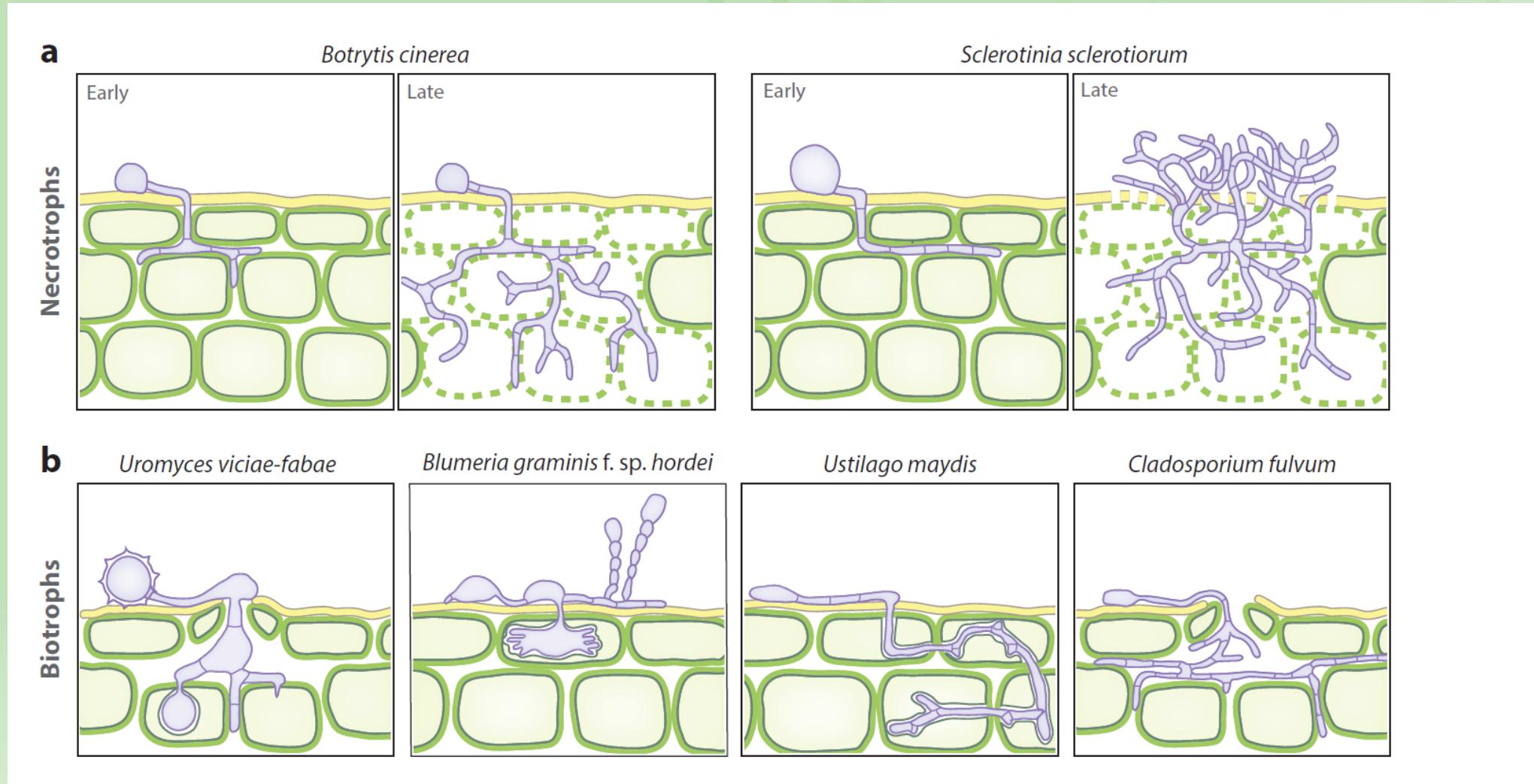


# Conceitos introdutórios da genética de interações patógeno-planta

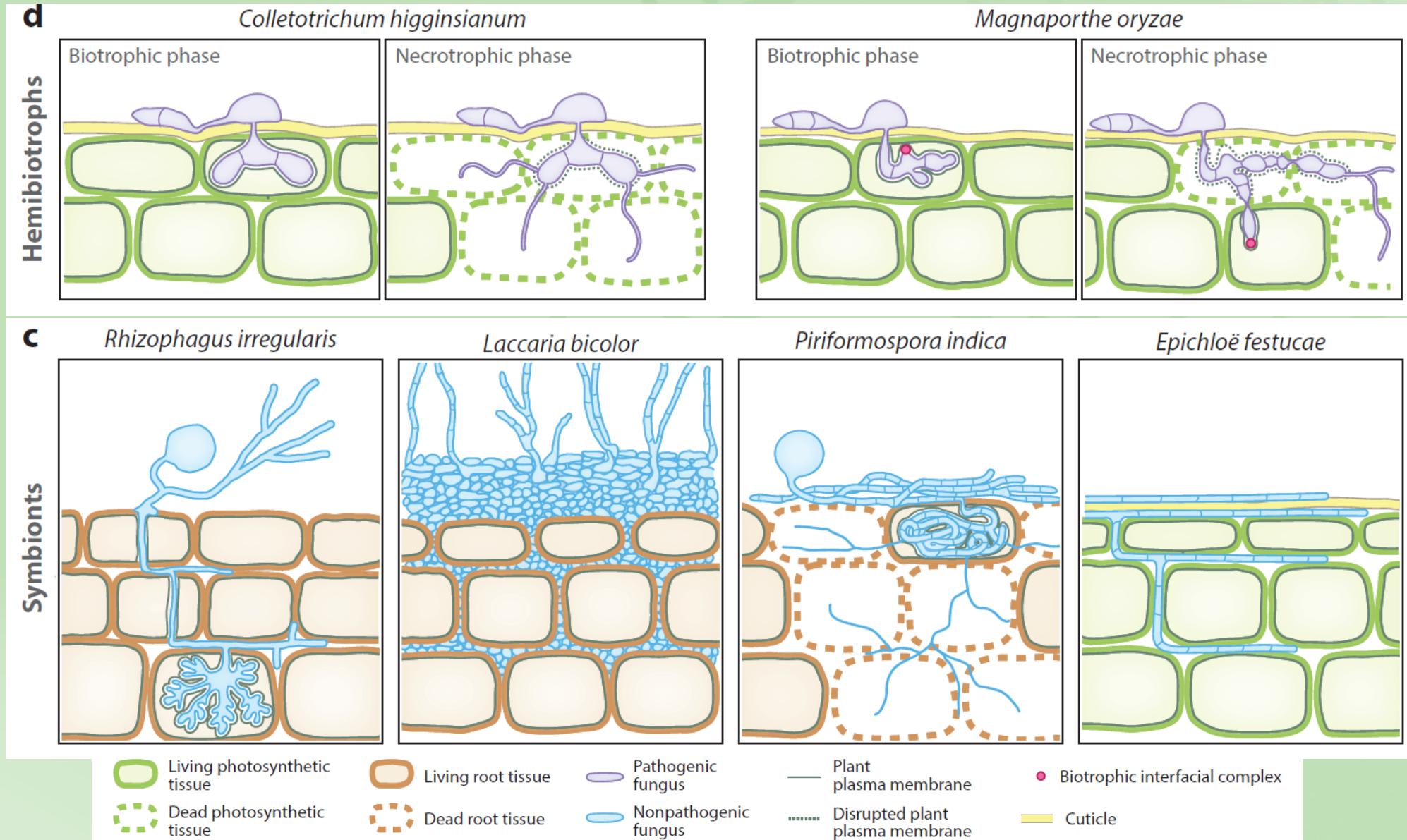
Profa. Dra. Chirlei Glienke  
BIOGEMM-UFPR

# Estratégias de interação de diferentes patógenos com os hospedeiros – modos de vida

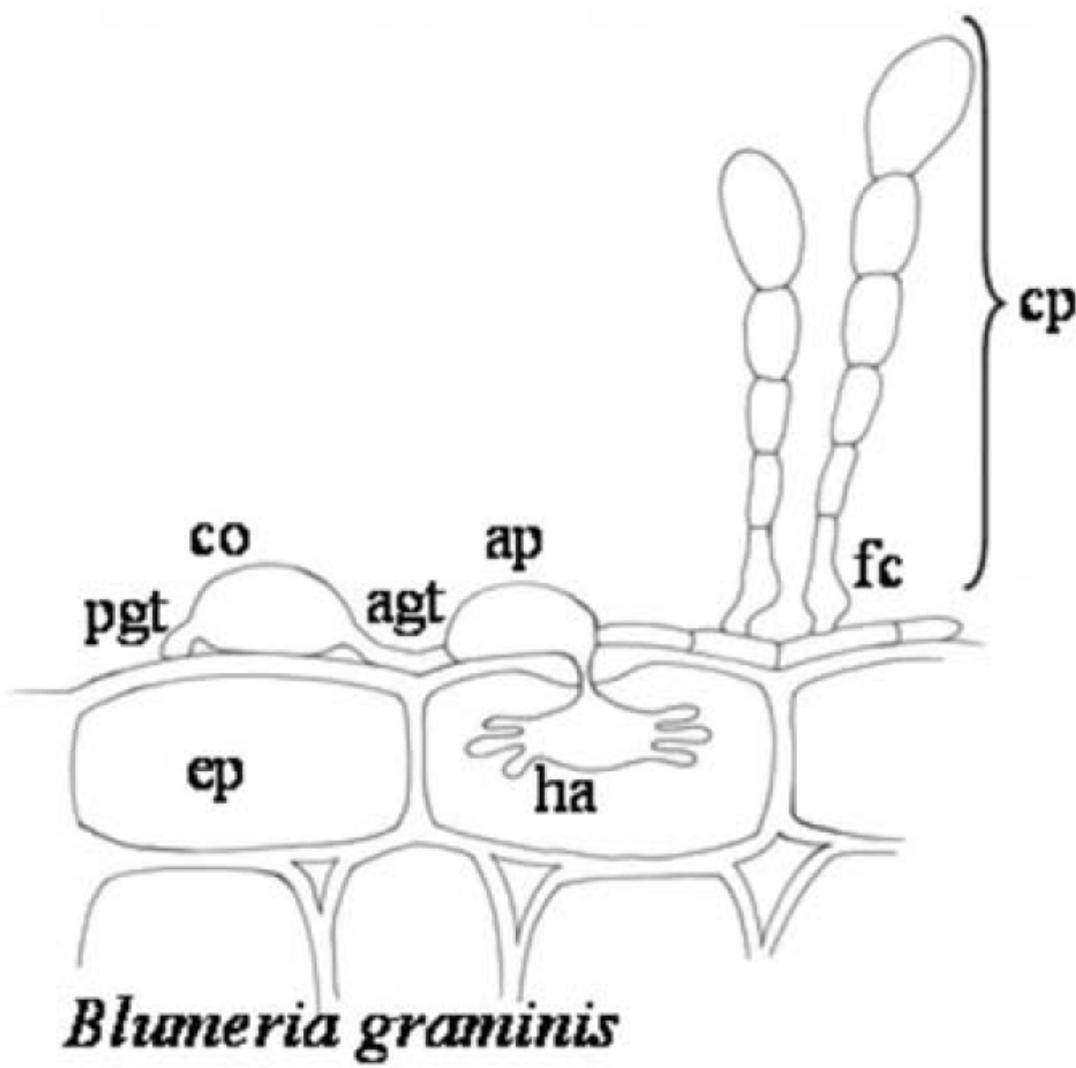


Living photosynthetic tissue      Living root tissue      Pathogenic fungus      Plant plasma membrane      Biotrophic interfacial complex  
Dead photosynthetic tissue      Dead root tissue      Nonpathogenic fungus      Disrupted plant plasma membrane      Cuticle

# Estratégias de interação de diferentes patógenos com os hospedeiros – modos de vida

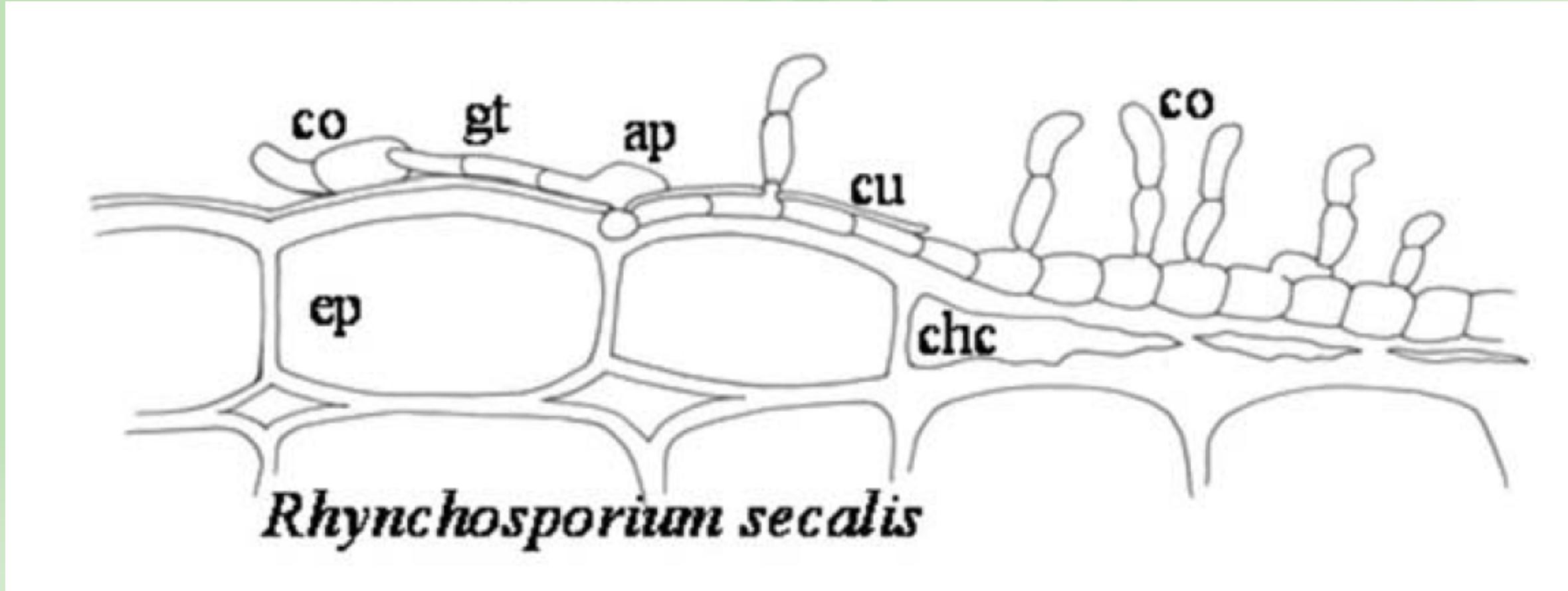


# Biotrófico obrigatório *Blumeria graminis*



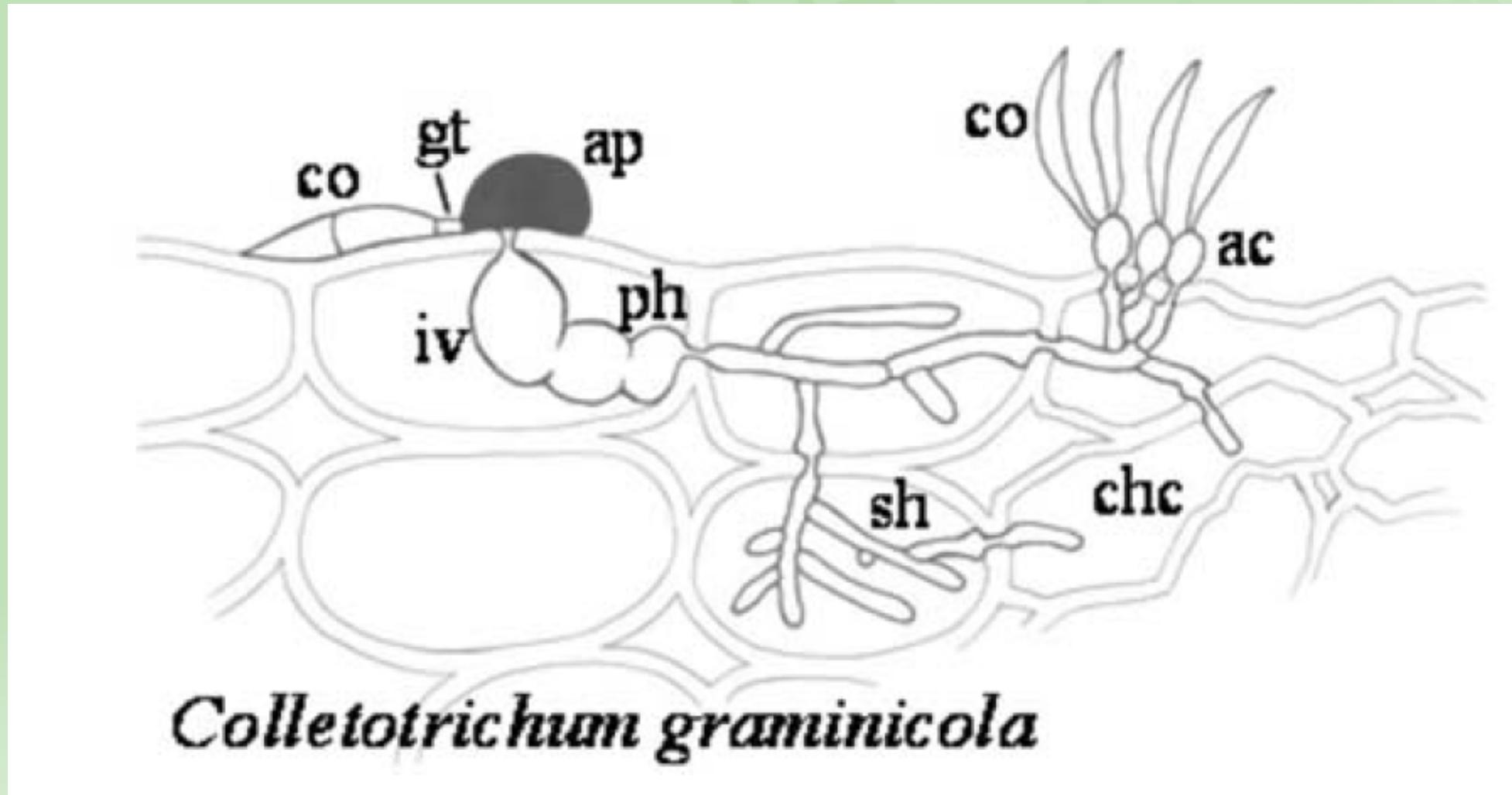
Primary and an appressorial  
germ tube (pgt; agt),  
Appressorium (ap)  
Epidermal cell of the host (ep)  
Haustorium (ha)  
Conidiophores (cp),  
Foot cell (fc).

# Necrotófico *Rhynchosporium secalis*



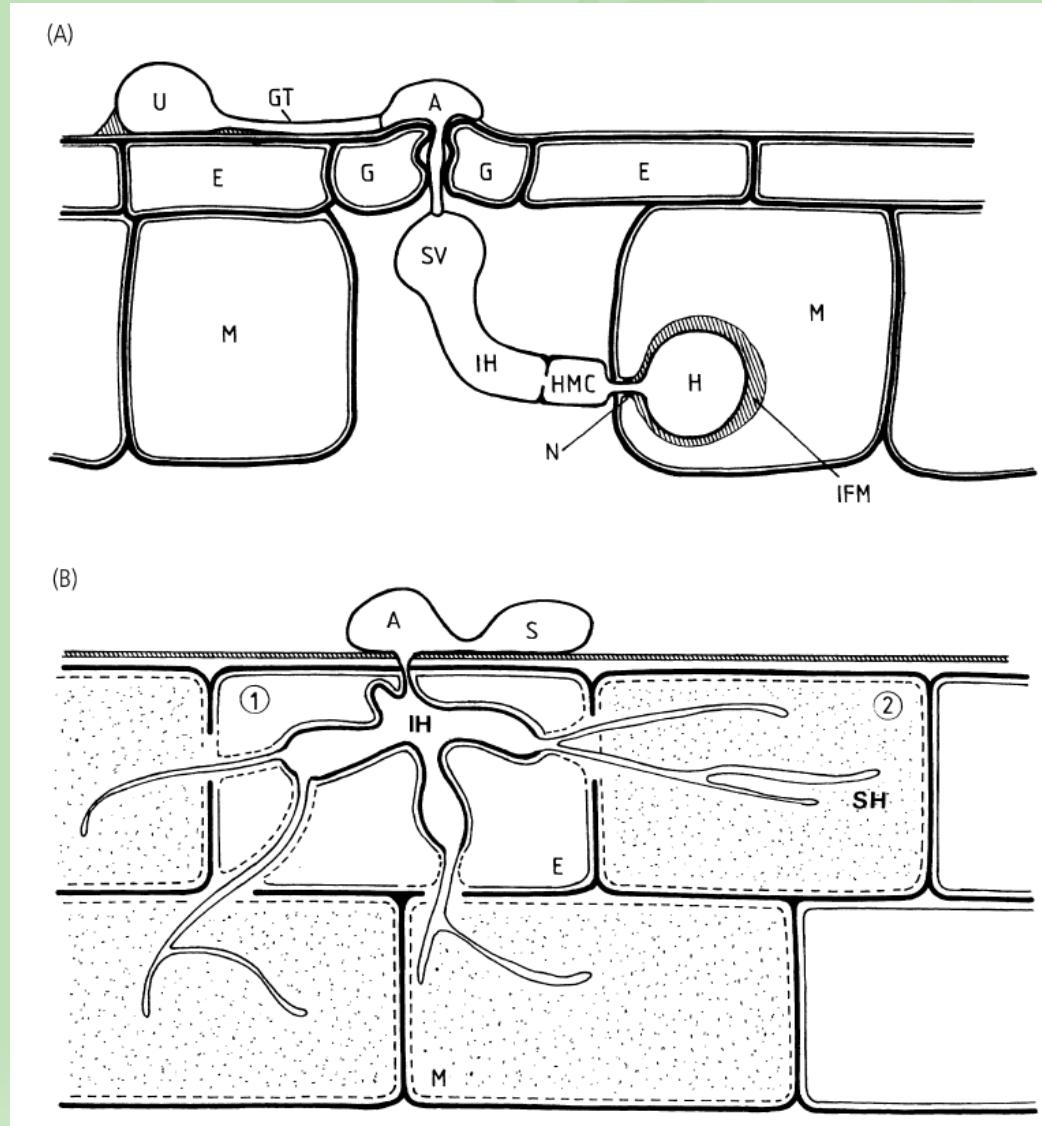
Germ tube (gt); appressorium (ap); cuticle (cu)

# Hemibiotrófico *Colletotrichum graminicola*

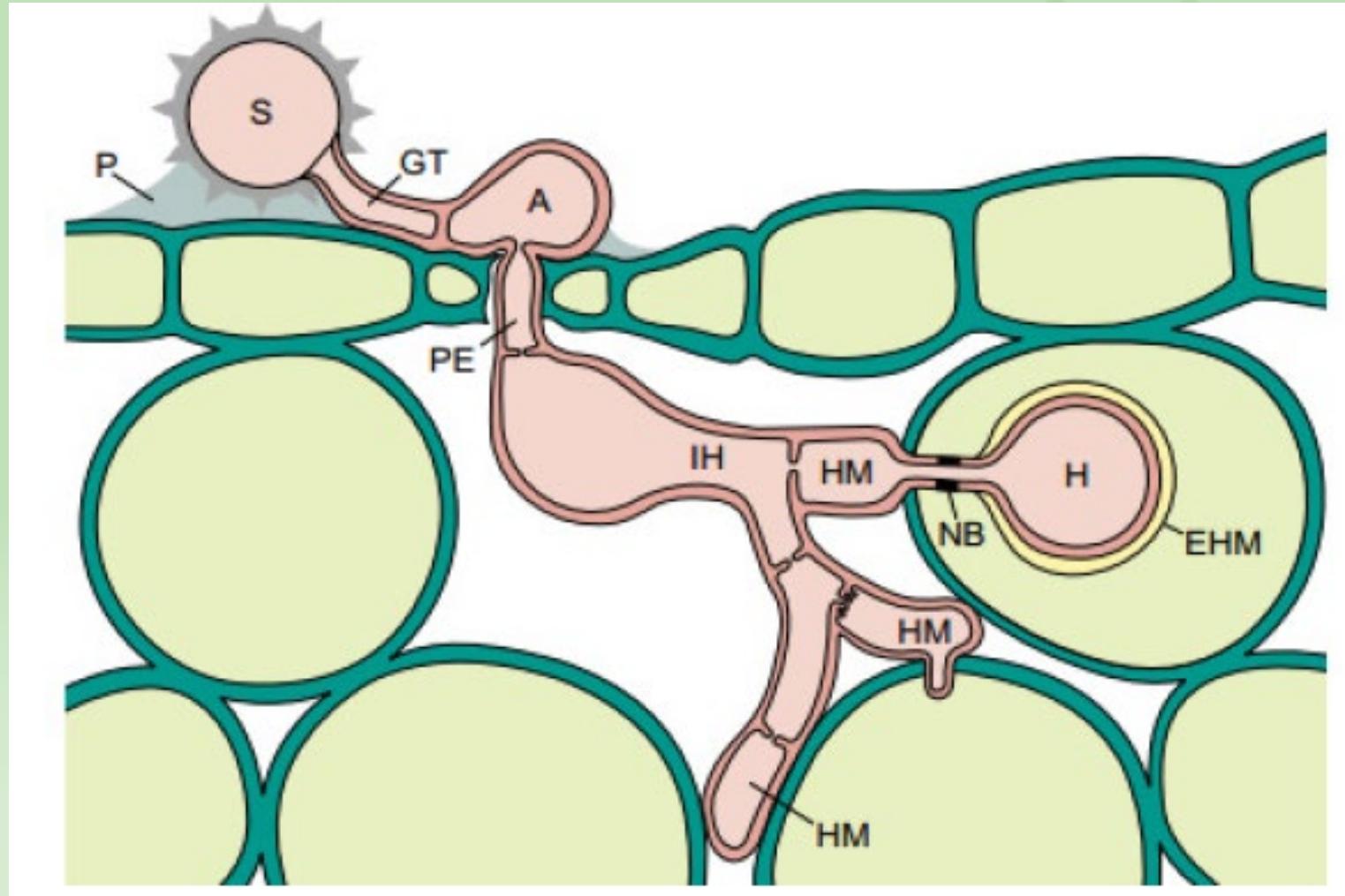


Conidium (co); germ tube (gt); melanized appressorium (ap); biotrophic infection vesicle (iv); primary hyphae (ph) necrotrophic secondary hyphae (sh); acervuli (ac)

# Biotróficos x Hemibiotróficos



# Infecção pelo fungo biotrófico *Uromyces fabae*

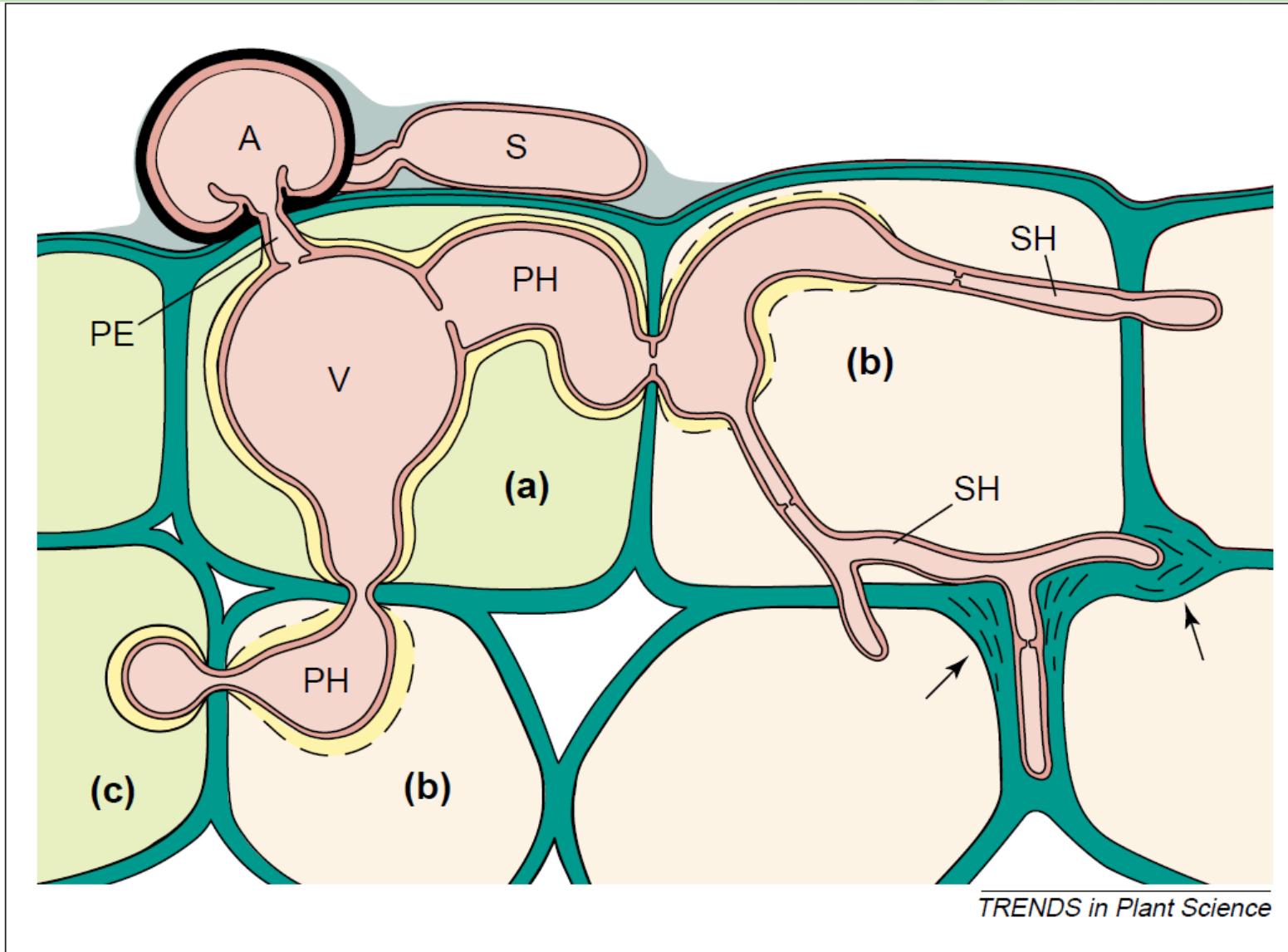


S: anurediospore  
GT: Tubo germinativo  
P: adhesion pad  
A: appressorium (entra pelo estômato)  
PE: Hifa de penetração  
IH: Hifa de penetração  
HM: haustorial mother cell (HM)  
H: haustorium  
NB: dark-staining neck-band  
EHM: extrahaustorial Membrane

# Fungos biotróficos de importância econômica

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

# Infecção pelo fungo hemibiotrófico *Colletotrichum lindemuthianum*



S: esporo

A: Apresso  
melanizado

PE: Hifa de penetração

V: Vesícula

PH: Hifa primária,  
a) Fase biotrófica

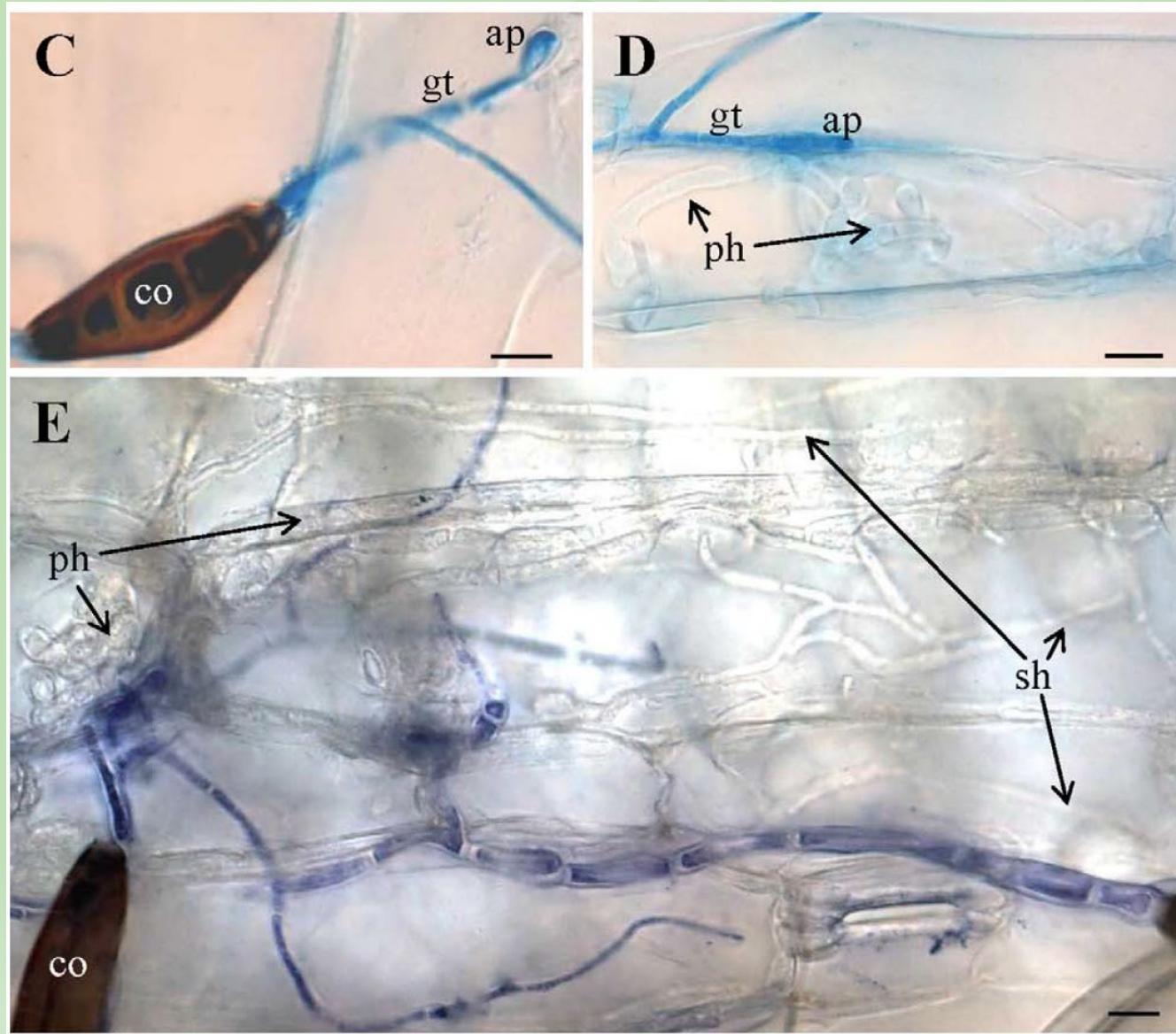
Yellow: Matrix de  
separação entre fungo e  
hospedeiro

b) Morte celular

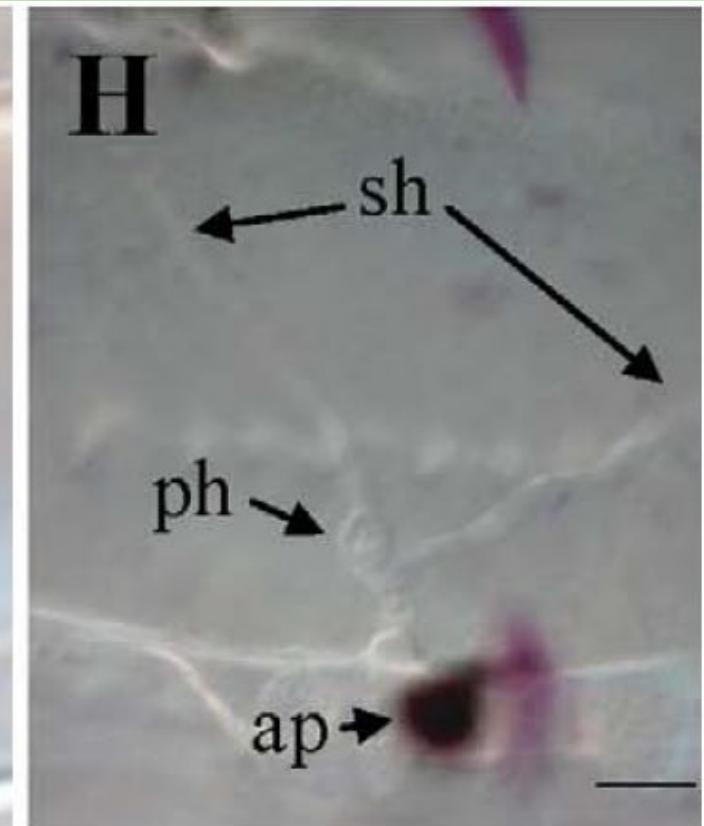
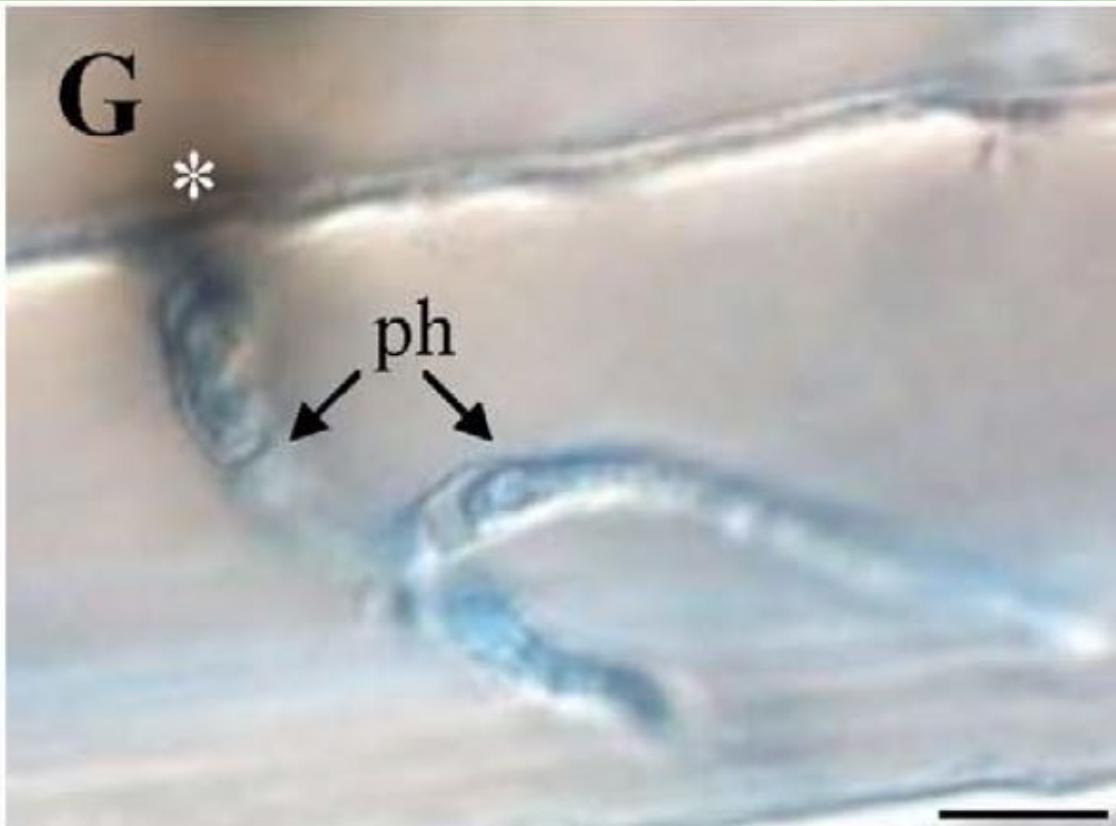
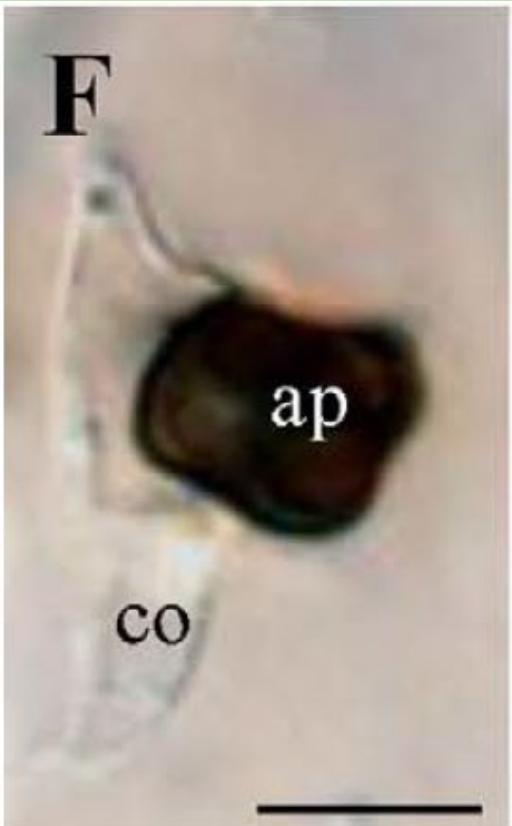
SH: Hifa secundária

Arrows: Quebra de  
parede celular mediada  
pela secreção de  
CWDEs pelas Hifas  
secundárias

# Hemibiotrófico *Bipolaris sorokiniana*



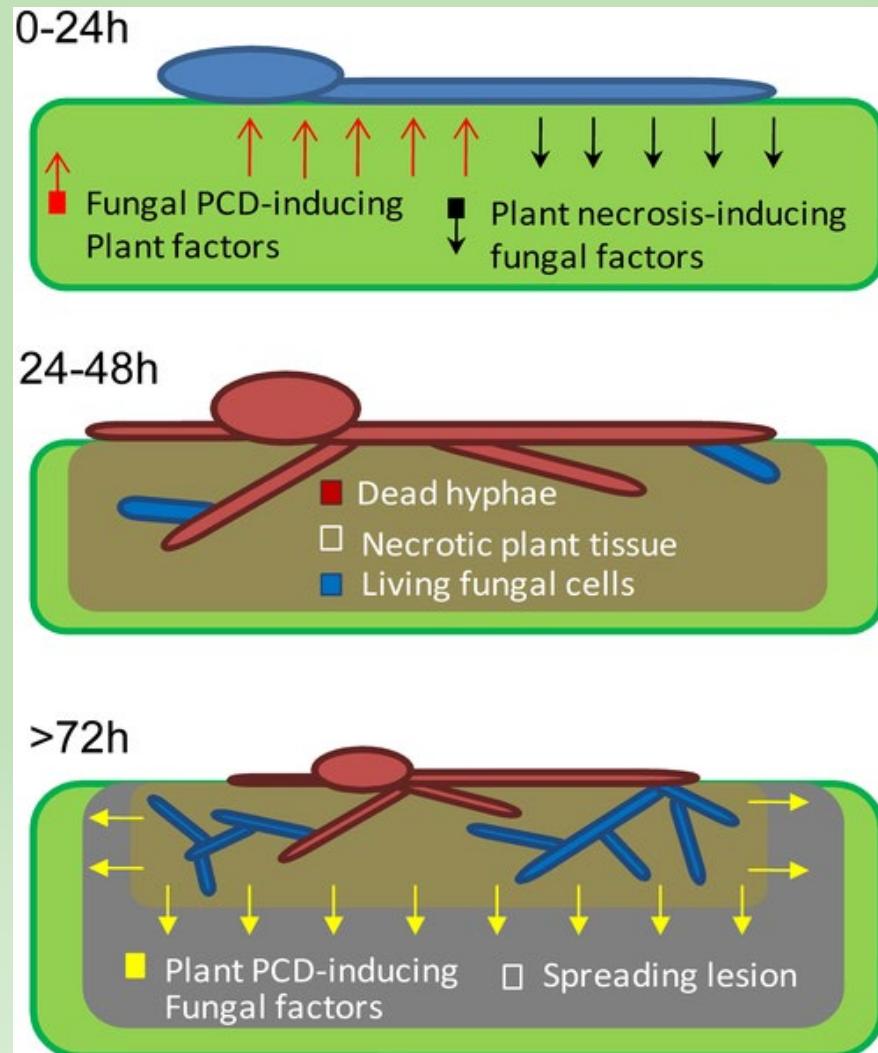
# Biotrófico *Colletotrichum graminicola*



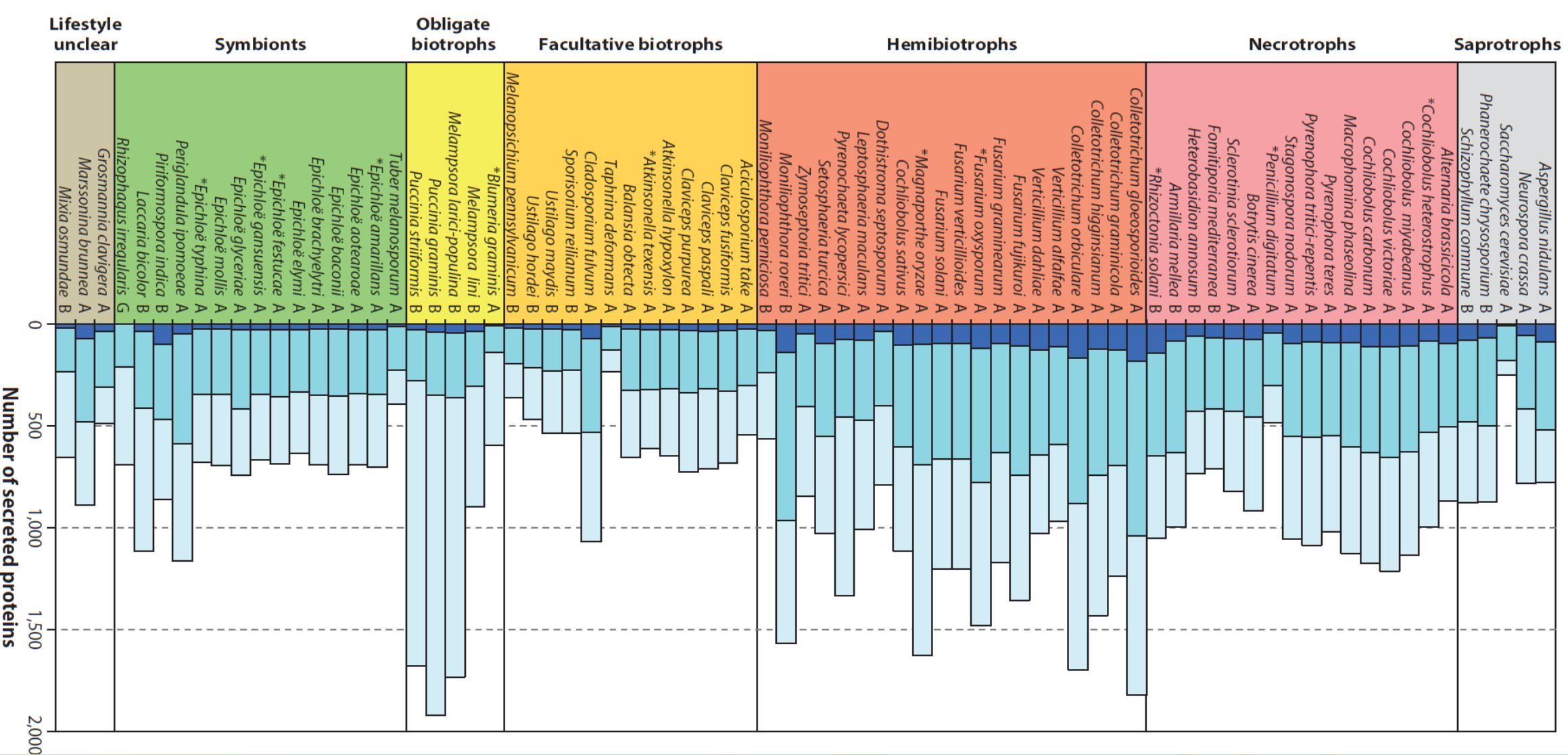
# Listo de patógenos biotróficos e hemibiotróficos

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

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



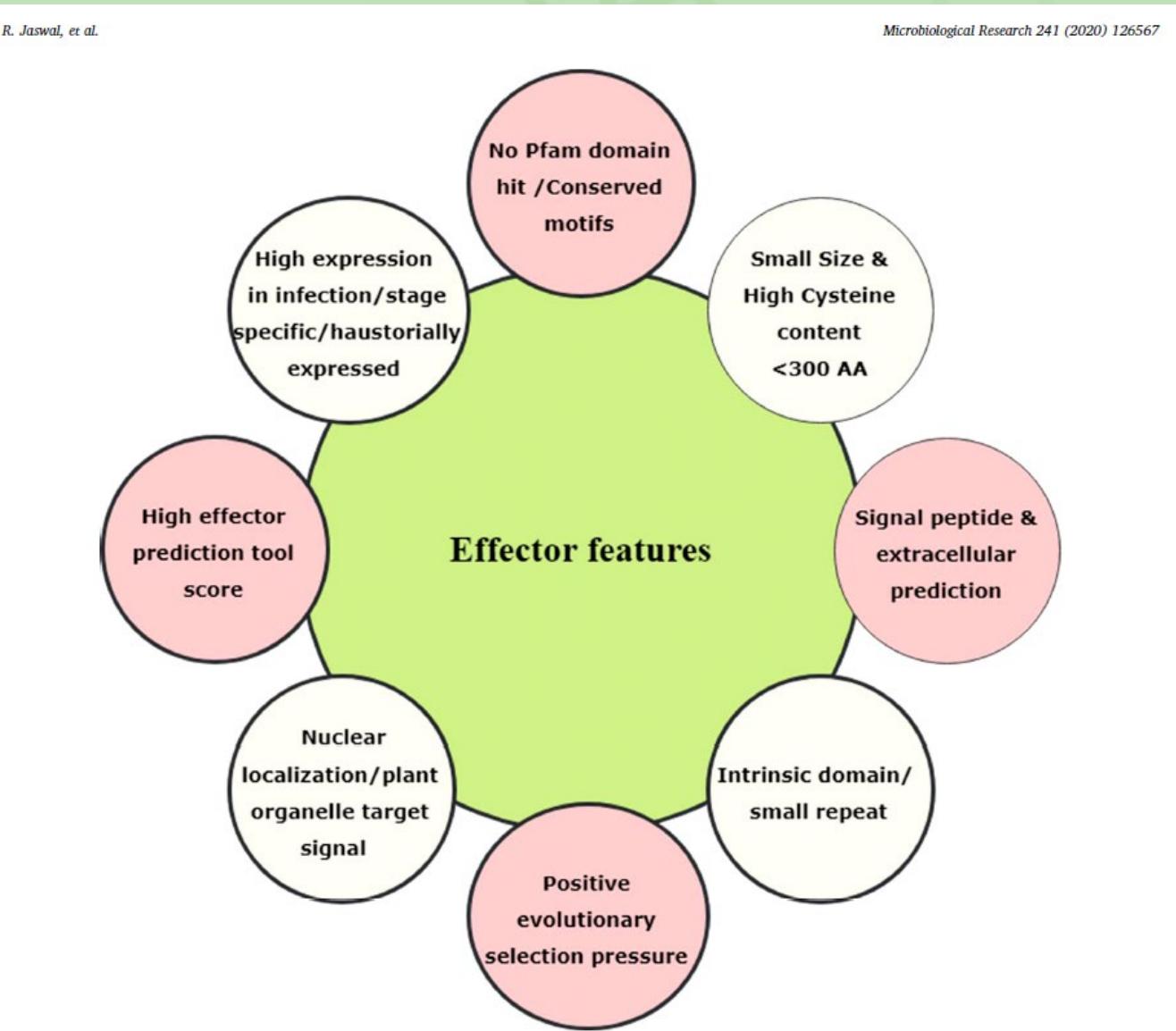
- **Necrotróficos não colonizam células vivas**
  - Morte celular programada do fungo induzida por fatores vegetais
  - Necrose de tecidos vegetais induzida por fatores fúngicos
  - PCD do vegetal induzido por fatores fúngicos



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

R. Jaswal, et al.

Microbiological Research 241 (2020) 126567

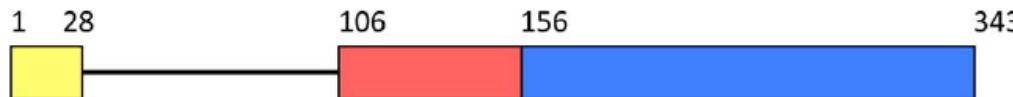


# Organização de efetores apoplásticos e citoplasmáticos

## Fungal effectors

N-terminal uptake domains without conserved motifs, no effector superfamily identified so far

**AvrM** - *Melampsora lini*



**AvrL567** - *Melampsora lini*



## Oomycete effectors

N-terminal targeting/translocation domains with conserved motifs (RXLR, LFLAK, CHXC) that define effector superfamilies

**AVR3a** - *Phytophthora infestans*



**AVR1b** - *Phytophthora sojae*



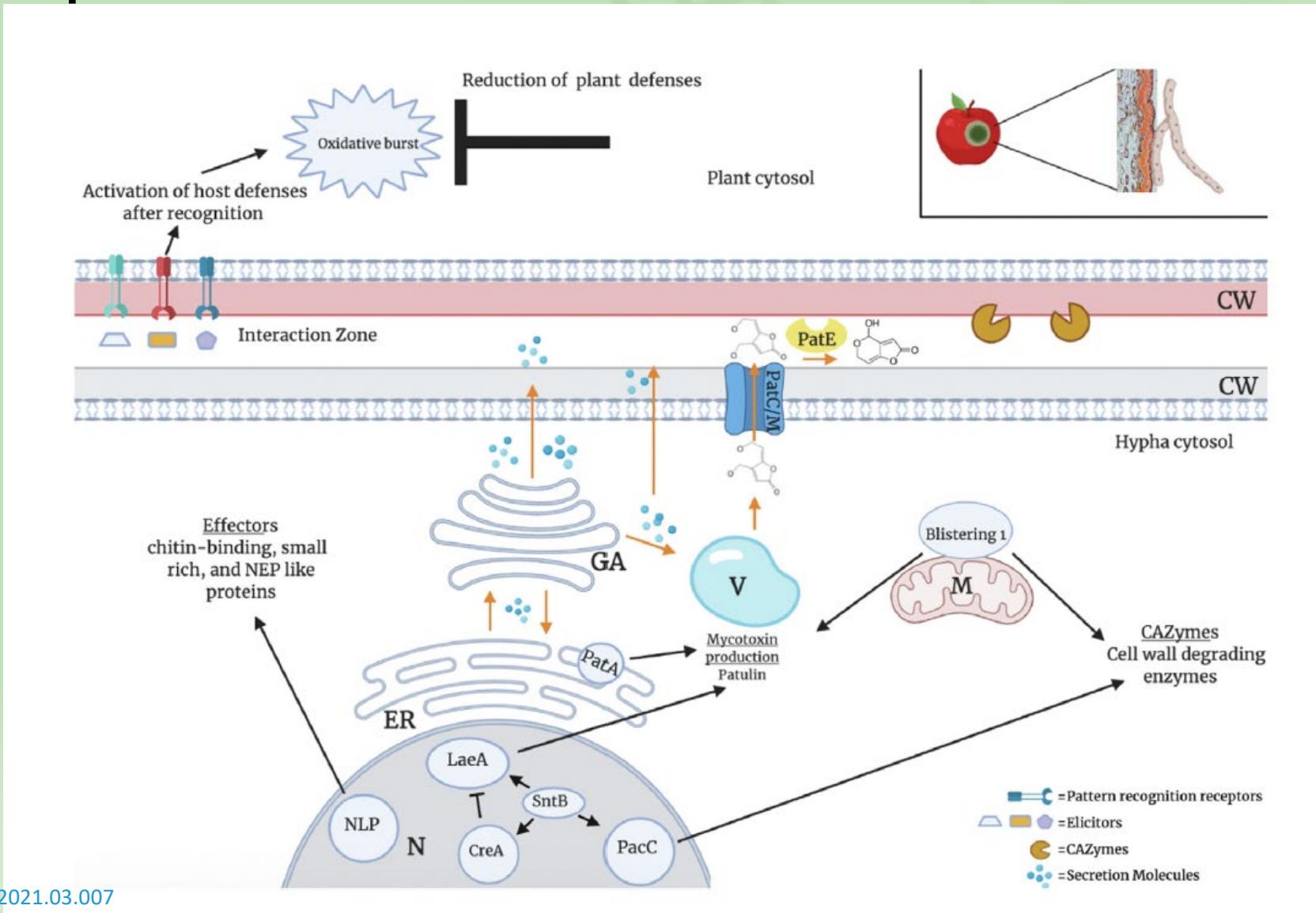
Signal peptide for secretion

Uptake or translocation domain

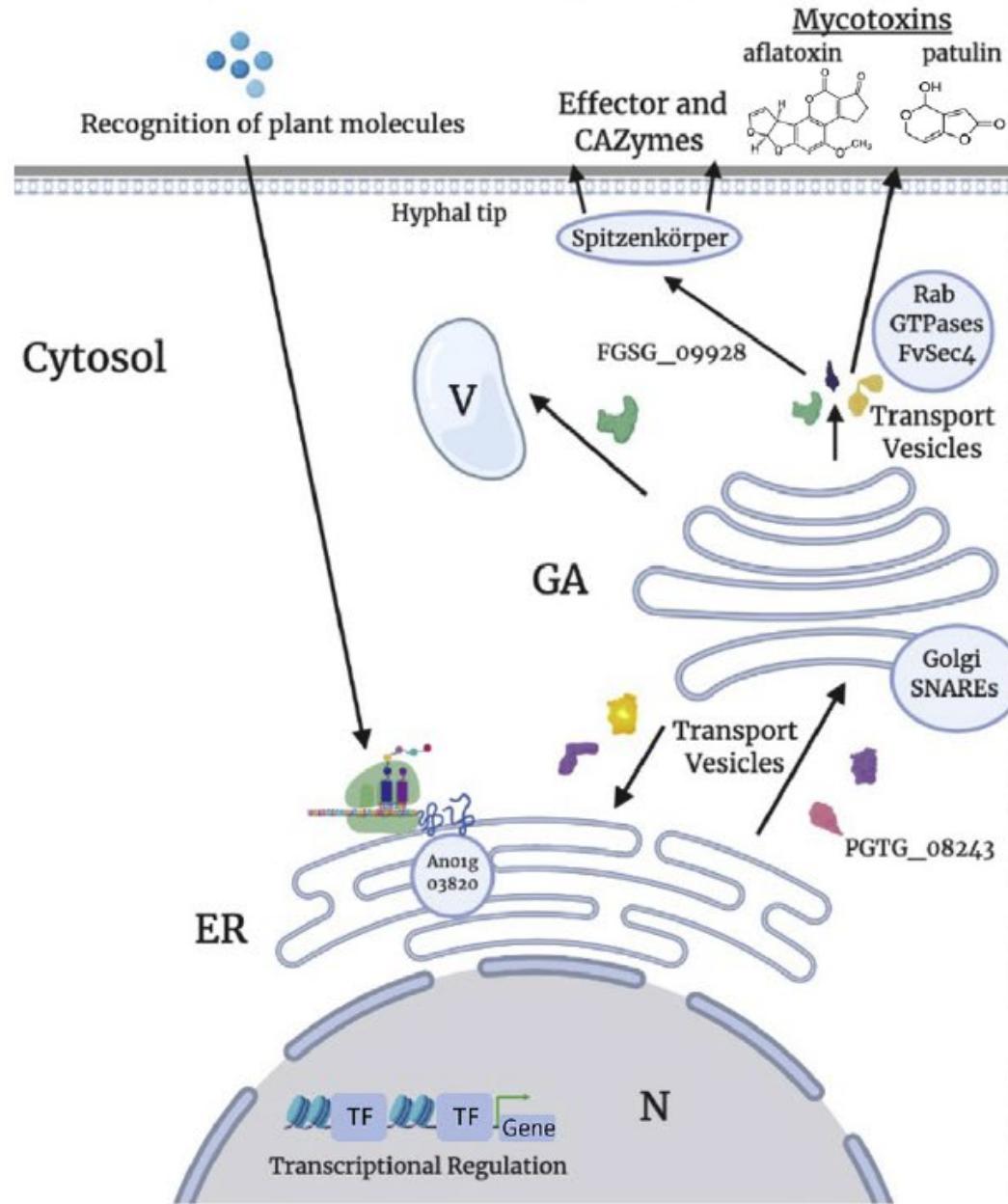
C-terminal region

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

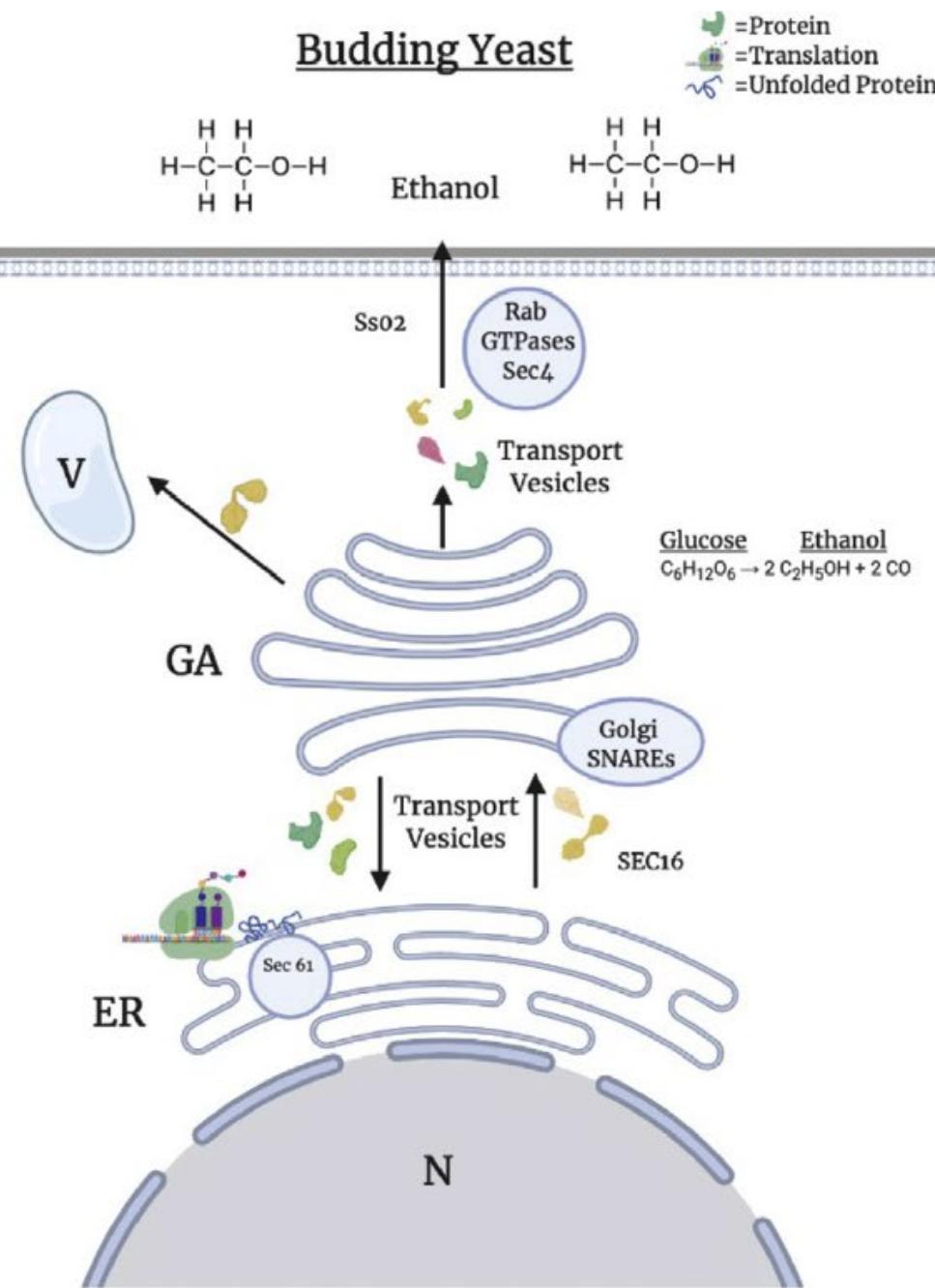
# Modelo conceitual atual exibindo as moléculas secretoras e componentes no patossistema *Penicillium expansum* e *Malus domestica* que estão associados à virulência



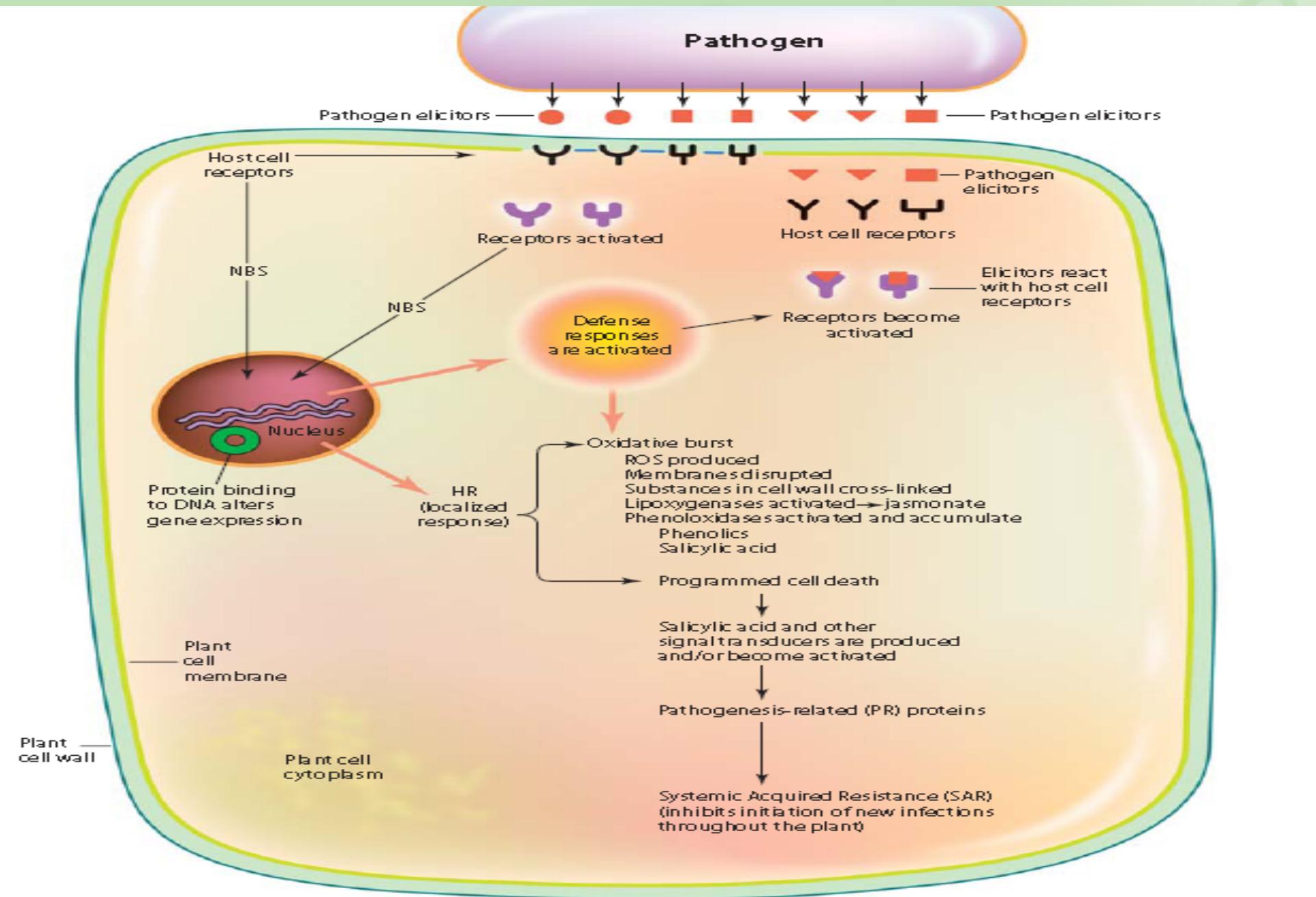
## Filamentous Phytopathogenic Fungi



## Budding Yeast



# Genes efetores como genes de avirulência



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

## Genetic model

Fungus	Resistance gene Avirulence gene	Plant	<i>R</i>	<i>r</i>
Avr			Resistance	Disease
<i>avr</i>			Disease	Disease

## Biochemical model

Plant: R protein

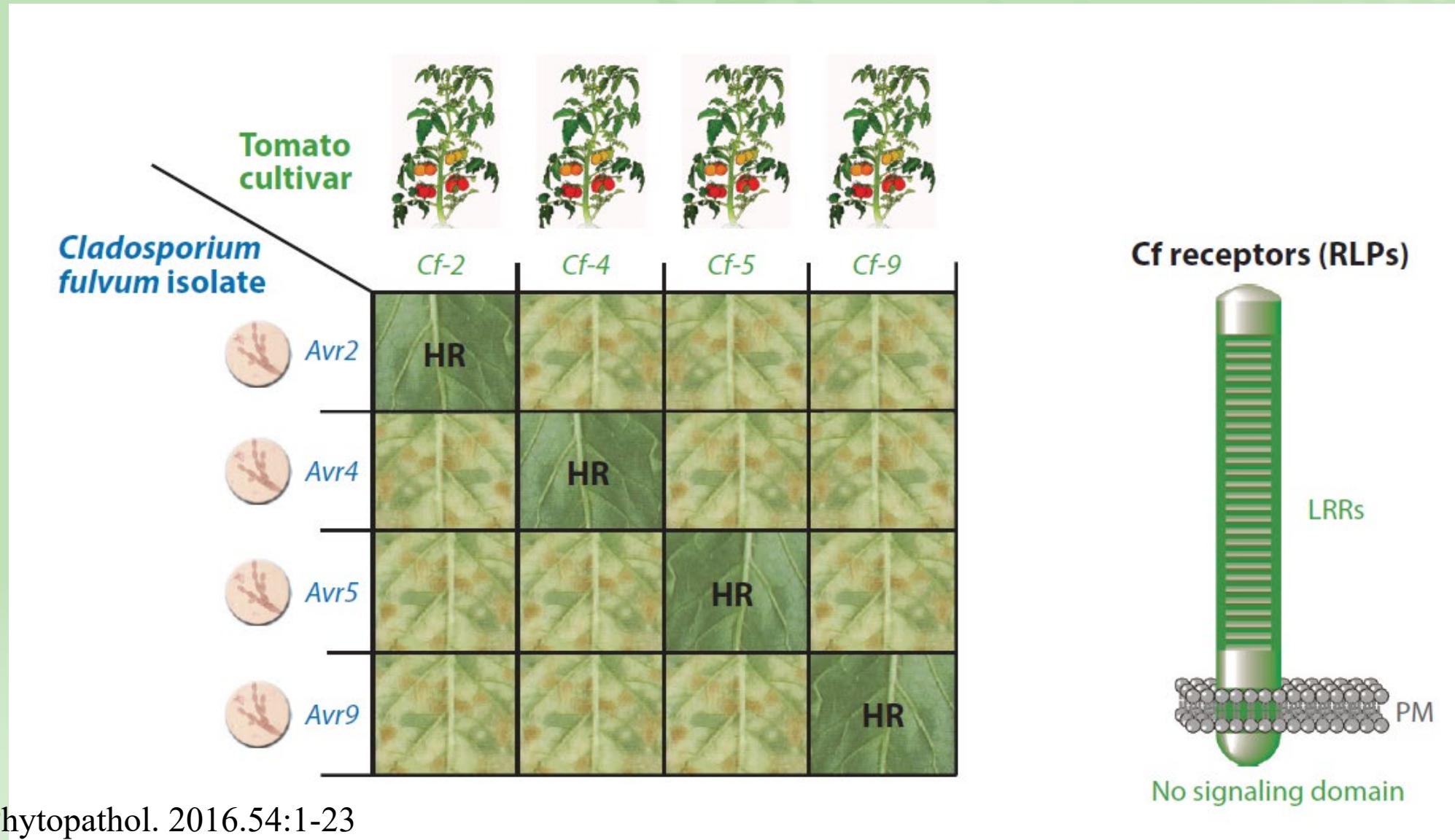


Resistance (HR)	Disease (no HR)

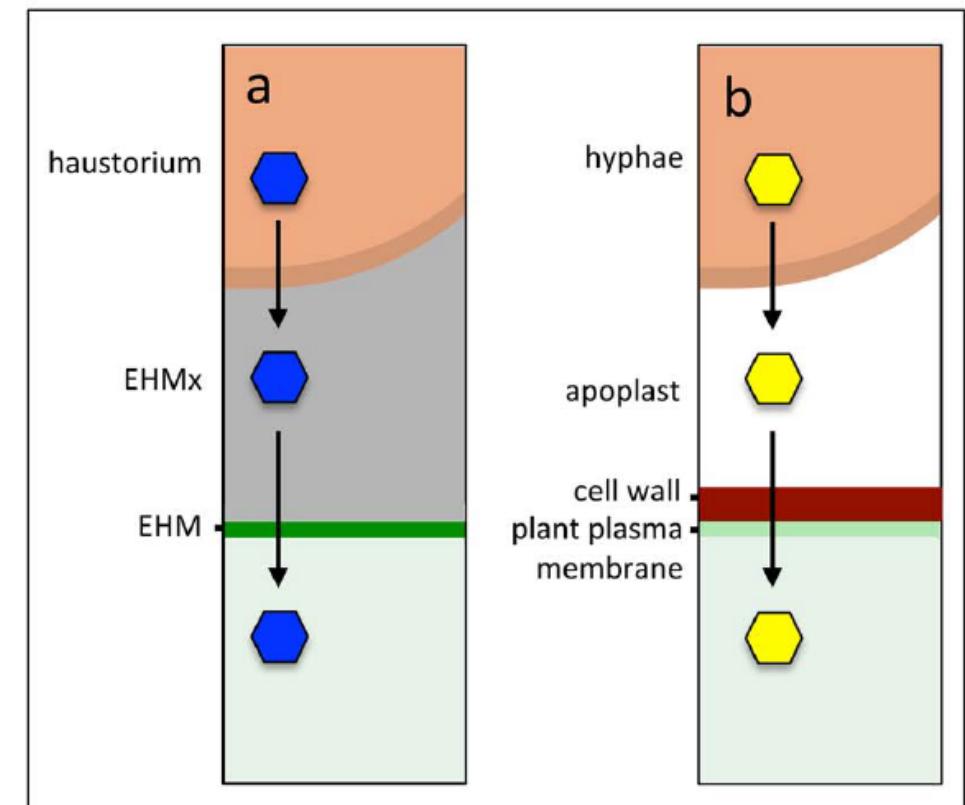
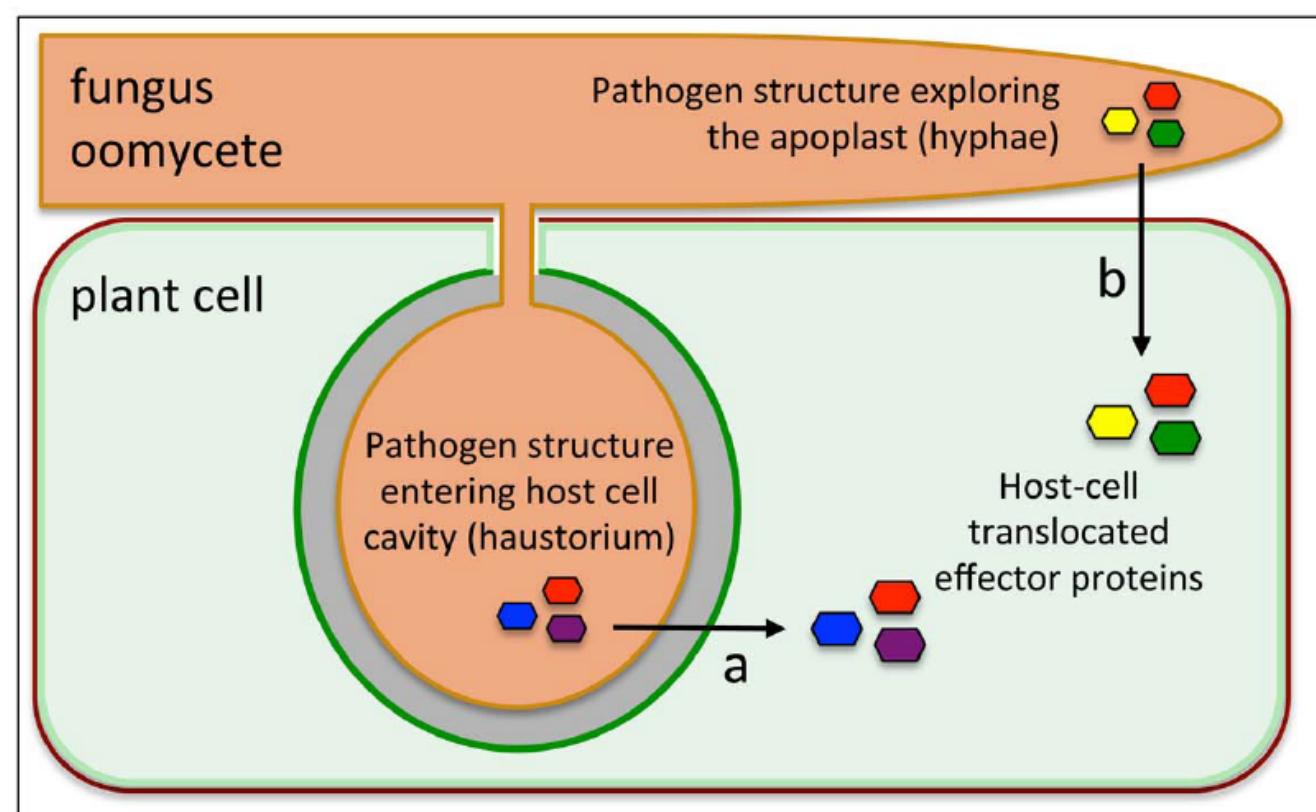
Fungus: Avr protein



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



# Estruturas de fungos e oomicetos para secreção de efetores



microbial plasma membrane

plant cell wall

plant plasma membrane

extra-haustorial membrane (EHM)

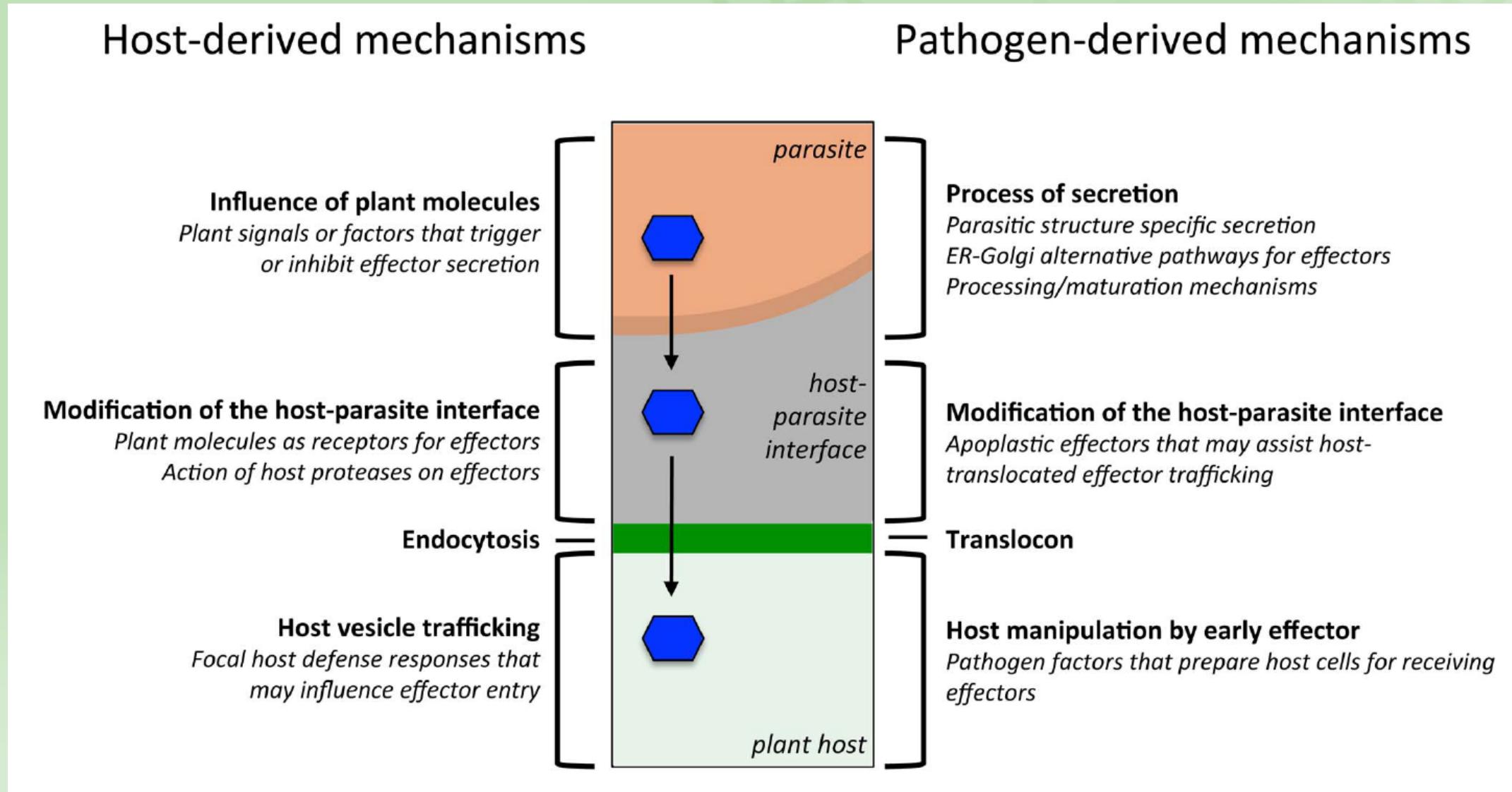
microbial cytoplasm

plant cytoplasm

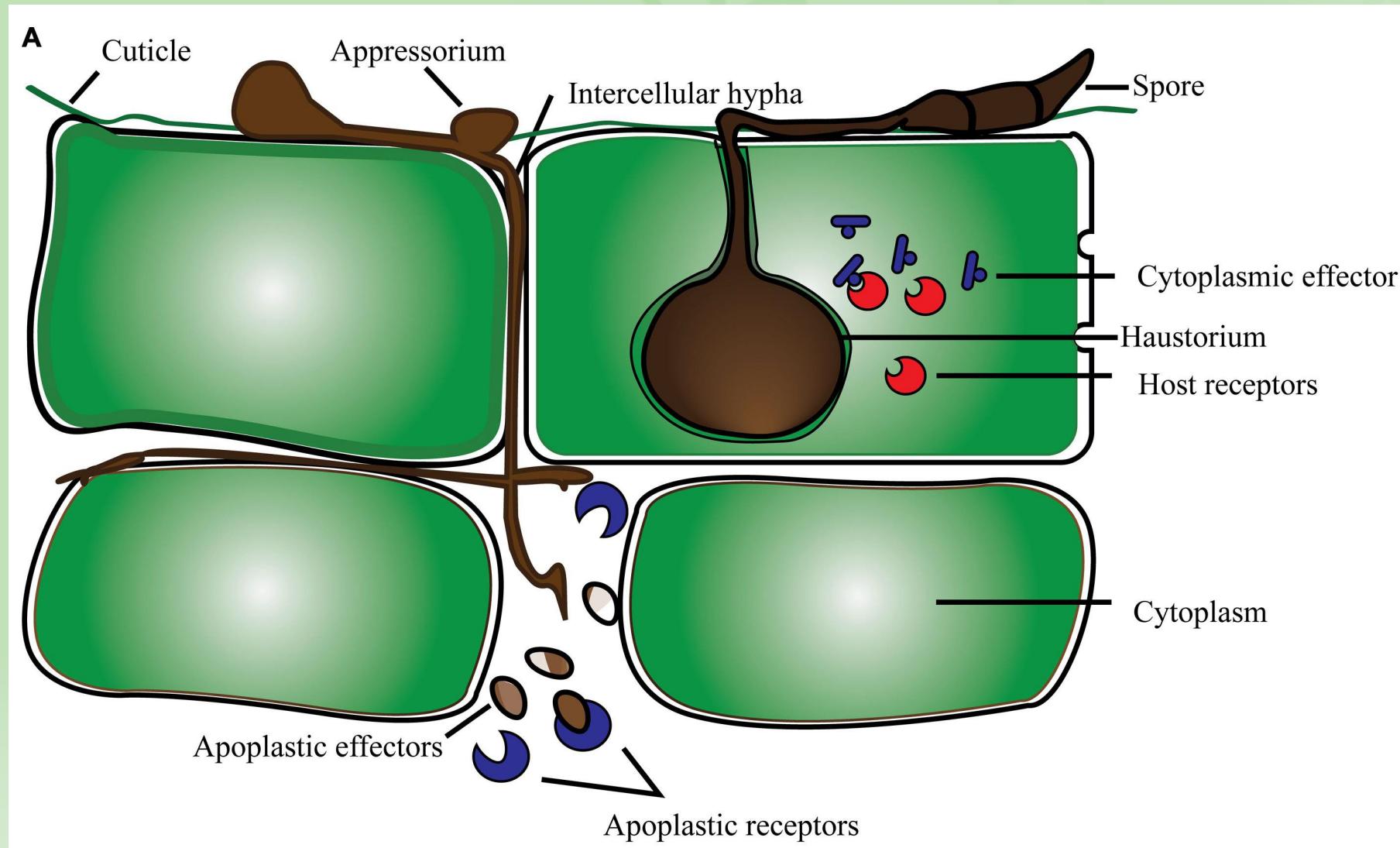
apoplast

extra-haustorial matrix (EHMx)

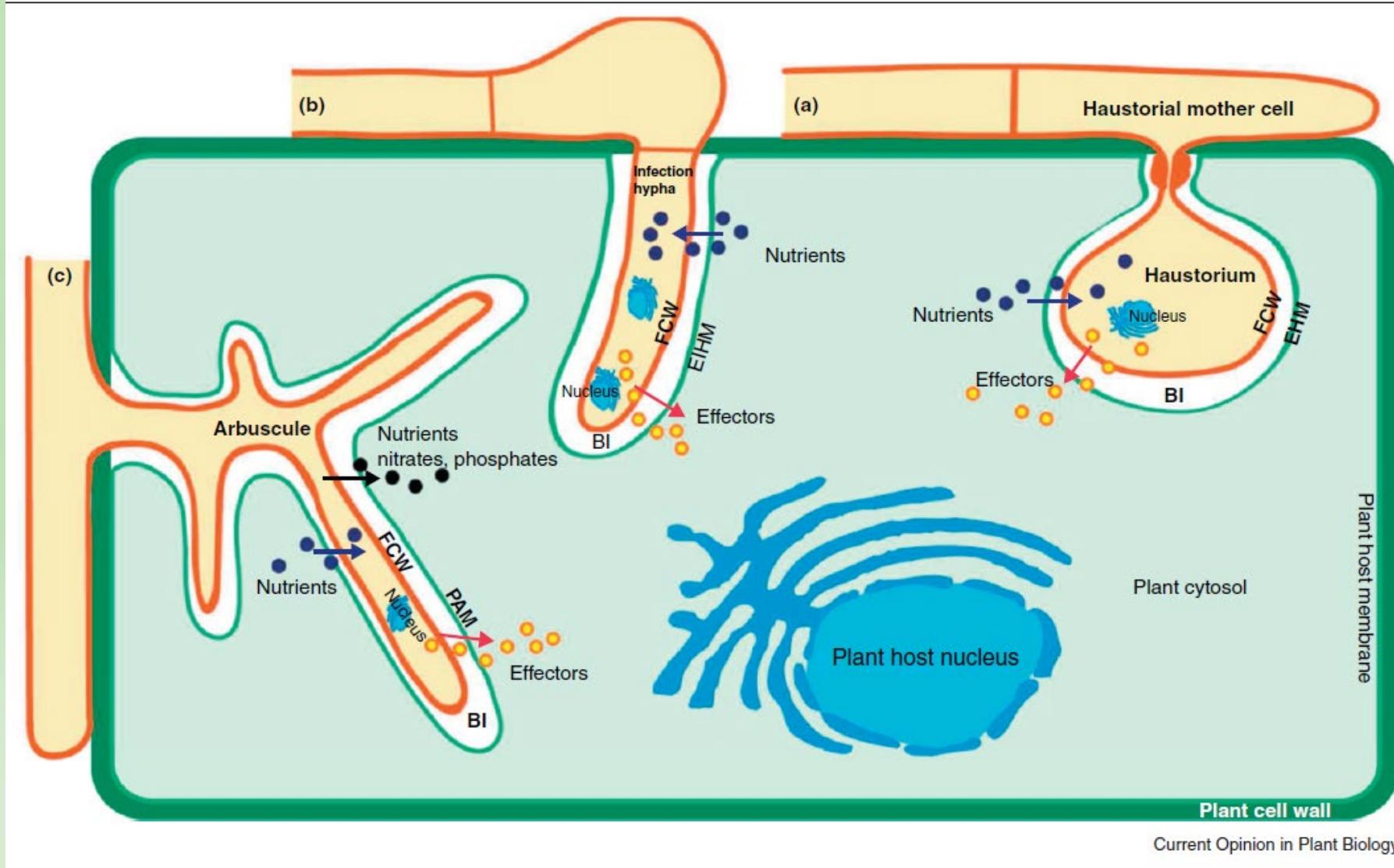
# Processo integrado da translocação dos efetores



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

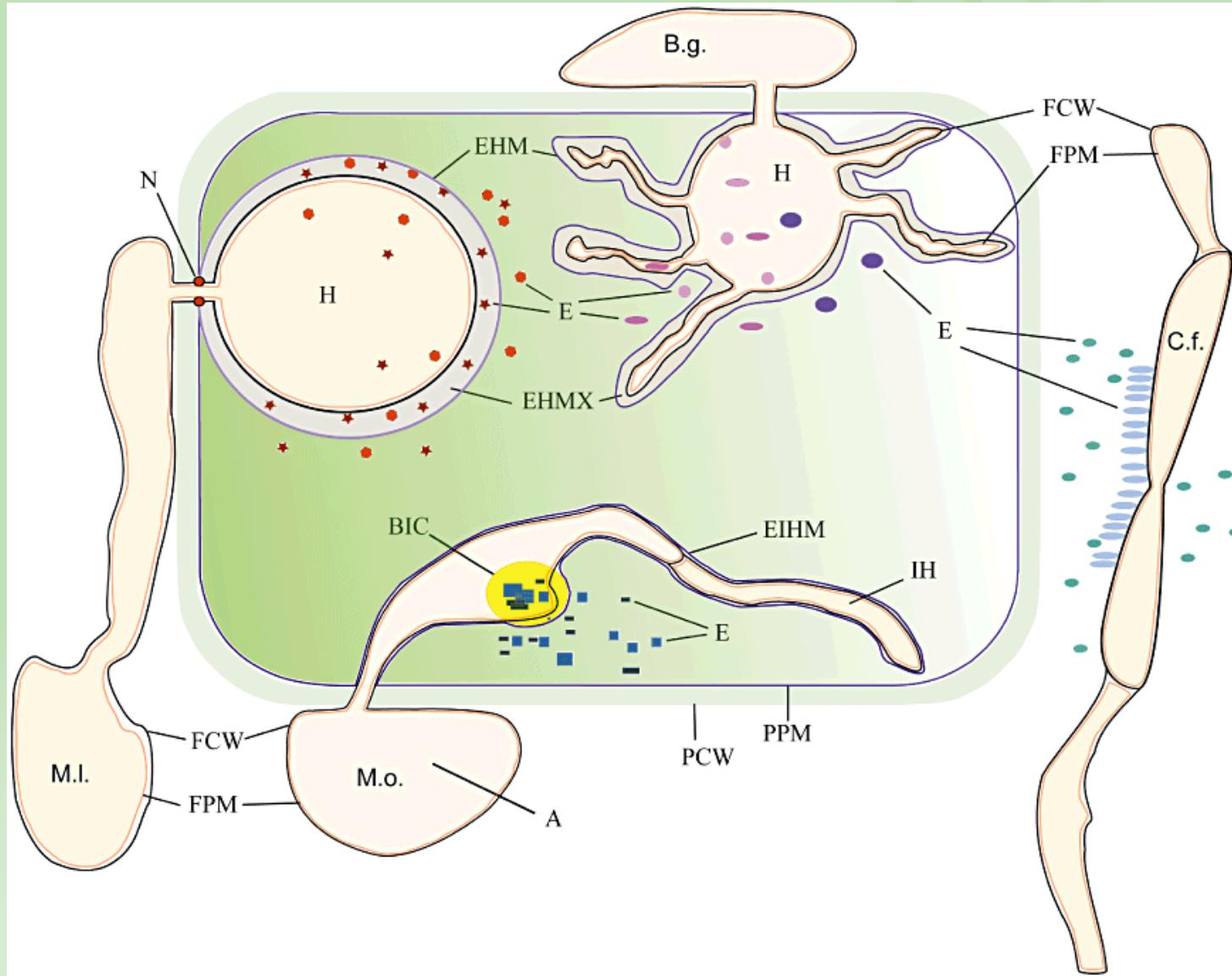


# Estruturas especializadas de infecção para secreção de efetores por fungos biotróficos



- 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:

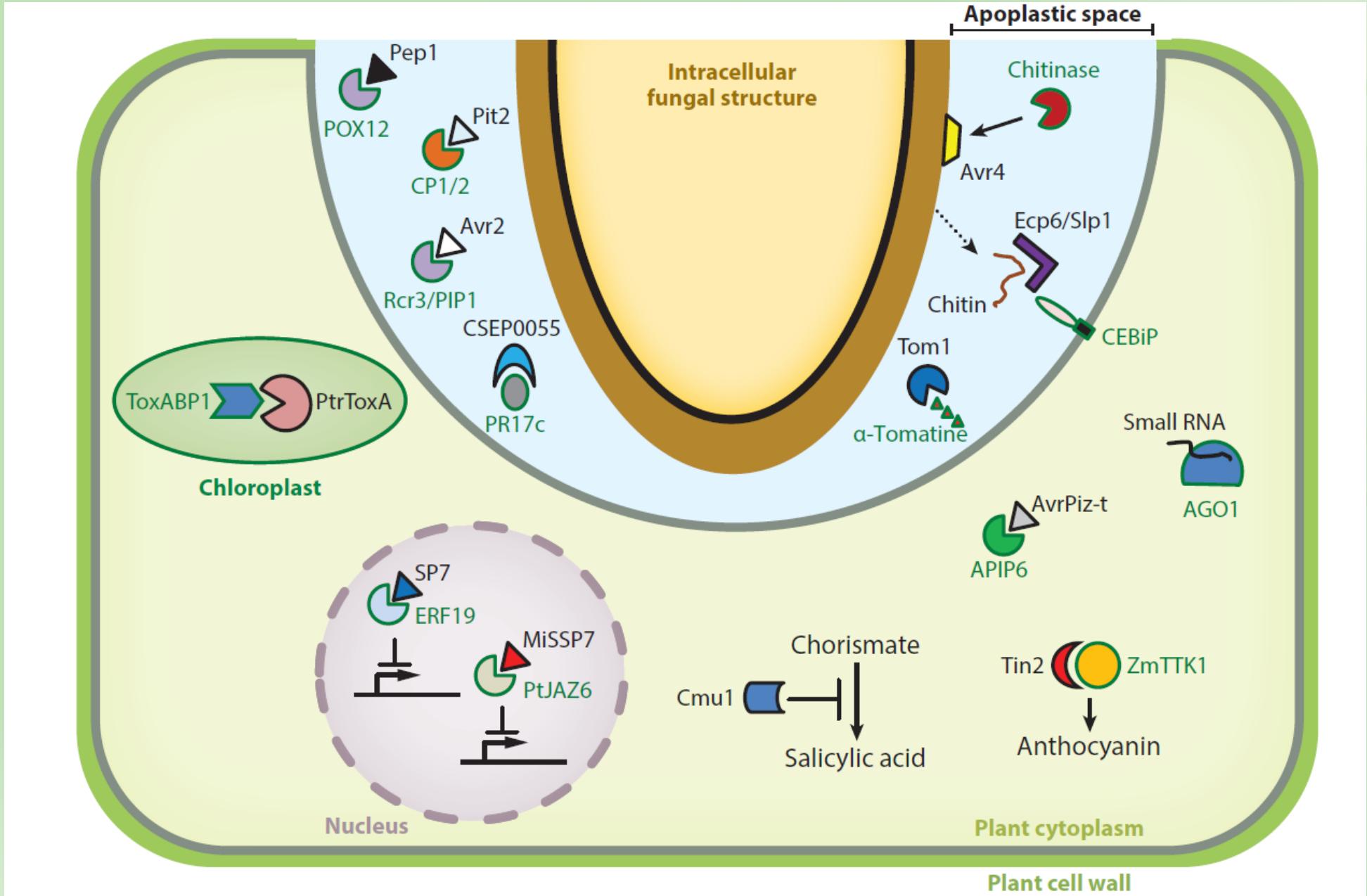
(*Magnaporthe oryzae*, M.o.)

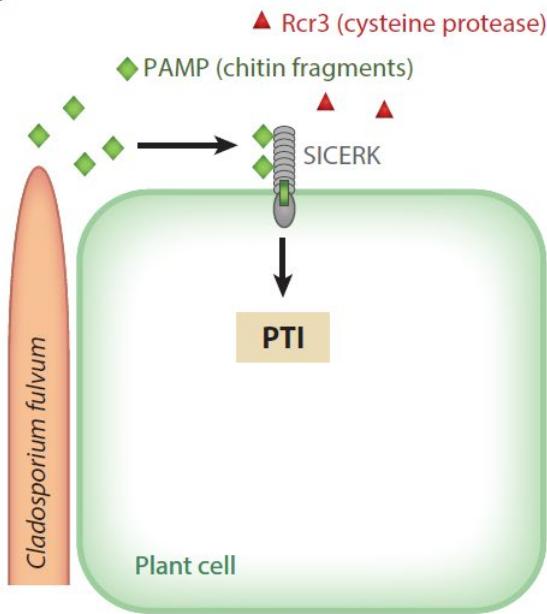
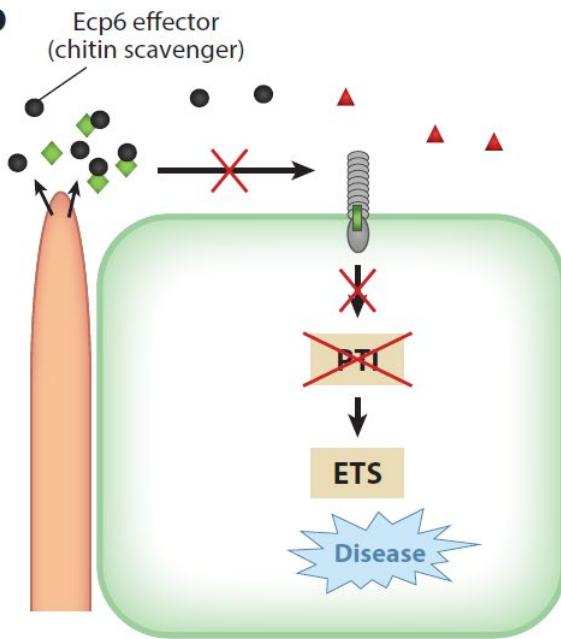
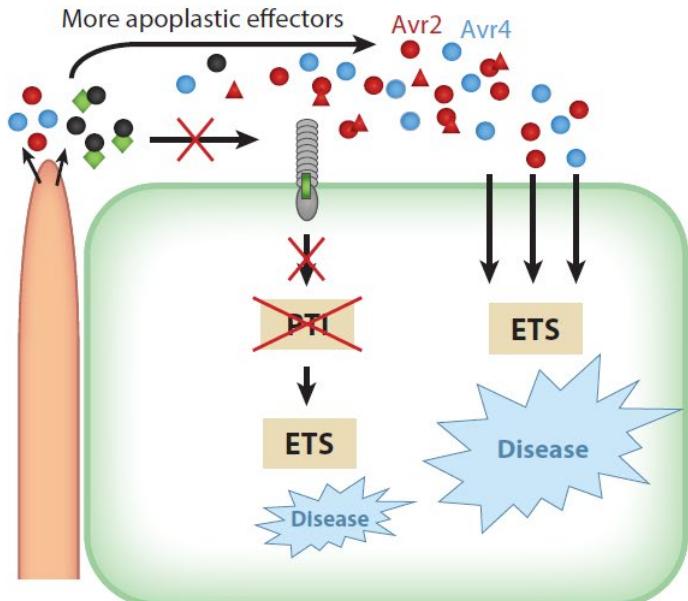
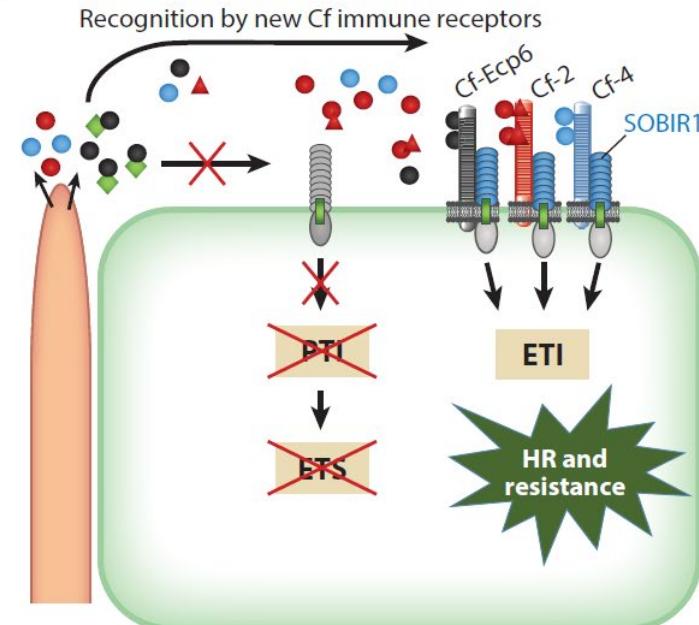
Penetração intracelular por

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.



**a****b****c****d**

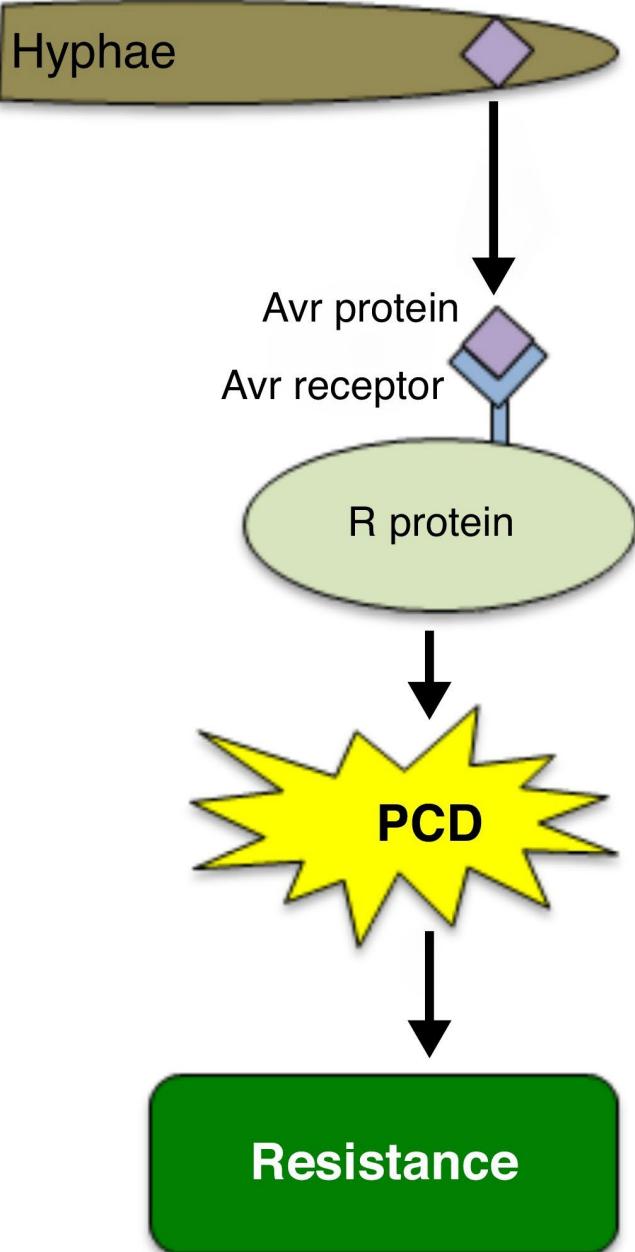
1. An evolutionary scenario of the arms race between *Cladosporium fulvum* and tomato. (a) Chitin fragments from *C. fulvum* are recognized by chitin receptor–inducing PAMP (pathogen-associated molecular pattern)-triggered immunity (PTI).

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).

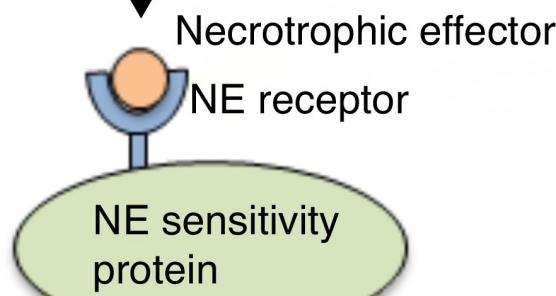
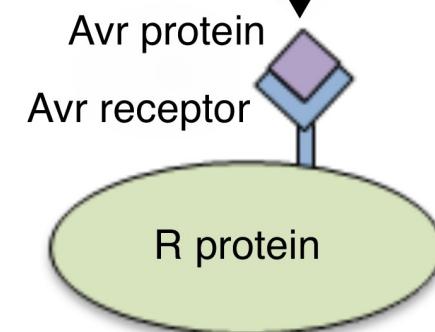
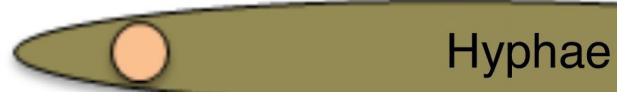
## Biotroph



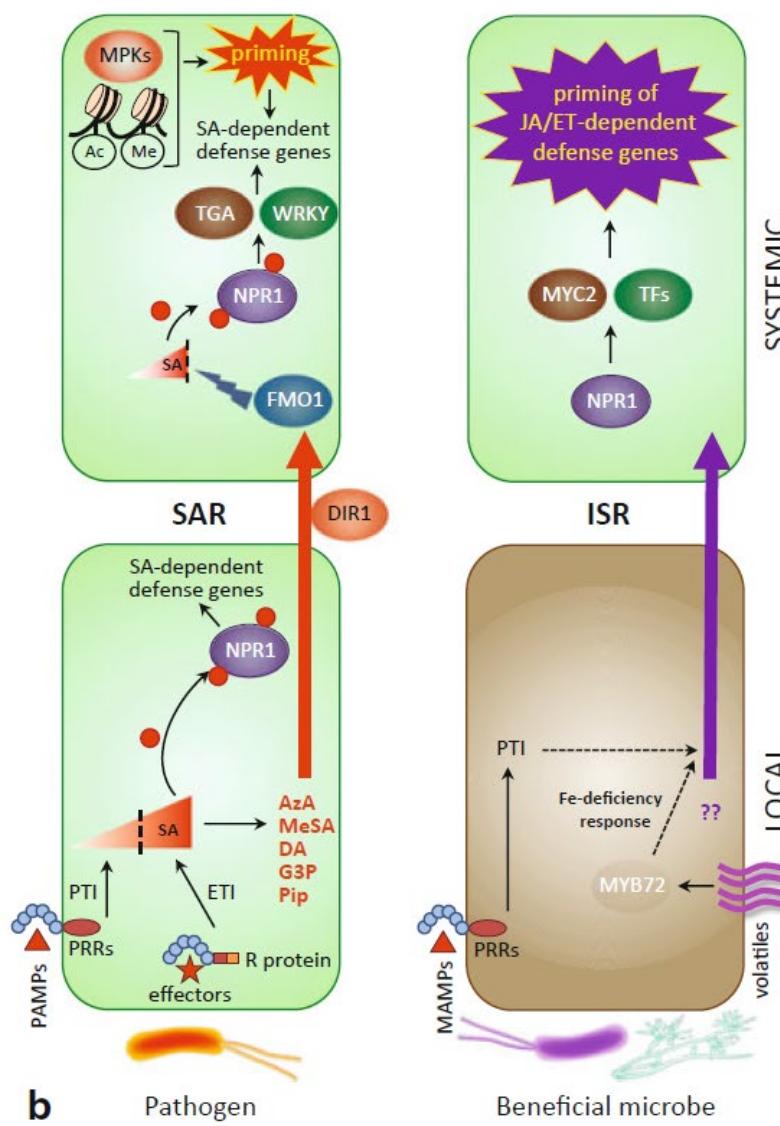
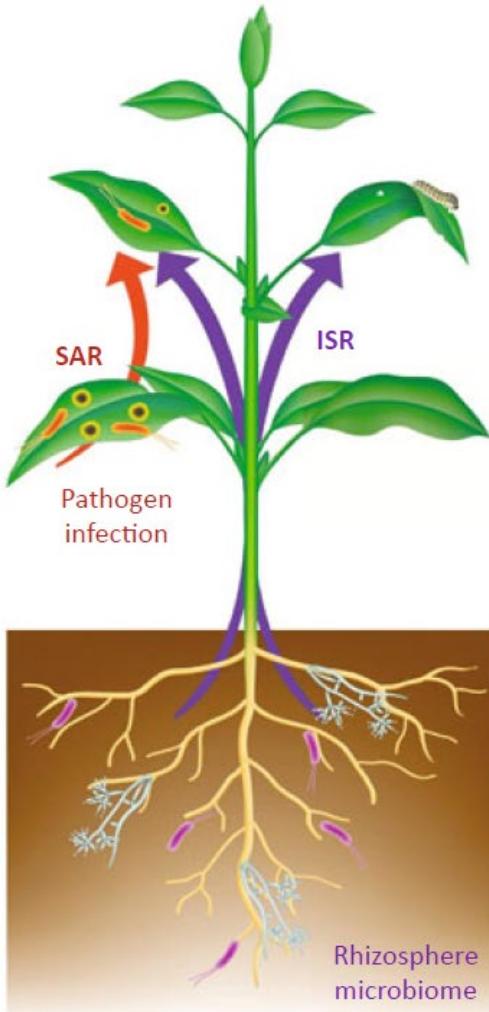
## Biotroph



## Necrotroph



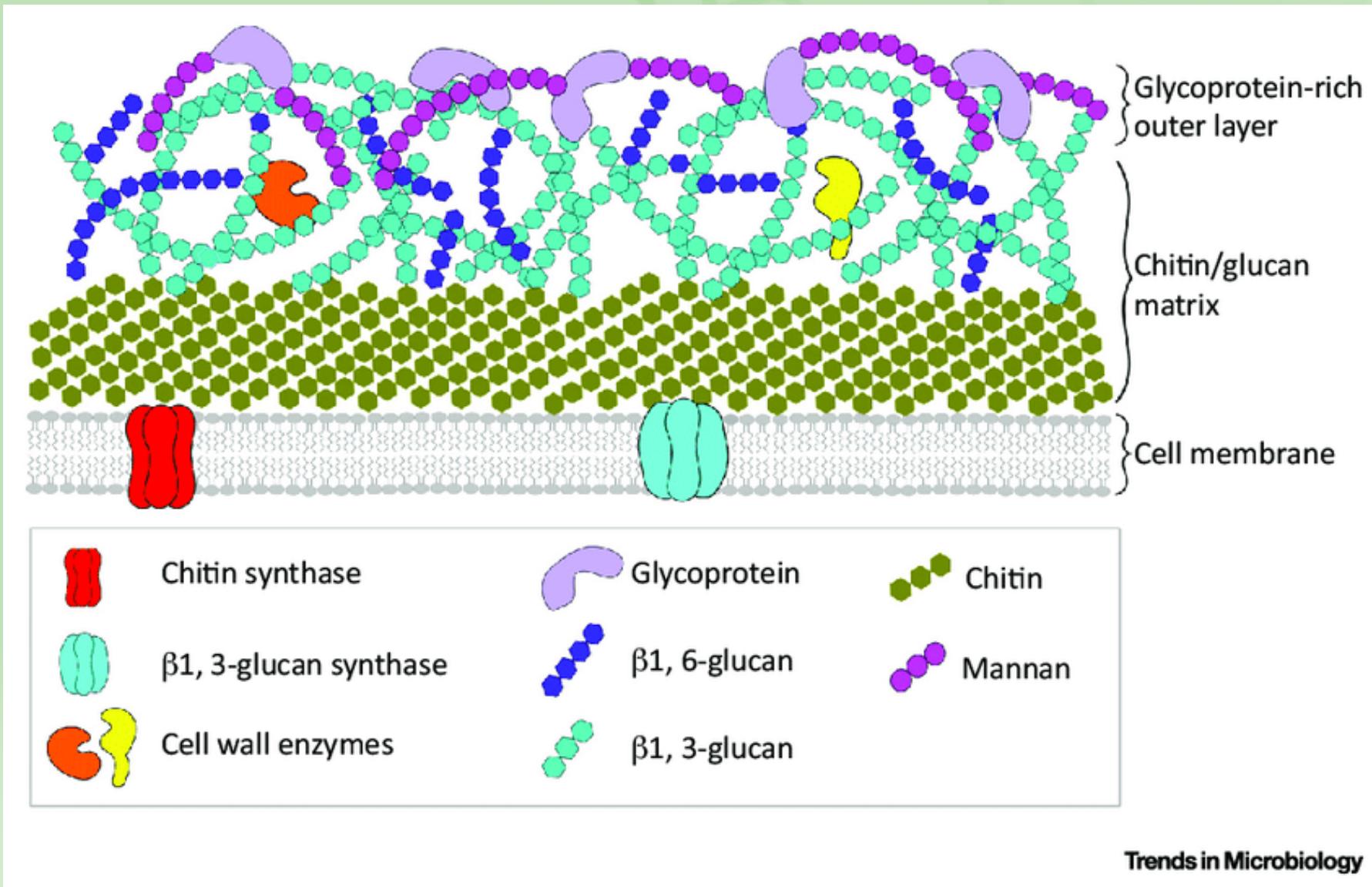
# Resistência sistêmica: Pathogen-Induced Systemic Acquired Resistance (SAR) x Induced Systemic Resistance (ISR) por microrganismos benéficos (Promotores de crescimento)



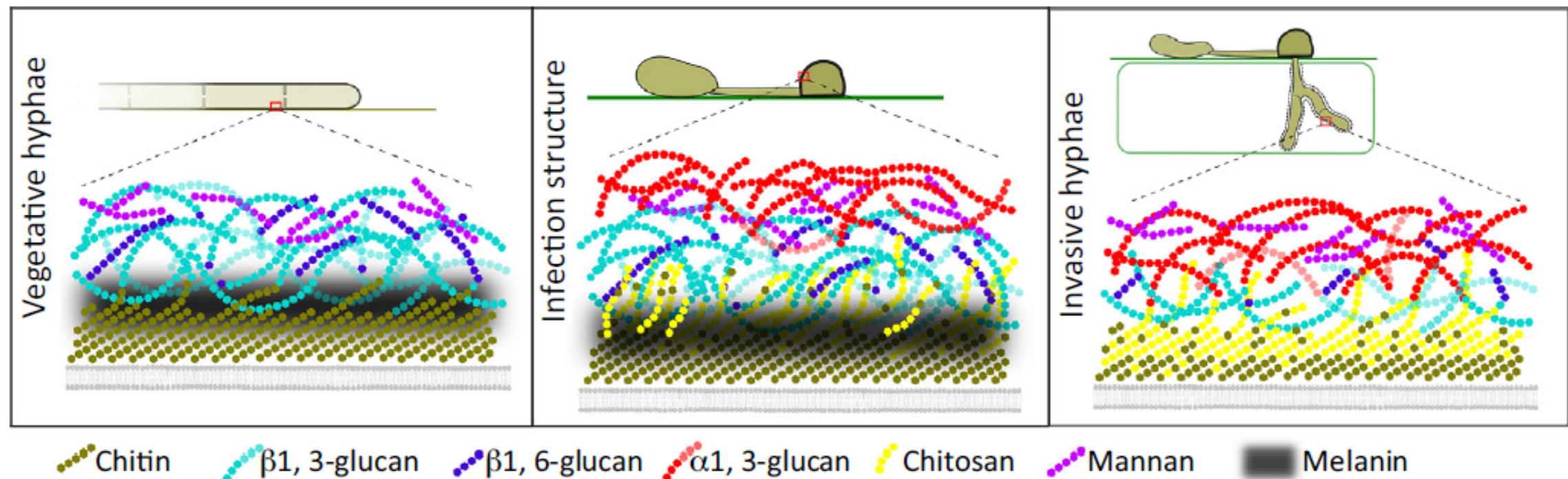
PAMPs (PTI) e Efetores (ETI)   
**SAR systemic acquired resistance:**   
 ↑ Salicylic acid (SA)  
 Proteínas NONEXPRESSOR OF PR GENES1 (NPR1)- fator transcripcional:  
 ↑ **PATHOGENESIS-RELATED (PR) genes**  
 MAMPs, lipopolysaccharideos (LPS), antibioticos, Sideroforos e voláteis   
**ISR induced systemic 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

# Estrutura básica da parede celular de fungos

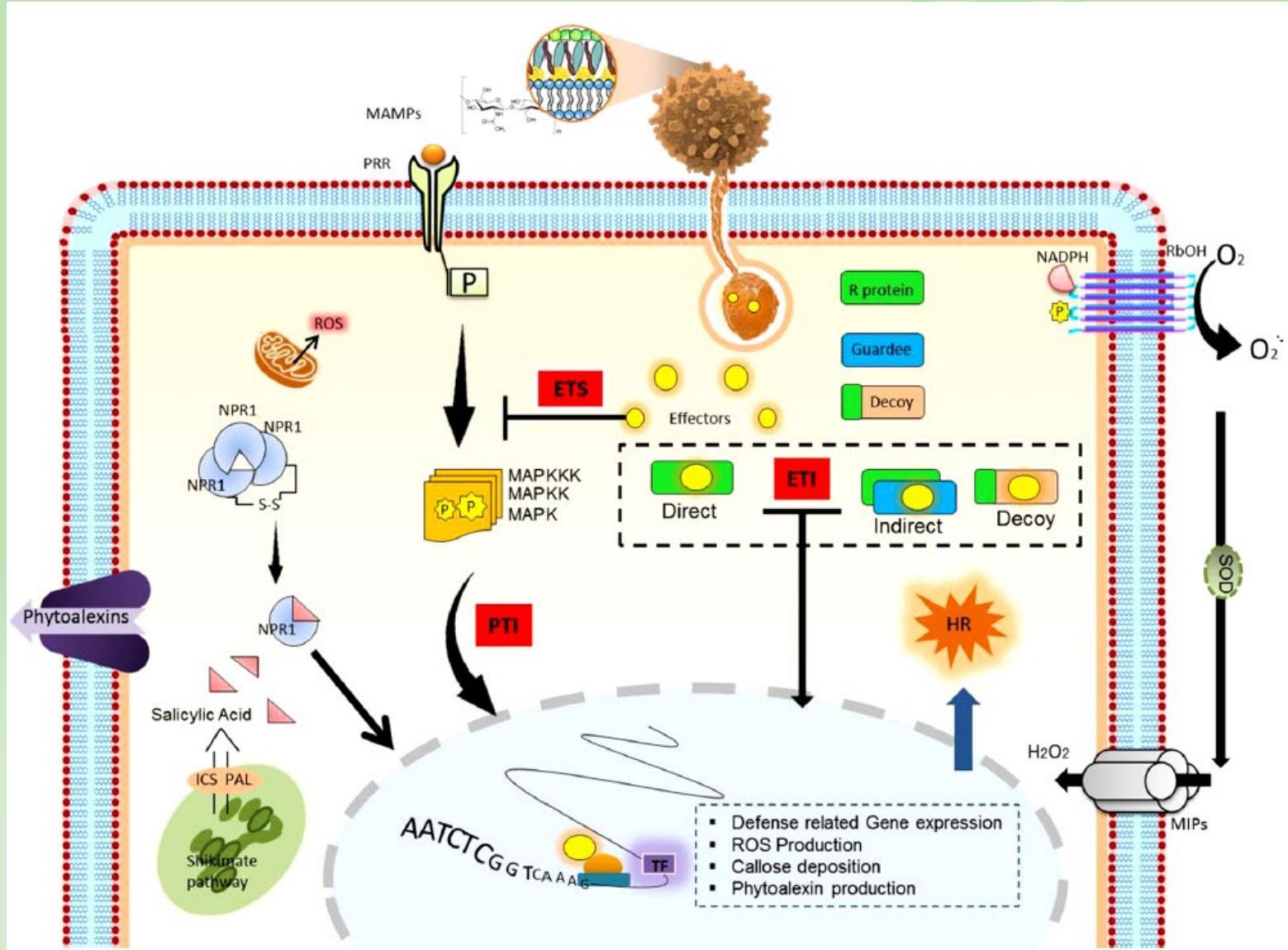


A liberação de PAMPs pode ser evitada alterando a composição da parede celular de estruturas de infecção, como por desacetilação da quitina (formando quitosana), redução do conteúdo de  $\beta$ -glucana, por síntese de alfa1,3-glucana, ou por secreção de proteínas efetoras, tanto para sequestrar PAMPs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



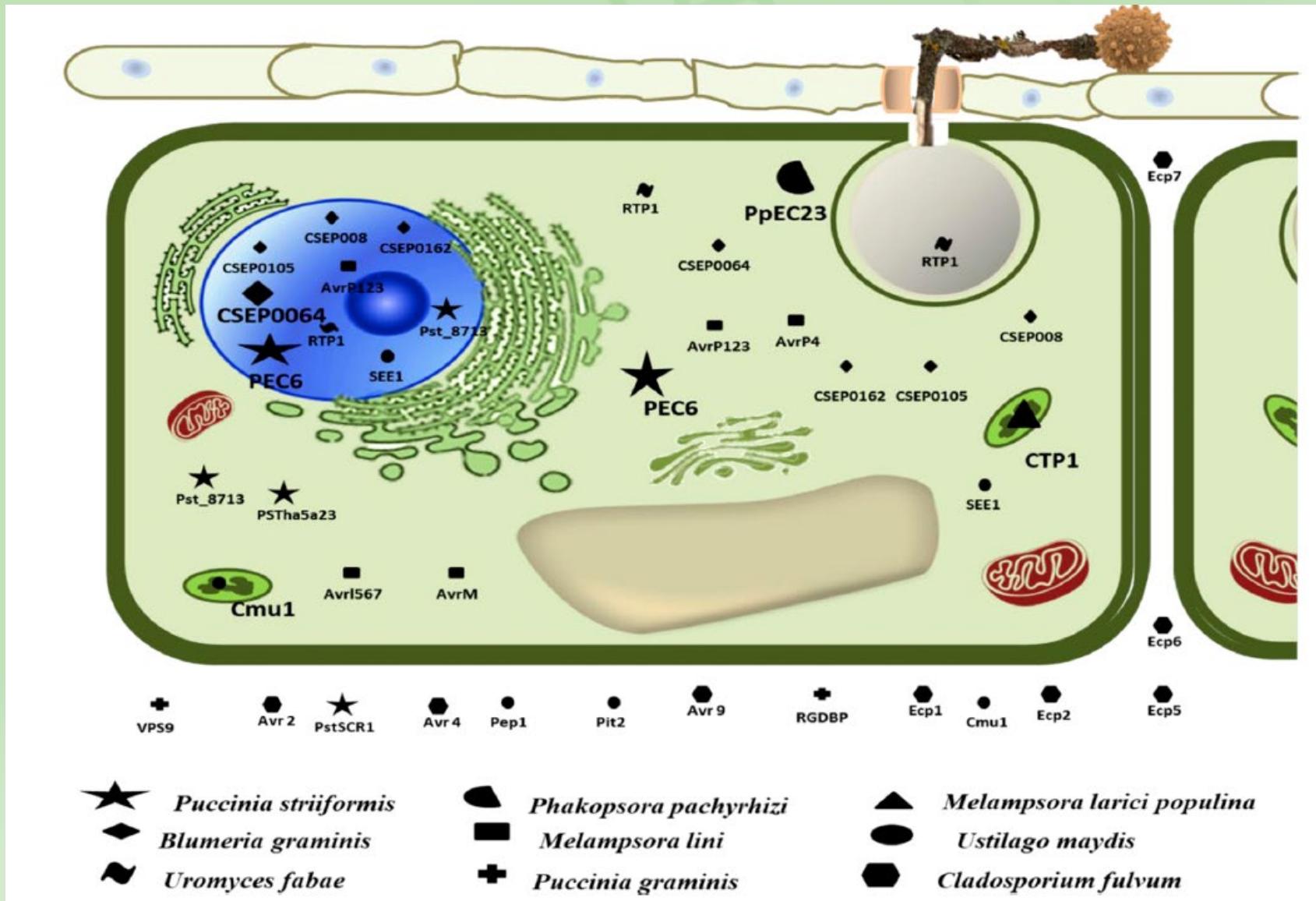
Trends in Microbiology

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

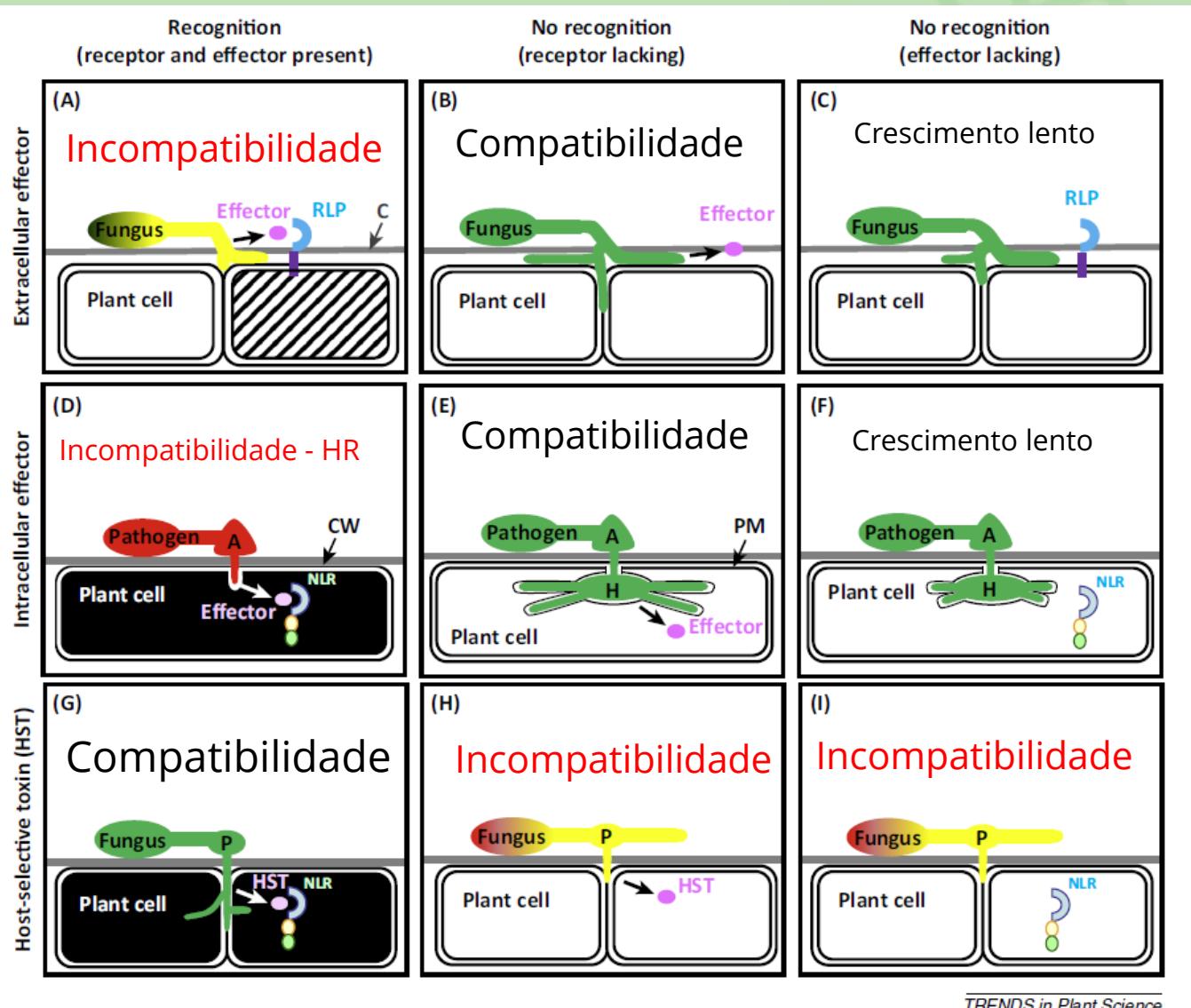


Resposta da planta: produção de compostos antimicrobianos: proteinases, quitinases e glucanases que danificam as estruturas do patógeno; inibidores de enzimas direcionados a moléculas produzidas pelo patógeno; e moléculas antimicrobianas não proteicas

# 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)

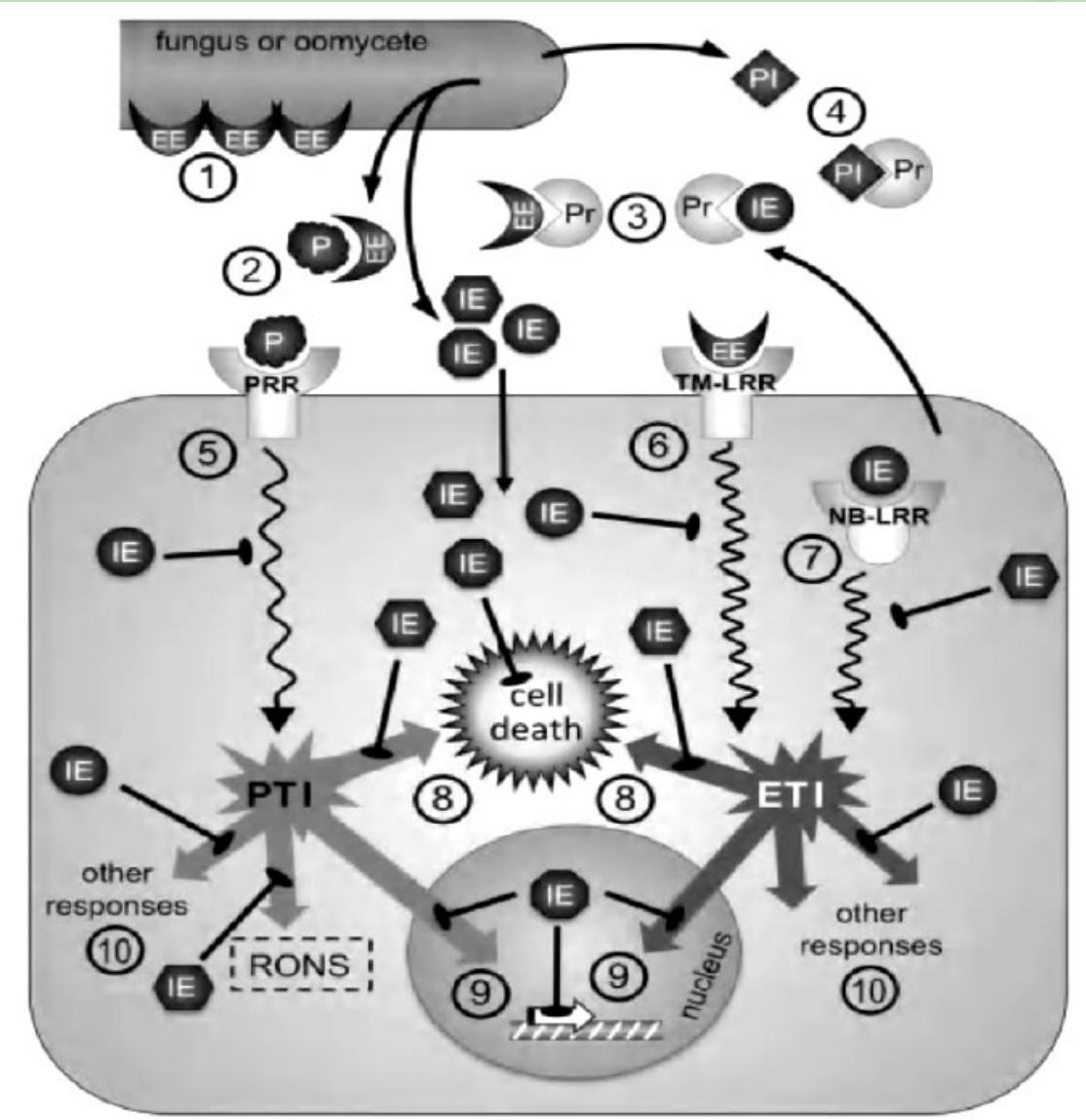
This recognition event triggers a rapid hypersensitive response that boosts host defence and usually results in host cell death and pathogen cell death.

(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;

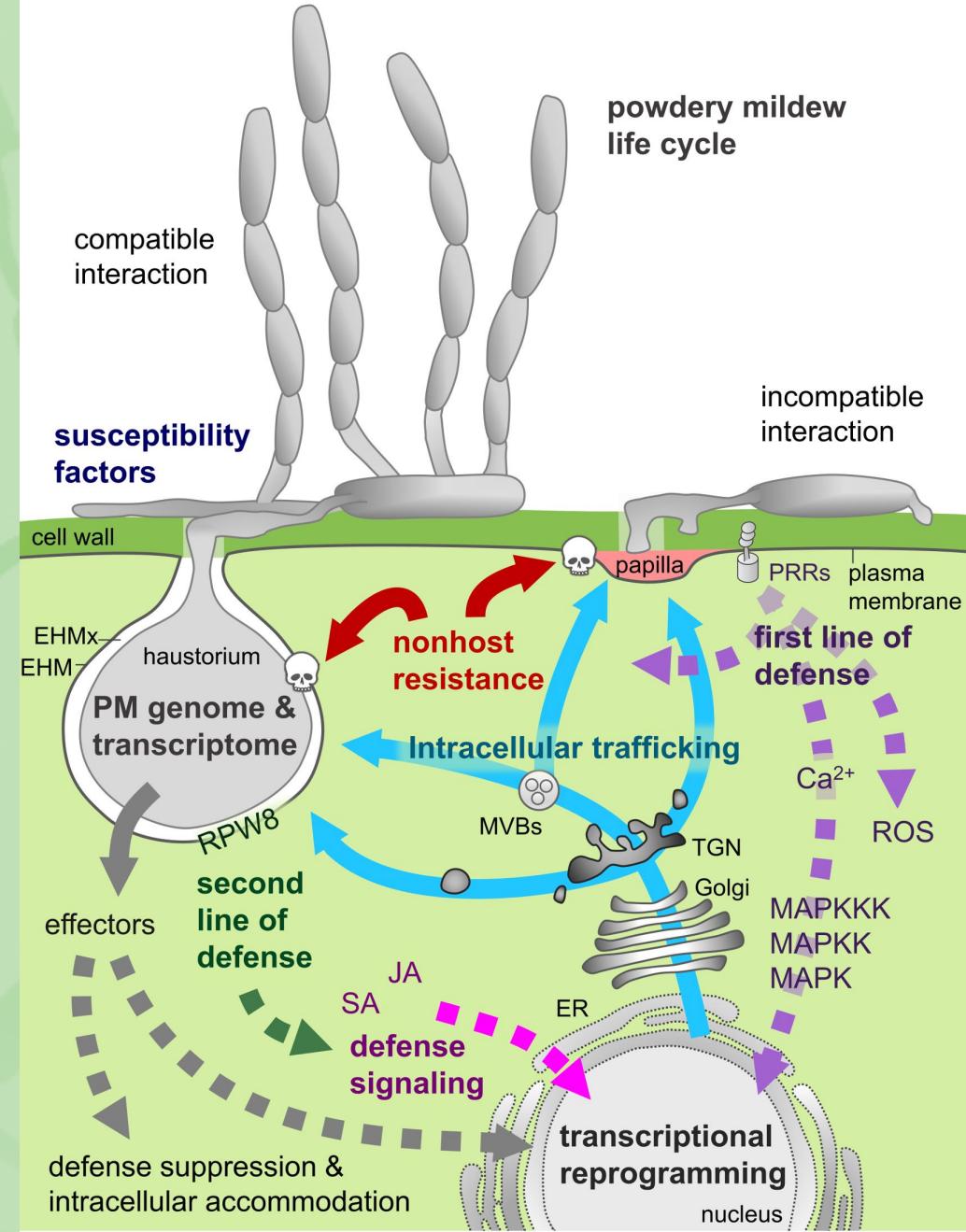
PRRs, pattern recognition receptors;

RPW8, RESISTANCE TO POWDERY MILDEW 8 protein;

ROS, reactive oxygen species;

SA, salicylic acid;

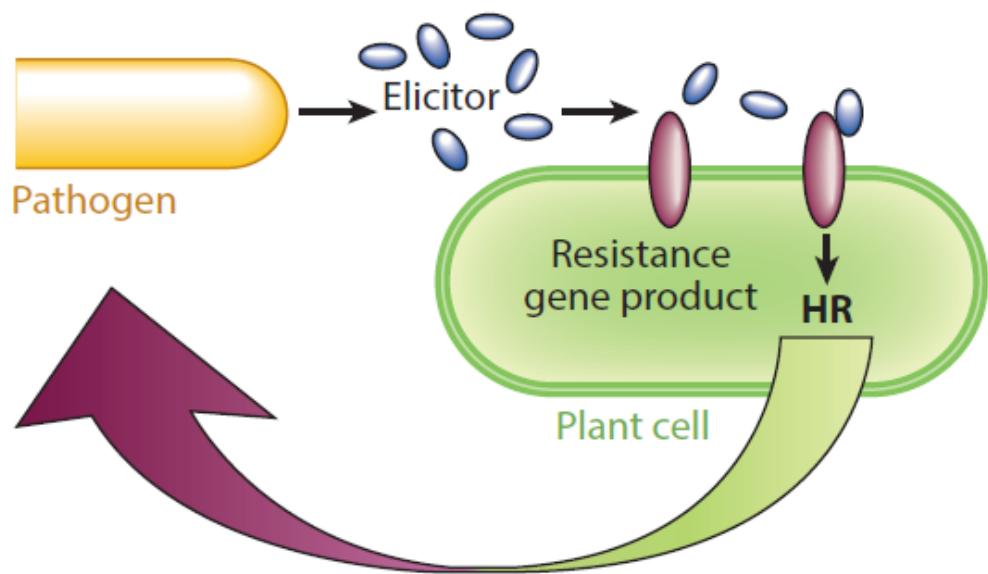
TGN, *trans*-Golgi network.



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

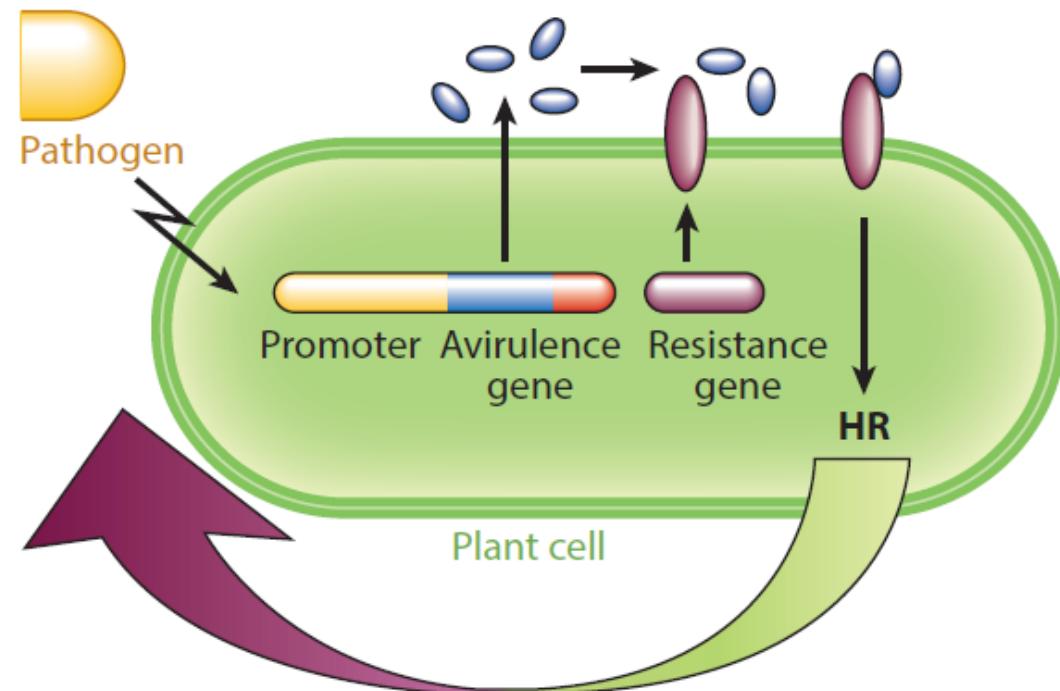
a

Avr (elicitor) produced by the pathogen itself induces a hypersensitive response (HR)

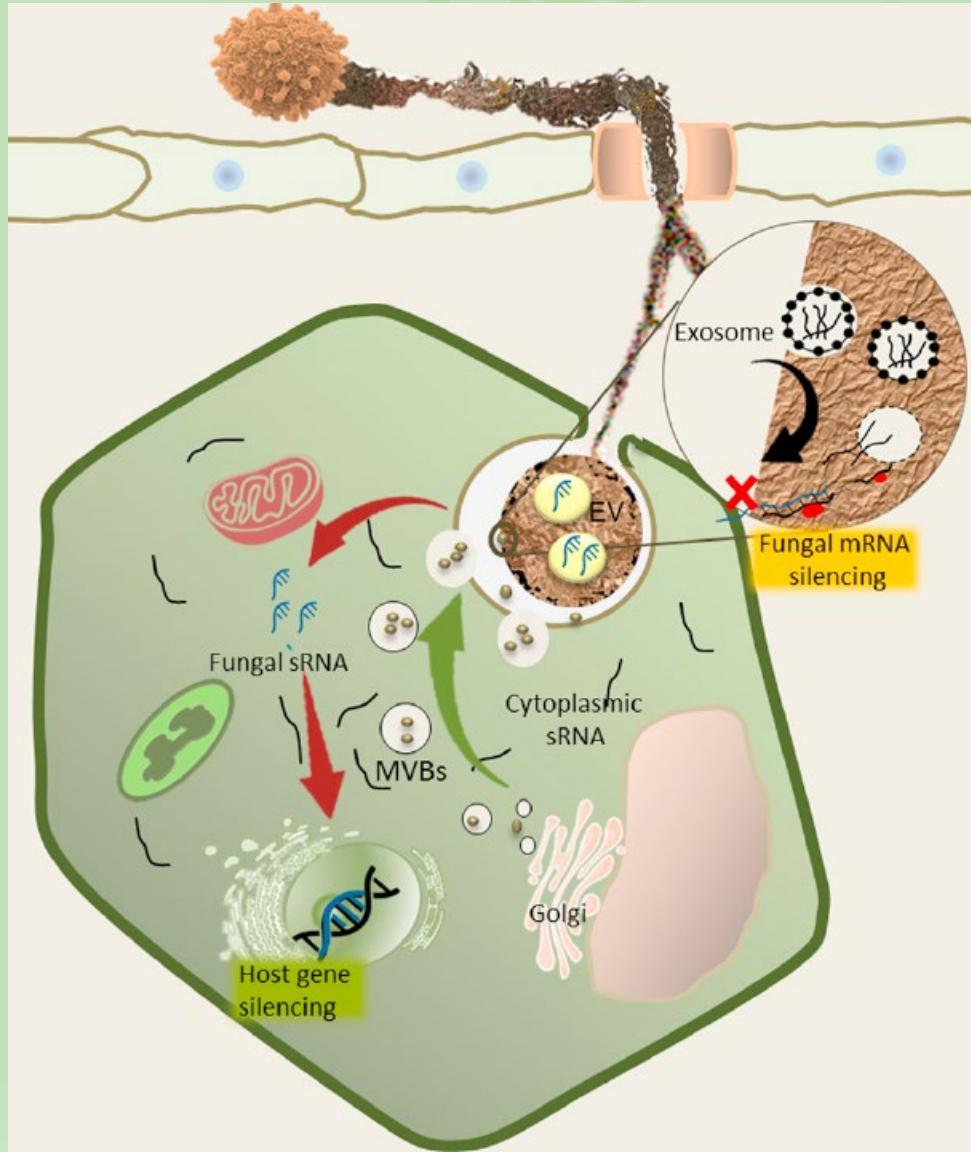


b

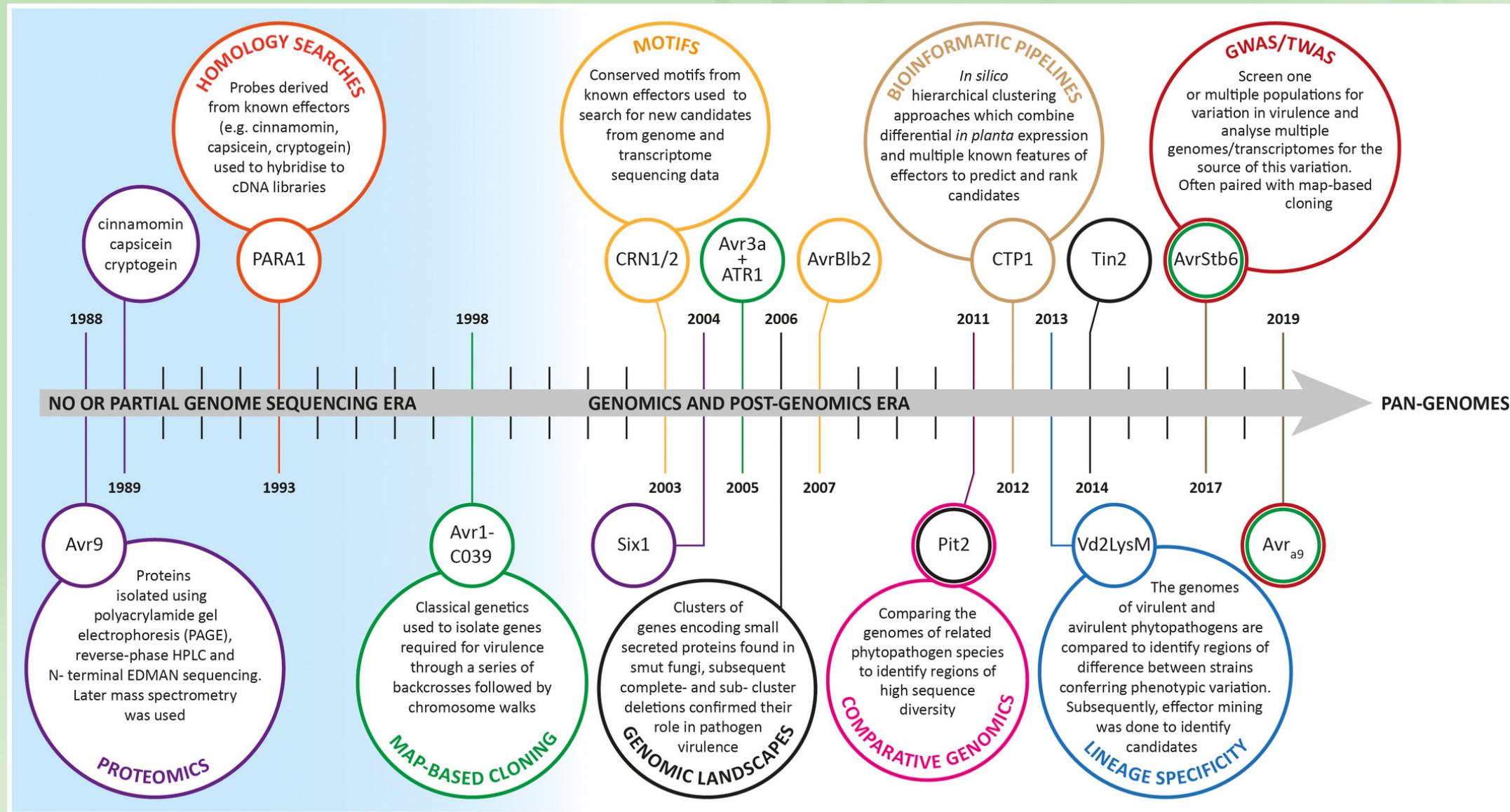
Pathogen-induced secretion of Avr by transgenic plants results in HR and provides broad-spectrum resistance



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



# Proteinaceous effector discovery and characterization in filamentous plant pathogens



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

Technique	Effector	Species	Reference
Proteomics	Avr9	<i>Cladosporium fulvum</i>	Schottens-Toma and de Wit (1988); van Kan et al. (1991)
	Six1	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	Rep et al. (2004)
Map-based cloning	Avr1-CO39	<i>Magnaporthe grisea</i>	Farman and Leong (1998)
	Avr3a	<i>Phytophthora infestans</i>	Armstrong et al. (2005)
	ATR1	<i>Hyaloperonospora parasitica</i>	Rehmany et al. (2005)
Homology searches	PARA1	<i>Phytophthora parasitica</i>	Kamoun et al. (1993)
	INF1	<i>P. infestans</i>	Kamoun et al. (1997)
Motifs/secretion peptides	Crn1 and Crn2	<i>P. infestans</i>	Torto et al. (2003)
	AvrBlb2	<i>P. infestans</i>	Win et al. (2007); Oh et al. (2009)
Genomic landscapes	Tin2	<i>Ustilago maydis</i>	Kämper et al. (2006); Brefort et al. (2014)
Comparative genomics	Pit2	<i>U. maydis</i>	Doeblemann et al. (2011)
Bespoke bioinformatic pipelines	CTP1	<i>Melampsora larici-populina</i>	Saunders et al. (2012b); Petre et al. (2015)
Lineage-specific	Vd2LysM	<i>Verticillium dahliae</i>	de Jonge et al. (2013); Kombrink et al. (2017)
GWAS/TWAS	AvrStb6	<i>Zymoseptoria tritici</i>	Zhong et al. (2017)
	Avr <sub>a9</sub>	<i>Blumeria graminis</i> f. sp. <i>hordei</i>	Saur et al. (2019a)

## 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