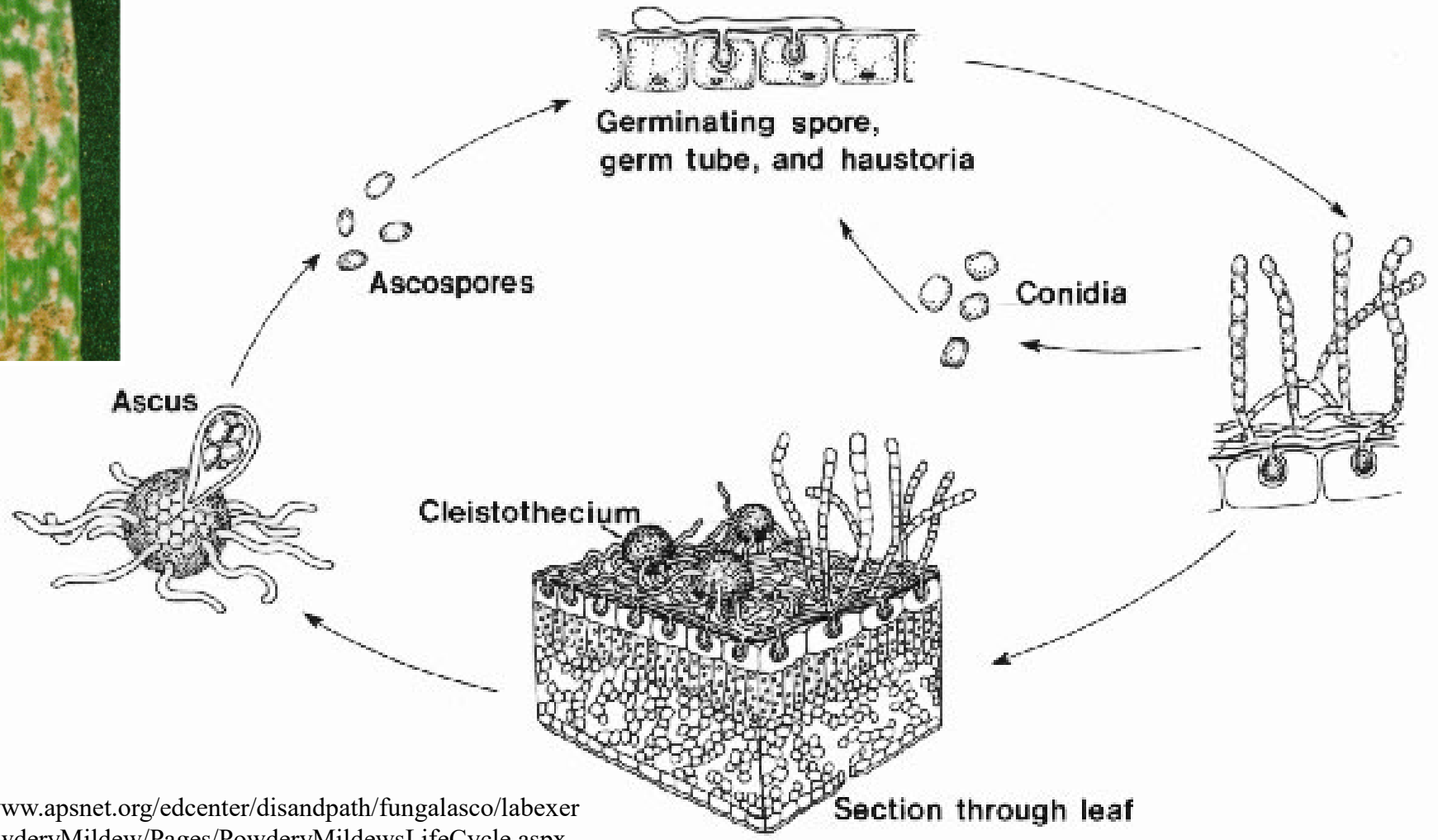
Biotrofia





Powdery mildew of cereals

Barley powdery mildew *Blumeria graminis* f. sp. *hordei*



https://www.apsnet.org/edcenter/disandpath/fungalasco/labexercises/PowderyMildew/Pages/PowderyMildewsLifeCycle.aspx

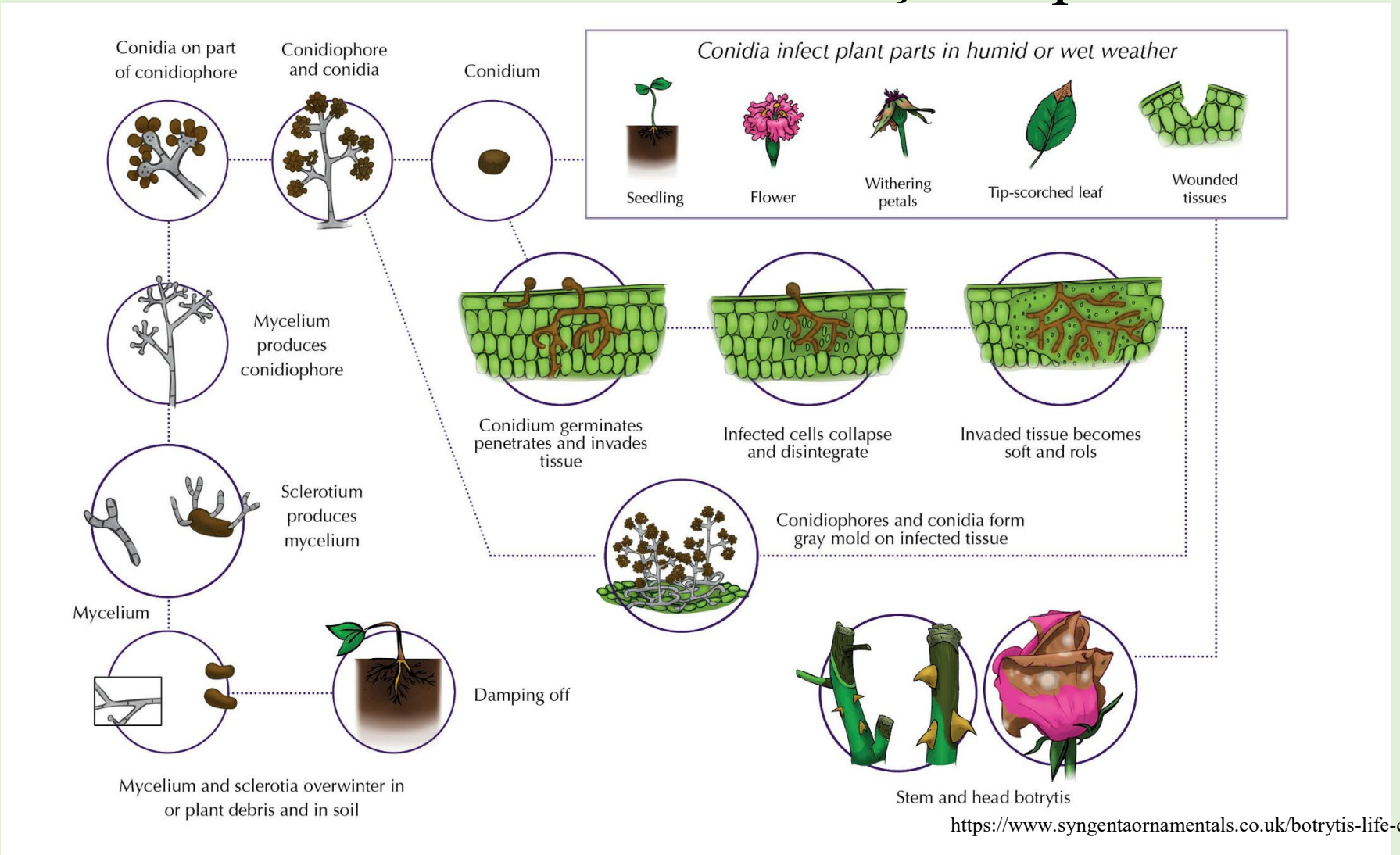
Necrotrofia

Necrotróficos matam o hospedeiro e se alimentam de céluars mortas

Botrytis cinerea

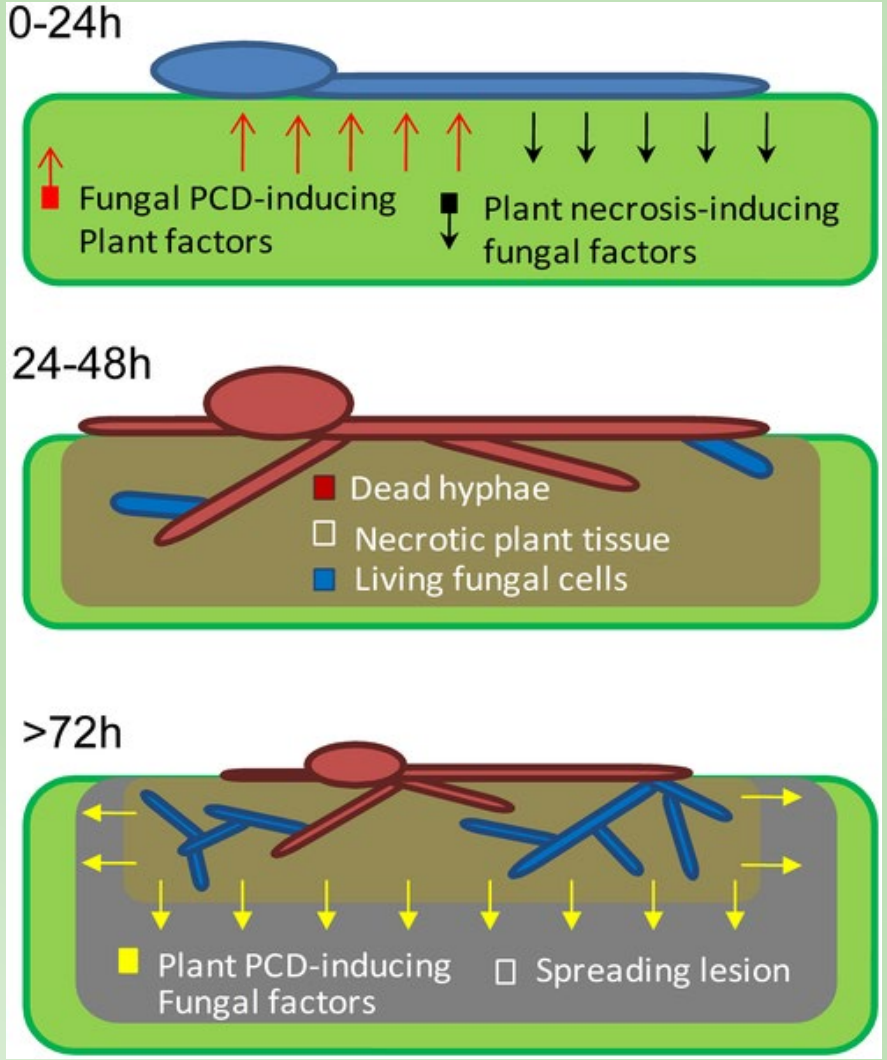
Necrotrofia

Não necessitam de estruturas de infecação especializadas



https://www.syngentaornamentals.co.uk/botrytis-life-cycle

Necrotróficos: Modelo mostrando o papel da PCD (morte celular programada) no início da infeção em Botrytis cinerea



- **Necrotróficos não colonizam células vivas**
- Morte celular programada do fungo induzida por fatores vegetais
- Necrose de tecidos vegetais induzida por fatores fúngicos
- PCD do vegetal induzido por fatores fúngicos

Shlezinger N, Minz A, Gur Y, Hatam I, Dagdas YF, et al. (2011) Anti-Apoptotic Machinery Protects the Necrotrophic Fungus Botrytis cinerea from Host-Induced Apoptotic-Like Cell Death during Plant Infection. PLOS Pathogens 7(8): e1002185. https://doi.org/10.1371/journal.ppat.1002185
https://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1002185

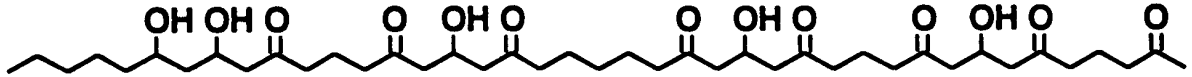
PLOS PATHOGENS

Necrotrofia

- Toxins / ROS kill host cells
- Nutrients become available
- Plant defense is prevented

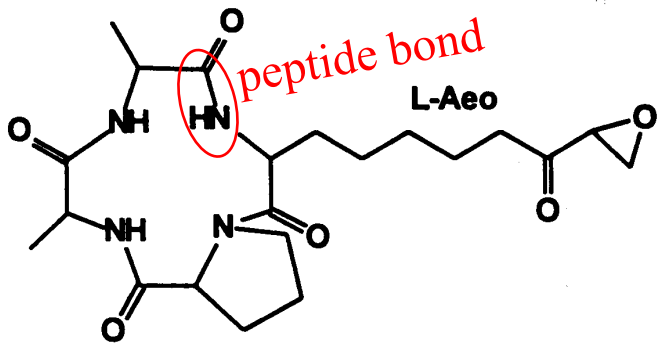
Northern Corn Leaf Blight
Cochliobolus carbonum
Helminthosporium carbonum

polyketide



T-toxin (*Cochliobolus heterostrophus* race T)

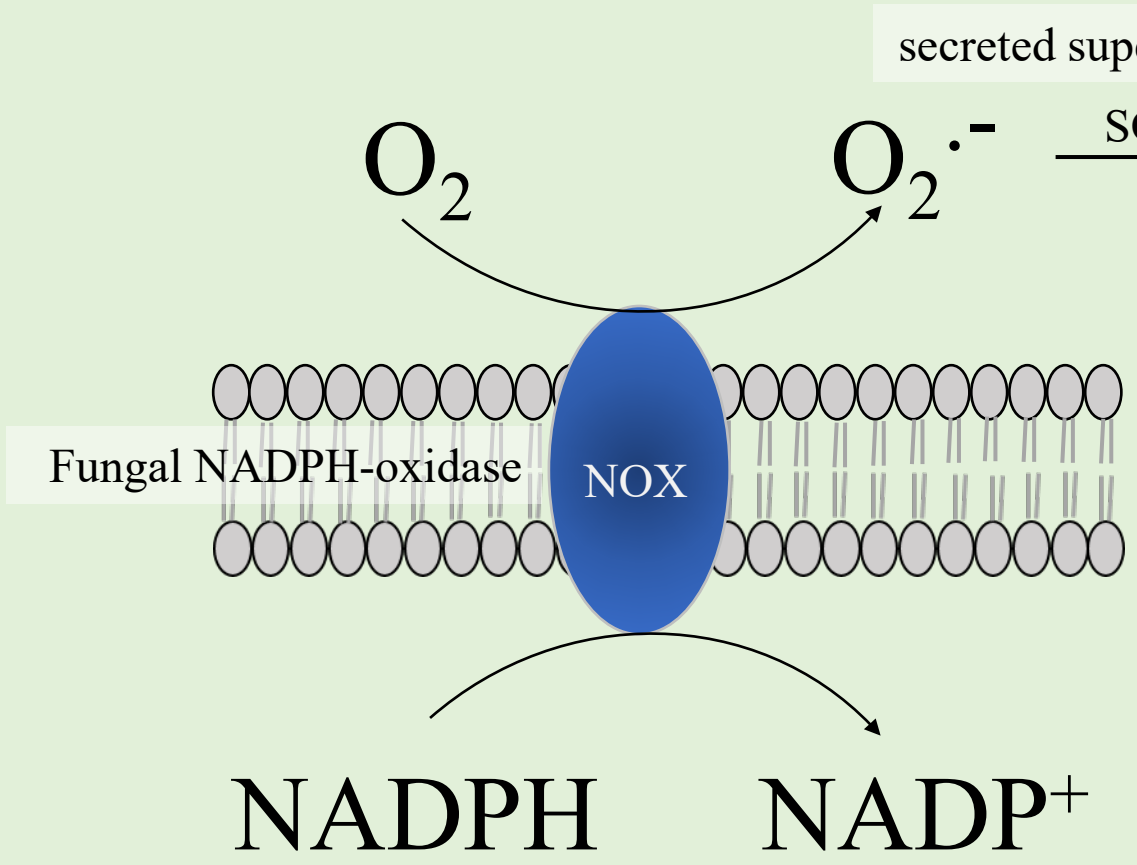
non-ribsomal peptide



HC-toxin (*Cochliobolus carbonum* race 1)

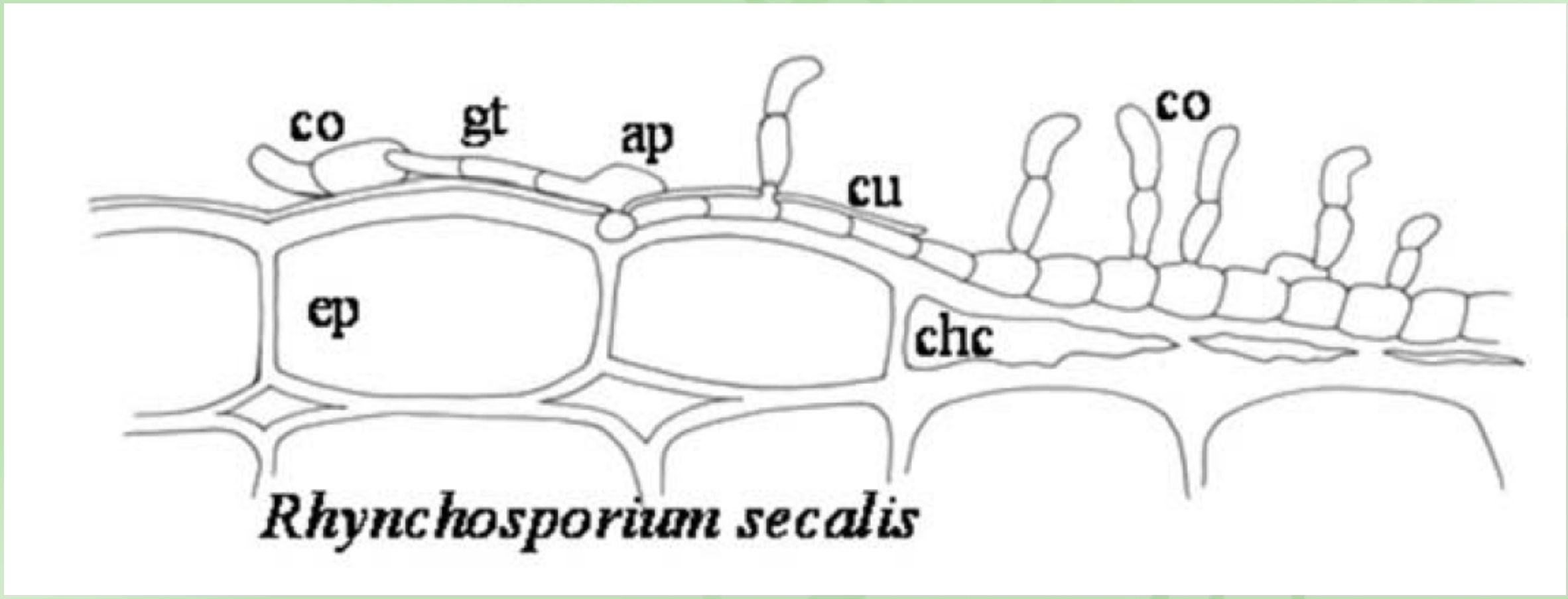
HC toxin: **cyclo(D-Pro-L-Ala-D-Ala-L-Aeo),**
Aeo = 2-amino-9,10-epoxy-8-oxodecanoic acid

Necrotrofia



- Toxins / ROS kill host cells
- Nutrients become available
- Plant defense is prevented

Necrotófico *Rhynchosporium secalis*



Germ tube (gt); appressorium (ap); cuticle (cu)

Hemibiotrofia

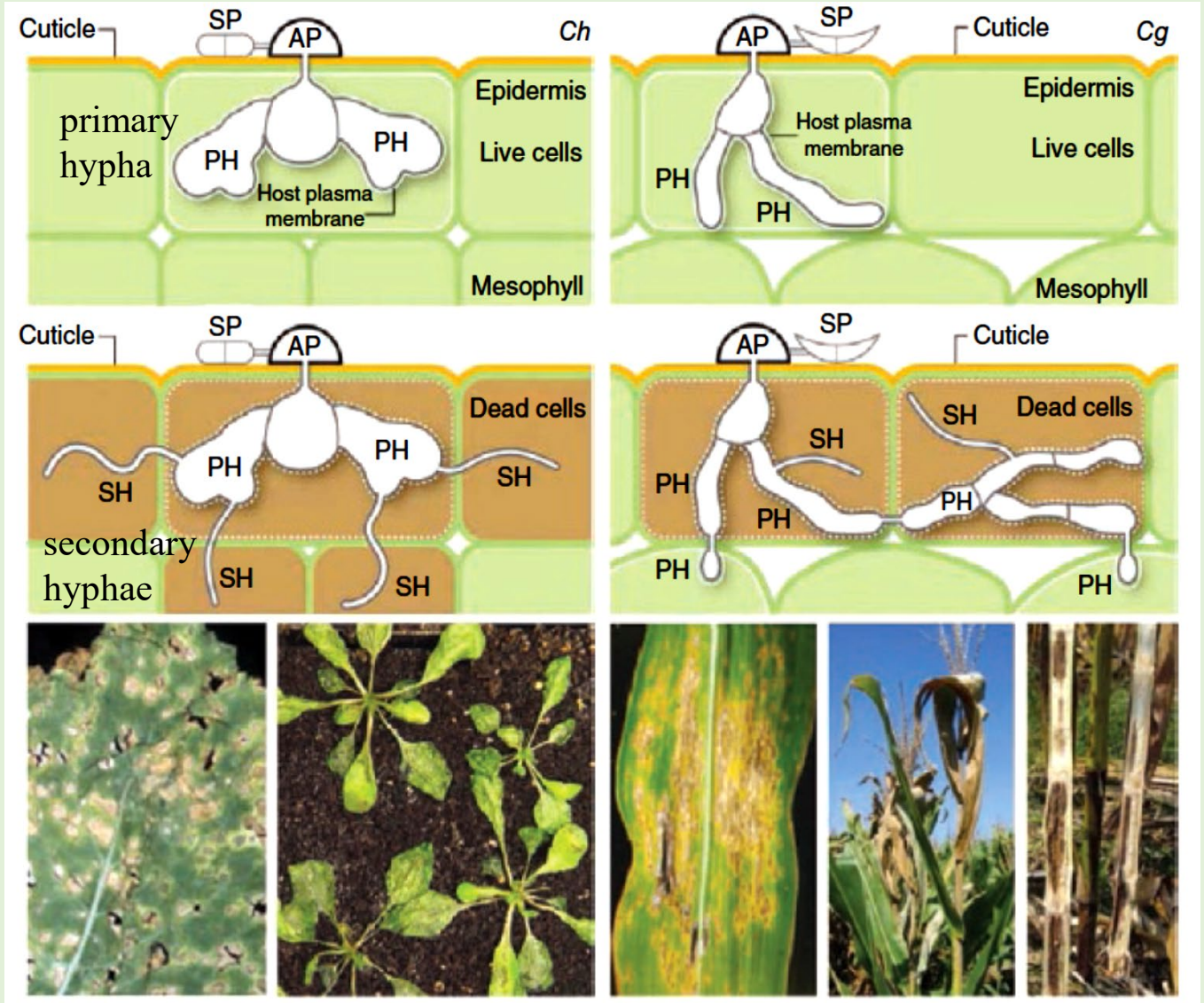
BIOTROFIA → NECROTROFIA

pathogens with a lifestyle switch

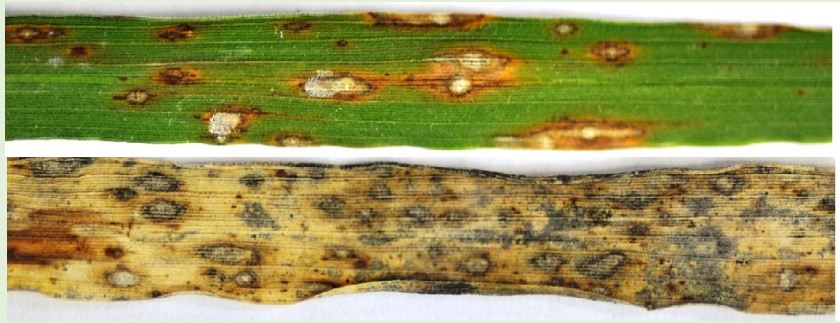
Diversas espécies de *Colletotrichum*:

C. higginsianum
C. graminicola

C. lindemuthianum
C. lagenarium
C. Gloeosporioides
C. abscissum

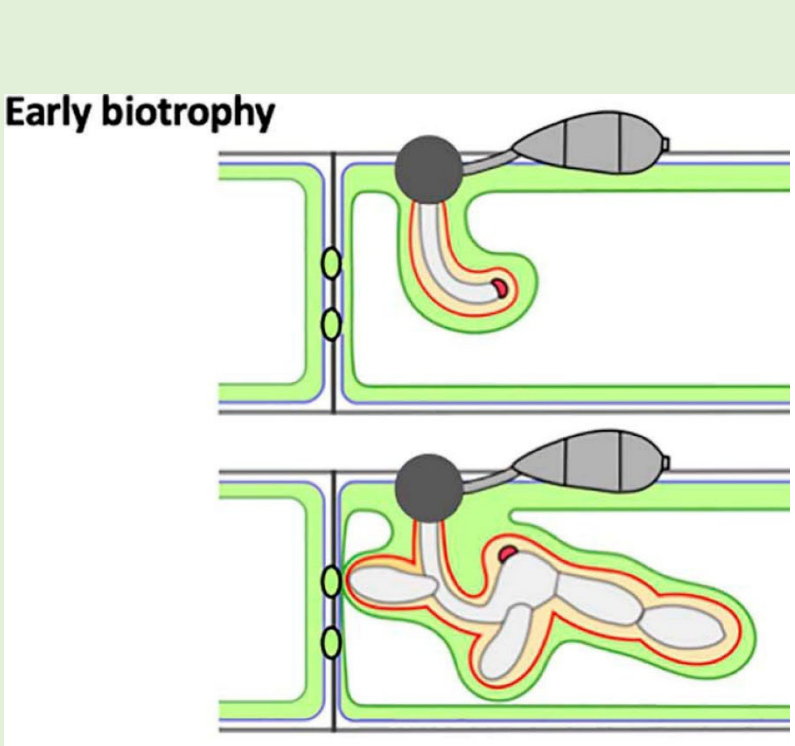


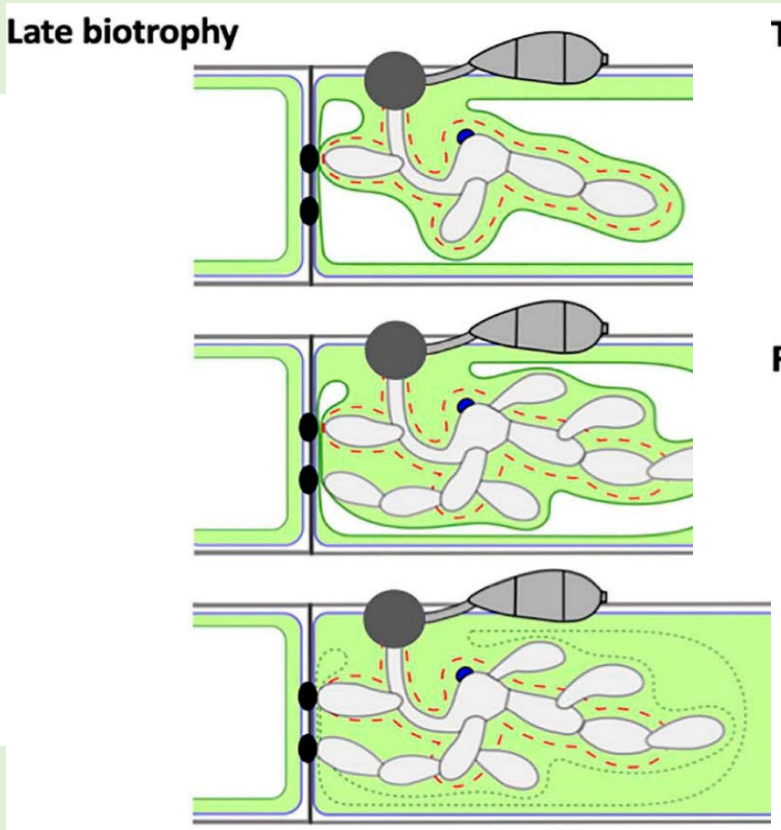
Hemibiotrofia

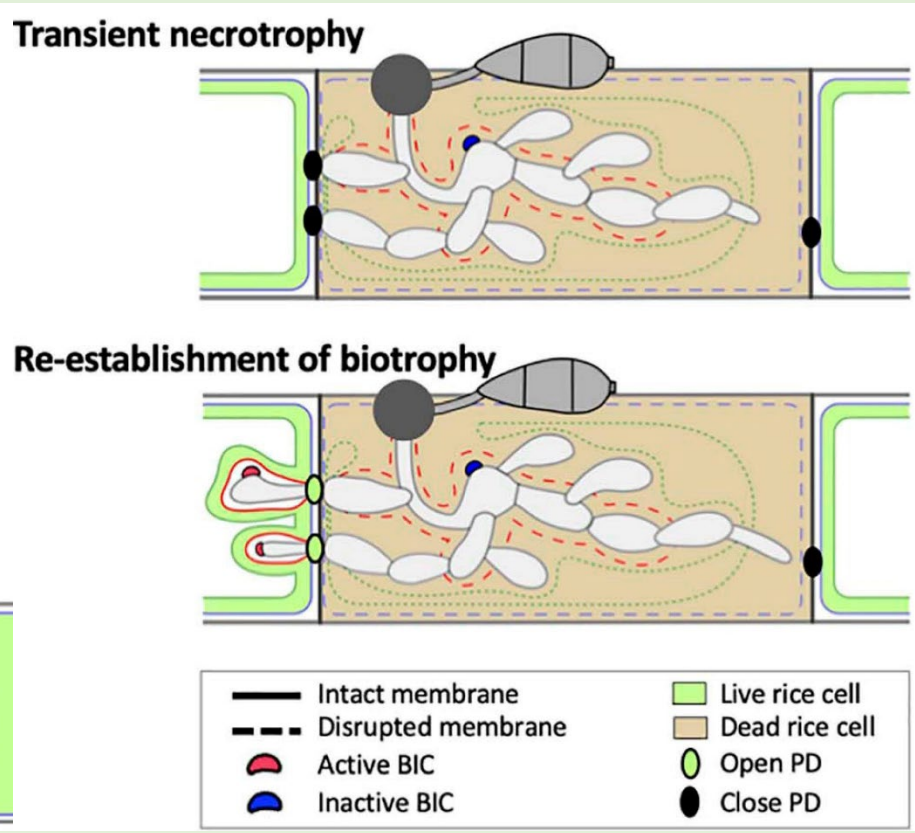


https://impb.ethz.ch/research/reseach-pb/research-pb/research-nkb/resistancessources.html

Magnaporthe oryzae

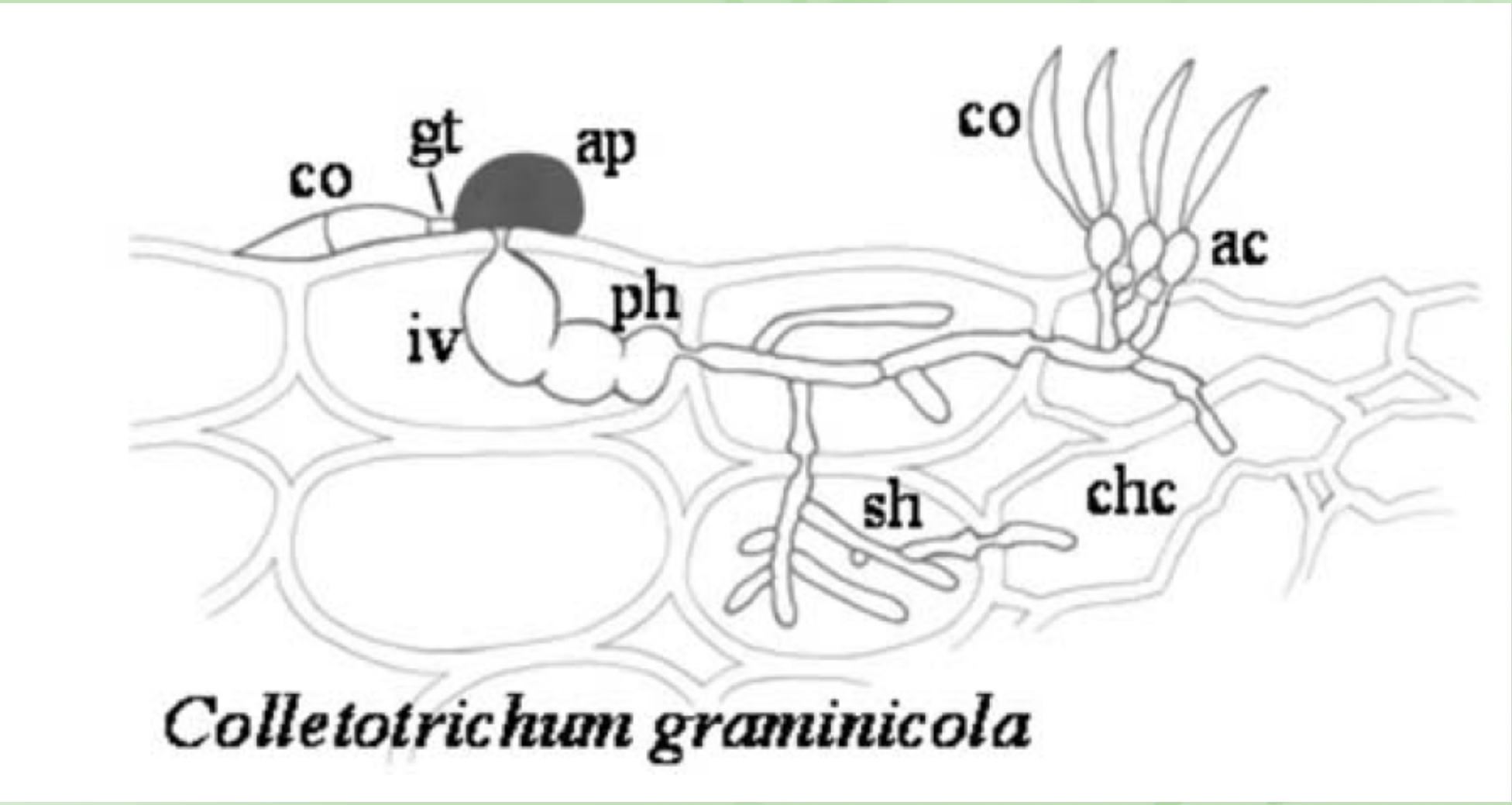






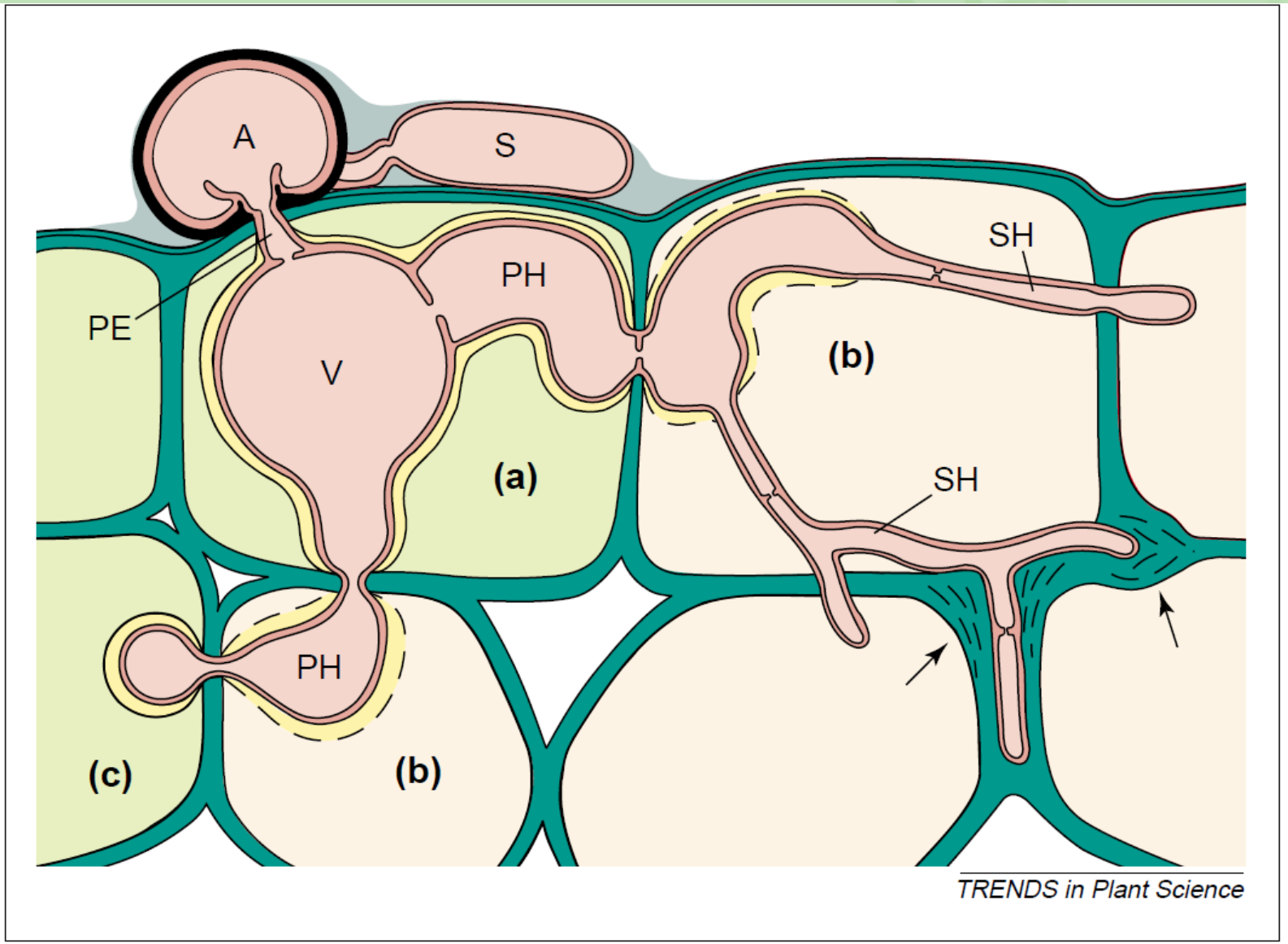
Jones et al. 2021 Front. Cell Dev. Biol. 9, 681734

Hemibiotrófico *Colletotrichum graminicola*



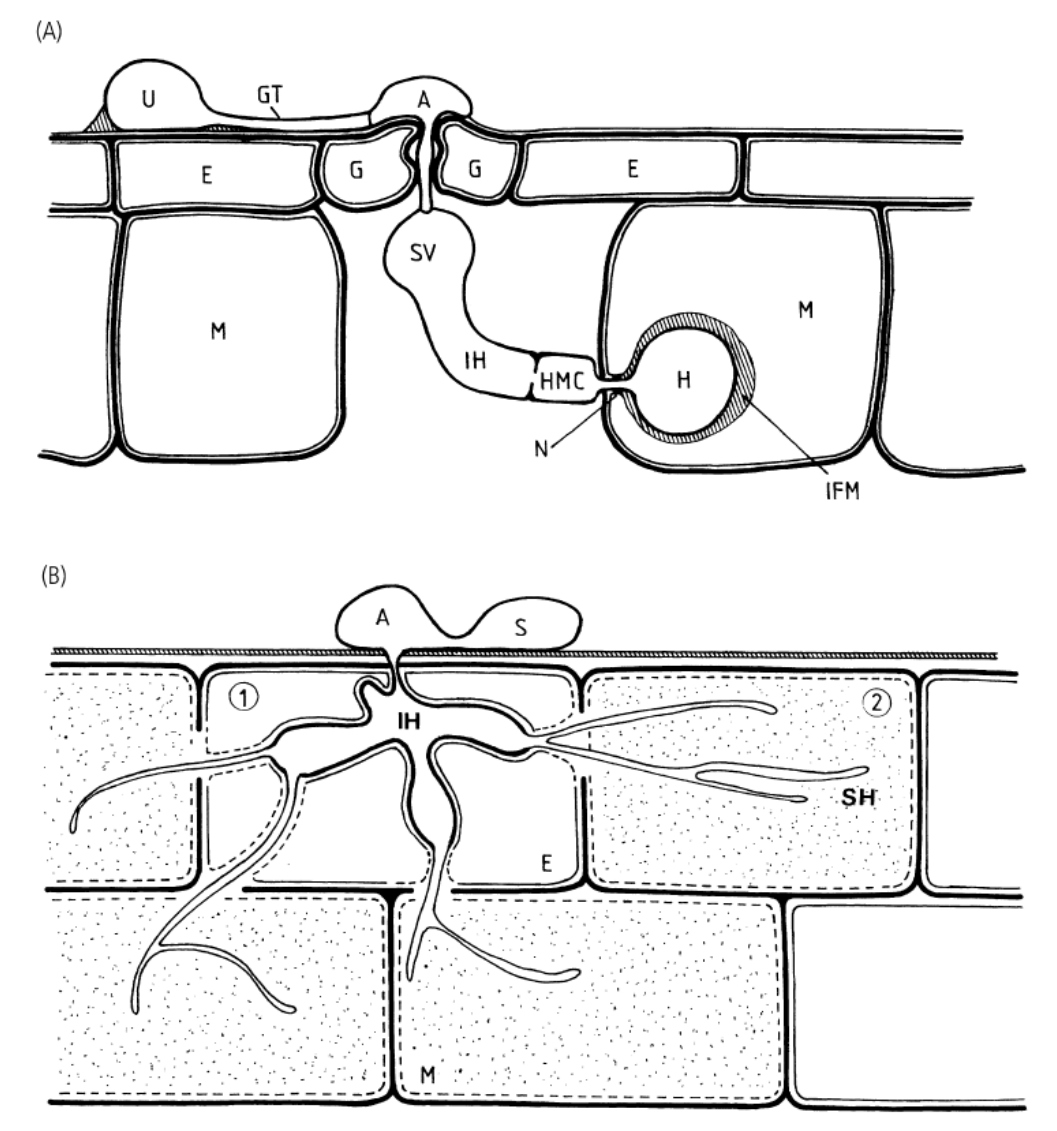
Conidium (co); germ tube (gt); melanized appressorium (ap); biotrophic infection vesicle (iv); primary hyphae (ph) necrotrophic secondary hyphae (sh); acervuli (ac)

Infecção pelo fungo hemibiotrófico *Colletotrichum lindemuthianum*

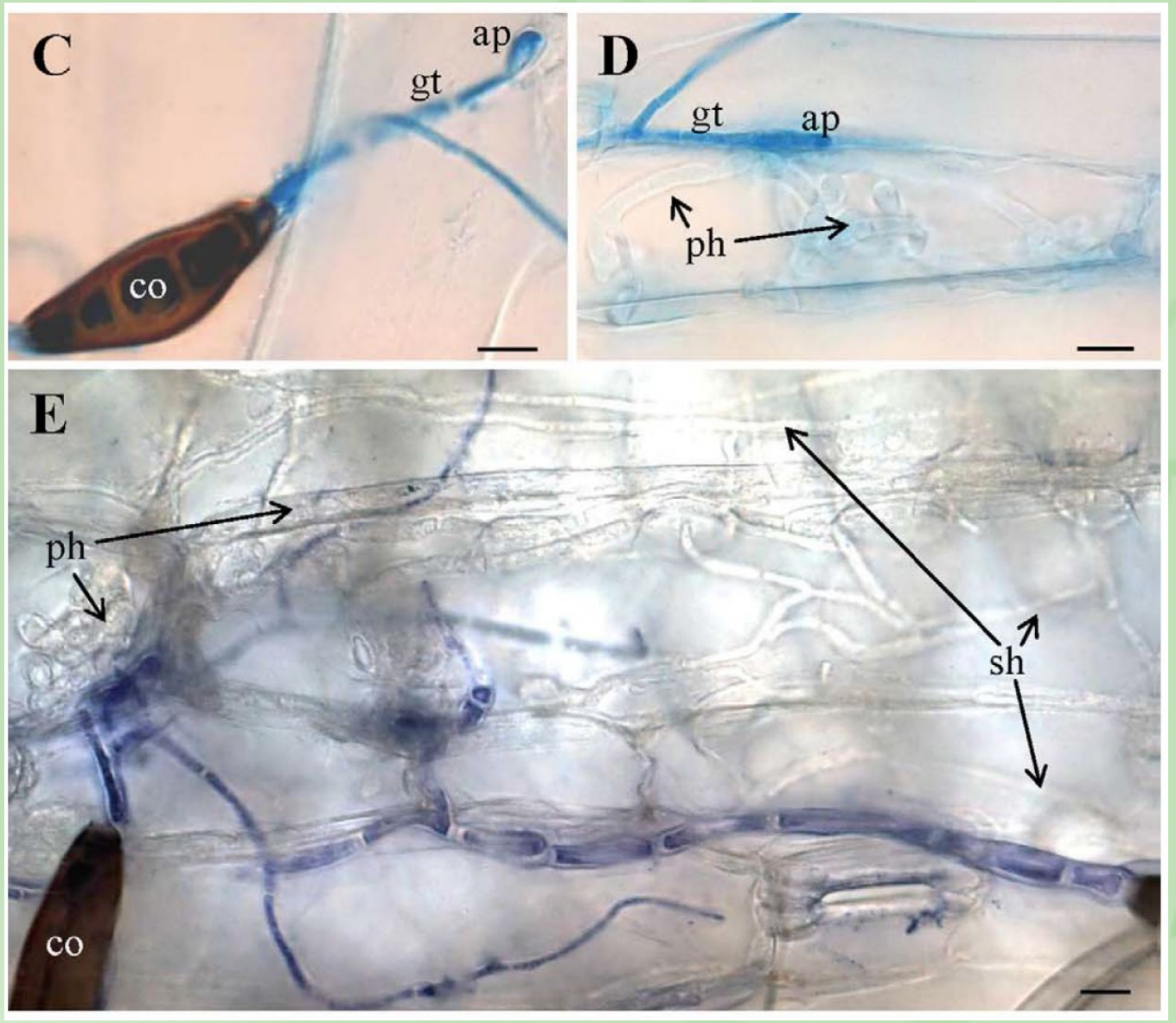


S: esporo
A: Apressório melanizado
PE: Hifa de penetração
V: Vesícula
PH: Hifa primária,
a) Fase biotrófica
Yellow: Matrix de separação entre fungo e hospedeiro
b) Morte cellular
SH: Hifa secundária
Arrows: Quebra de parede cellular mediada pela secreção de CWDEs pelas Hifas secundárias

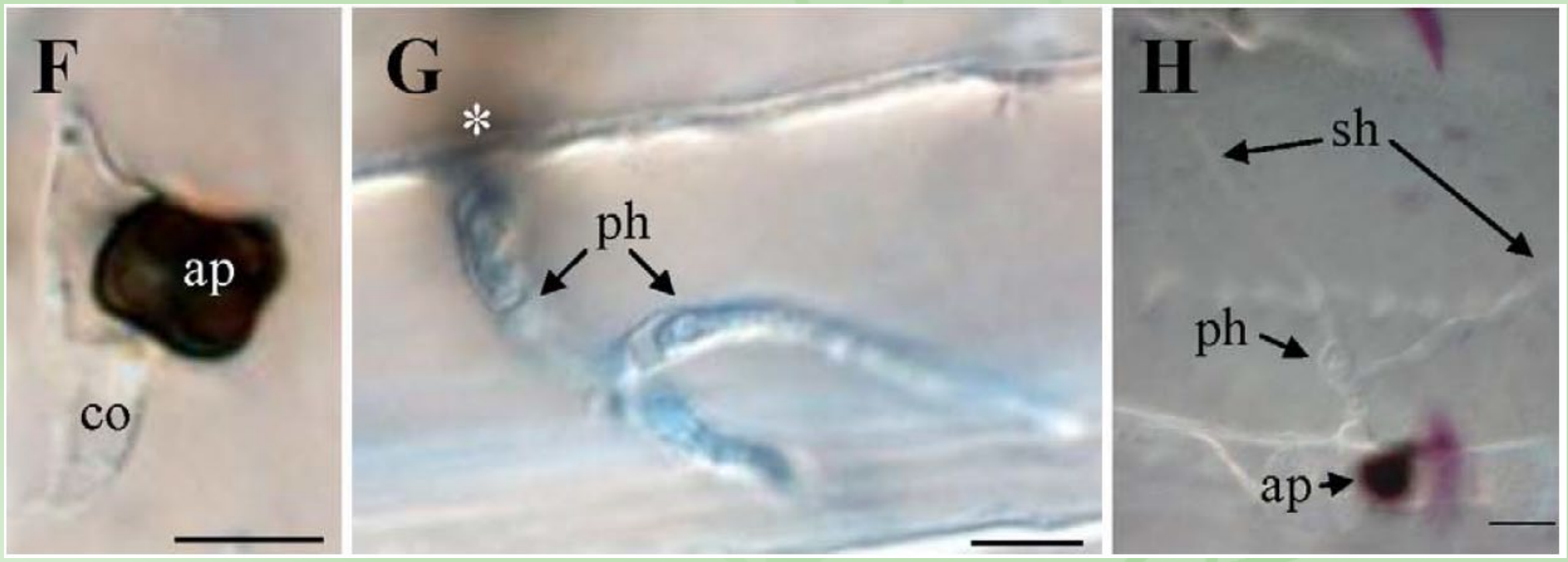
Biotróficos x Hemibiotróficos



Hemibiotrófico *Bipolaris sorokiniana*

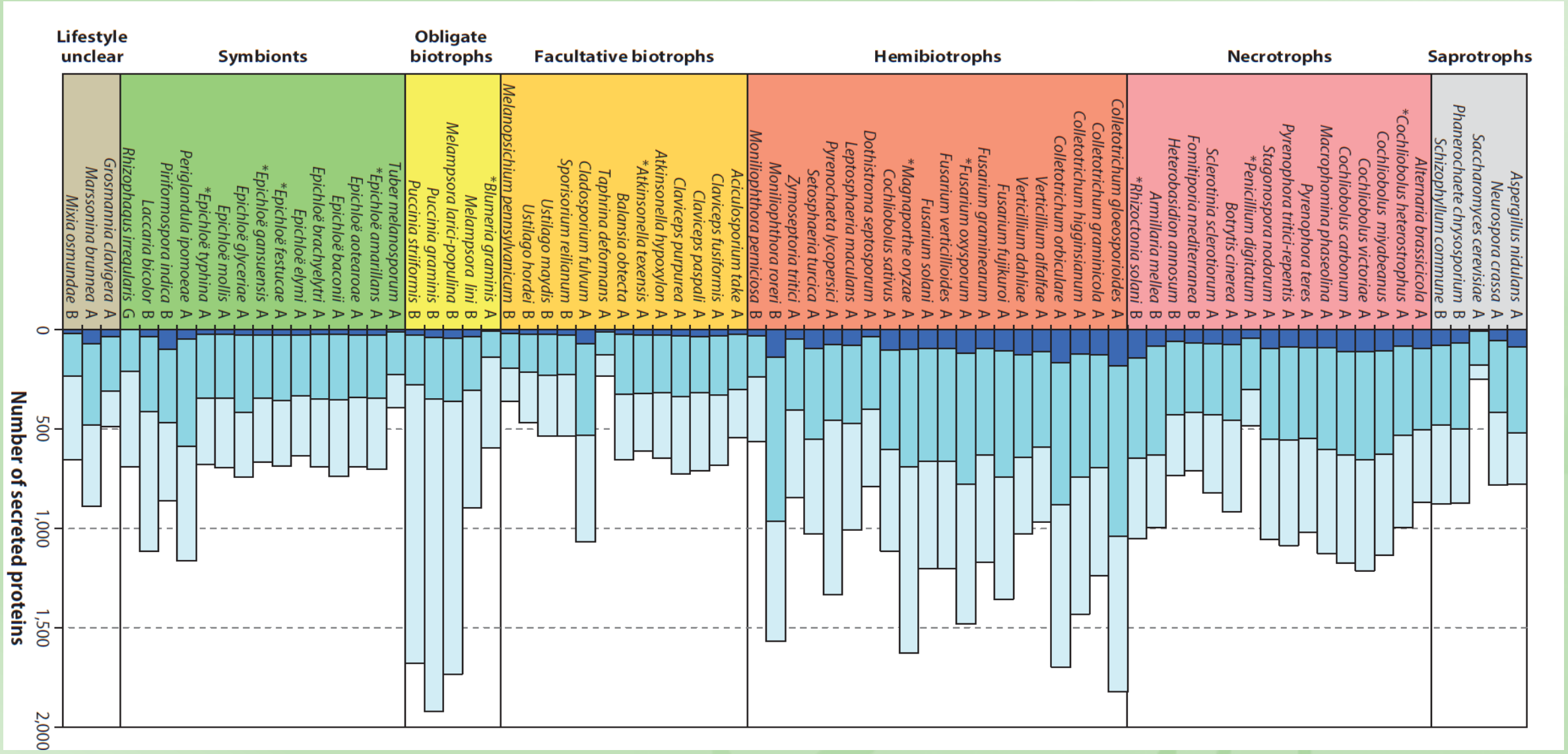


Hemibiotrófico *Colletotrichum graminicola*



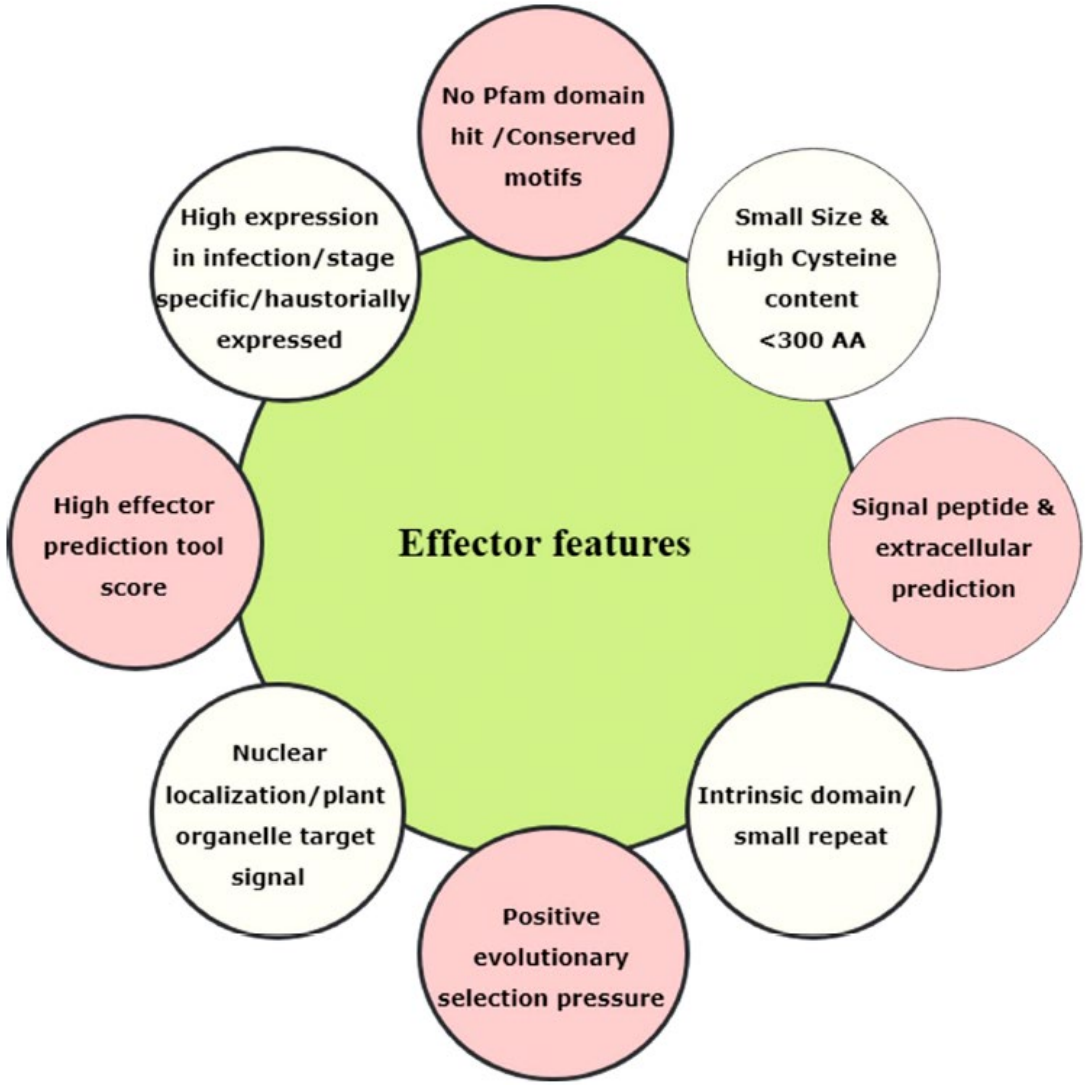
Lista de patógenos biotróficos e hemibiotróficos

Fungal spp.	Growth habit	Mode of nutrition	Biotrophy restricted to single cell?[a]	Neckbands[b]	ATPase activity[c]	PACP labelling[d]	Intramembrane particles[e]
Erysiphe pisi[1]	epiphytic haustorial	obligate biotroph	yes	yes	no activity	no labelling	absent
Blumeria graminis[2]	epiphytic haustorial	obligate biotroph	yes	yes	no activity	no labelling	ND
Puccinia poarum —dikaryotic form[2]	intercellular and haustorial	obligate biotroph	yes	yes	no activity	weak labelling	ND
Puccinia poarum —monokaryotic form[2]	intercellular and intracellular hyphae	obligate biotroph	no	no	activity in neck region only	weak labelling	ND
Uromyces appendiculatus —dikaryotic form[3,4]	intercellular and haustorial	obligate biotroph	yes	yes	no activity	weak labelling	absent
Albugo candida[5,6]	intercellular hyphae haustorial	obligate biotroph	yes	yes, relatively simple	no activity	weak labelling	ND
Cladosporium fulvum[7]	intercellular hyphae	hemibiotroph	N/A	N/A	N/A	N/A	N/A
Colletotrichum lindemuthianum[8]	intracellular hyphae	hemibiotroph	no	no	normal activity	labelling	present
Colletotrichum destructivum[9]	intracellular hyphae	hemibiotroph	yes	no	ND	ND	ND
Phytophthora infestans[2,10]	intercellular hyphae haustorial	hemibiotroph	yes	no	ND	ND	ND
VA mycorrhizae[3,11]	intercellular and intracellular hyphae	mutualistic symbiont	no	no	high activity	labelling	ND

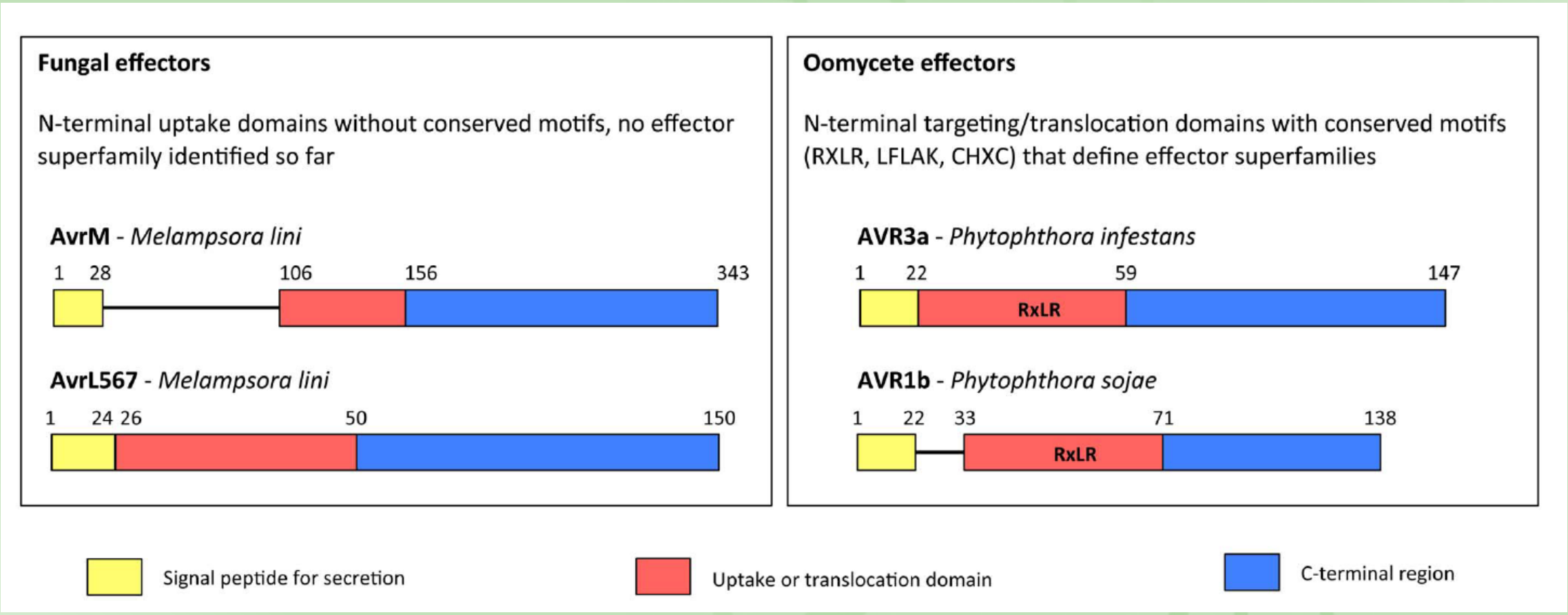


Annu. Rev. Plant Biol. 2015.66:513-545.

Características típicas de candidate secretory effector proteins (CSEPs) em vários fungos fitopatogênicos

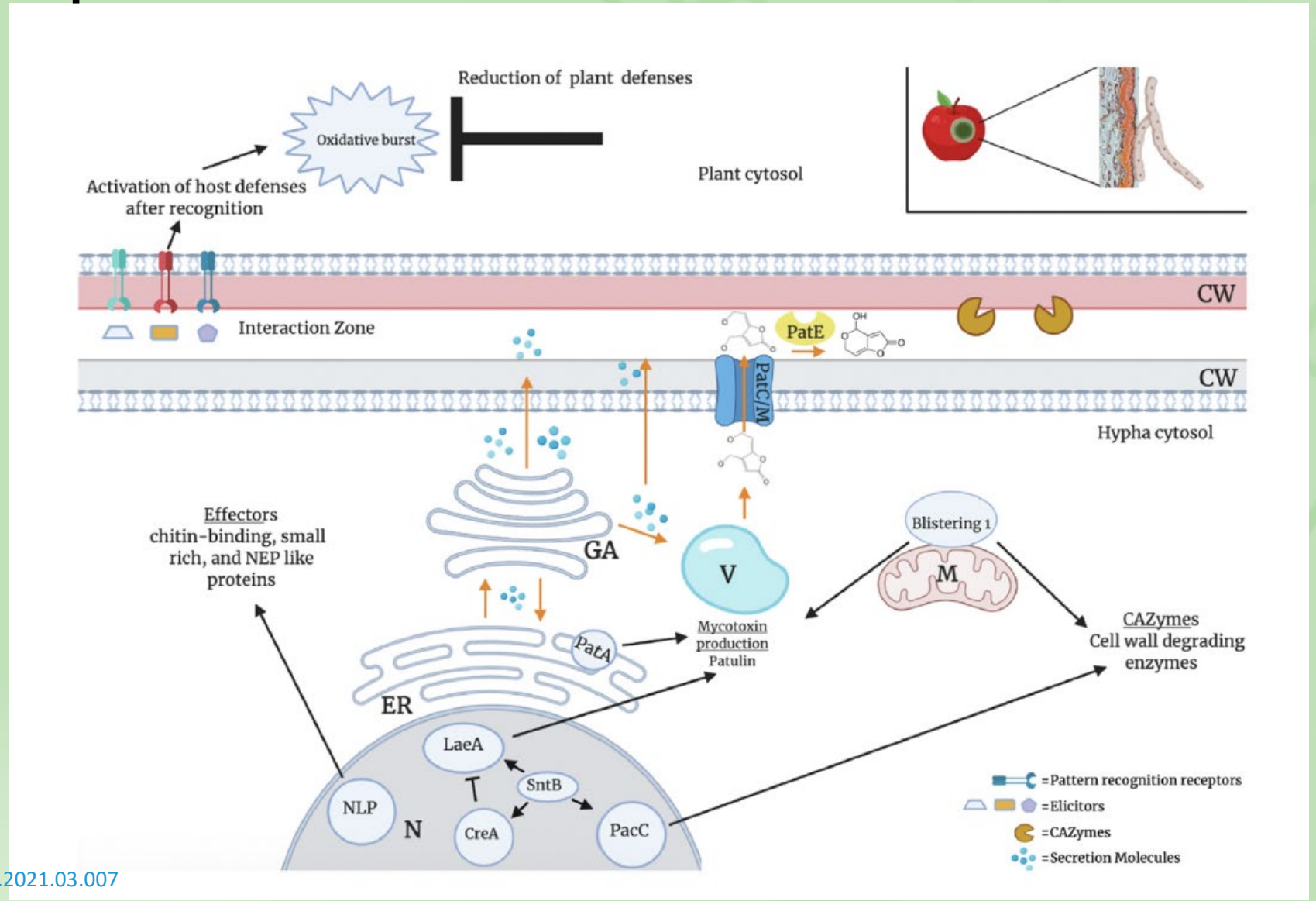


Organização de efetores apoplásticos e citoplasmáticos

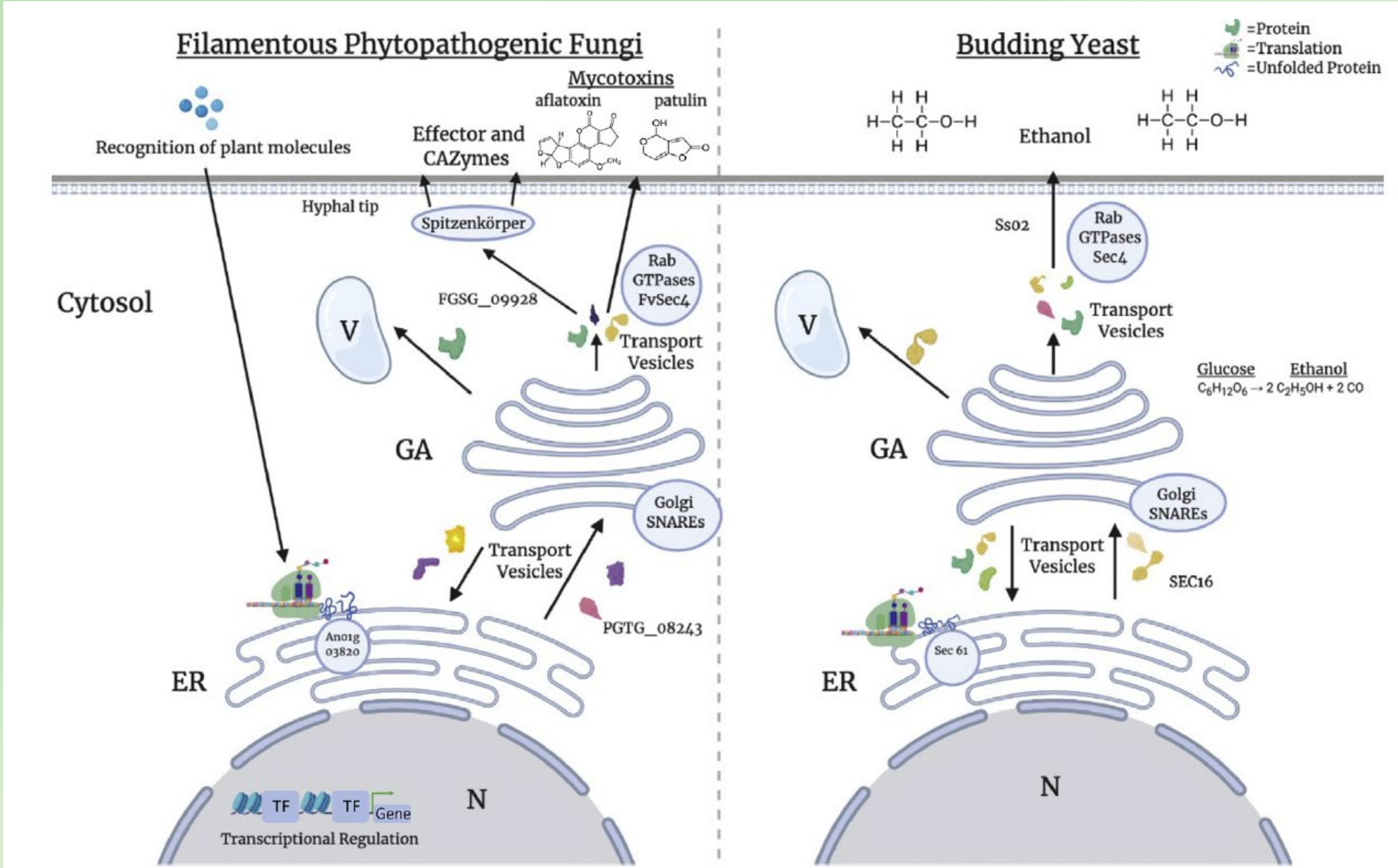


Dominios ‘‘uptake’’ ou ‘targeting/translocation’’: mediam a entrada dos efetores na célula hospedeira

Modelo conceitual atual exibindo as moléculas secretoras e componentes no patossistema *Penicillium expansum* e *Malus domestica* que estão associados à virulência

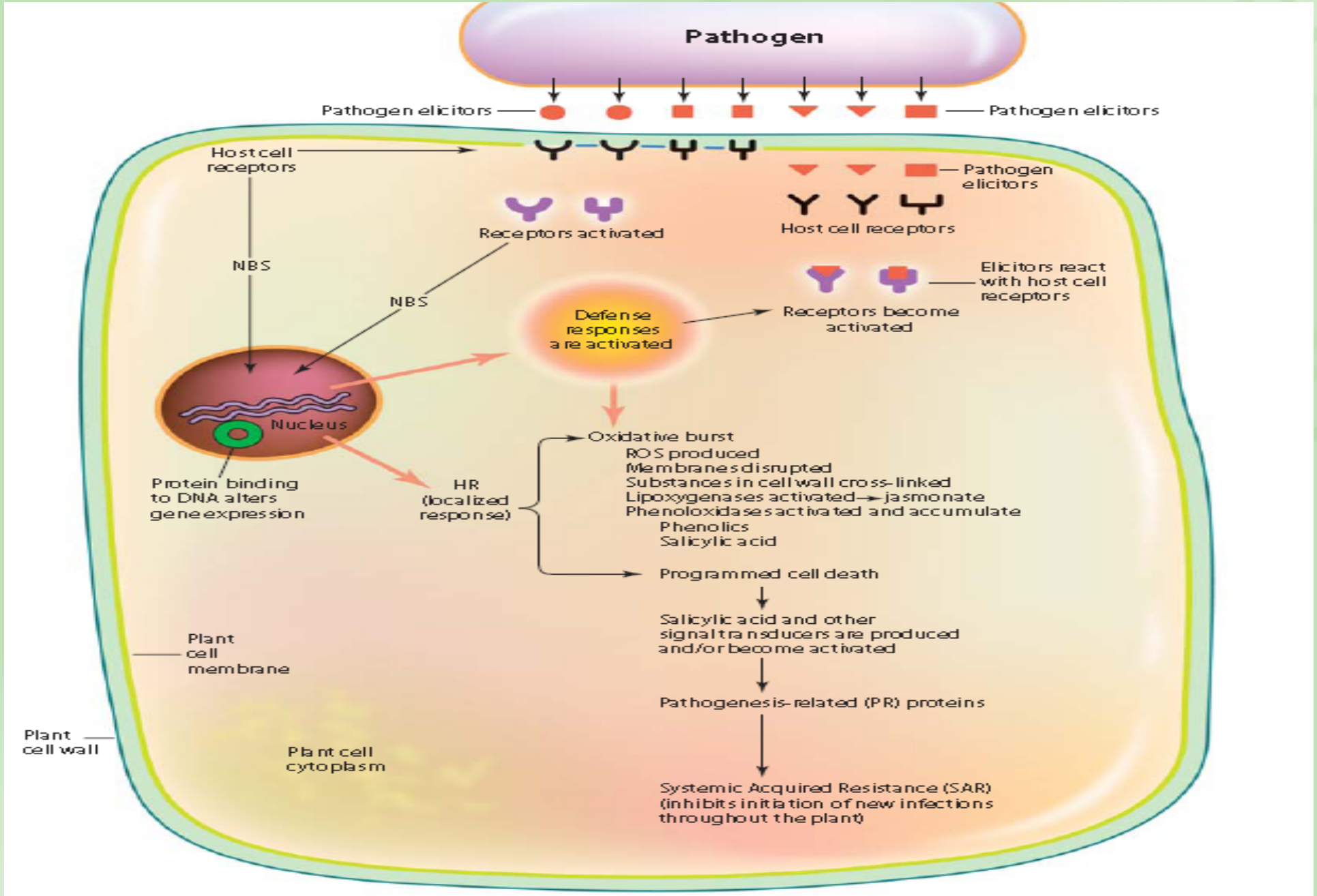


https://doi.org/10.1016/j.fbr.2021.03.007



https://doi.org/10.1016/j.fbr.2021.03.007

Genes efetores como genes de avirulência

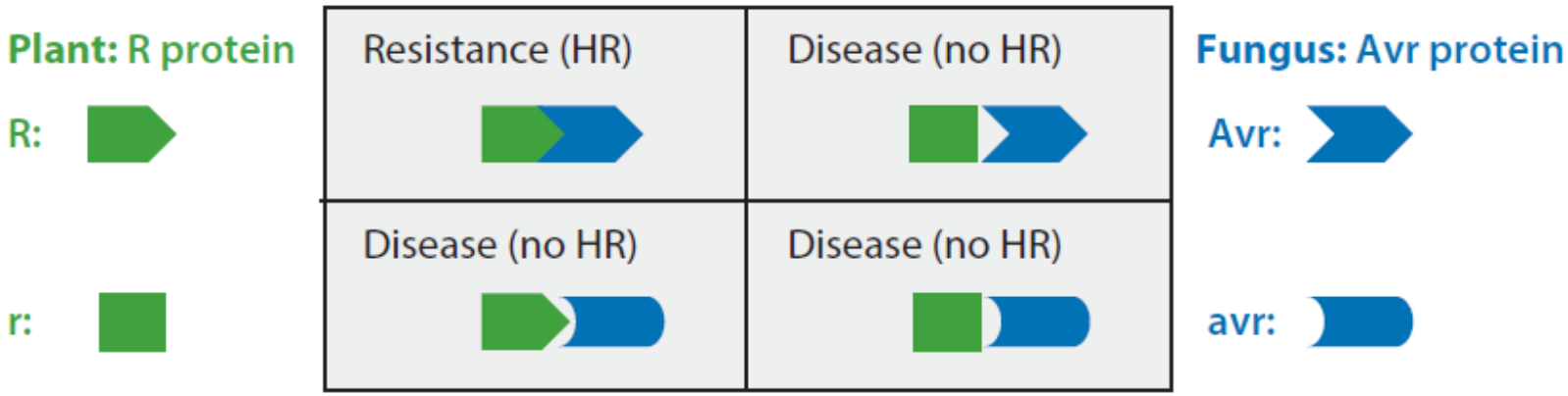


Modelos genético e bioquímico para a hipótese gene a gene de Flor

Genetic model

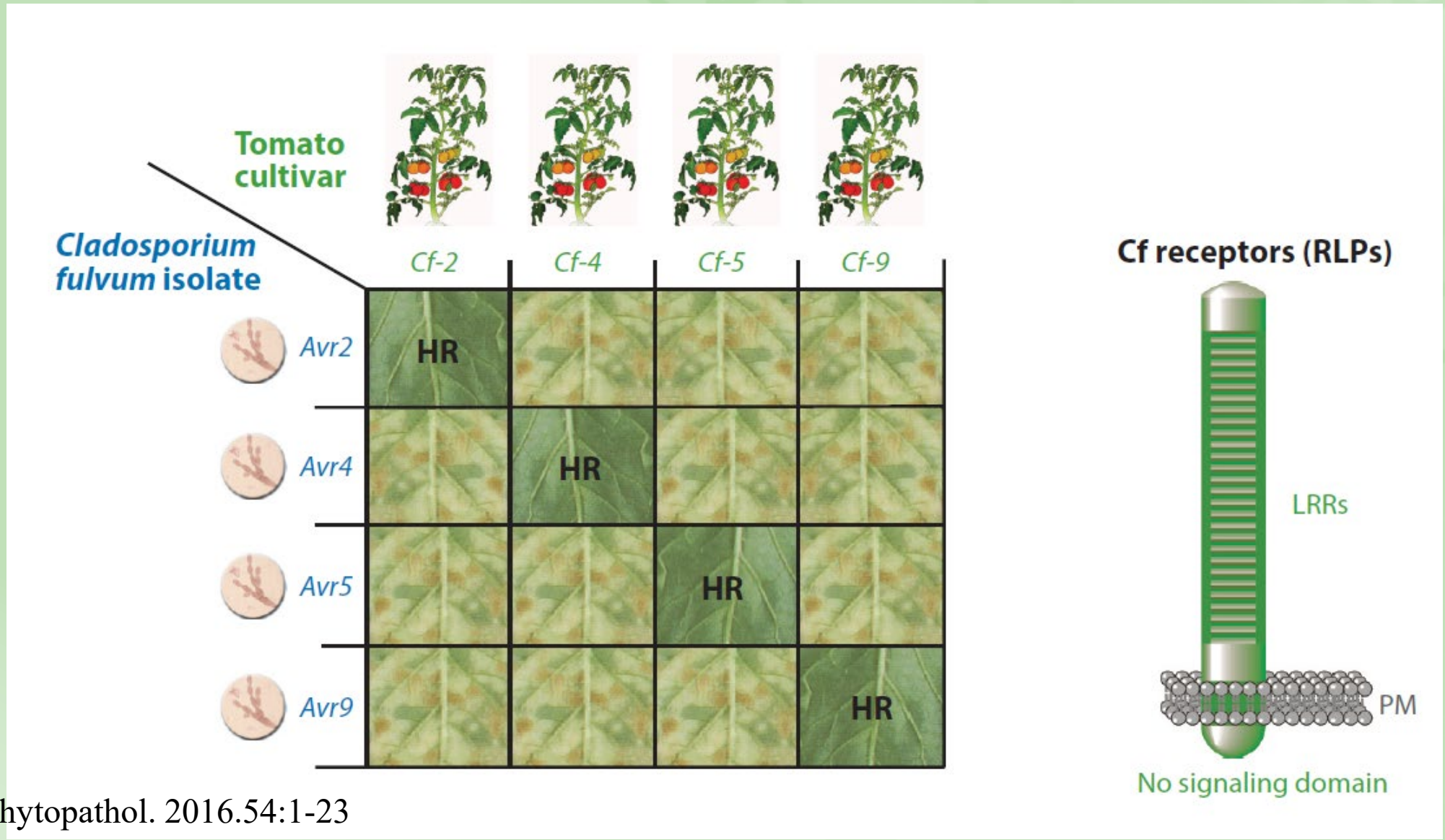
Fungus: Avirulence gene / Plant: Resistance gene	*R*	*r*
Avr	Resistance	Disease
avr	Disease	Disease

Biochemical model



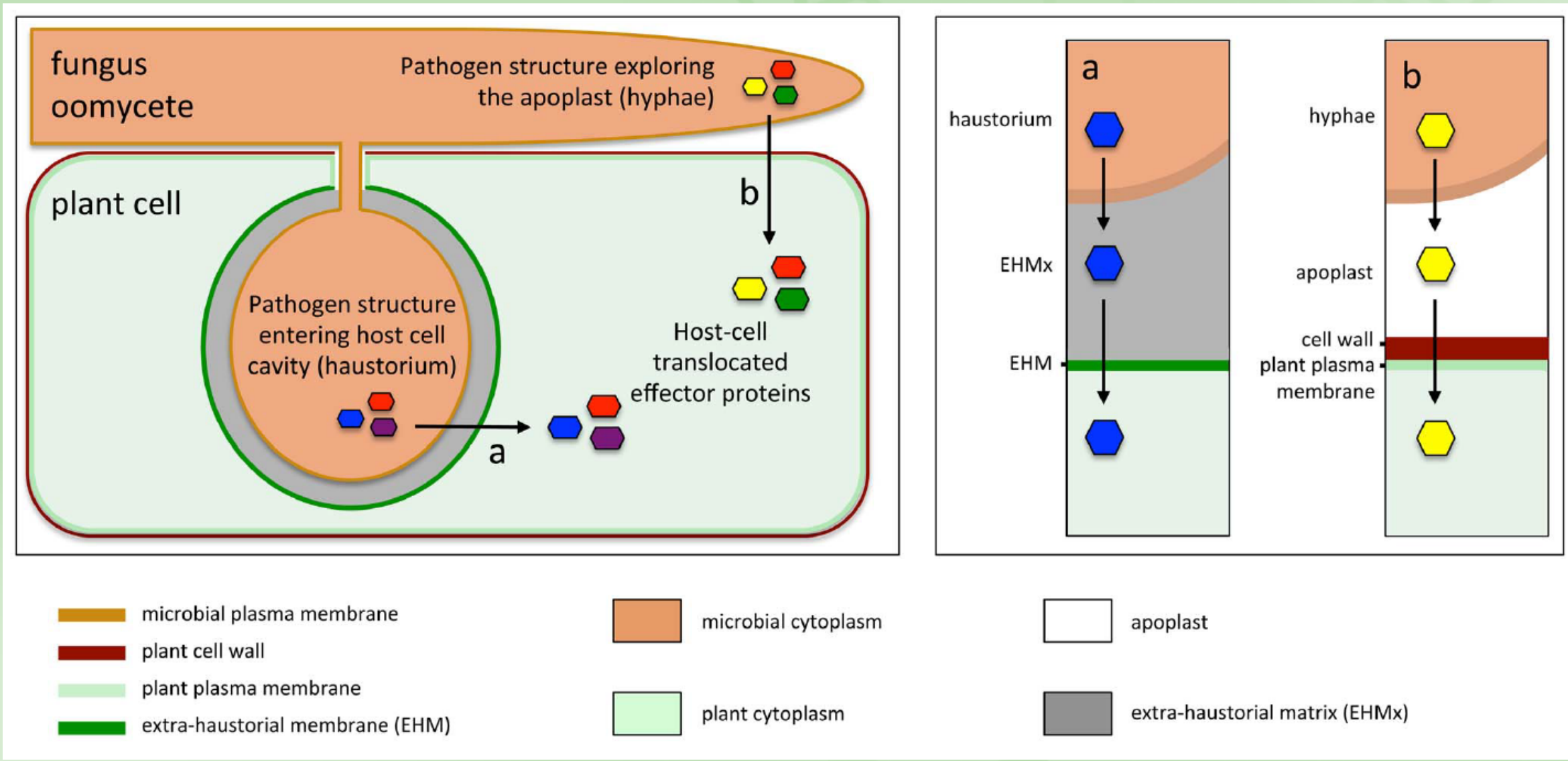
HR: hypersensitive response

Receptores LRR-membrane receptor-like: proteínas que reconhecem as proteínas Avr de Cladosporium fulvum: HR

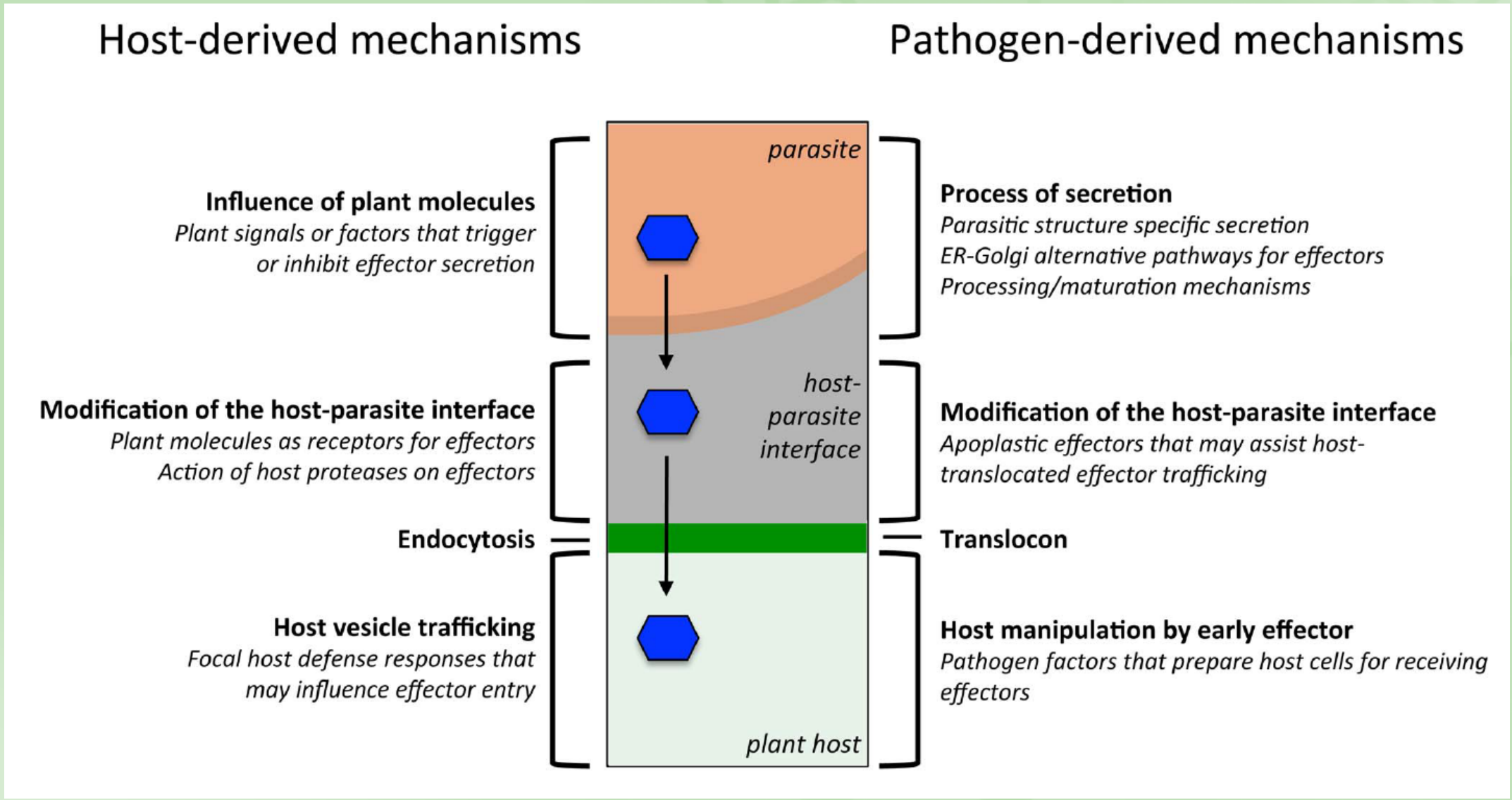


Annu. Rev. Phytopathol. 2016.54:1-23

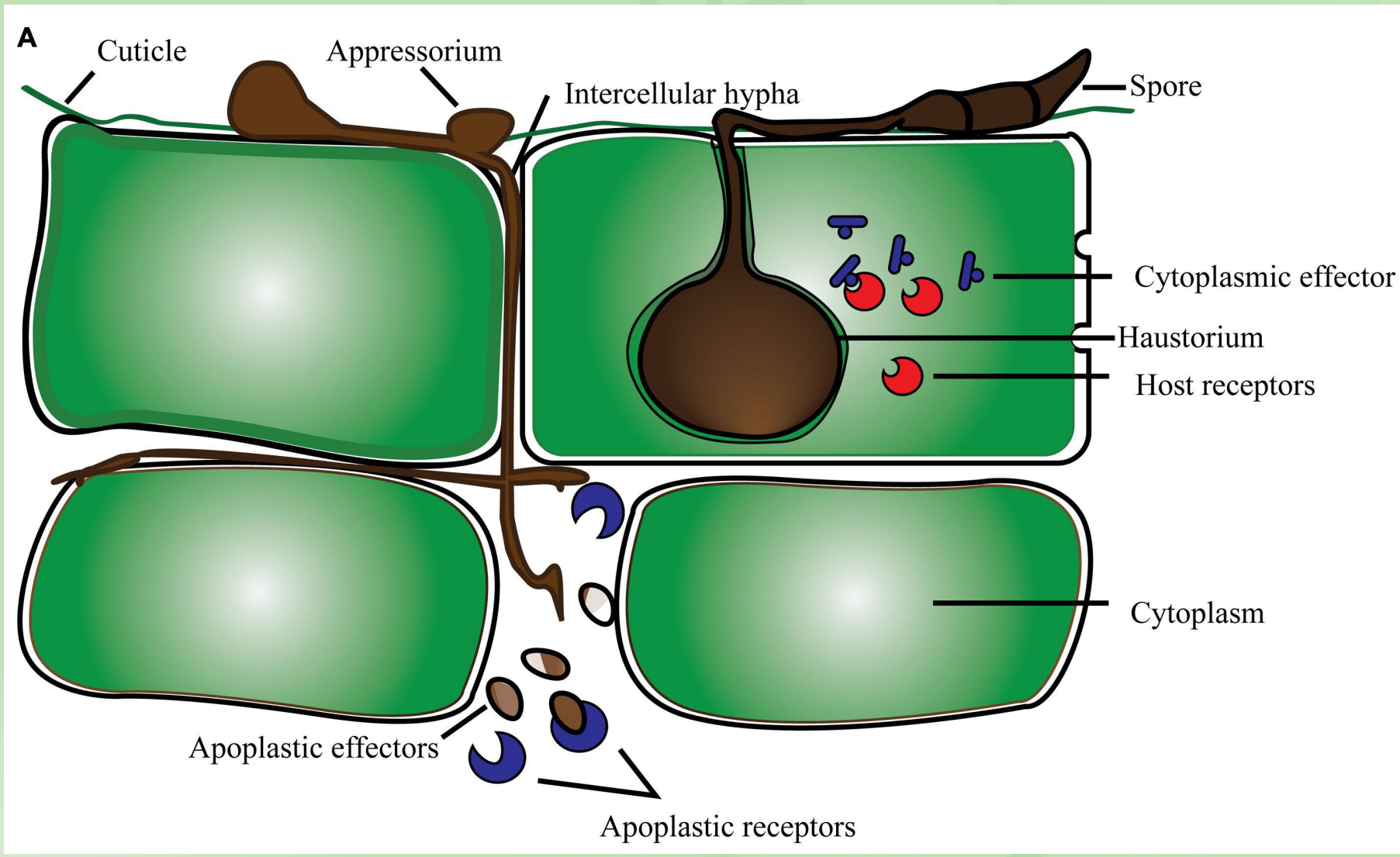
Estruturas de fungos e oomicetos para secreção de efetores



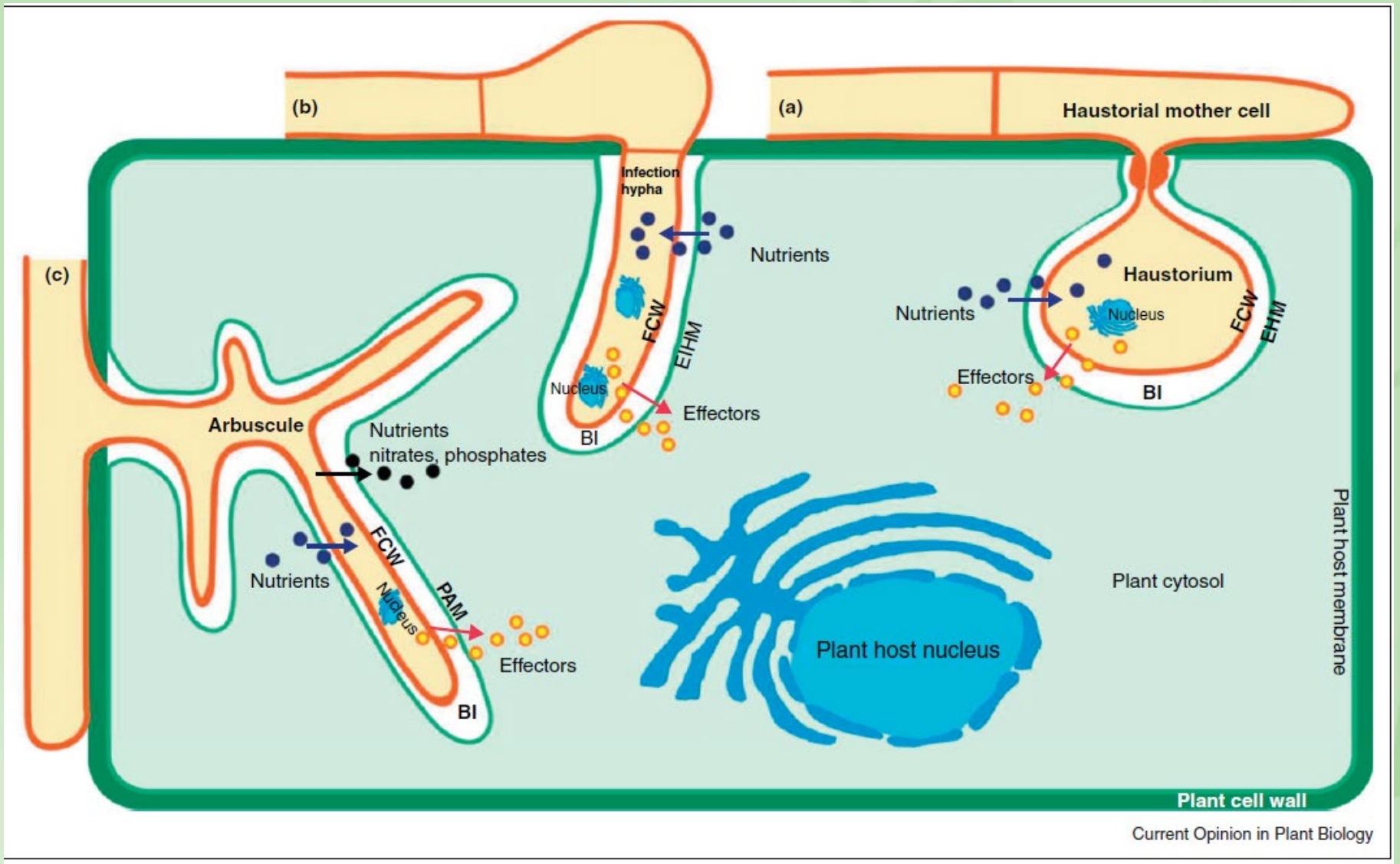
Processo integrado da translocação dos efetores



Efetores secretados por fungos e oomicetos no citoplasma ou na região apoplástica da célula vegetal

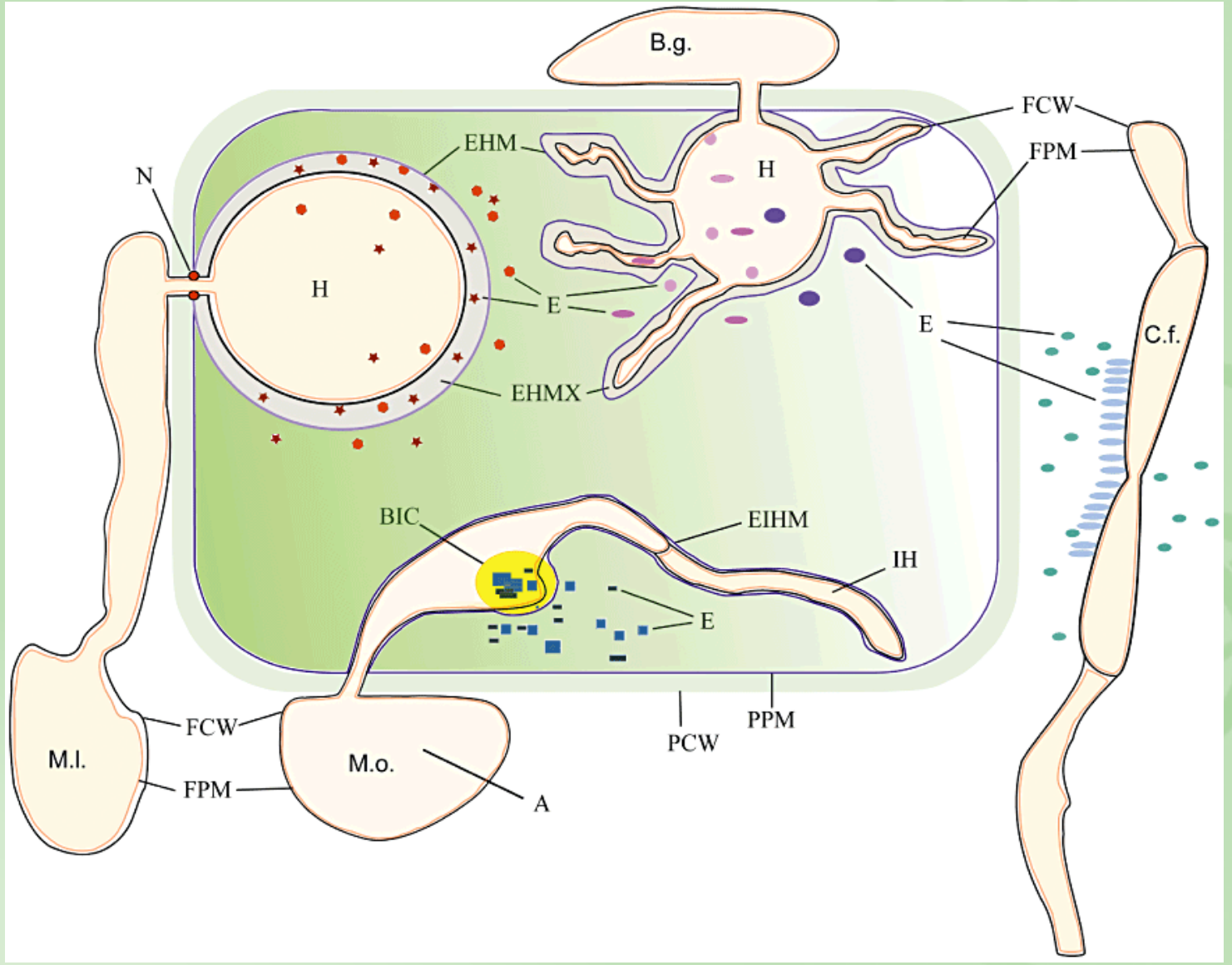


Estruturas especializadas de infecção para secreção de efetores por fungos biotróficos



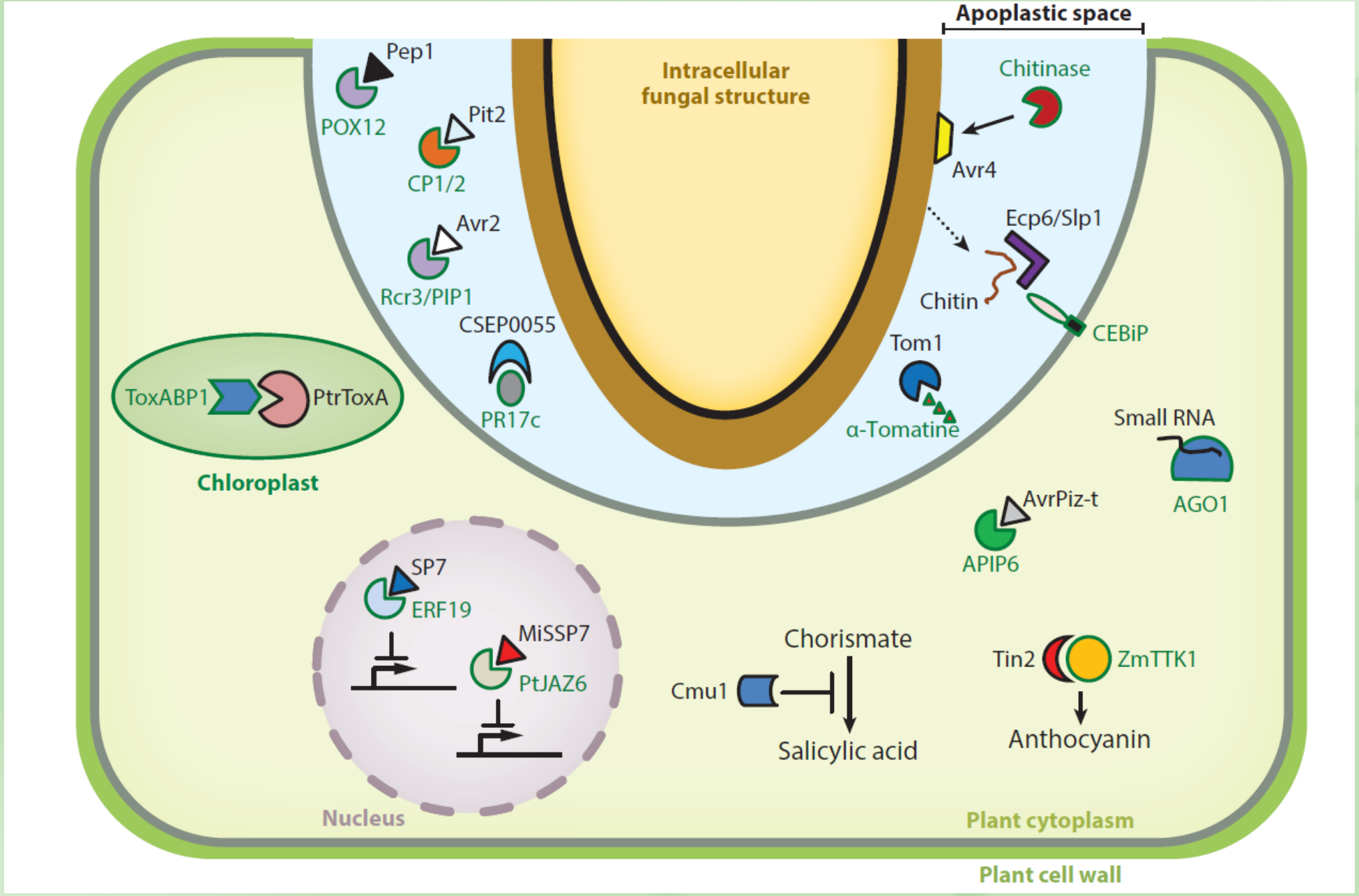
A. Haustoria-forming fungal pathogens
B. non haustoria-forming fungal pathogens
C. arbuscular mycorrhiza

O papel de efetores na infecção de fungos biotróficos e hemibiotróficos

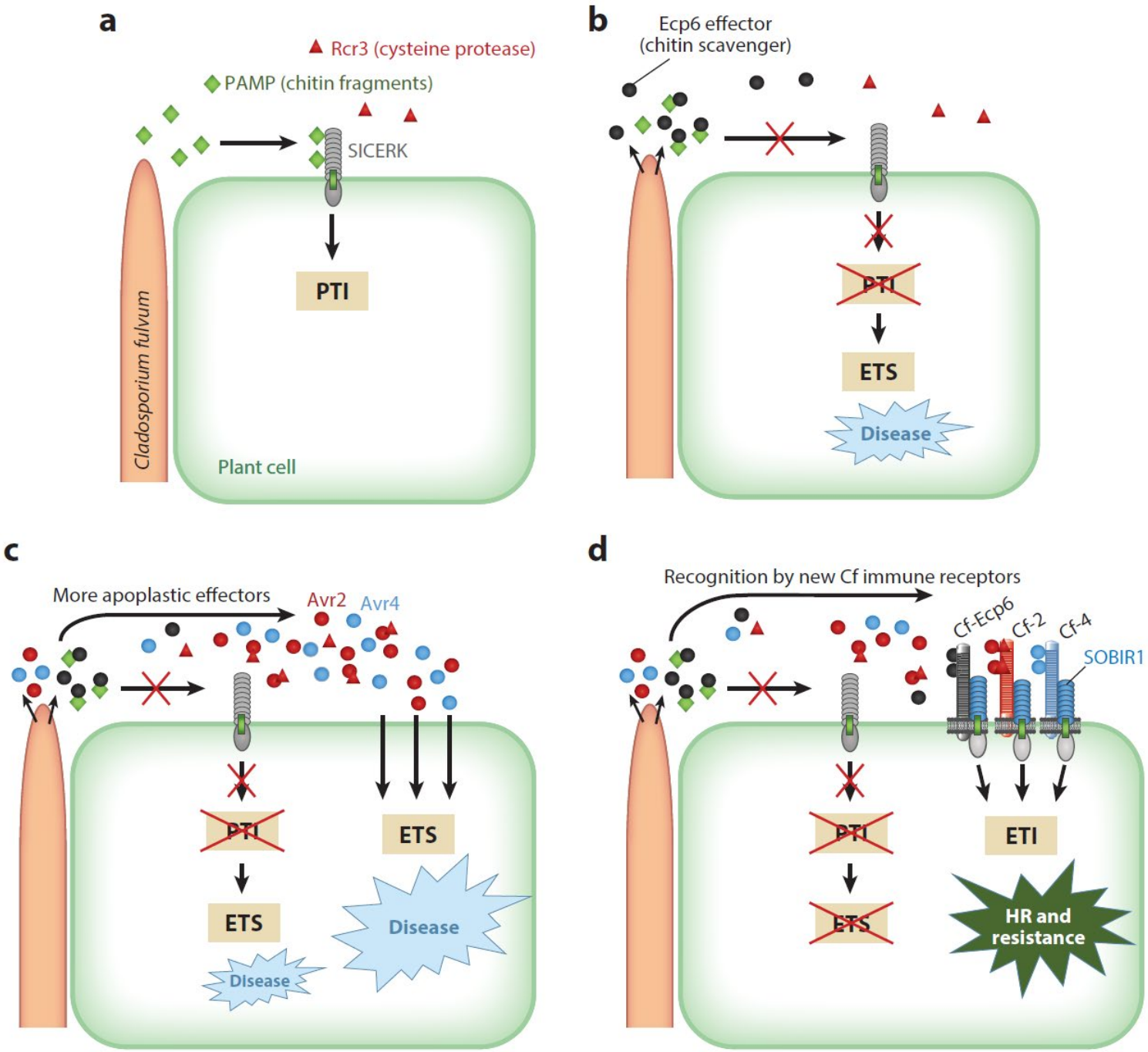


Quatro fungos patogênicos com diferentes estratégias de infecção:
Espaço intercelular: (*Cladosporium fulvum*, C.f.),
Hifas intracelulares: (*Magnaporthe oryzae*, M.o.)
Penetração intracellular por haustórios: (*Blumeria graminis*, B.g and *Melampsora lini*, M.l.
A, appressorium; BIC, biotrophic interfacial complex; E, effector; EHM, extrahaustorial membrane; EHMX, extrahaustorial matrix; EIHM, extrainvasive hyphal membrane; FCW, fungal cell wall; FPM, fungal plasma membrane; H, haustorium; IH, invasive hypha; N, neckband; PCW, plant cell wall; PPM, plant plasma membrane.

Cellular Microbiology, Volume: 13, Issue: 12, Pages: 1849-1857, First published: 16 August 2011, DOI: (10.1111/j.1462-5822.2011.01665.x)

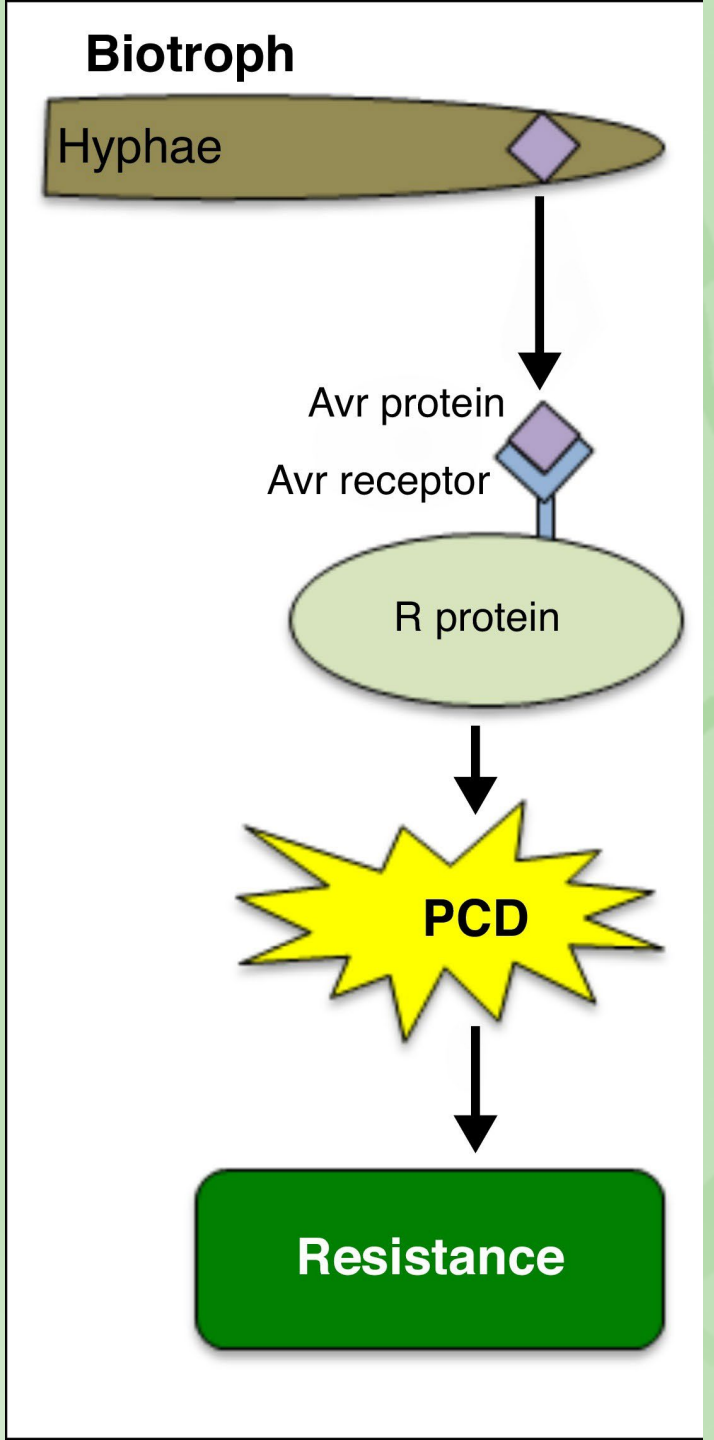


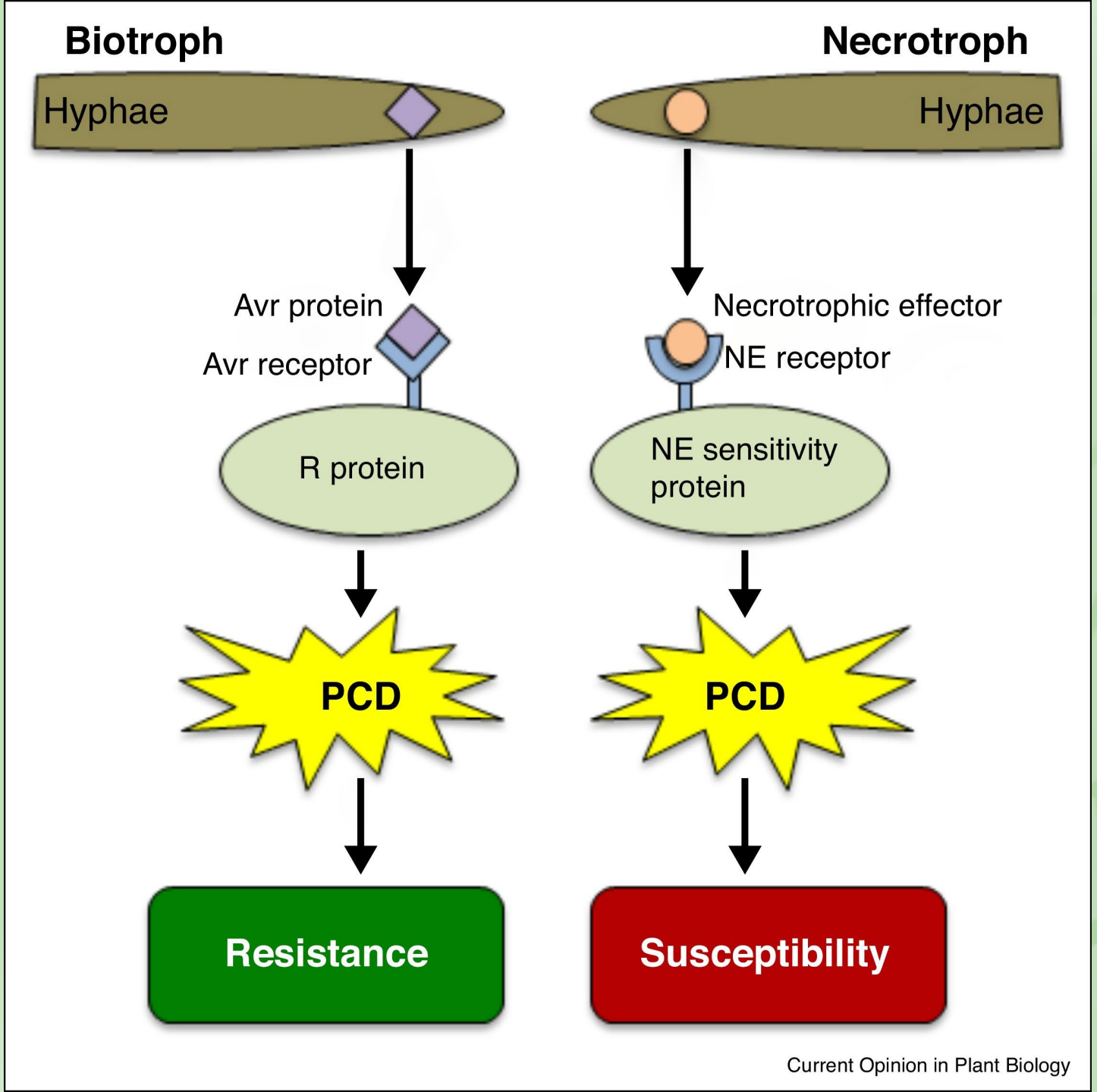
Annu. Rev. Plant Biol. 2015.66:513-545.



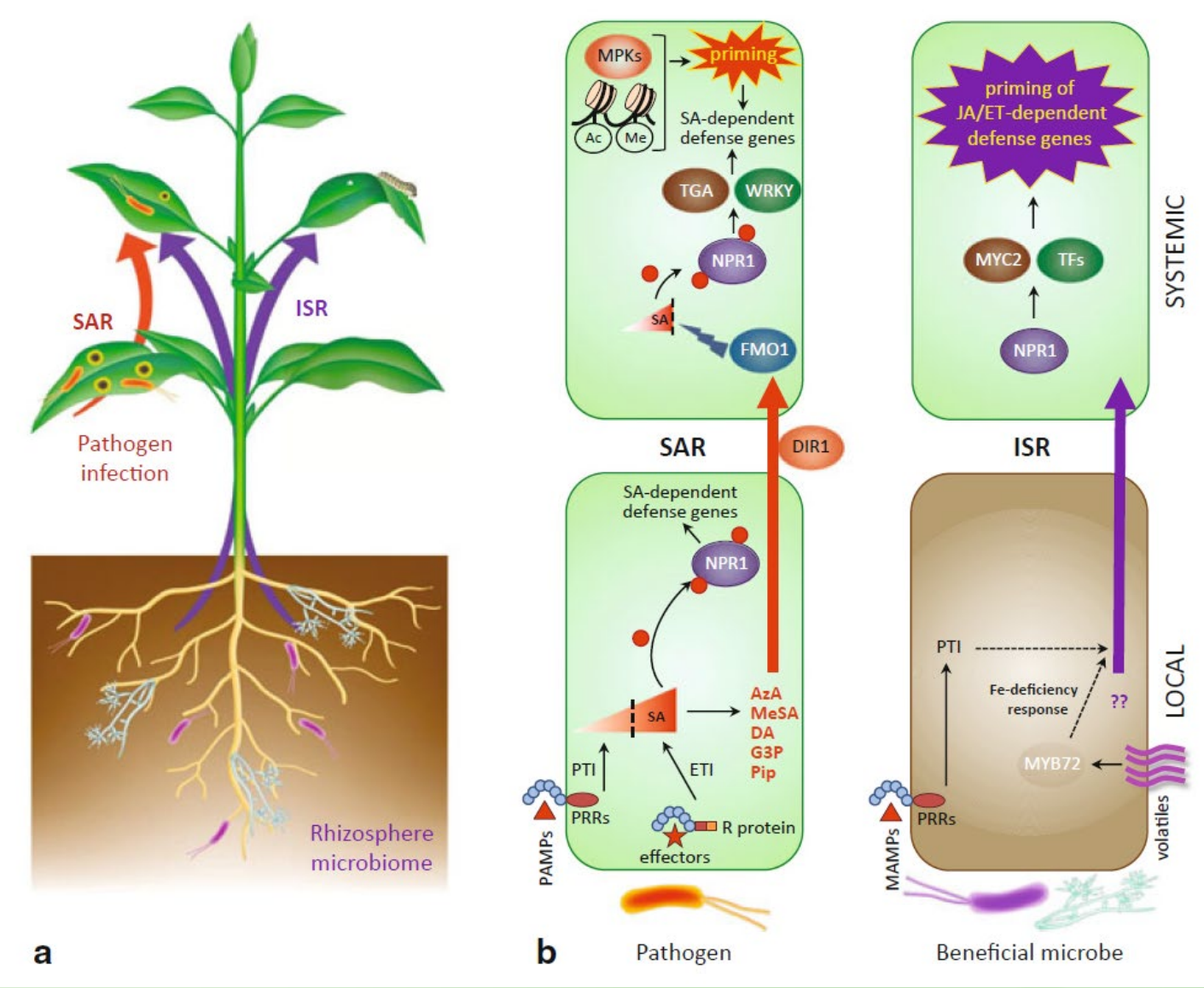
1. An evolutionary scenario of the arms race between *Cladosporium fulvum* and tomato. (*a*) Chitin fragments from *C. fulvum* are recognized by chitin receptor–inducing PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI).
2. (*b*) *C. fulvum* secretes the Ecp6 effector that scavenges chitin fragments to prevent PTI, leading to a weak form of effector-triggered susceptibility (ETS).
3. (*c*) *C. fulvum* secretes additional effectors (Avr2) targeting apoplastic host targets (cysteine protease Rcr3) and Avr4 (protecting against plant chitinases) to increase virulence.
4. (*d*) Tomato develops new Cf immune receptors to recognize new effectors to induce effector-triggered immunity (ETI).

Annu. Rev. Phytopathol. 2016.54:1-23


Biotroph
Hyphae
Avr protein
Avr receptor
R protein
PCD
Resistance


Biotroph
Necrotroph
Hyphae
Hyphae
Avr protein
Avr receptor
R protein
Necrotrophic effector
NE receptor
NE sensitivity protein
PCD
PCD
Resistance
Susceptibility
Current Opinion in Plant Biology

Resistência sistêmica: Pathogen-Induced Systemic Acquired Resistance (SAR) x Induced Systemic Resistance (ISR) por microrganismos benéficos (Promotores de crescimento)



PAMPs (PTI) e Efetores (ETI)

SAR systemic acquired resistance:

↑ Salicylic acid (SA)

Proteínas NONEXPRESSOR OF *PR* GENES1 (NPR1)- fator transcricional:

↑ *PATHOGENESIS-RELATED* (*PR*) genes

MAMPs, lipopolysaccharideos (LPS), antibioticos, Sideroforos e voláteis

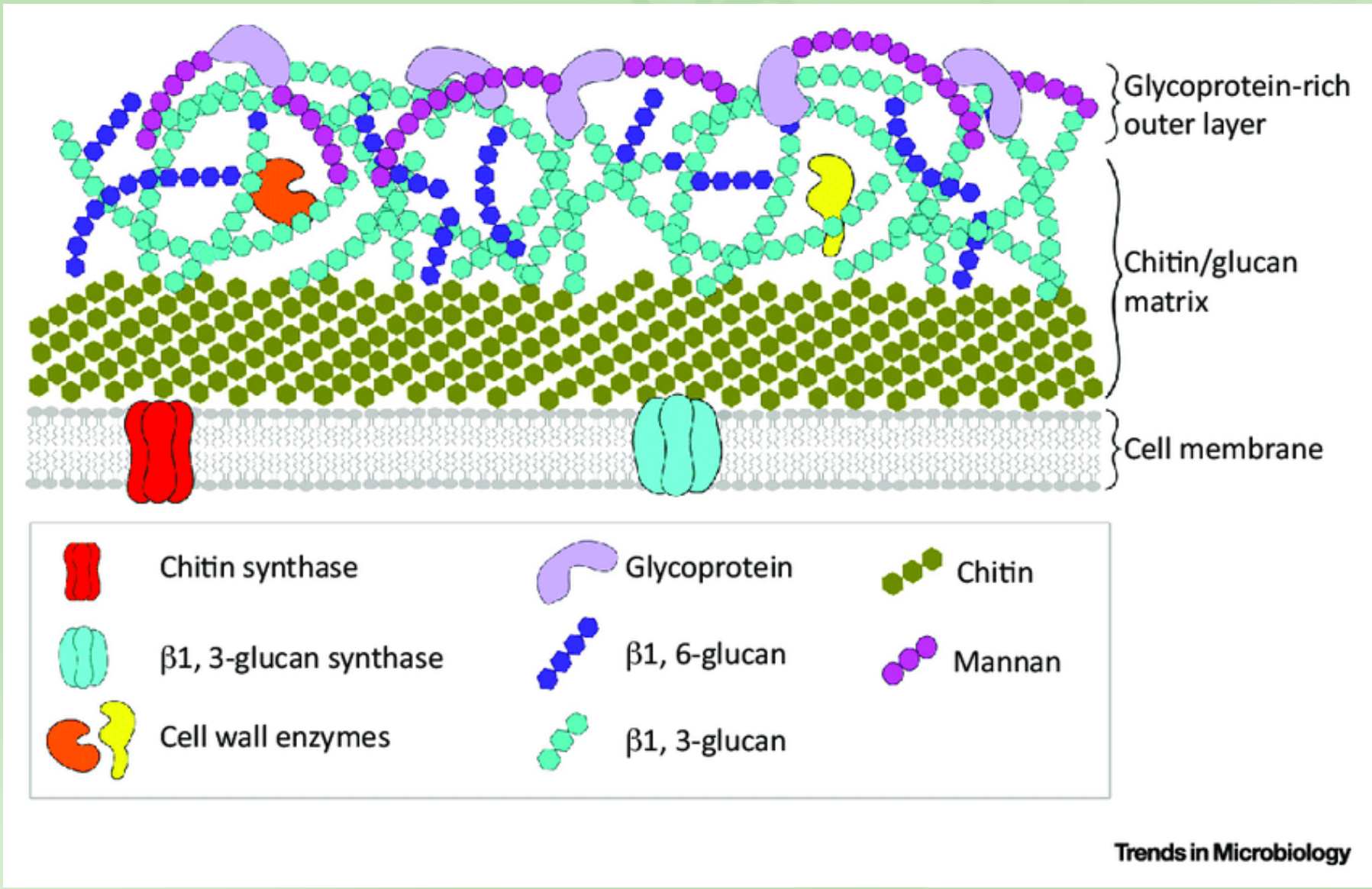
ISR induced systemic resistance

JA e ET ➡ genes de defesa

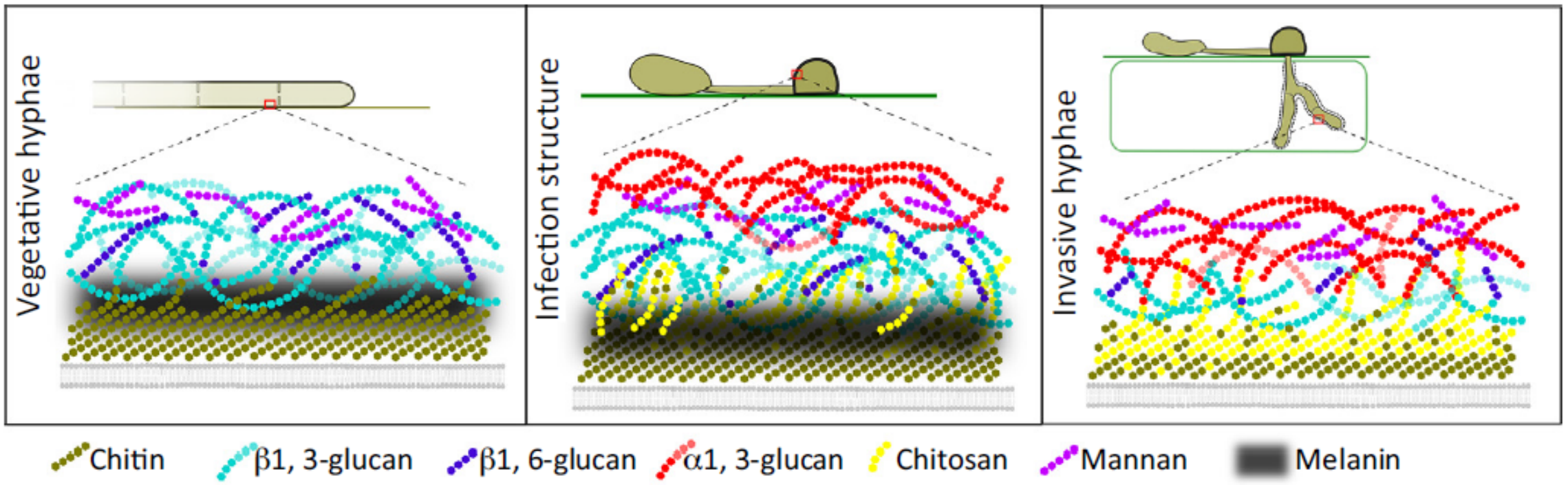
Ac acetylation,
ET ethylene,
ETI effector-triggered immunity,
Fe iron,
ISR induced systemic resistance,
JA jasmonic acid,
MAMP microbe-associated molecular pattern,
Me methylation,
PAMP pathogen-associated molecular pattern, *PRR* pattern-recognition receptor,
PTI PAMP-triggered immunity,
R protein Resistance protein,
SA salicylic acid,
SAR systemic acquired resistance,
TF transcription factor
PATHOGENESIS-RELATED (*PR*) genes

Principles of Plant-Microbe Interactions, DOI 10.1007/978-3-319-08575-3_14

Estrutura básica da parede cellular de fungos

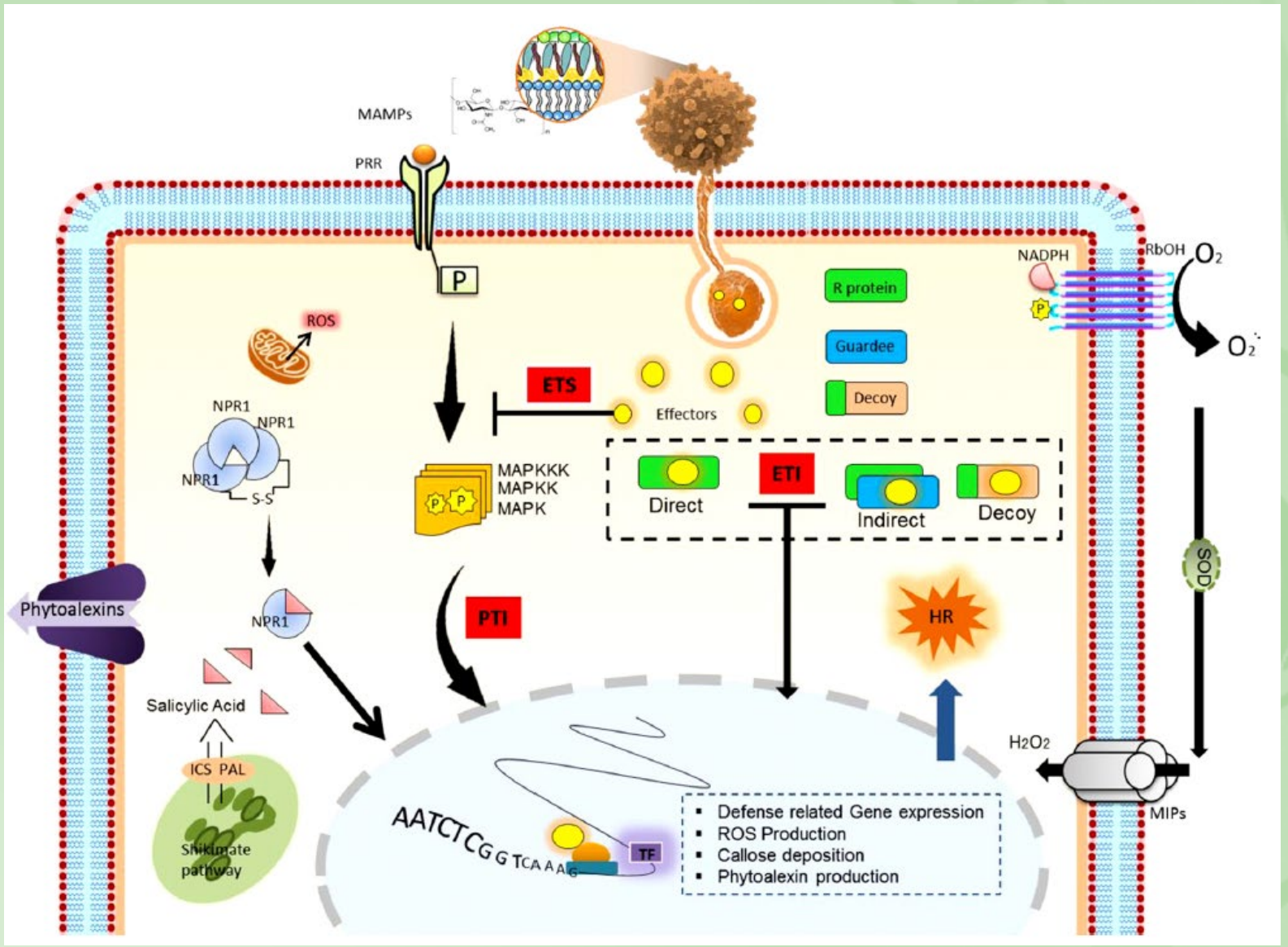


A liberação de PAMPs pode ser evitada alterando a composição da parede celular de estruturas de infecção, como por desacetilação da quitina (formando quitosana), redução do conteúdo de b-glucana, por síntese de alfa1,3-glucana, ou por secreção de proteínas efetoras, tanto para sequestrar PAMPs ou para supressão do sistema de defesa da planta



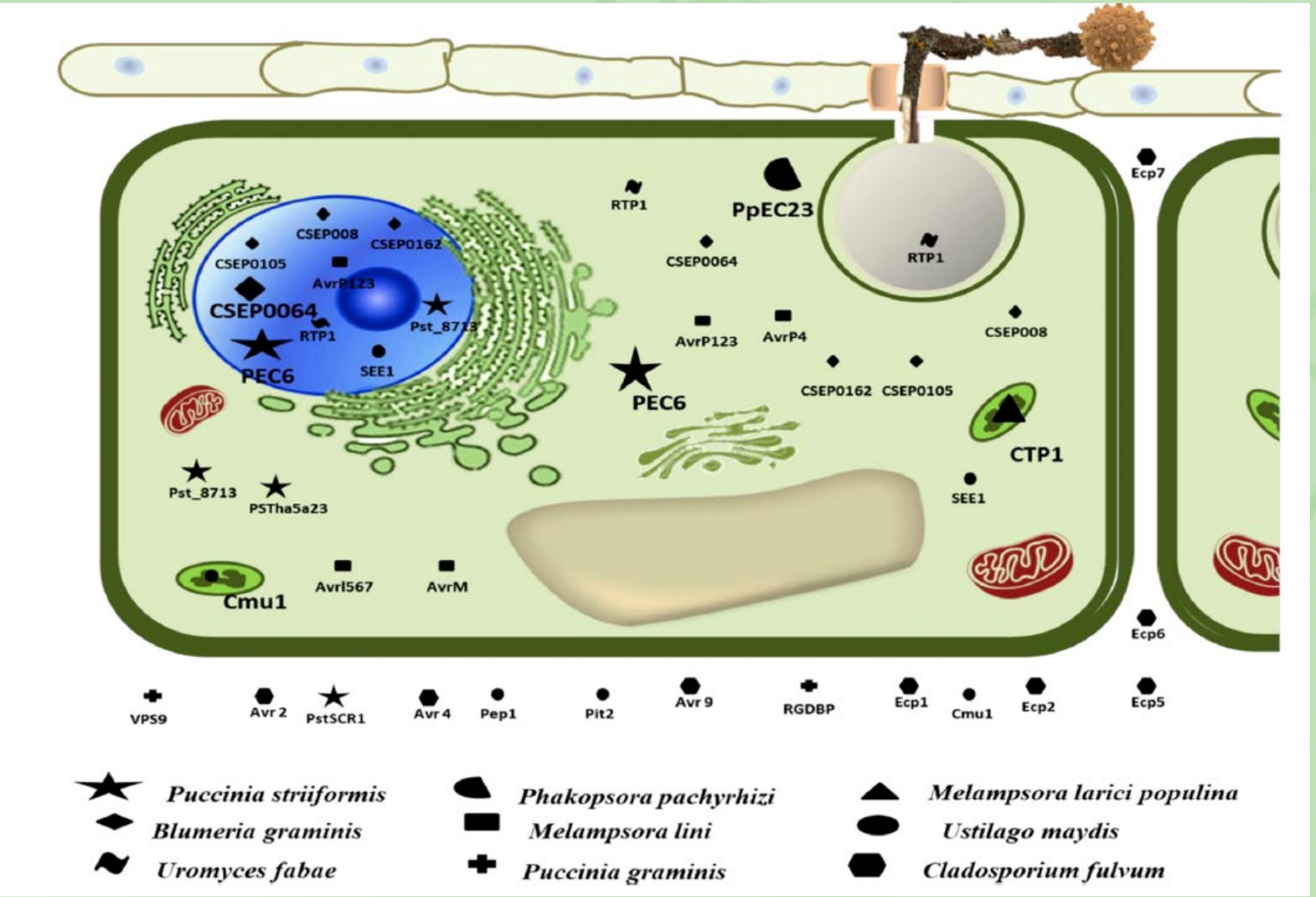
http://dx.doi.org/10.1016/j.tim.2017.05.015

Comunicação entre o sistema imunológico da planta hospedeira, efetores de fungos e proteínas de virulência

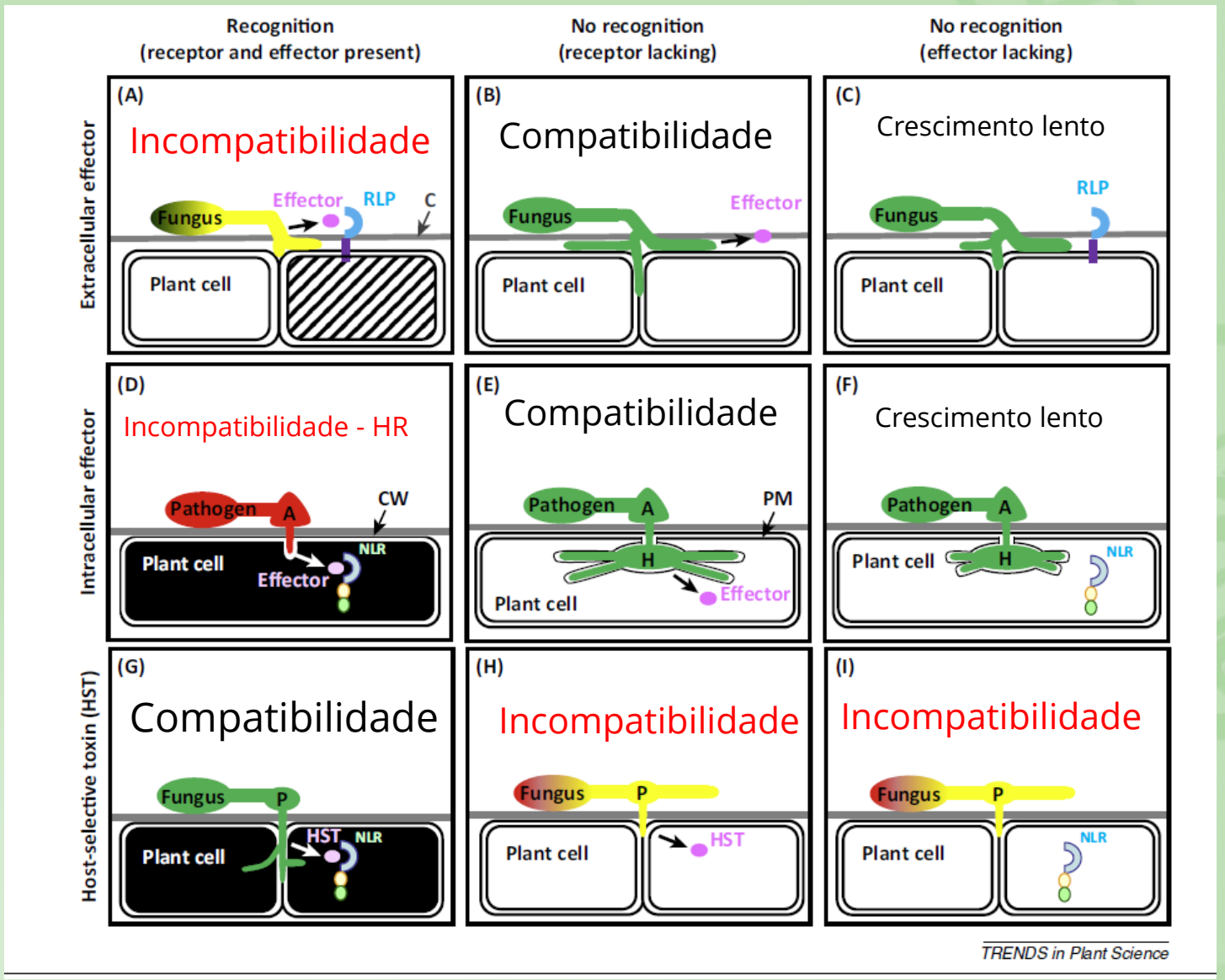


Resposta da planta: produção de compostos antimicrobianos: proteinases, quitinases e glucanases que danificam as estruturas do patógeno; inibidores de enzimas direcionados a moléculas produzidas pelo patógeno; e moléculas antimicrobianas não proteicas

Localização subcellular de várias proteínas efetoras de fungos patogênicos biotróficos



Três tipos de resposta hospedeira à patógenos foliares

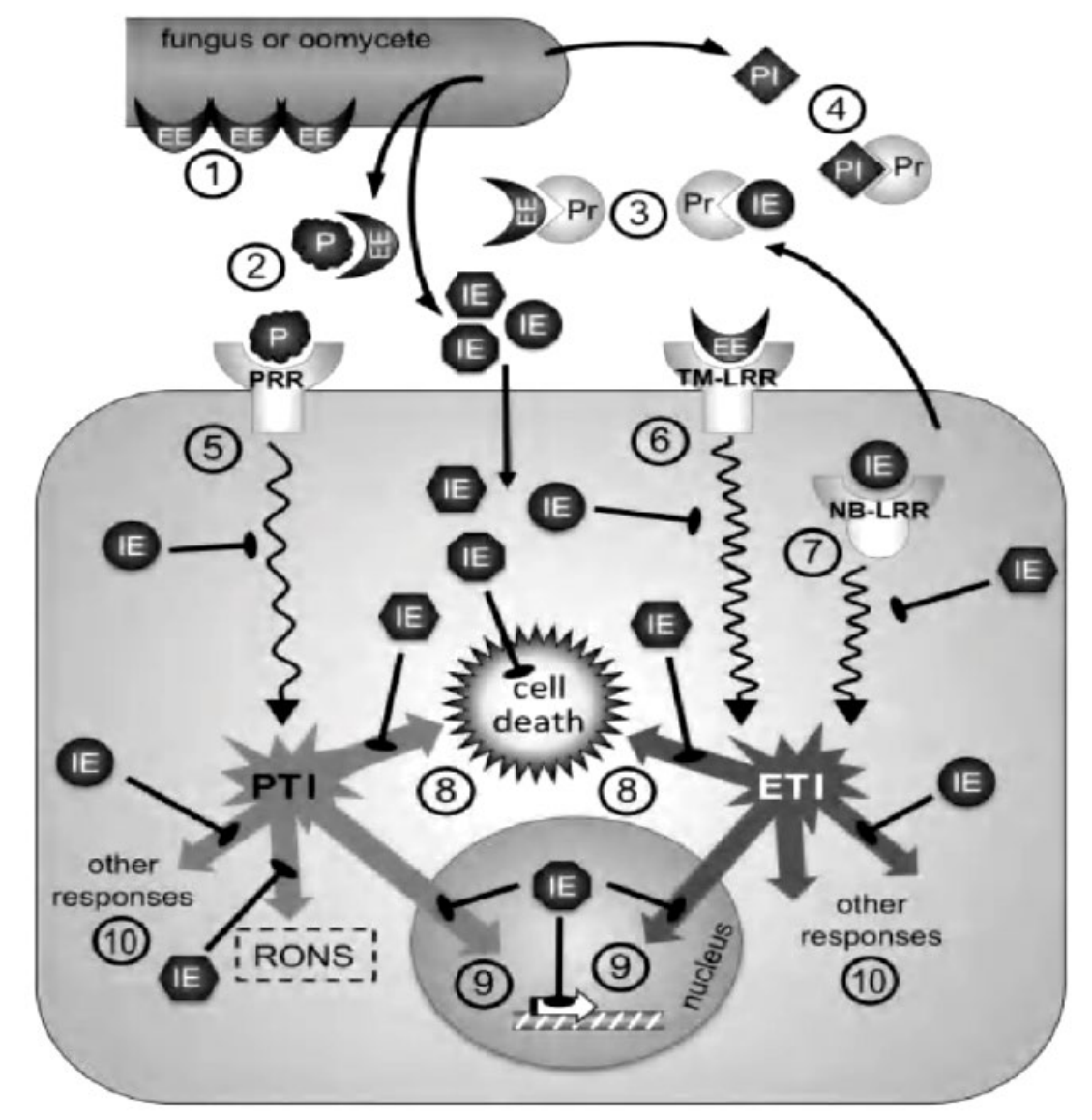


(A) Resistance (R) gene-mediated effector-triggered defence (ETD)
Host cell death typically occurs in only a few cells several days (C. fulvum and L. maculans) or weeks (R. commune and P. brassicae) after infection.
The pathogen does not die but can resume growth after host senescence begins or after the immune response is otherwise compromised.
RLPs: Receptor-like proteins

(B) Interação compatível por ausência de receptor
fungo está vivo
(C) Sem efetores, pouca proliferação

(D) R gene-mediated effector-triggered Immunity (ETI) results in incompatible interactions Nucleotide binding site (NBS) leucine-rich repeat (LRR) receptors (NLRs)
This recognition event triggers a rapid hypersensitive response that boosts host defence and usually results in host and pathogen cell death ( )
(G) Effector-triggered susceptibility (ETS) results in compatible interactions with necrotrophic fungal pathogens that secrete host-selective toxins (HSTs)
http://dx.doi.org/10.1016/j.tplants.2014.04.009

Diversas vias de entrada de efetores na célula hospedeira



(EE=Extracellular effectors outside apoplast. IE= intracellular effectors. (1) Prevent effector from cell walls or (2) prevent recognition receptors (PRR). Plants secrete proteases (Pr) (3) to degrade IE or EE, but pathogens (4) secrete protease inhibitors (PI) to block those proteases. Recognition of PAMPs by PRRs (5) produces signaling events that trigger PAMP-triggered immune responses (PTI). Recognition of EE by trans-membrane leucine-rich receptors (TM-LRRs) (6) or recognition of IE by nucleotide-binding leucine-rich receptors (NB-LRRs) (7) leads to effector-triggered immune responses (ETI). Signaling events for both PTI and ETI may be inhibited by intracellular effectors. PTI and ETI both produce programmed cell death (8), and effectors may inhibit the triggering of cell death or the cell death machinery itself. PTI and ETI both involve transcriptional changes (9), and nuclear-targeted effectors may interfere with signaling within the nucleus or transcriptional events directly. PTI and ETI involve numerous other responses (10), including the production of reactive oxygen and nitrogen species (RONS), and effectors may interfere with those responses as well).

Patossistema Arabidopsis-Powdery Mildew

EHM, extrahaustorial membrane;

EHMx, extrahaustorial matrix;

ER, endoplasmic reticulum;

JA, jasmonic acid;

MAPK(KK/KKK), mitogen-activated protein kinase (kinase kinase/kinase kinase kinase);

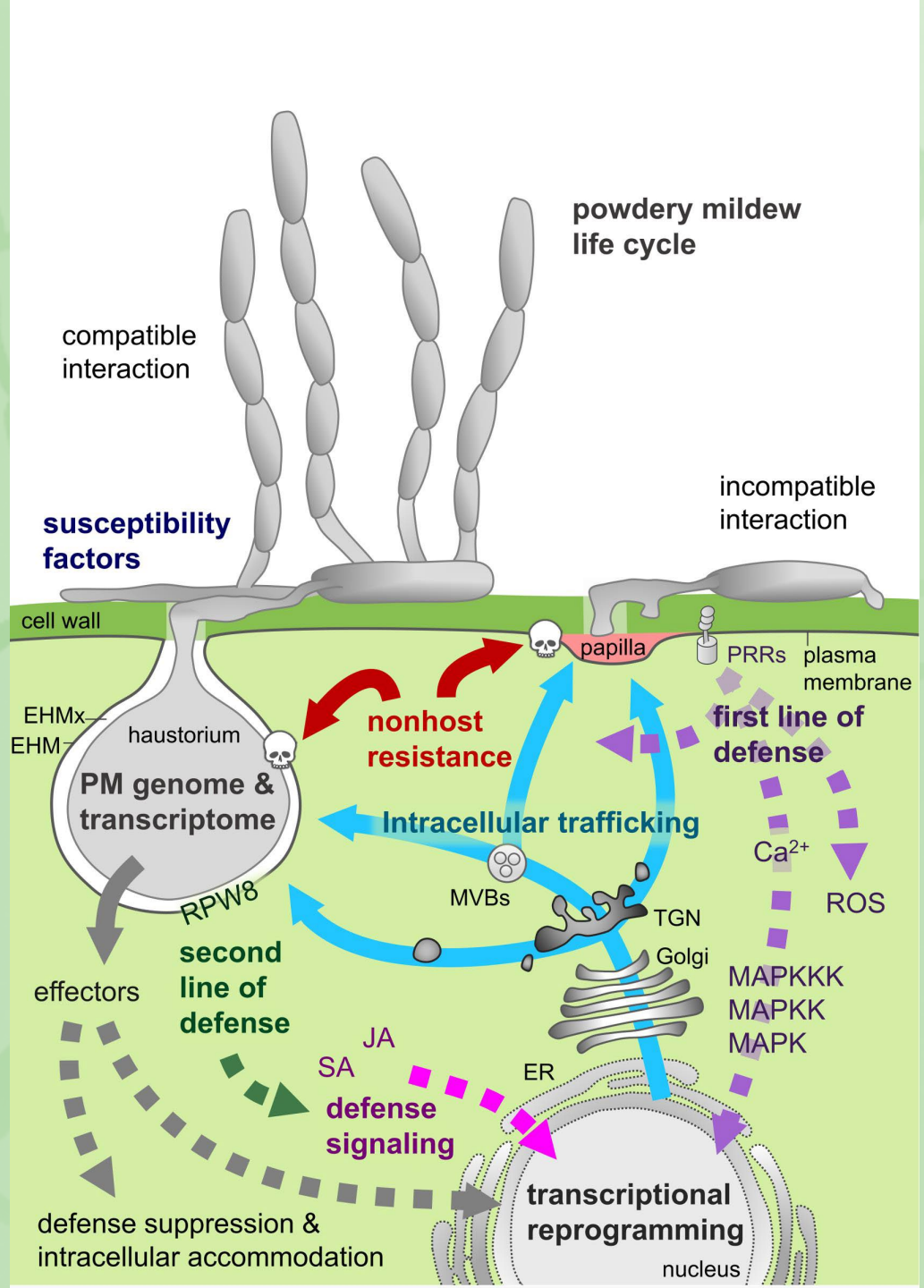
MVBs, multivesicular bodies;

PRRs, pattern recognition receptors;

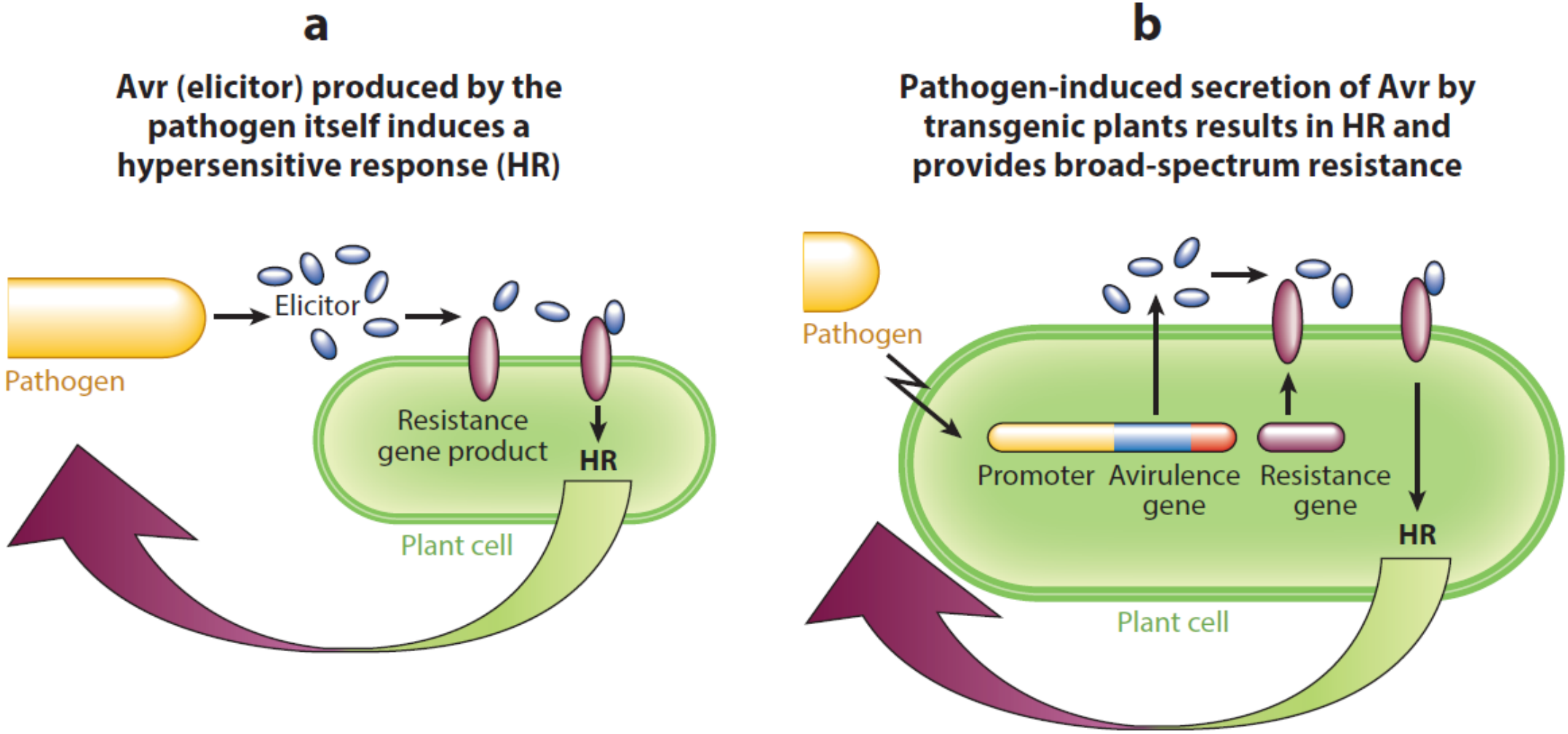
RPW8, RESISTANCE TO POWDERY MILDEW 8 protein;
ROS, reactive oxygen species;

SA, salicylic acid;

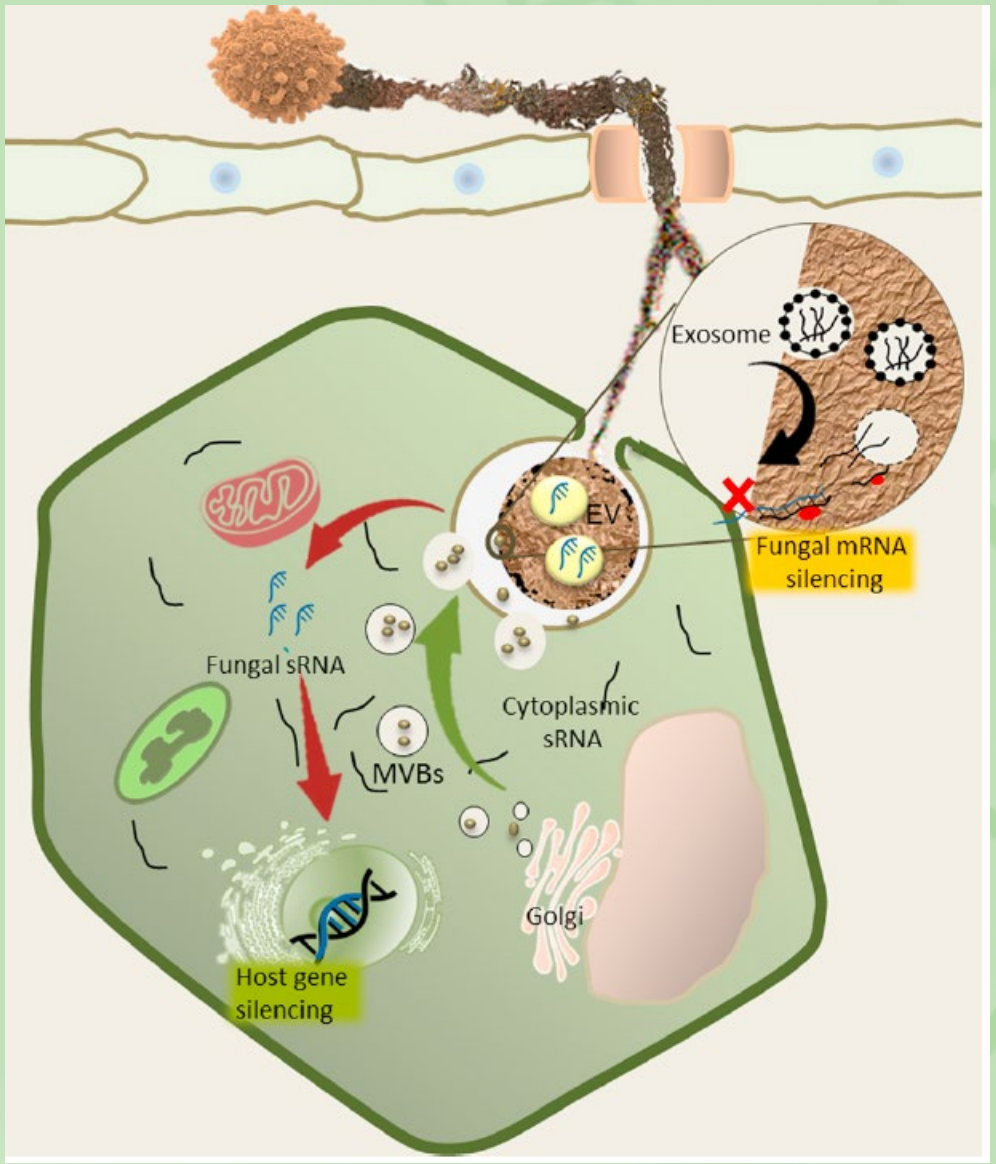
TGN, *trans*-Golgi network.



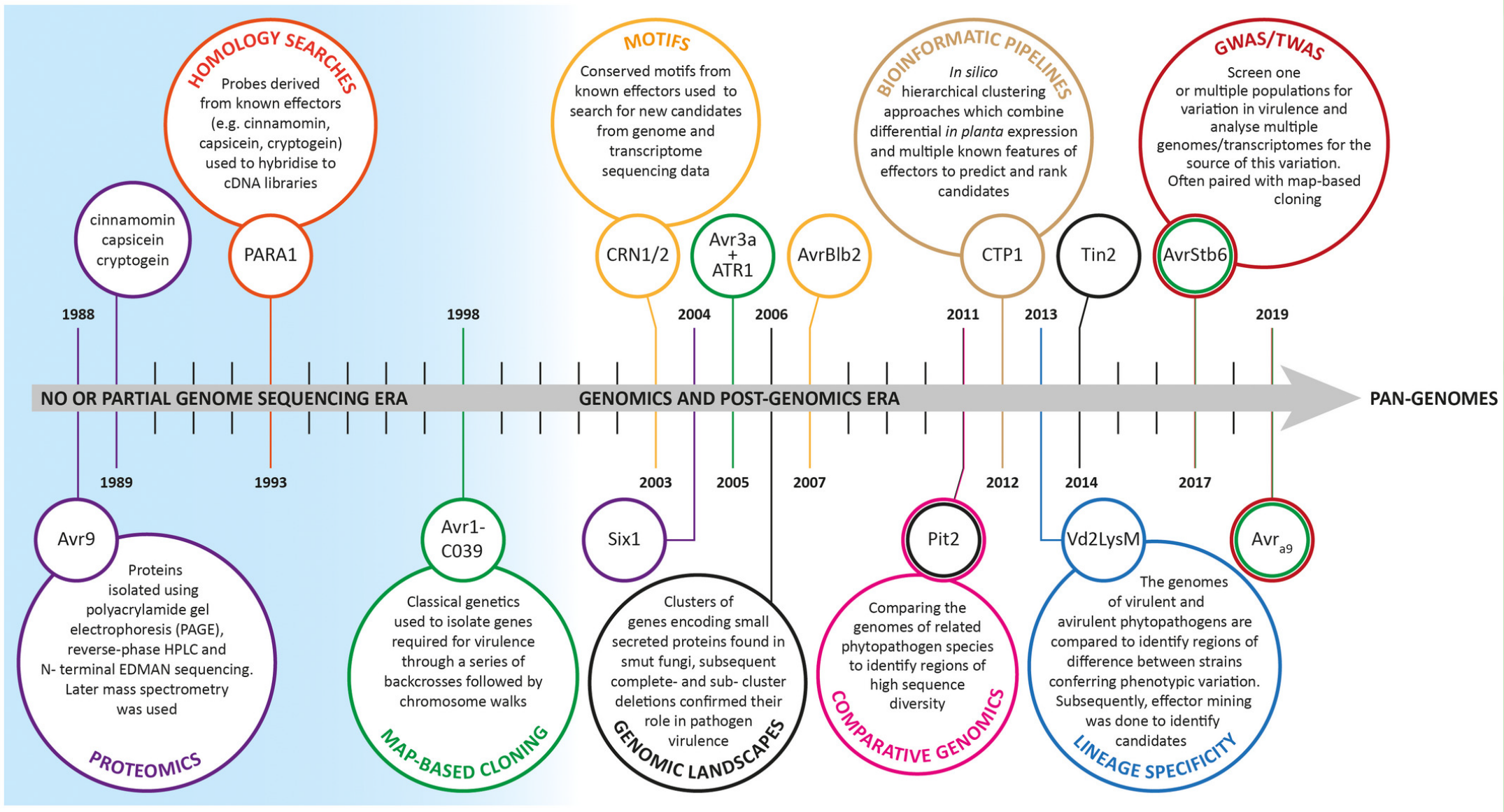
Natural e engenheirado mecanismos de resistência via reconhecimento Avr e R proteínas - HR



Troca de sRNA entre o hospedeiro e os patógenos fúngicos durante o processo de infecção



Proteinaceous effector discovery and characterization in filamentous plant pathogens



Molecular Plant Pathology, Volume: 21, Issue: 10, Pages: 1353-1376, First published: 07 August 2020, DOI: (10.1111/mpp.12980)

TABLE 2 Approaches and techniques deployed for effector discovery and the initial proteins/genes successfully isolated

Technique	Effector	Species	Reference
Proteomics	Avr9	*Cladosporium fulvum*	Schottens-Toma and de Wit (1988); van Kan et al. (1991)
	Six1	*Fusarium oxysporum* f. sp. *lycopersici*	Rep *et al.* (2004)
Map-based cloning	Avr1-CO39	*Magnaporthe grisea*	Farman and Leong (1998)
	Avr3a	*Phytophthora infestans*	Armstrong *et al.* (2005)
	ATR1	*Hyaloperonospora parasitica*	Rehmany *et al.* (2005)
Homology searches	PARA1	*Phytophthora parasitica*	Kamoun *et al.* (1993)
	INF1	*P. infestans*	Kamoun *et al.* (1997)
Motifs/secretion peptides	Crn1 and Crn2	*P. infestans*	Torto *et al.* (2003)
	AvrBIb2	*P. infestans*	Win *et al.* (2007); Oh et al. (2009)
Genomic landscapes	Tin2	*Ustilago maydis*	Kämper *et al.* (2006); Brefort *et al.* (2014)
Comparative genomics	Pit2	*U. maydis*	Doehlemann *et al.* (2011)
Bespoke bioinformatic pipelines	CTP1	*Melampsora larici-populina*	Saunders *et al.* (2012b); Petre *et al.* (2015)
Lineage-specific	Vd2LysM	*Verticillium dahliae*	de Jonge *et al.* (2013); Kombrink *et al.* (2017)
GWAS/TWAS	AvrStb6	*Zymoseptoria tritici*	Zhong *et al.* (2017)
	Avr_{a9}	*Blumeria graminis* f. sp. *hordei*	Saur *et al.* (2019a)

Molecular Plant Pathology, Volume: 21, Issue: 10, Pages: 1353-1376, First published: 07 August 2020, DOI: (10.1111/mpp.12980)

ABREVIATURAS E SÍMBOLOS

ETI.....Imunidade ativada por efetores
HR.....Resposta hipersensitiva
ISR.....Resistência sistêmica induzida
LPS.....Lipopolissacarídeos
LRR.....Receptor rico em leucina
MAMP.....Padrões moleculares associados a microrganismos
MAPK.....Proteína quinase ativada por mitogênese
NO.....Óxido nítrico
NPP.....Proteínas indutoras de necrose
PAMP.....Padrões moleculares associados a patógenos
PR.....Proteínas de resistência
PRR.....Receptores para padrão de reconhecimento
PTI.....Imunidade ativada por PAMPs
SA.....Ácido salicílico
SAR.....Resistência sistêmica adquirida
ROS.....Espécies reativas de oxigênio
T3SS.....Sistema de secreção tipo III
TLR.....Receptores toll-like