

A grayscale electron micrograph showing a plant cell with a large, dark, irregularly shaped structure, likely a pathogen, inside. The plant cell wall and internal organelles are visible.

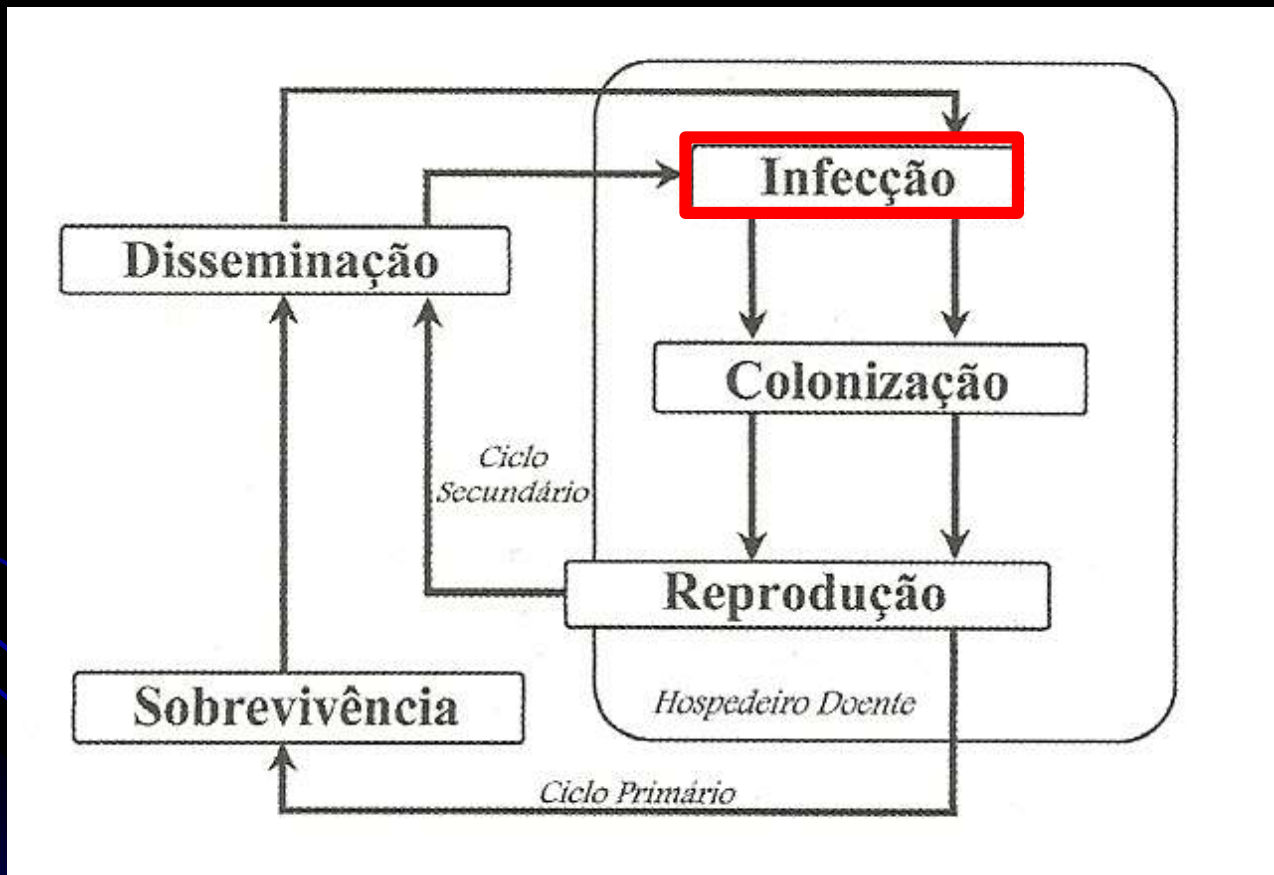
Fisiologia e Bioquímica Fitopatológica

**Atração, adesão, germinação
de propágulos e penetração
das plantas por patógenos**

Atividades importantes na vida de um patógeno durante a interação

- Localizar
- Aderir
- Penetrar
- Colonizar
- Supressão das defesas do hospedeiro
- Reprodução
- Dispersão

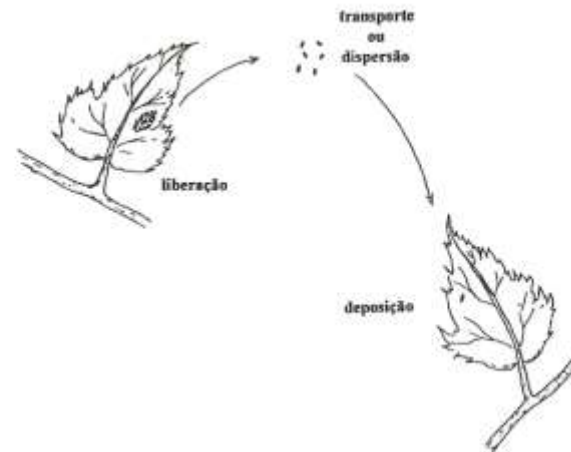
Processos no ciclo das relações patógeno-hospedeiro



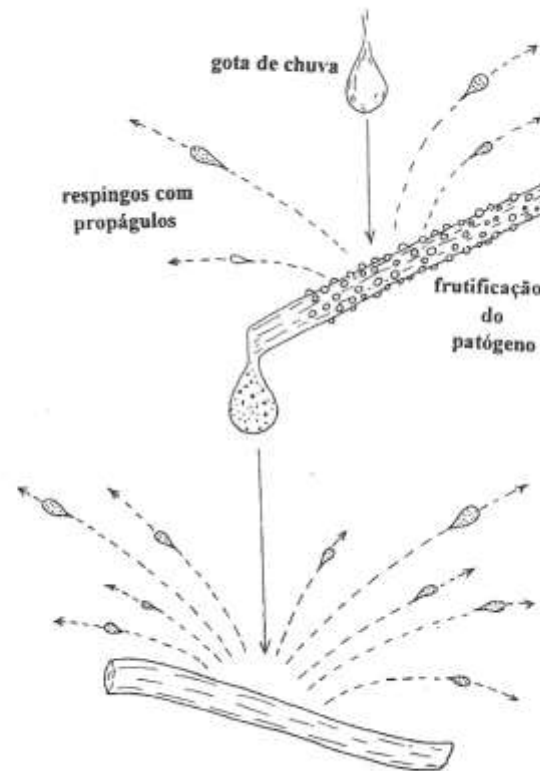
Processos, subprocessos e mecanismos envolvidos no ciclo das relações patógeno-hospedeiro

Processos	Subprocessos	Mecanismos
Sobrevivência		Estruturas especializadas Atividades saprofiticas Plantas Vetores
	Liberação	Ativa (projeção de ascósporos, balistósporos, etc.) Passiva (vento, água, etc.)
Disseminação	Dispersão	Ar Água Homem Insetos
	Deposição	Sedimentação Impacto Turbulência
	Pré-penetração	
Infecção	Penetração	Aberturas naturais Ferimentos Direta
	Estabelecimento de relações parasitárias	
Colonização		Intercelular Intracelular
	Reprodução	Sexuada Assexuada

O processo “Disseminação”



Processos envolvidos na disseminação de um patógeno foliar



Liberação de esporos por respingos de chuva

O processo “Disseminação”

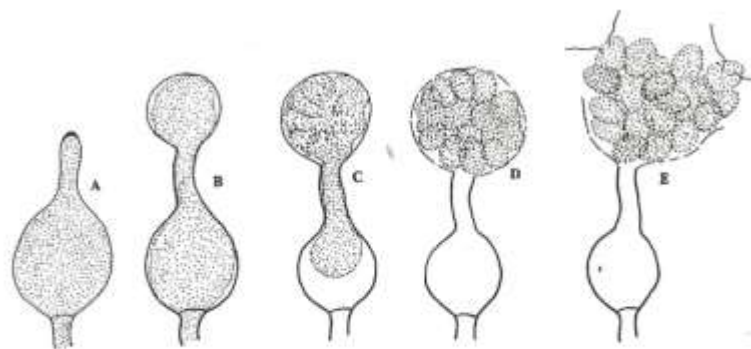


Figura 14.4 - Liberação de zoósporos de *Pythium middletonii* (A) movimentação do protoplasma pela estrutura tubular; (B) formação de vesícula; (C) migração do protoplasma para a vesícula; (D) formação de zoósporos; (E) liberação de zoósporos (Ingold, 1971).

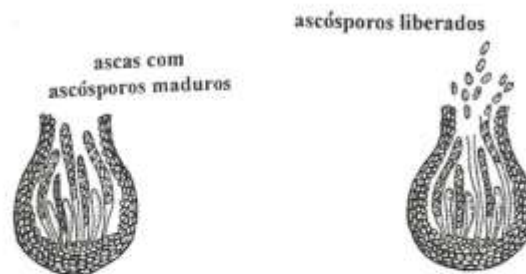


Figura 14.2 - Liberação de ascósporos do fungo *Venturia inaequalis* pelo mecanismo de ejeção.



Figura 14.5 - Dispersão de teliospores de *Ustilago nuda*, agente causal do carvão da cevada, pelo vento. O diagrama representa um campo de cevada com inflorescências infectadas projetadas acima do nível das inflorescências saudáveis, uma estratégia que favorece a disseminação do patógeno (Ingold, 1971).

Processos, subprocessos e mecanismos envolvidos no ciclo das relações patógeno-hospedeiro

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Reprodução		Sexuada Assexuada

Deposição

Pré-
penetração

Penetração

Relações
parasitárias
estáveis

Colonização

Infecção

Gauman, 1950; Hirst & Schein, 1965; Butt & Royle, 1980;
Lucas, 1998; Schumann & D'Arcy, 2006

Infecção

Roberts &
Boothroid, 1972;
Gonzales, 1976;
Trigiano et al. 2004

Infecção

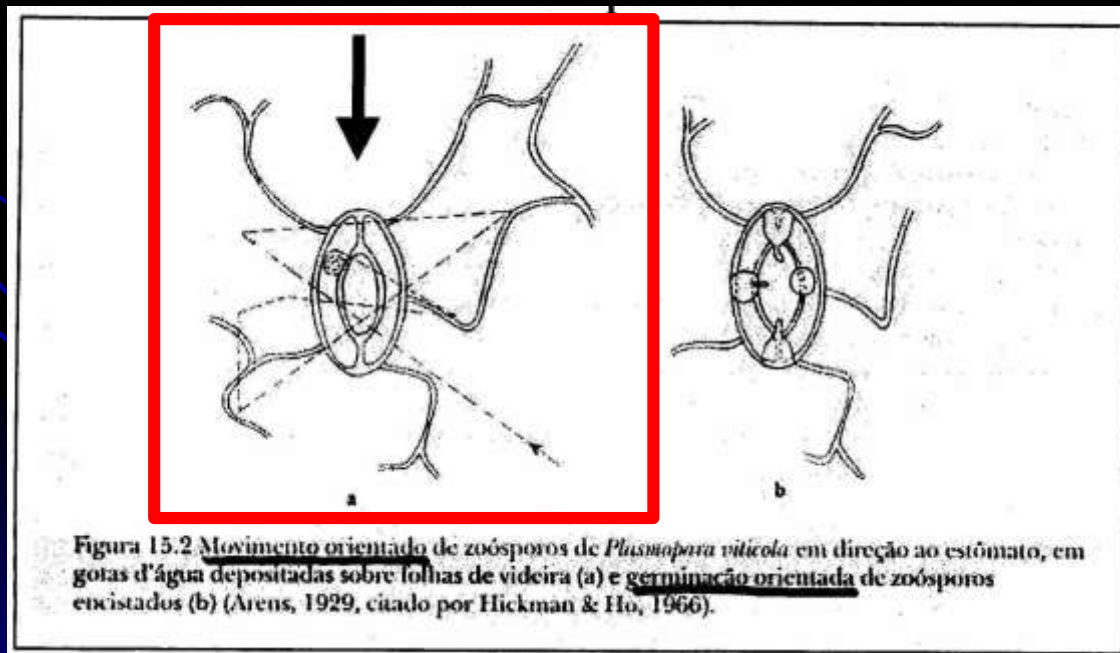
Strobel & Mathre, 1970; Agrios, 1988; 1997

Infecção – processo que tem início na pré-penetração e termina com o estabelecimento de relações parasitárias estáveis entre patógeno e hospedeiro

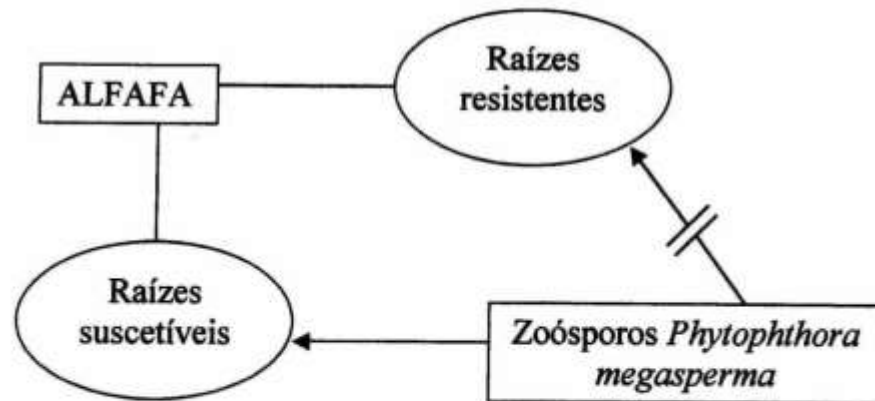
O SUB-PROCESSO “PRÉ-PENETRAÇÃO”

- A) Tatismo (= taxia)** – movimento direcionado do patógeno em relação ao hospedeiro
- Resposta de patógenos e/ou estágios móveis na água do solo/ filme d'água na superfície das plantas. Ex. zoósporos / bactérias.

Quimiotatismo – movimento em resposta à um estímulo químico



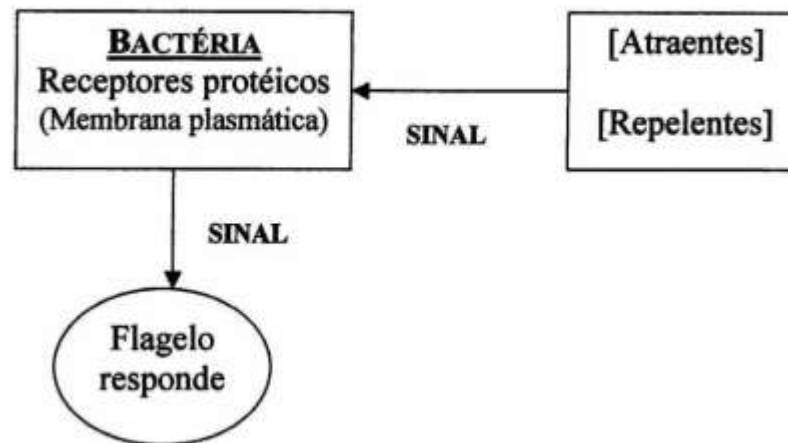
Quimiotatismo

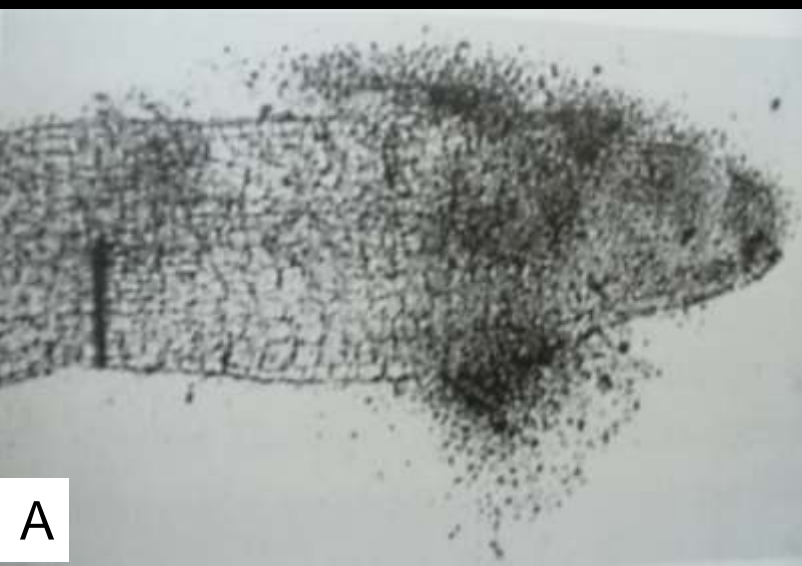


(Chi/Sabo, 1978)

Quimiotatismo

- Específico x Não-específico
- Quimioatraentes x quimiorepelentes
 - ▶ amino ácidos, açúcares, nucleotídeos, vitaminas





Susceptível

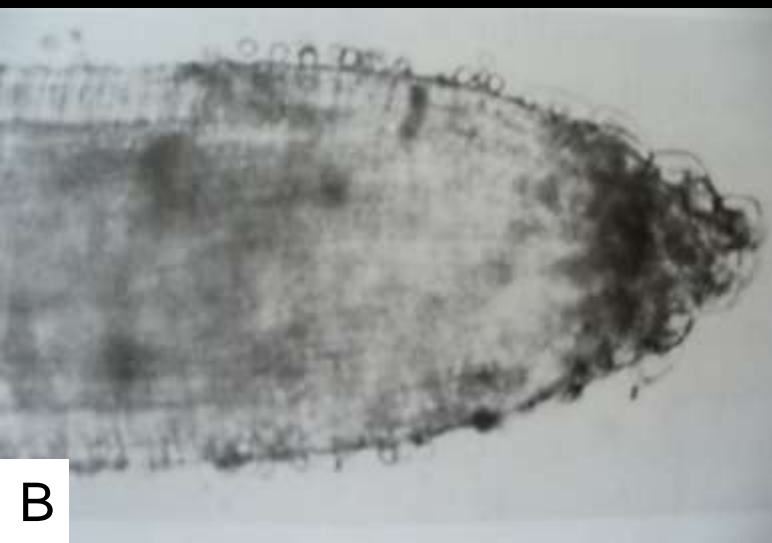


A

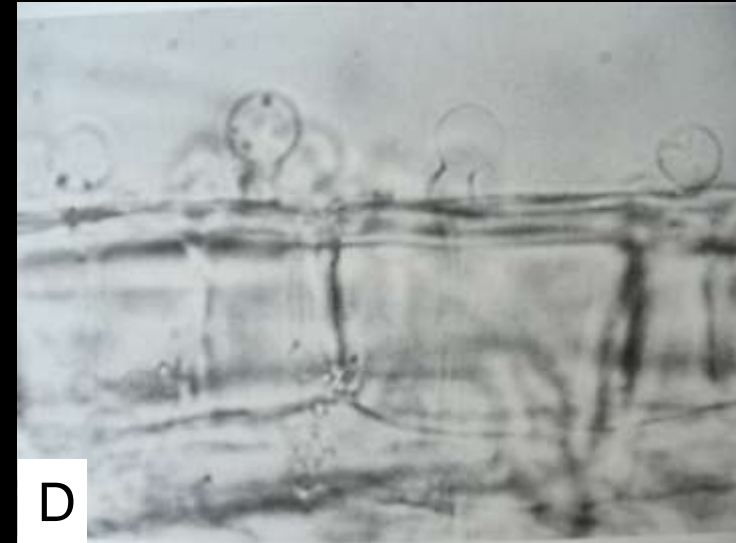
C

1 hora

24 horas



Resistente



B

D

Atração de zoósporos de *Phytophthora cinnamomi* em direção a raízes susceptíveis (A e C) e resistente (B e D). Atração - 1 hora após inoculação (A e B) e infecção e colonização - 24 horas após inoculação (C e D) (Agrios, 2005)

(Vídeo)

Videos

Zoospores of *Phytophthora nicotianae* x tobacco root tip

<https://www.youtube.com/watch?v=PxF8OwDtJh0>

***Phytophthora plurivora* zoospores attracted to Beech root exudates**

<https://www.youtube.com/watch?v=F4sITLkhwuY>



Dois grupos de zoósporos de mildio da videira (*Plasmopara viticola*) encistados junto a dois estômatos (Agrios, 2005)



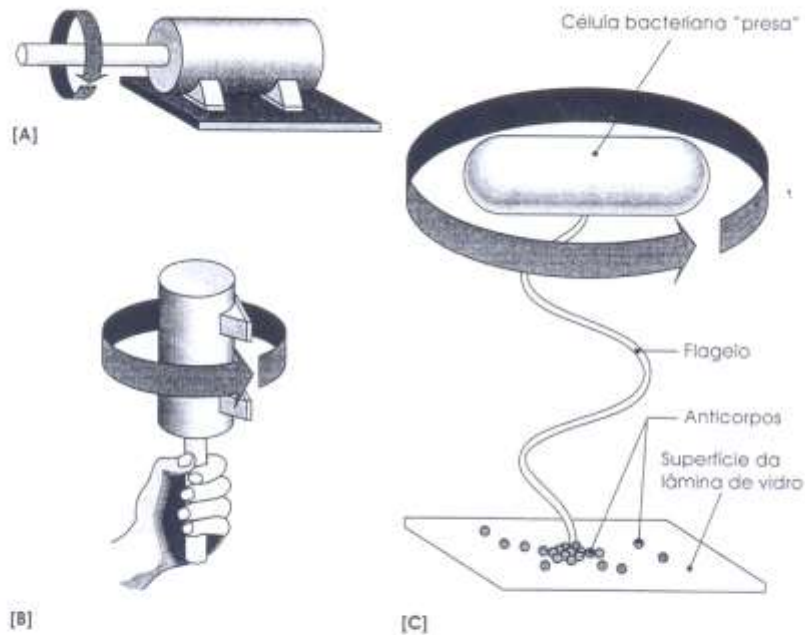
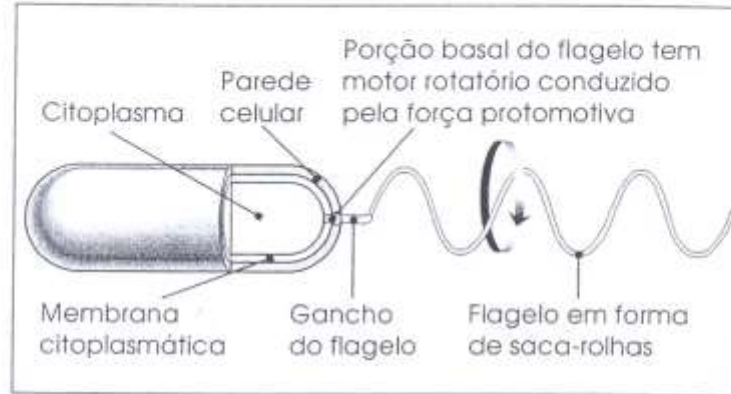
Células de *Pseudomonas syringae*, agente causal da mancha bacteriana, vistas ao redor de um estômato de folha de cerejeira

BACTÉRIAS

Movimentação

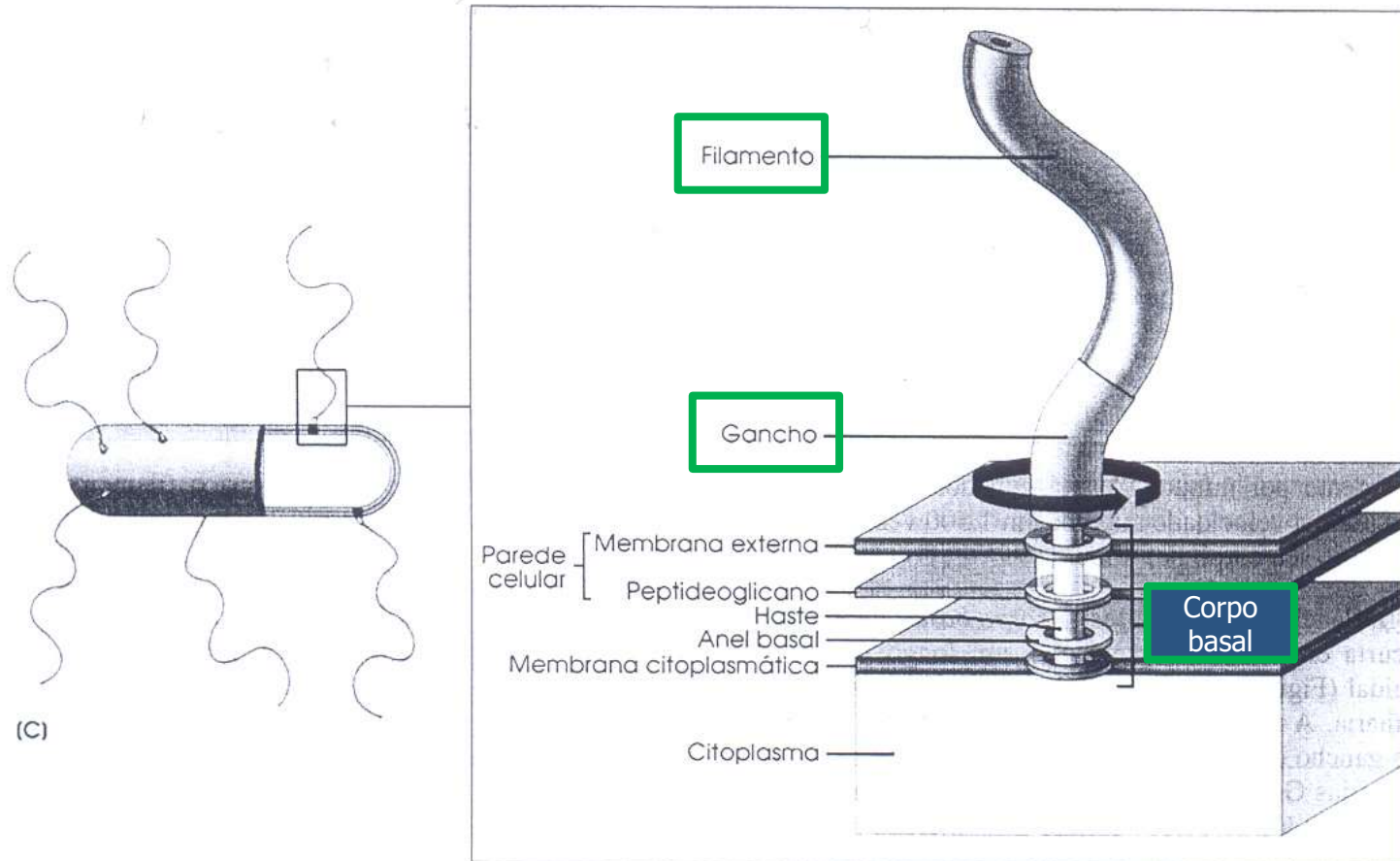


Figura 12.13 O motor rotatório que movimenta o flagelo bacteriano está associado com os discos da porção basal do flagelo e é conduzido pela força protomotiva.



Princípio dos experimentos da "célula-presa", mostrando que o flagelo bacteriano realmente gira em uma direção. [A] Quando um motor elétrico é colocado sobre uma mesa, o eixo gira enquanto o motor permanece parado. [B] Se o motor for segurado pelo eixo, o corpo do motor girará, enquanto o eixo permanecerá parado. [C] Uma célula bacteriana com um único flagelo pode ser presa a uma lâmina de vidro que tenha sido coberta com anticorpos para imobilizar o flagelo. Tal célula girará como um cata-ventos, como o corpo do motor em [B].

Bactérias - Fixação do flagelo



Desenho de um corpo basal ilustrando sua estrutura e fixação a bactérias Gram-negativas. O flagelo de bactérias Gram-positivas tem somente dois anéis (um par) que fixam o flagelo à membrana plasmática e a parede celular.

Blastocladiella emersonii

Phytophthora palmivora

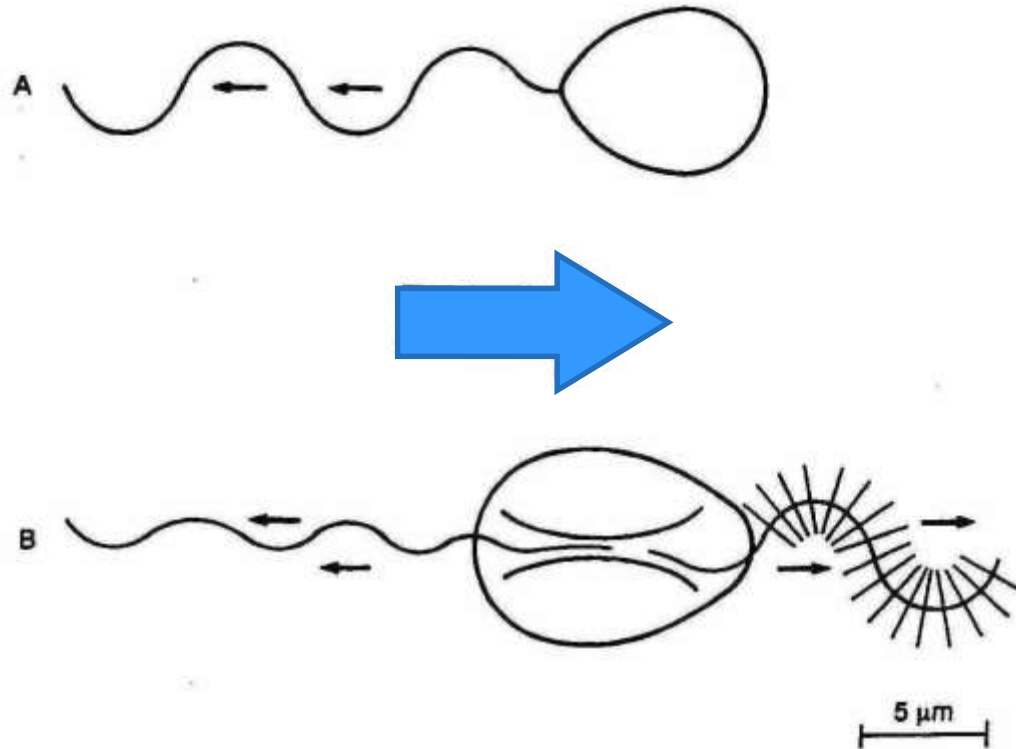
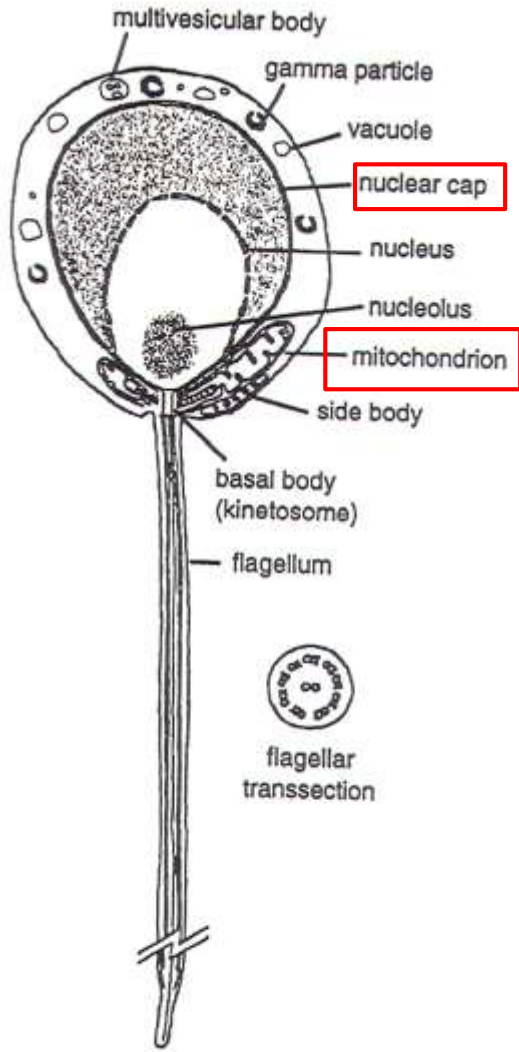


Figure 4.15 The motility of fungal zoospores. The large arrow indicates the direction in which the zoospores are swimming and the small arrows the direction that waves travel along the flagella – away from the body of the zoospore. **A.** Zoospore of the Chytridiomycete *Blastocladiella emersonii*. Waves travelling backward along the single smooth posterior flagellum generate a thrust that pushes the zoospore forwards. A change in the direction of movement occurs by the development of a sharp bend at the flagellum base, changing the direction in which the head points; in effect, the flagellum has acted as a rudder. **B.** Zoospore of the Oomycete *Phytophthora palmivora*, with two flagella emerging from a deep groove on the ventral surface of the zoospore. The smooth posterior 'whiplash' flagellum behaves in a similar way to that of *Blastocladiella*. The anterior flagellum is a 'rinsel' flagellum – it carries stiff hairs, mastigonemes. Although the waves travel forward, the resulting movement of the mastigonemes generates a thrust that contributes to the forward movement of the zoospore. (After Carlile, M. J. (1985). The zoospore and its problems. In P. G. Ayres & L. Boddy, eds., *Water, Fungi and Plants. Eleventh Symposium of the British Mycological Society*, pp. 105–118. Cambridge University Press, Cambridge.)

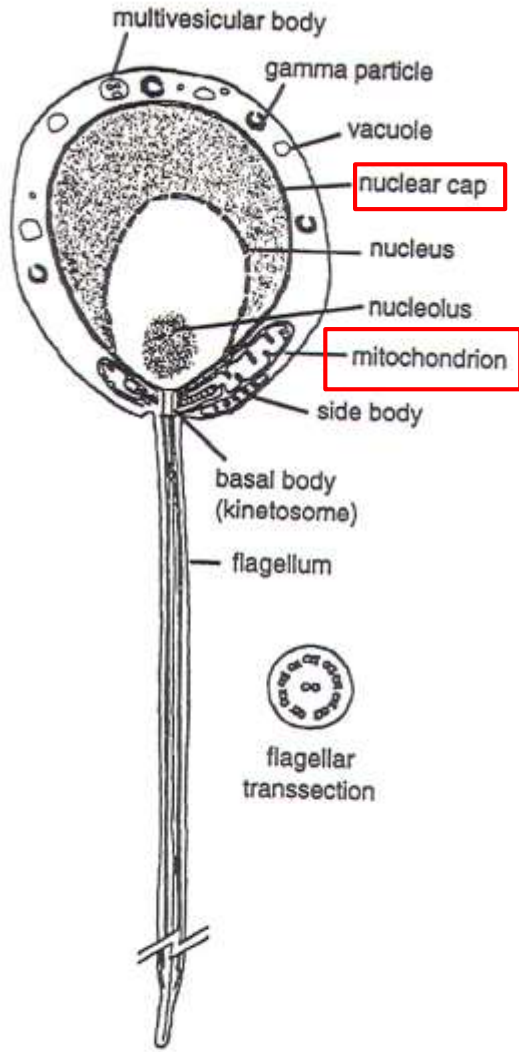
Movimentação
dos zoósporos



Zoospore of *Blastocladia emersonii*

Um único mitocôndrio ocorre próximo ao corpúsculo basal

(respiração alta devido ao flagelo) – esporos dormentes não sintetizam macromoléculas



Zoospore of *Blastocladia emersonii*

Ribossomos ficam empacotados dentro da “nuclear cap” separados do citoplasma

(um inibidor de síntese proteica preso aos ribossomos explica a não síntese de proteínas)

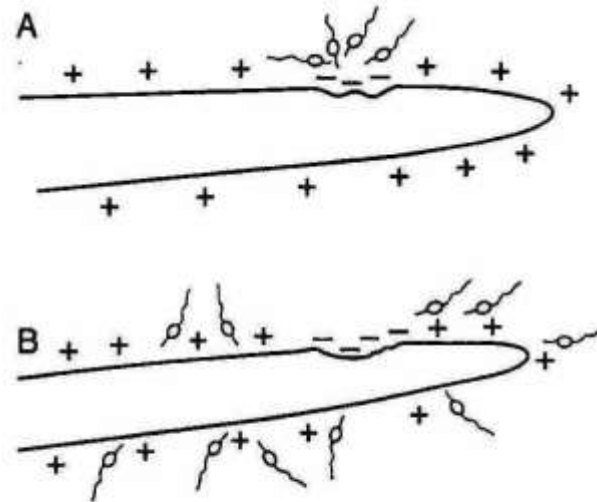


30 min após o encistamento, a capa nuclear desintegra para liberar os ribossomos

Um único mitocôndrio ocorre próximo ao corpúsculo basal

(respiração alta devido ao flagelo) – esporos dormentes não sintetizam macromoléculas

Electrotaxismo – movimento em resposta à carga elétrica



Pythium aphanidermatum

Phytophthora palmivora

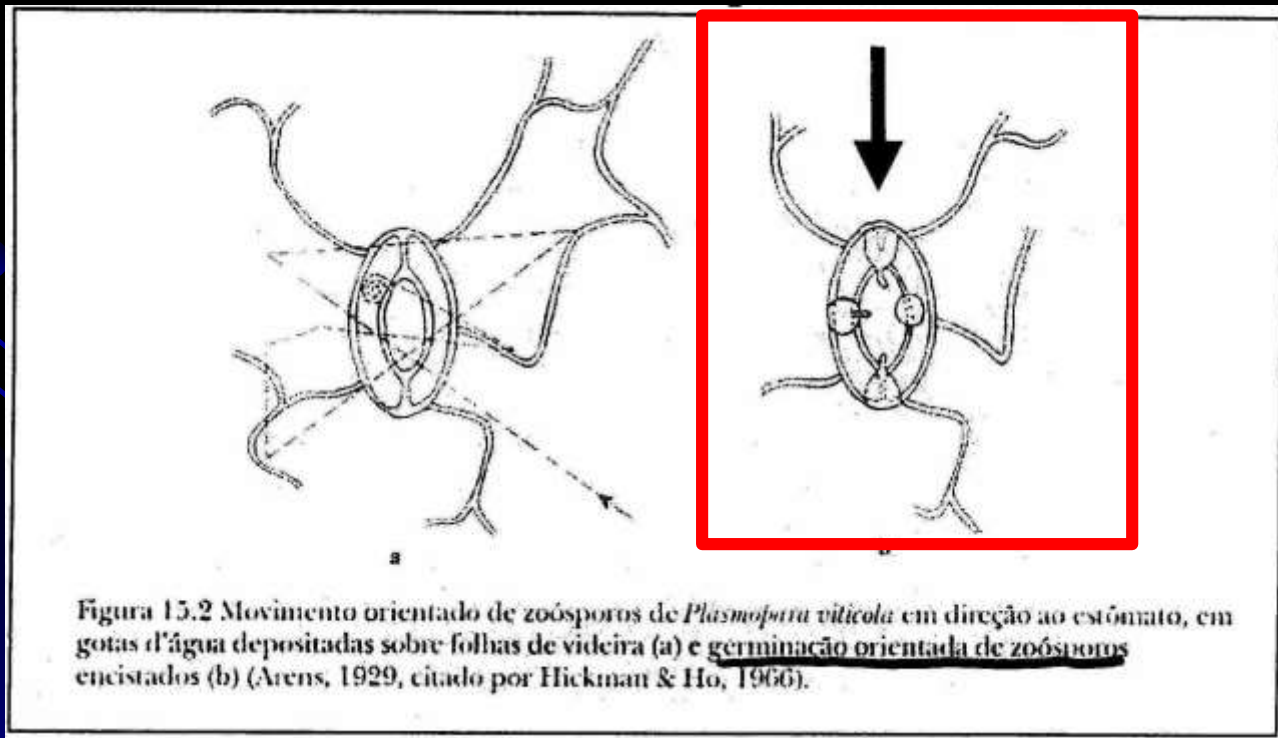
Figure 7.9 Electrotaxis of Oomycete zoospores to plant roots. The direction of swimming of zoospores placed in an electrical field was found to vary between species, with *Pythium aphanidermatum* zoospores moving towards the cathode and *Phytophthora palmivora* towards the anode. Plant roots bear a small electrical charge on their surface, normally positive, but changing to negative in parts of the root that are damaged. In an experiment to test for a role of electrotaxis in the attraction of zoospores to plant roots, damaged roots were placed in suspensions of zoospores of either *Pythium* or *Phytophthora*. *Pythium* spores (A) were differentially attracted to the negatively charged damaged area whereas *Phytophthora* zoospores (B) aggregated all over the positively charged intact parts of the root. (Redrawn from Morris, B. M. & Gow, N. A. R. (1993). *Phytopathology* 83, 877–882.)

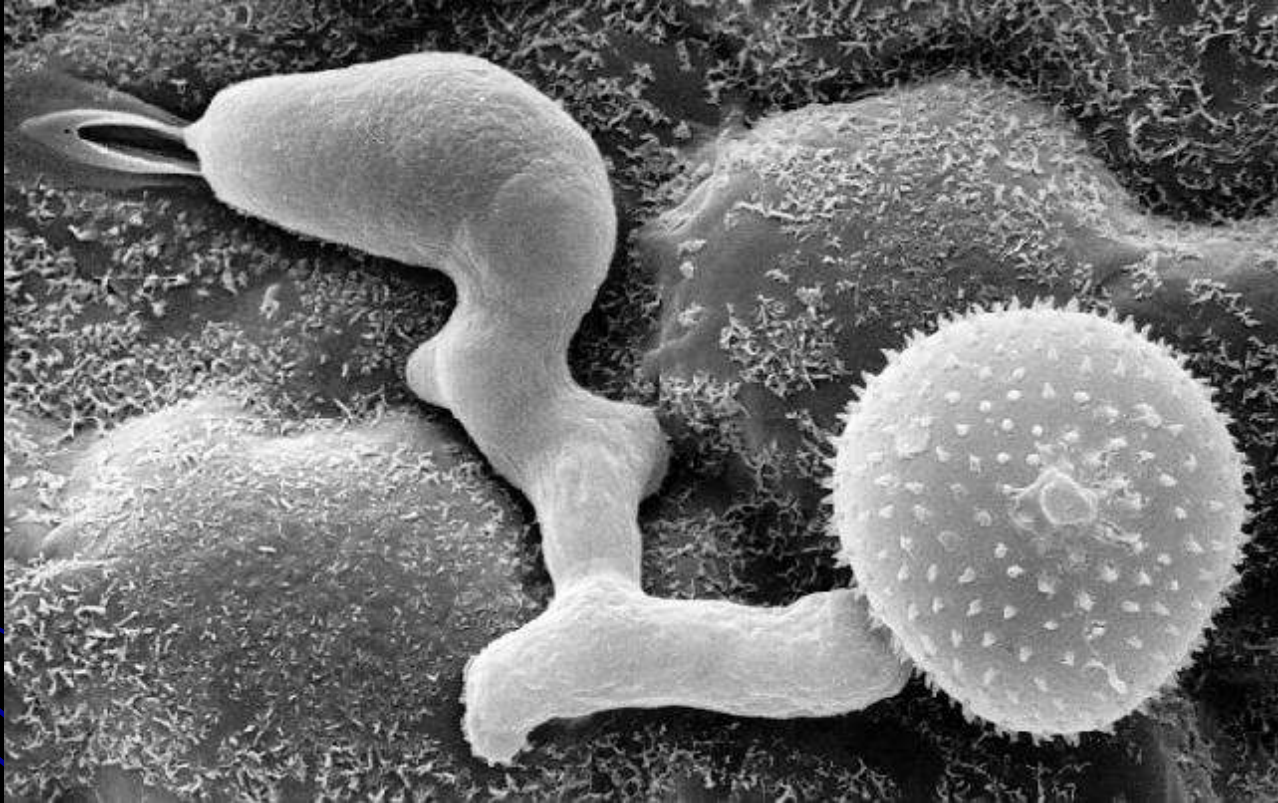
O SUB-PROCESSO “PRÉ-PENETRAÇÃO”

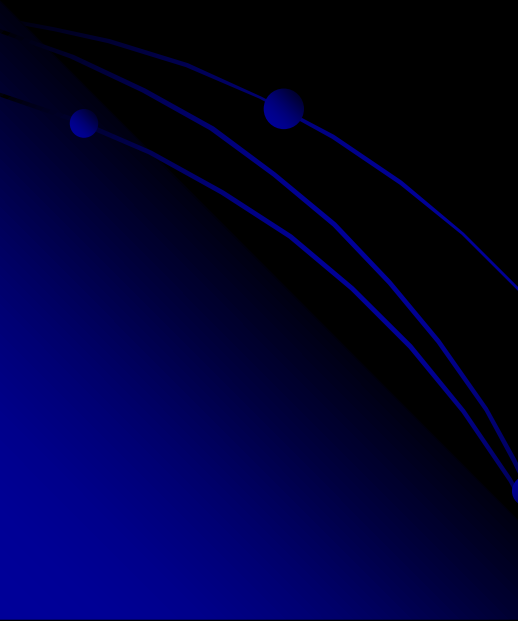
B) Tropismo – crescimento direcionado do patógeno na superfície do hospedeiro

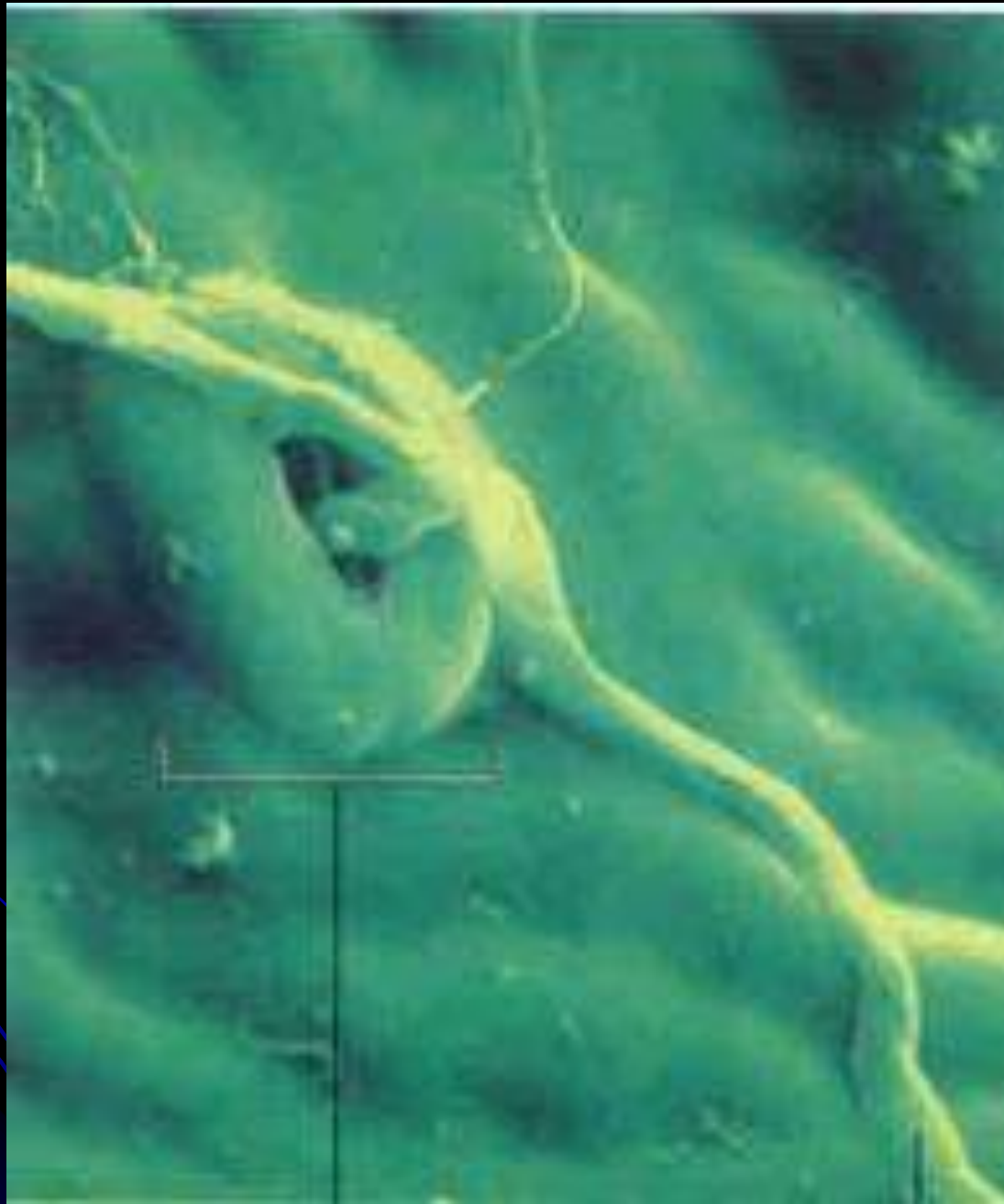
- Resposta de tubos germinativos e hifas de fungos filamentosos.

Quimiotropismo – crescimento em resposta à um estímulo químico









Tigmotropismo

Crescimento em
resposta à topografia
de uma superfície

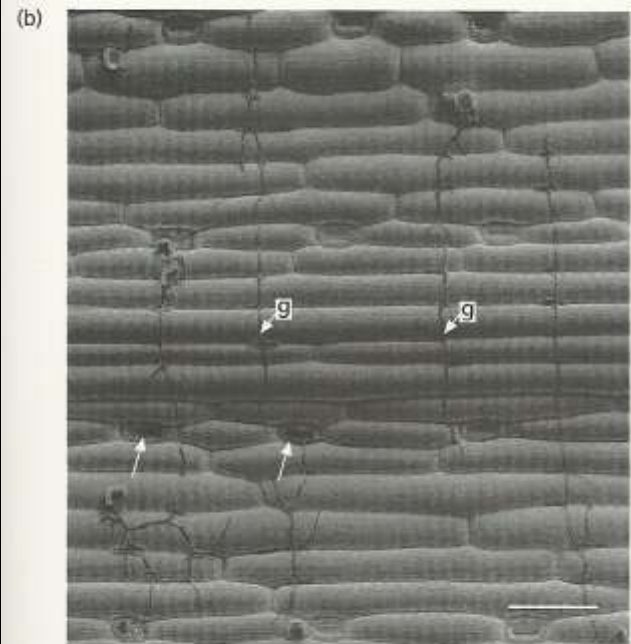
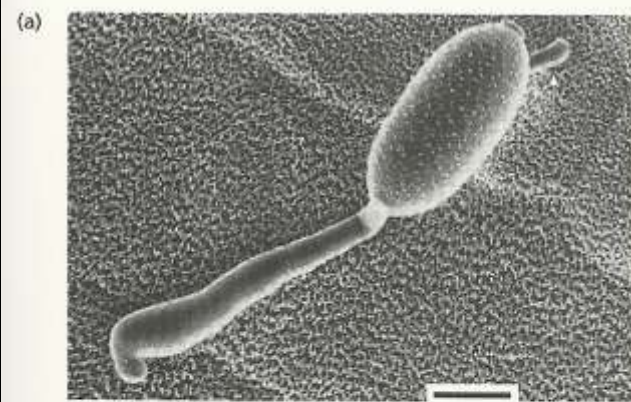
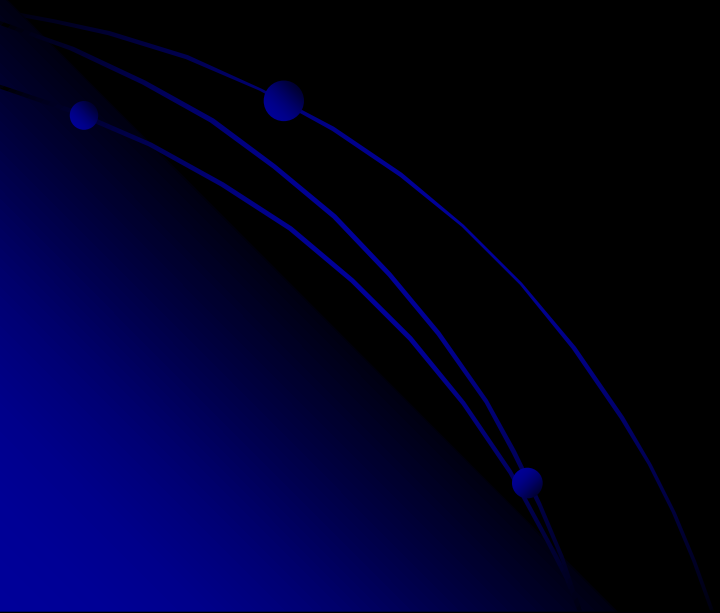
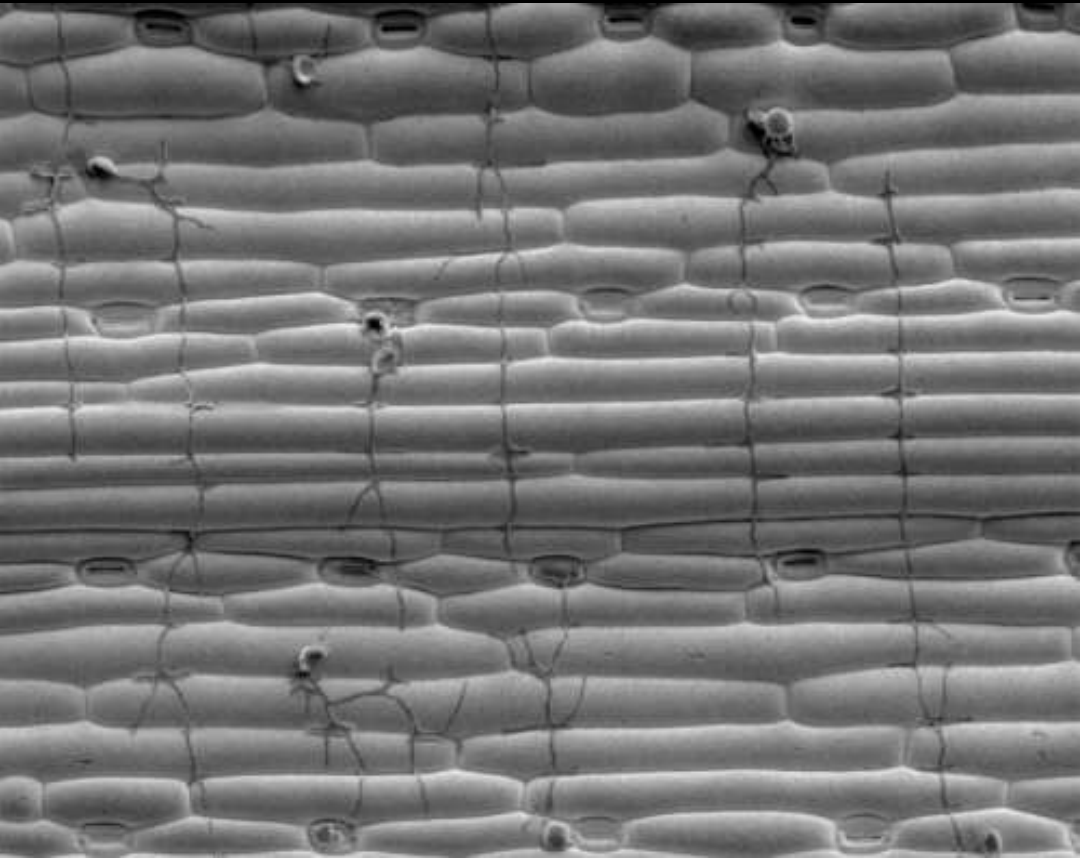
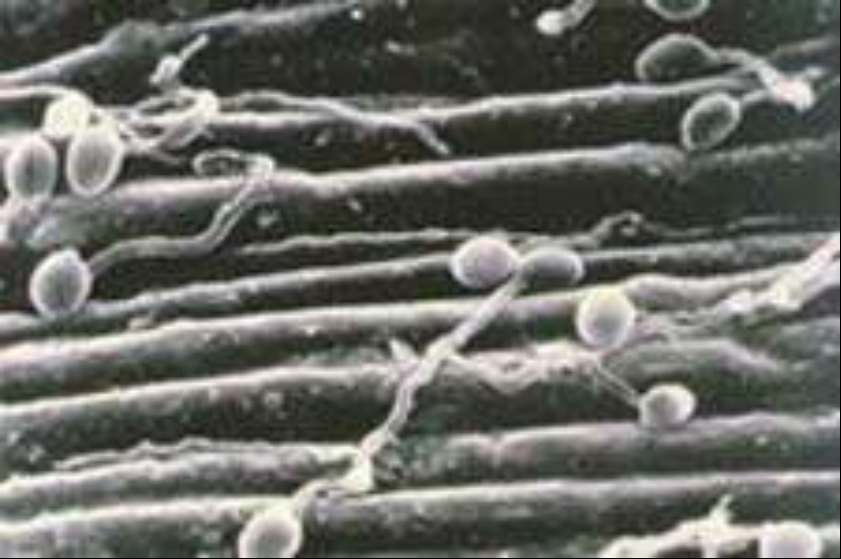
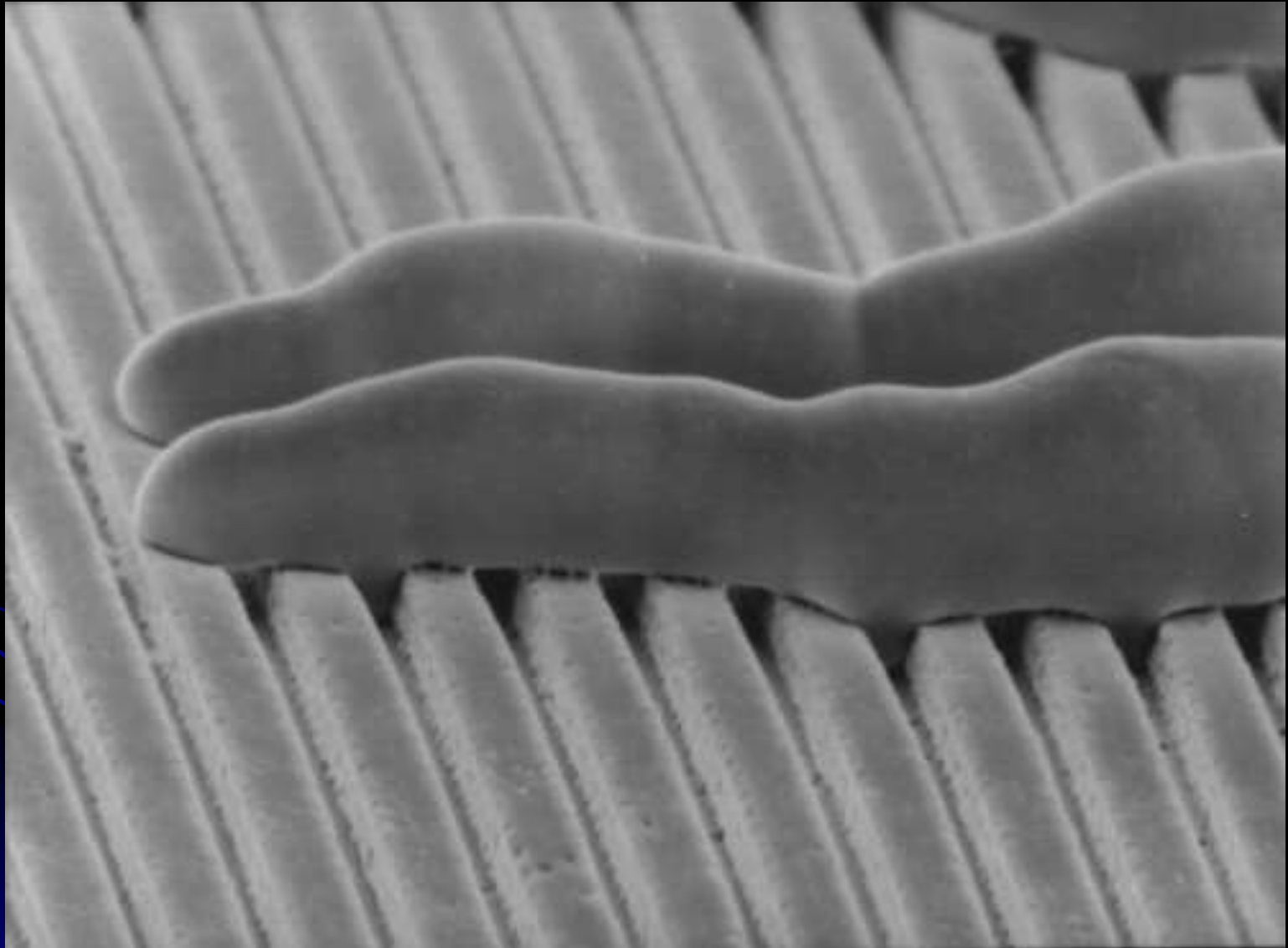
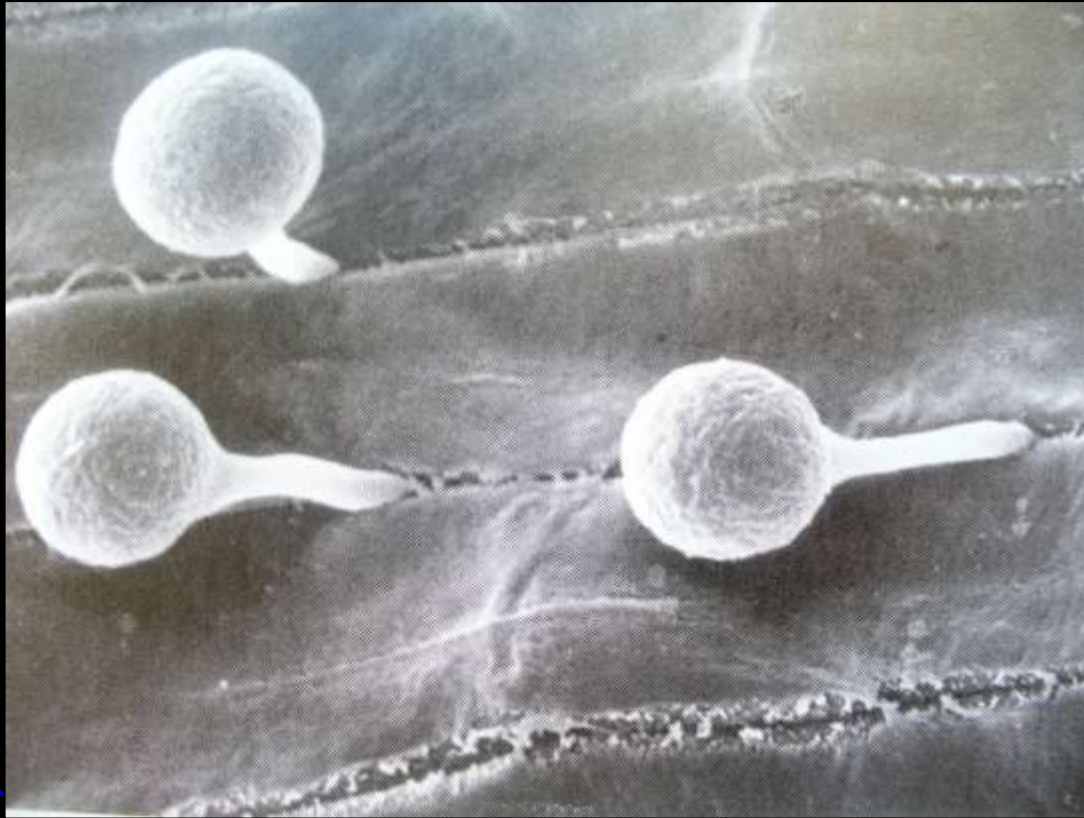


Fig. 6.4 Early development of fungal pathogens on the surface of barley leaves viewed by scanning electron microscopy. (a) Germinating spore of the powdery mildew *Erysiphe graminis* showing small, primary germ tube (arrow) and larger appressorial germ tube with hooklike tip. Scale bar = 10 μm . (Courtesy of Tim Carver.) (b) Germ tubes (g) of brown rust (*Puccinia hordei*) showing growth perpendicular to the orientation of epidermal cells, short branches formed at cell junctions, and appressoria (arrows) formed over stomata, which occur in rows. Scale bar = 100 μm . (From Read *et al.* 1992.)

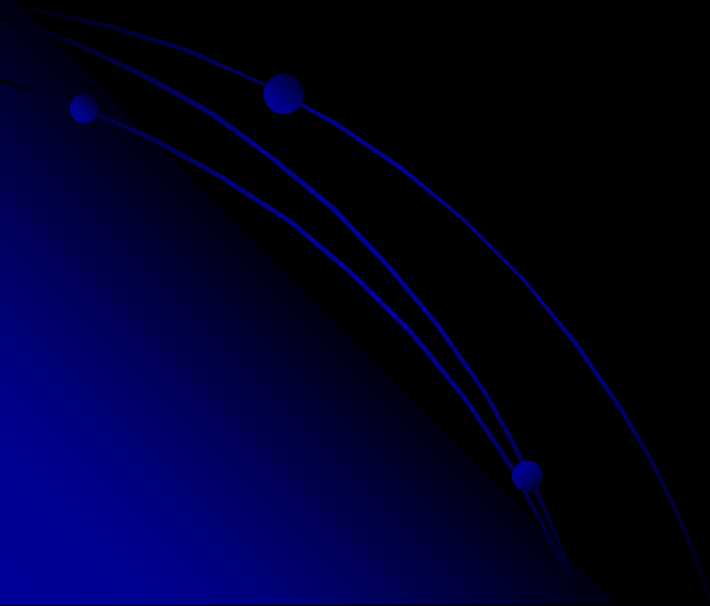






Zoósporos de *Phytophthora sojae* encistados e penetrando em raíz (Agrios, 2005)

ESTÍMULOS PARA AS RESPOSTAS DE MOVIMENTO OU CRESCIMENTO

- Substâncias químicas – quimiotropismo/ quimiotatismo
 - Cargas elétricas – eletrotropismo/ eletrotatismo
 - Topografia de uma superfície - tigmotropismo
 - Água – hidrotropismo/ hidrotatismo
 - Luz - fototropismo/ fototatismo
- 

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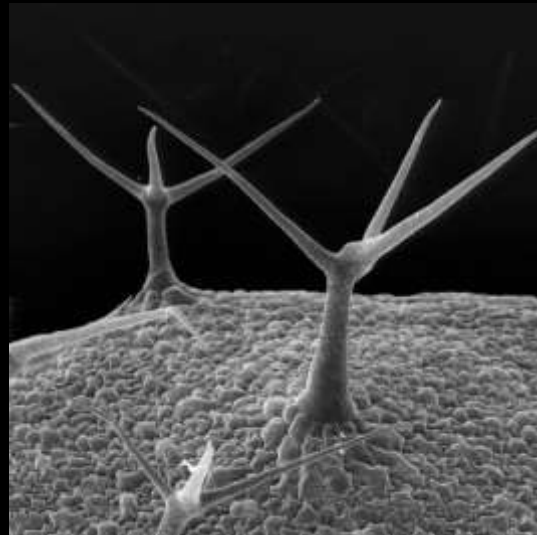
MAIS COMUNS NA FASE DE PRÉ-PENETRAÇÃO

- Quimiotatismo - patógenos veiculados pelo solo
- Tigmotropismo – patógenos de parte aérea

ADESÃO

Esporo x Chance (Hospedeiro)

- **Fixação** – mecanismos através dos quais os propágulos fúngicos ficam presos na superfície do hospedeiro (Ex. tricomas).



ADESÃO

➤ Adesão – mecanismo mais refinado, requerendo a presença de materiais adesivos ou alterações da cutícula pelo fungo, para a retenção dos propágulos fúngicos na superfície do hospedeiro.

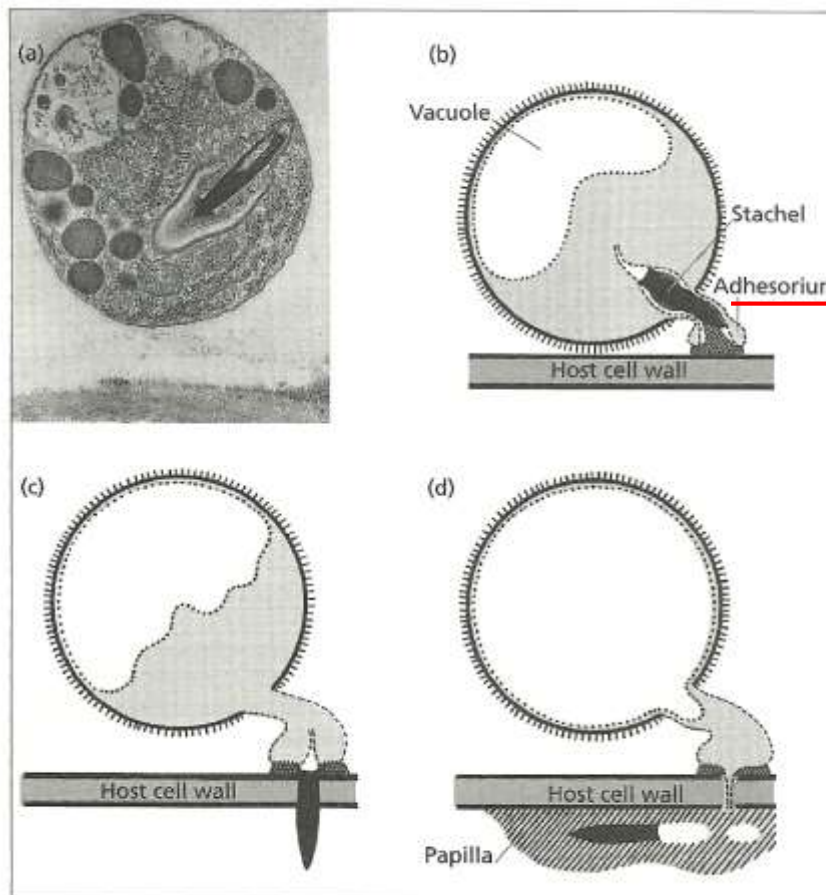


Fig. 6.6 (a) Electron micrograph section of a *Plasmidiophora brassicae* zoospore encysted on a root hair, showing bullet-like stachel ($\times 26\,500$). (b–d) Diagrammatic summary of the penetration of a root hair: (b) vacuole enlarges and small adhesorium appears; (c) stachel punctures host wall; (d) penetration has occurred and the host protoplast has deposited a papilla at the penetration site. (From Williams *et al.* 1973.)

Mucilagem, matriz, envelopes, substâncias de ligação

Material adesivo – mistura de:

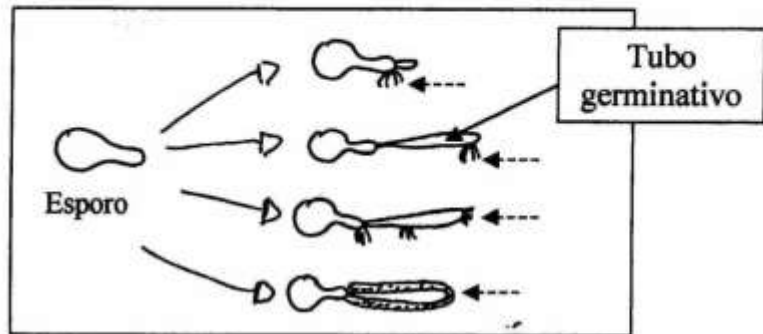
- Polissacarídeos
- Glicoproteínas
- Polímeros hexoaminas
- Material fibrilar

Molhado

Visguento (pegajoso)

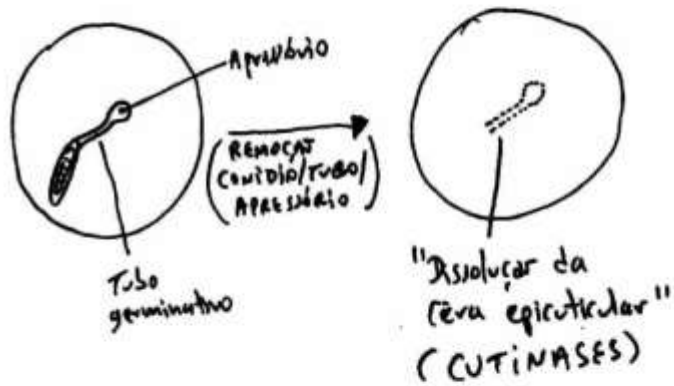
Propágulo fica aderido ao hospedeiro

Deposição de material adesivo:



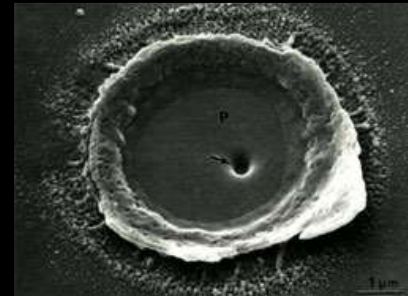
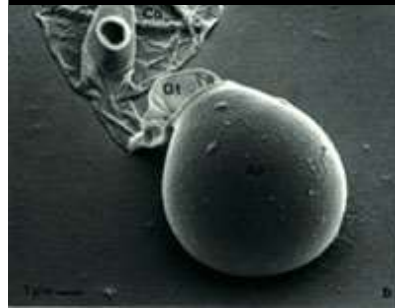
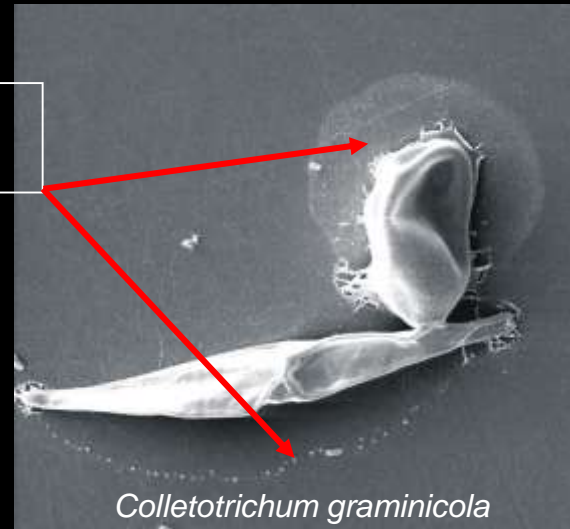
“A presença de mucilagens é frequentemente associada com apressórios”

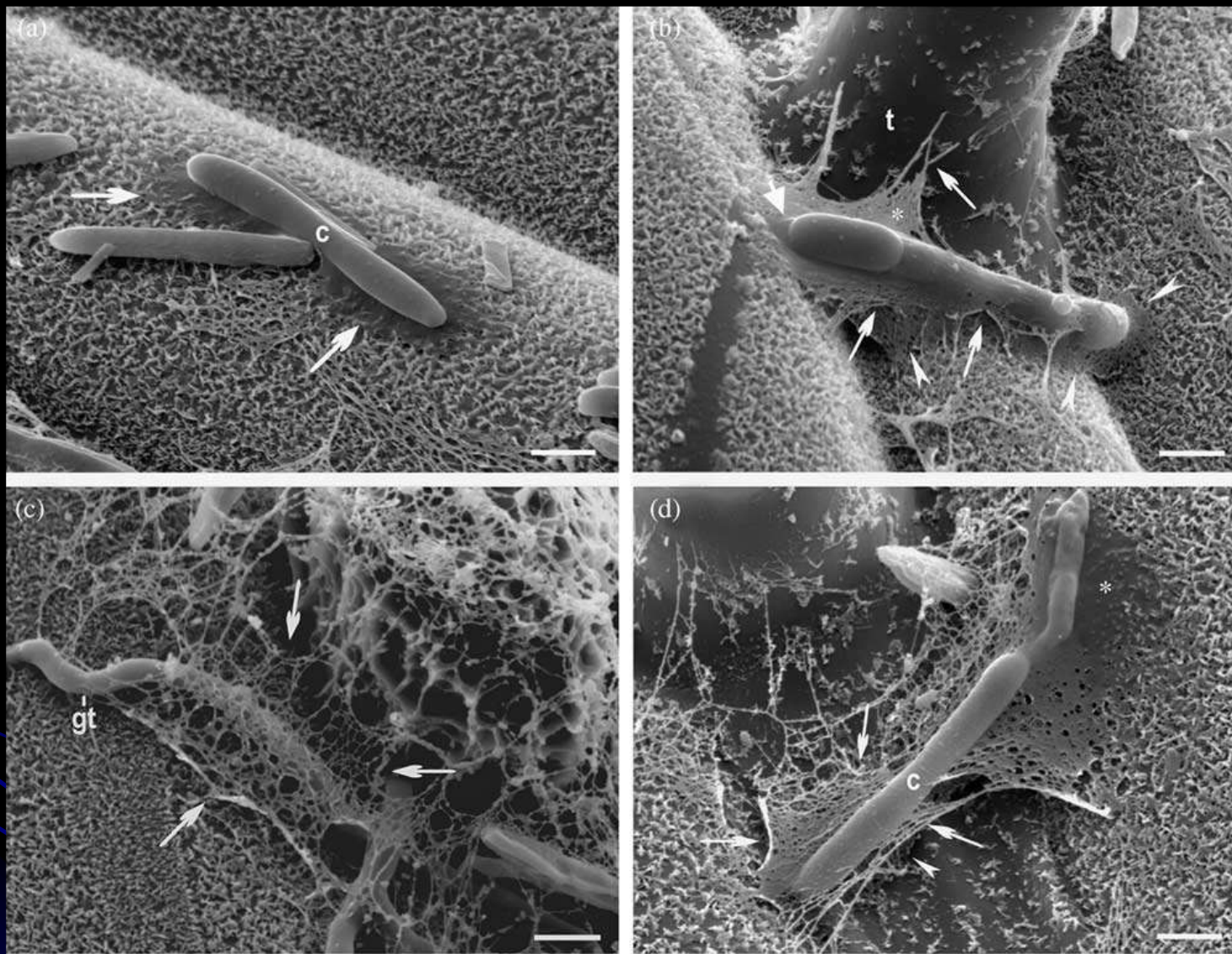
Alterações cutícula – “Impressões cuticulares” / “Erosão”



“Adesão pode ser um processo ativo requerendo estímulos específicos do hospedeiro”

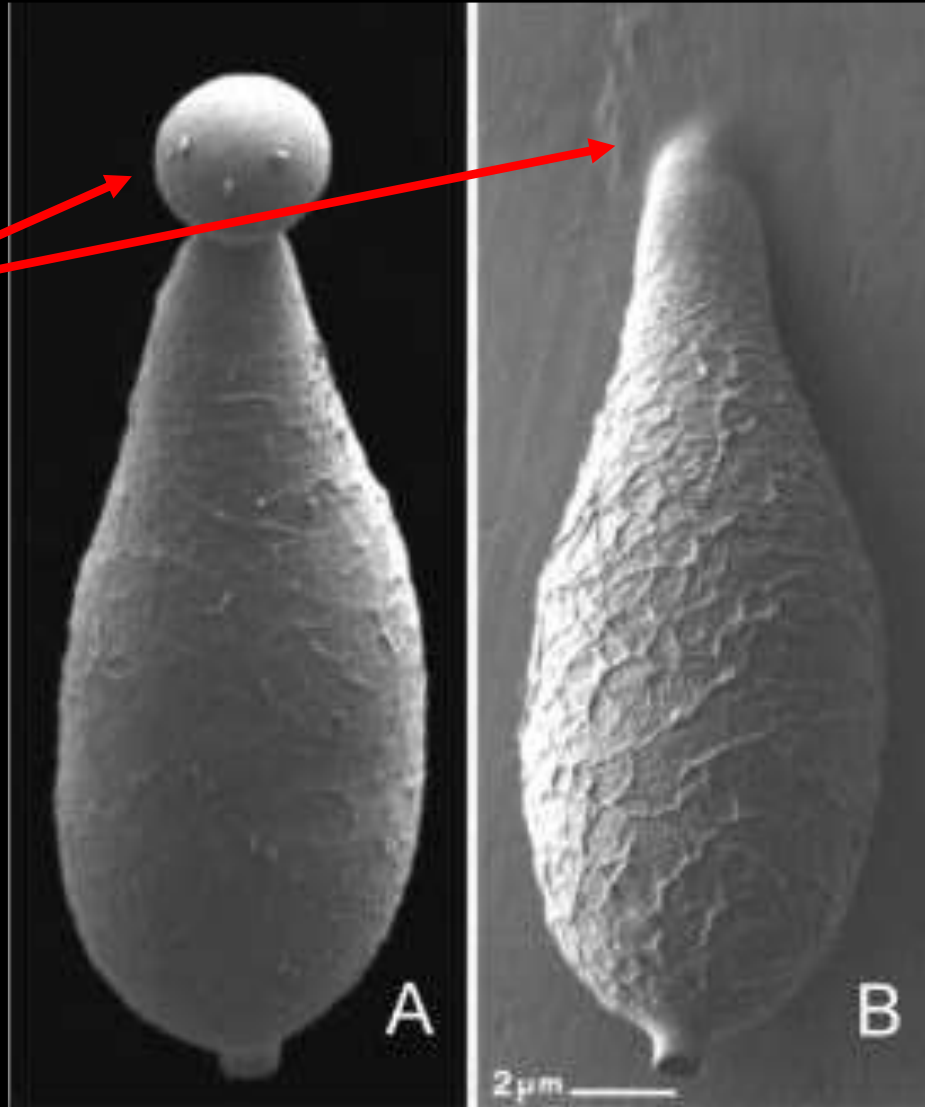
Material adesivo



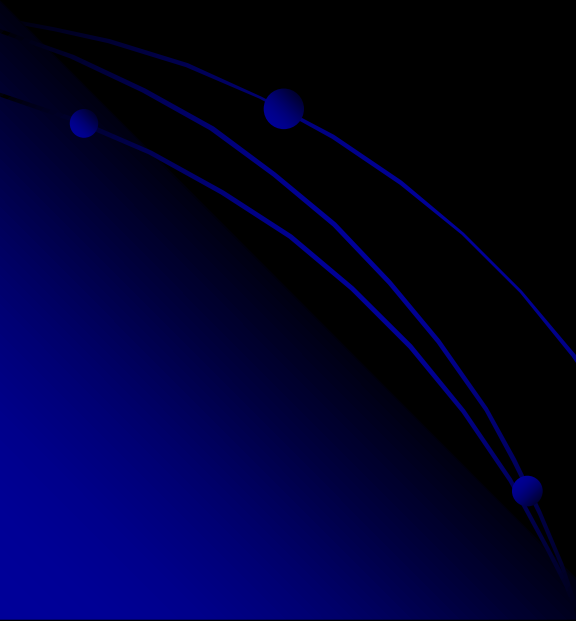
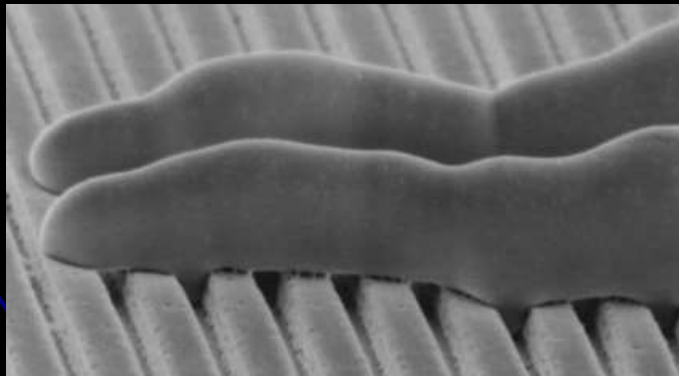
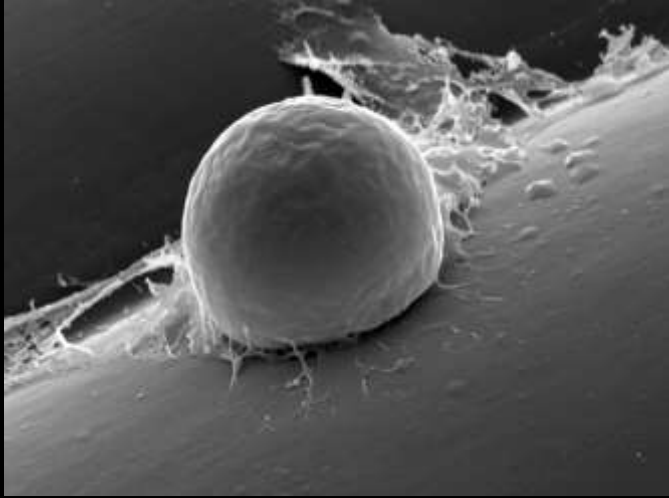


Esposos de *Stagonospora nodorum* (mancha da gluma) aderidos em folha de trigo (a) imediatamente após a inoculação, (b) 3 horas após, (c) 5 horas após e (d) 8 horas após.

Mucilagem



Magnaporthe grisea





Bactérias

- Adesão

Streptococcus mutans - usa a glicana (polímero de glicose) presente na cápsula para aderir na superfície do dente e causar cárie.

Glicocálice - Camada de material viscoso (polissacarídeos / polipeptídeos) que circunda algumas células bacterianas.

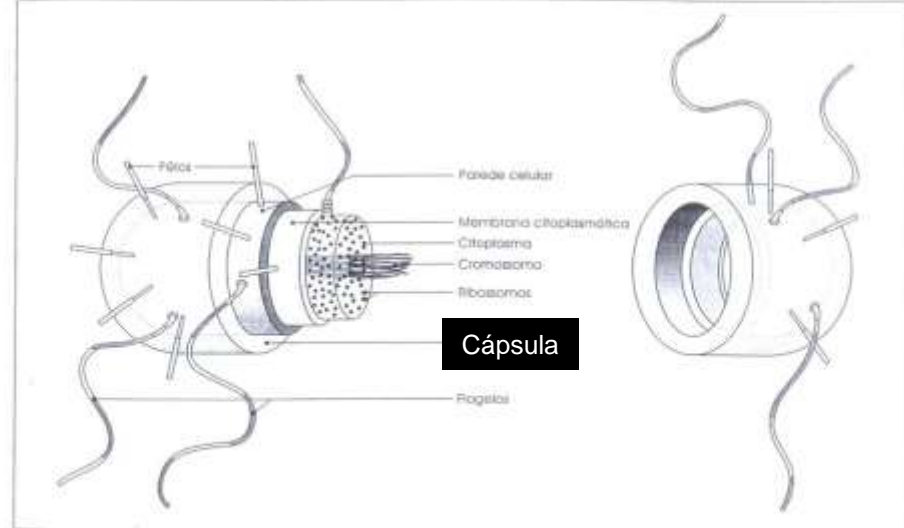
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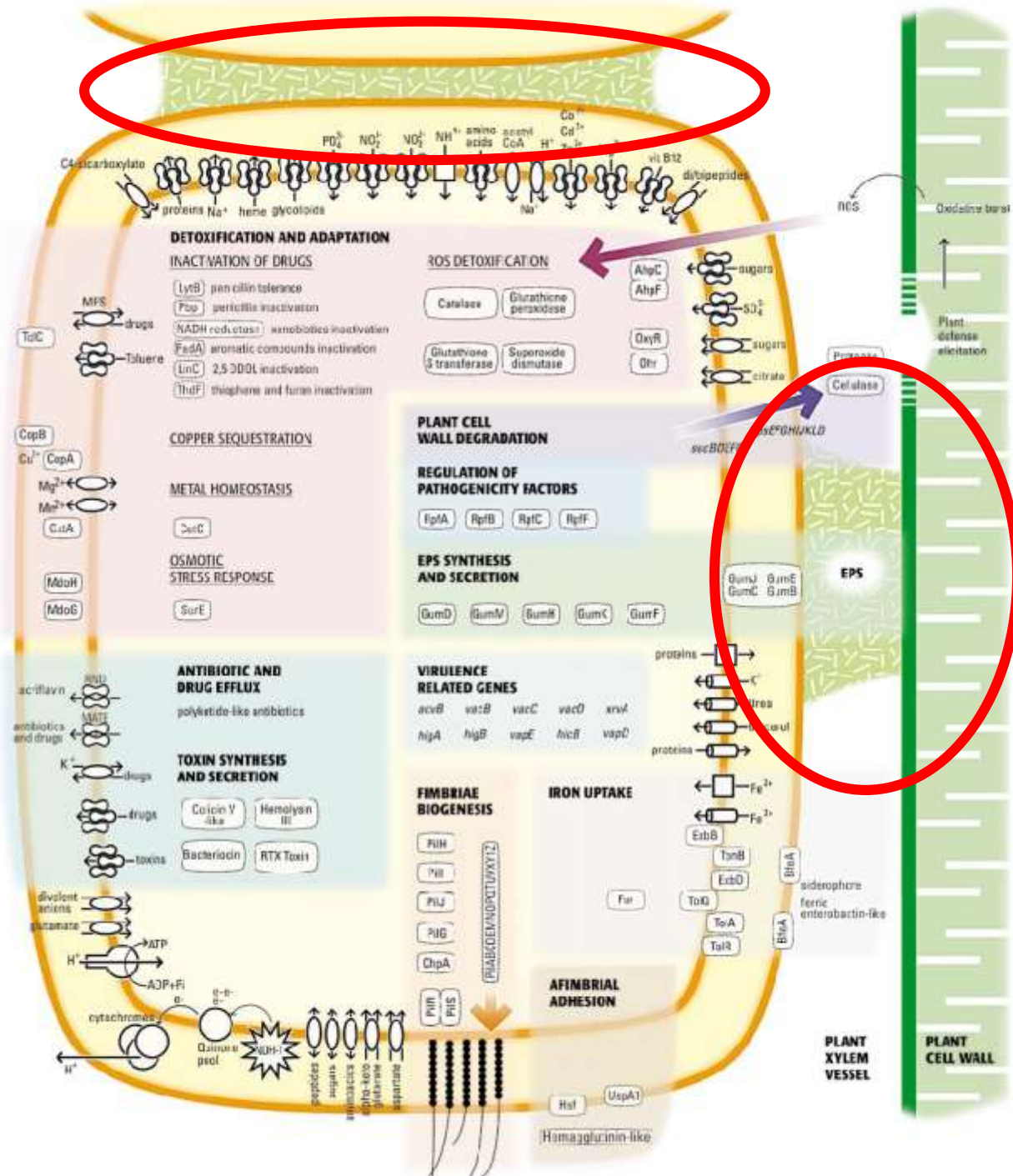
Cápsula – organizado de maneira definida / acoplado firmemente à parede celular

Camada limosa – desorganizado / acoplado frouxamente à parede celular (solúvel em água)

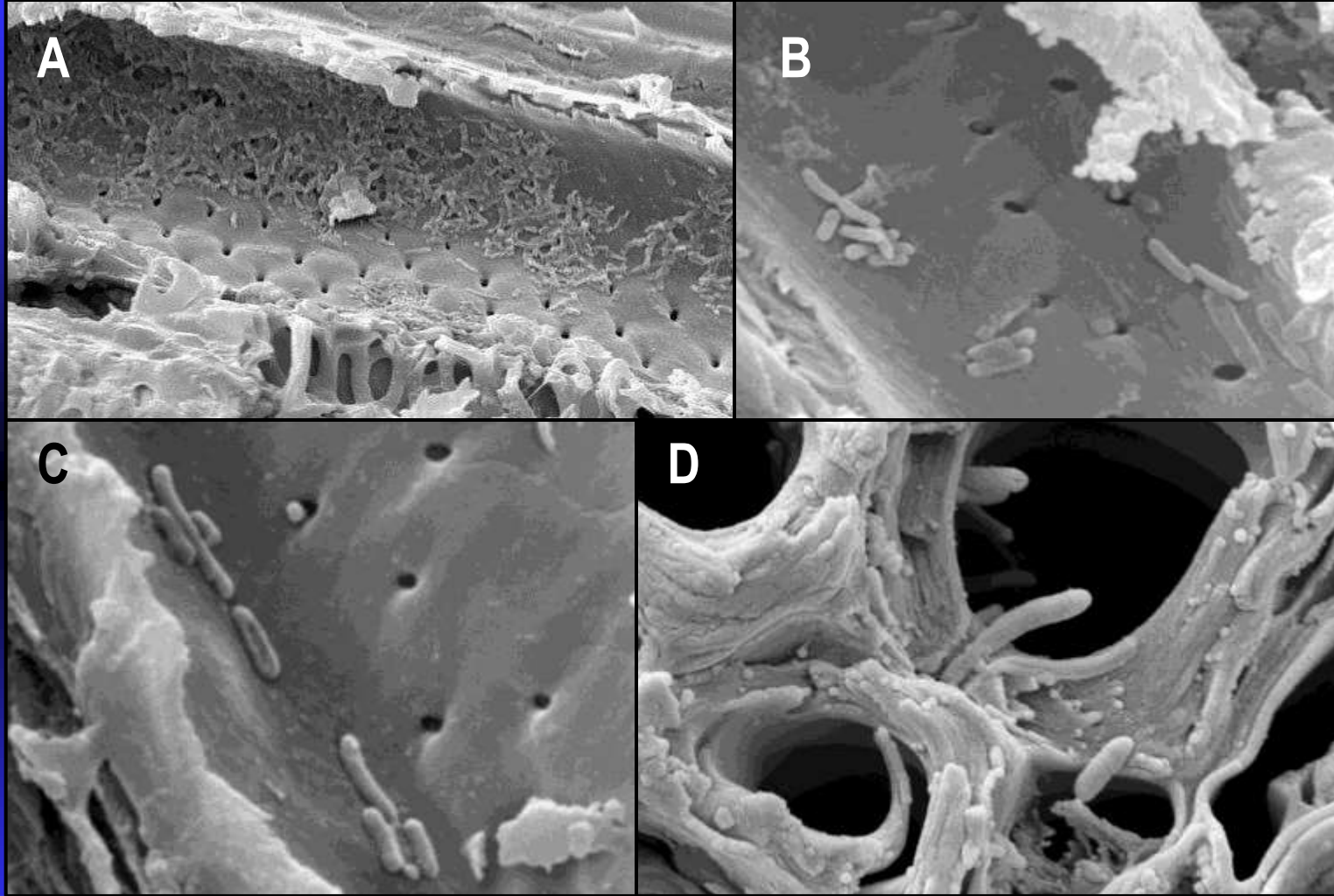
- Função – aderência / proteção contra dessecação / reservatório de nutrientes

Representação esquemática da estrutura geral de uma célula bacteriana





MEV de *X. fastidiosa* em xilema de laranjeira



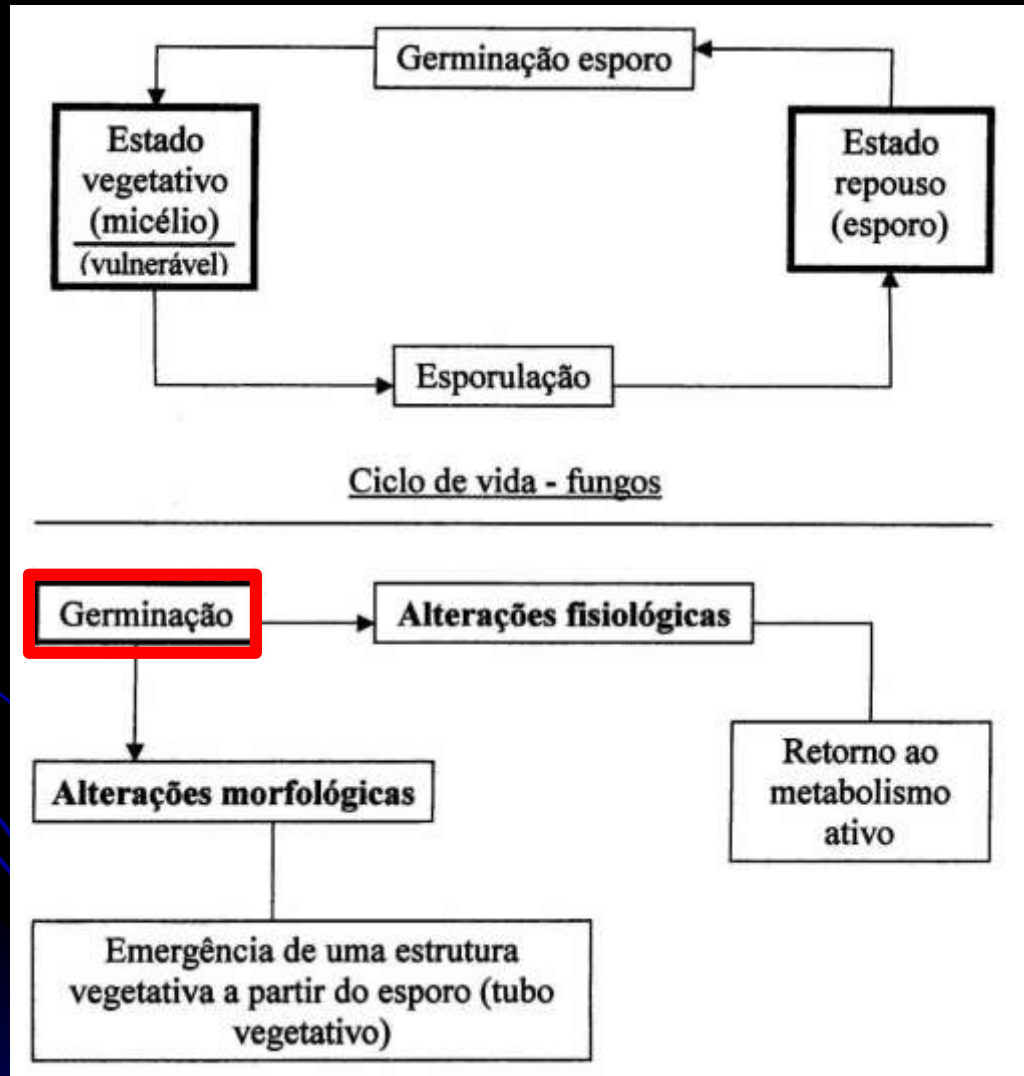
Germinação

“O esporo fúngico é geralmente um propágulo unicelular, algumas vezes multicelular, com uma baixa taxa de metabolismo.”



Germinação

“O esporo fúngico é geralmente um propágulo unicelular, algumas vezes multicelular, com uma baixa taxa de metabolismo.”



Germinação – condições

“ De maneira geral, os esporos tem controle sobre os seus destinos”

Germinação – estágios

- I. Início da germinação
- II. Crescimento esférico
- III. Emergência do tubo germinativo

I. Início da germinação

Entre a esporulação e o início da germinação o esporo está em um estado quiescente (denominado de período de dormência)



- Este período pode durar horas até anos
- Durante a dormência, mudanças morfológicas não ocorrem e a taxa metabólica é baixa

**Dormência exógena
(ambiental)**

**Dormência endógena
(constitutiva)**

I. Início da germinação

➤ Dormência exógena (ambiental)

- Falta de ambiente adequado para a germinação. (Ex. temperatura inadequada ou nutrientes essenciais em falta).
- É provável que o momento e o local de germinação dos esporos de dispersão seja frequentemente controlado por este tipo de dormência.

“É comum fungos produzirem inibidores voláteis para impedir que seus competidores ao redor tenham esporos germinados”

Temperature optima for germination and growth of fungi
for which the optima differ

Fungus	Spore	Optimum (°C)	
		Germination	Growth
<i>Plasmodiophora brassicae</i>	Oospore	27-30	16-21
<i>Peronospora schachtii</i>	Sporangium	4-7	12
<i>P. parasitica</i>	Sporangium	8-12	3-4
<i>P. sinaciae</i>	Sporangium	9	16
<i>Rhizopus delamar</i>	Zygospor	26-38	41-45
<i>Valsa japonica</i>	Ascospore	18-23	23-25
<i>Pseudopeziza ribis</i>	Ascospore	12	24
<i>Urocystis occulta</i>	Spore	15	24
<i>Ustilago hordei</i>	Teliospor	22-30	16-20
<i>Botrytis fabae</i>	Conidium	15-20	20-25
<i>Ceratophorum setosum</i>	Conidium	20-28	10-15
<i>Coniothyrium pirinum</i>	Conidium	25	18-20
<i>Alternaria brassicae</i>	Conidium	33-35	25-27

I. Início da germinação

➤ Dormência endógena (constitutiva)

- Depende de características estruturais ou metabólicas do esporo e pode requerer condições específicas ou incomuns para o seu término
- Pode evitar a germinação de esporos de sobrevivência em condições que pareçam ser favoráveis para a germinação

Dormancy and Nutritional Requirements for Spore Germination*			
Nutrient Status	Spore Type	Germination Medium	Dormancy Type
Insufficient	Conidia Sporangiospores	Carbon and/or nitrogen source	Environmental
Self-sufficient	Zoospores Oospores Ascospores Zygospores Uredospores Basidiospores	Distilled water or inorganic salt solutions	Constitutive

*Note: Exceptions are likely.

Characteristics of fungal spores - germination

Spore	Characteristics	Life Cycle Role	Classes
Zoospore	Wall-less, one-celled, flagellated	Asexual or meiotic ^a	Chytridiomycetes Oomycetes Hyphochytridiomycetes
Oospore	Thick-walled, one-celled, poor germination	Zygotes	Oomycetes
Sporangiospore	Thin-walled, one-celled, rapid germination	Asexual or meiotic ^b	Zygomycetes
Zygospor	Thick-walled, one-celled, poor germination	Zygotes	Zygomycetes
Conidium	Thin-walled, one- to many-celled, usually rapid germination	Asexual	Ascomycetes Basidiomycetes Deuteromycetes
Ascospore	Thick- or thin-walled, one- to several-celled, germination various	Meiotic	Ascomycetes
Basidiospore	Thin-walled, one-celled, rapid germination	Meiotic	Basidiomycetes
Teliospore	Thick-walled, one- or two-celled, form basidia, not mycelia on germination	Zygotes	Basidiomycetes
Urediniospore	Thin-walled, one-celled rapid germination	Asexual	Basidiomycetes
Aeciospore	Thin-walled, one-celled, rapid germination	Asexual	Basidiomycetes

^aZoospores are produced in sporangia and may also be thought of as sporangiospores. Some sporangia are sites of meiosis, and the spores produced are therefore meiotic.

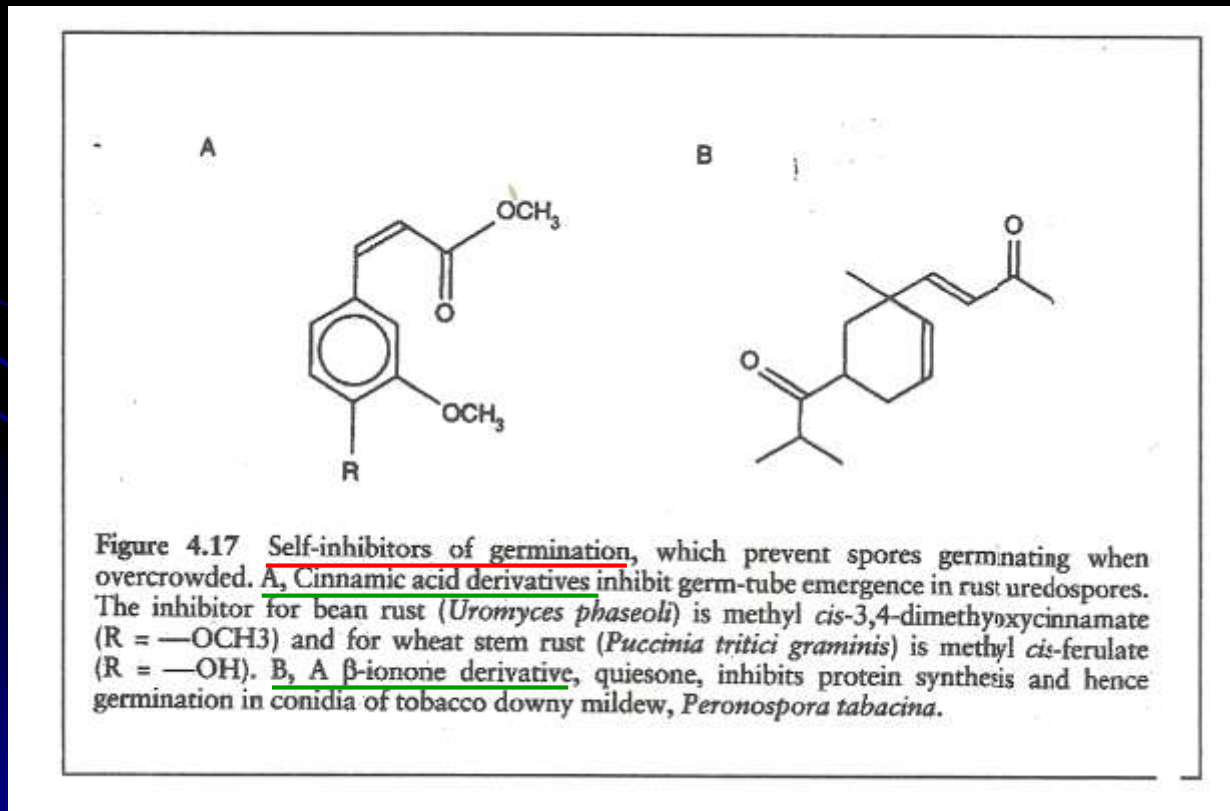
^bSpores produced in germ sporangia from zygospor are meiotic.

I. Início da germinação

➤ Auto-inibidores (Self-inhibitors)

- Evitam que os esporos germinem todos de uma vez em uma mesma condição

- Exemplo - *Colletotrichum graminicola* produz micosporina-alanina e ácido acético



Lavagem



Remoção do
inibidor

I. Início da germinação

➤ Ativação (quebra da dormência)

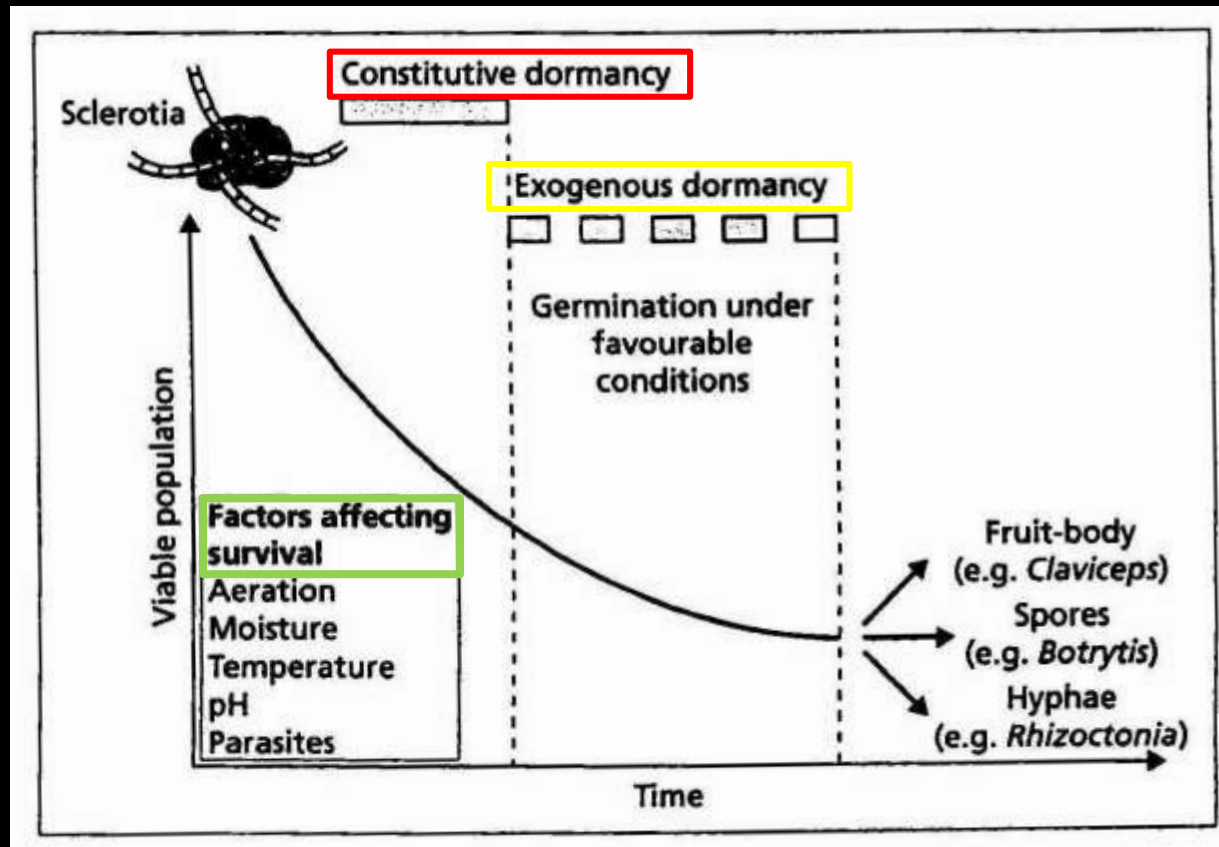
Ativação (quebra da dormência)

- Temperaturas – entre 27 °C e 95 °C (5 min a 30 hr)
- Químicos – alguns servem como agente umectante

Table 4. Chemical Activators of Fungal Spores [43]

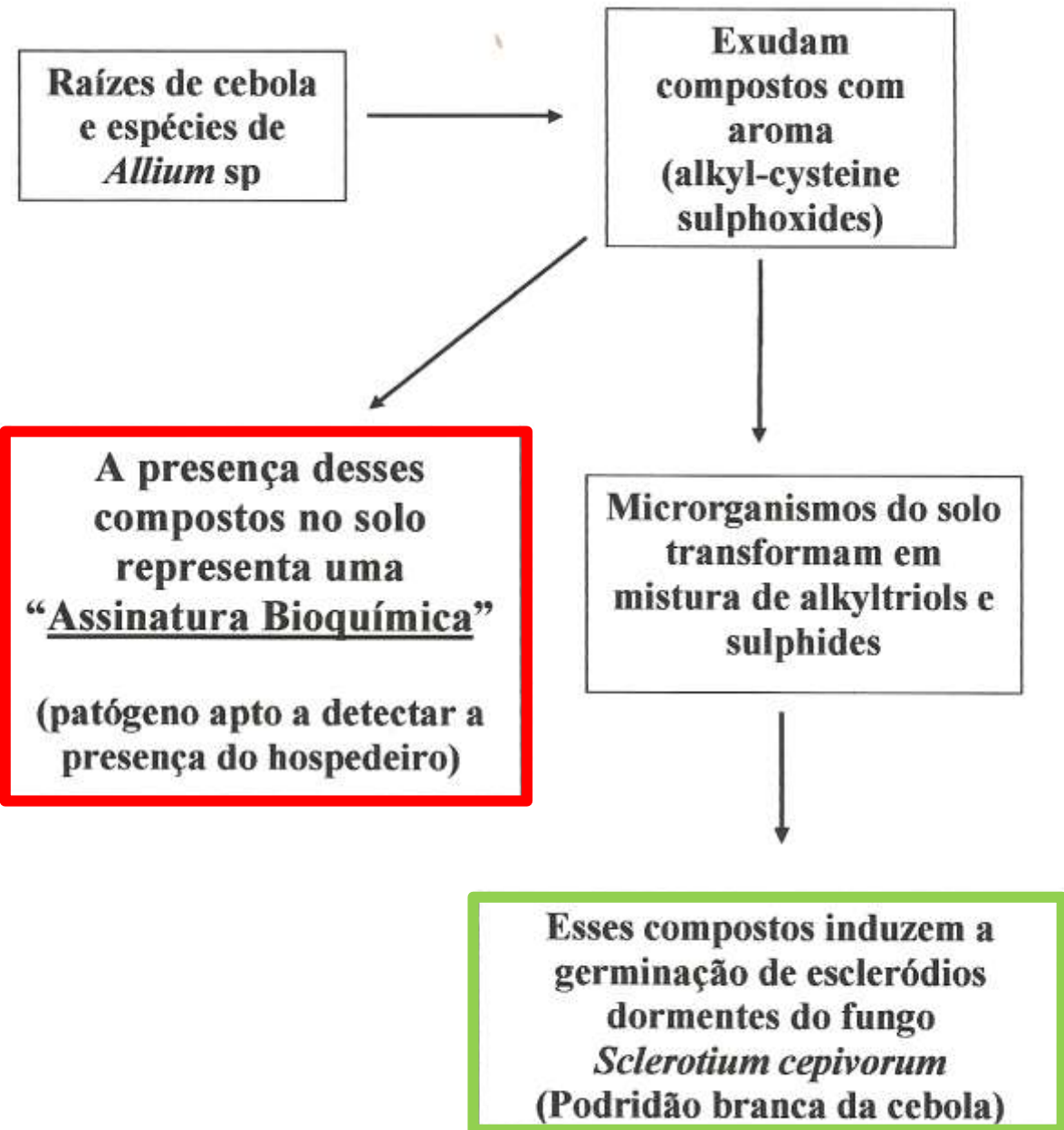
Fungi	Activator
<i>Myxomycetes</i>	Detergents
<i>Colletotrichum trifolii</i>	Tween-20 (a detergent)
<i>Phycomyces blakesleeanus</i>	Organic acids (acetic, propionic, butyric)
<i>Penicillium frequentans</i>	Ethanol, acetone
<i>Neurospora</i> spp. ascospores	Low concentrations: furans, thiophenes, pyrrole, aromatic alcohols High concentrations: ethanol, acetone, other organic solvents
<i>Coprinus radiatus</i>	Furfural and other heterocyclic <u>compounds</u> , but <u>heat essential</u> for maximal germination
<i>Anthracoïdia</i> spp.	Detergents

Survival and germination of fungal sclerotia under natural conditions



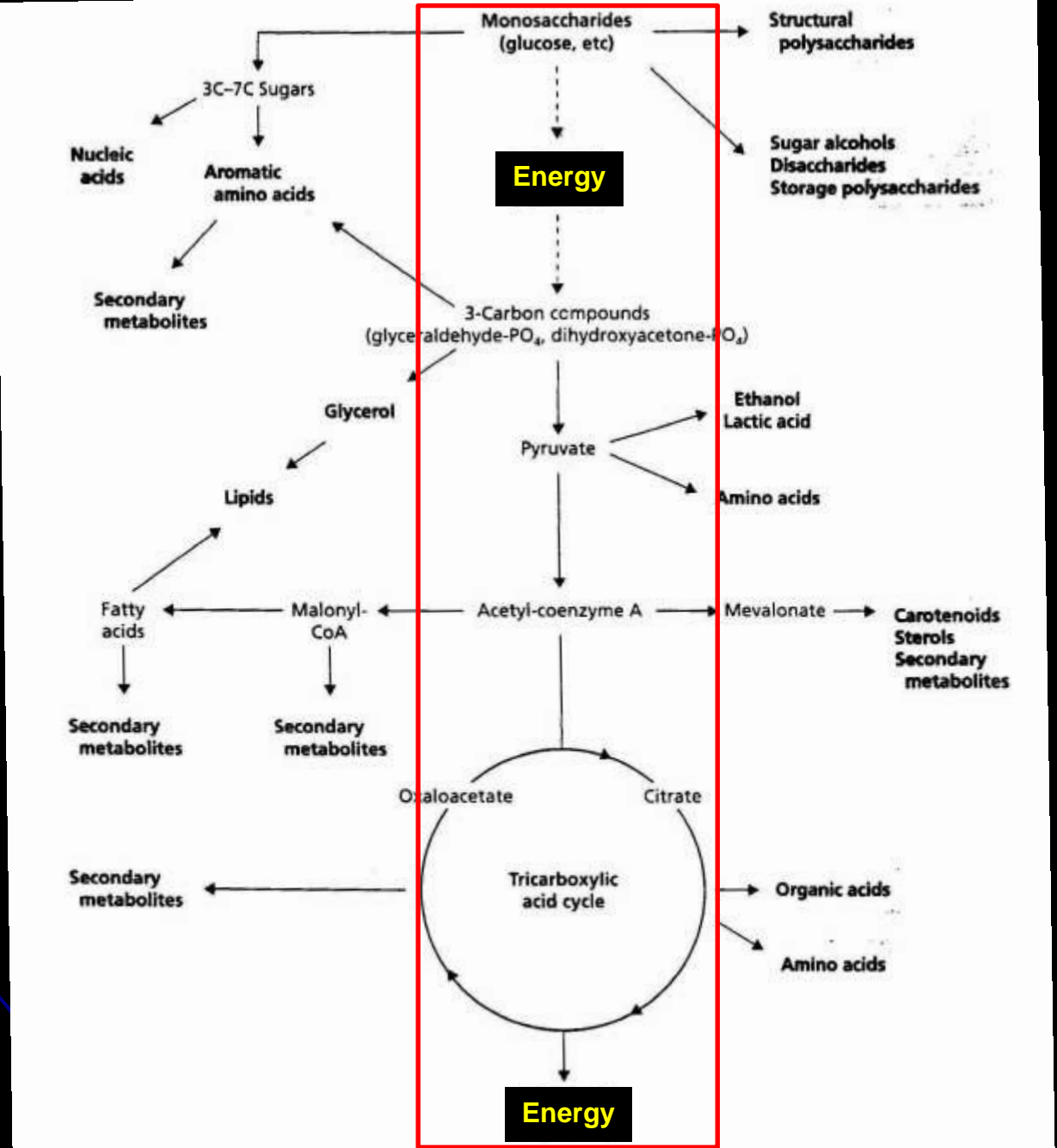
- População inicial diminui devido aos efeitos de diferentes fatores externos
- Inicialmente, a germinação é evitada por fatores constitutivos (auto-inibidores, permeabilidade - barreiras)
- Após os fatores constitutivos serem ultrapassados, existe a necessidade de uma combinação favorável de fatores exógenos

Sinais específicos do hospedeiro podem auxiliar na quebra da dormência



I. INÍCIO DA GERMINAÇÃO

Alterações Metabólicas



Overview of the basic metabolic pathways of fungi, showing how the main energy-yielding pathways provide the precursors for products used in growth and biosynthesis

I. INÍCIO DA GERMINAÇÃO

Alterações Metabólicas

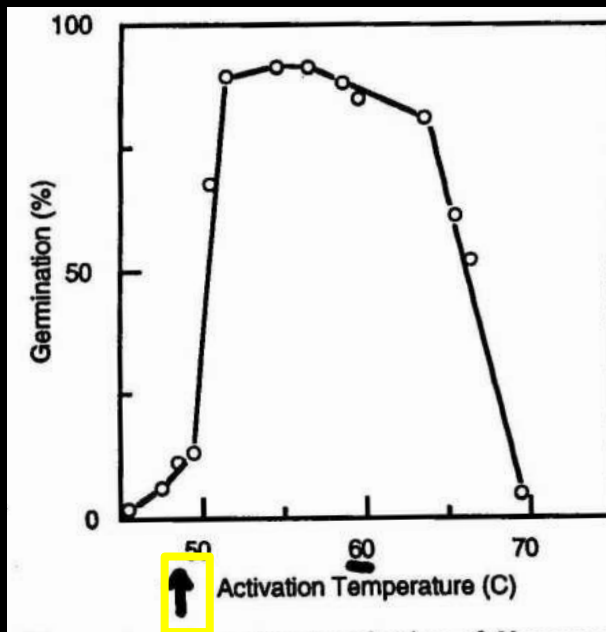


Figure 4. Effect of heat activation of *Neurospora tetrasperma* ascospores at different temperatures on subsequent germination at room temperature. Re-drawn from the data of Goddard [72].

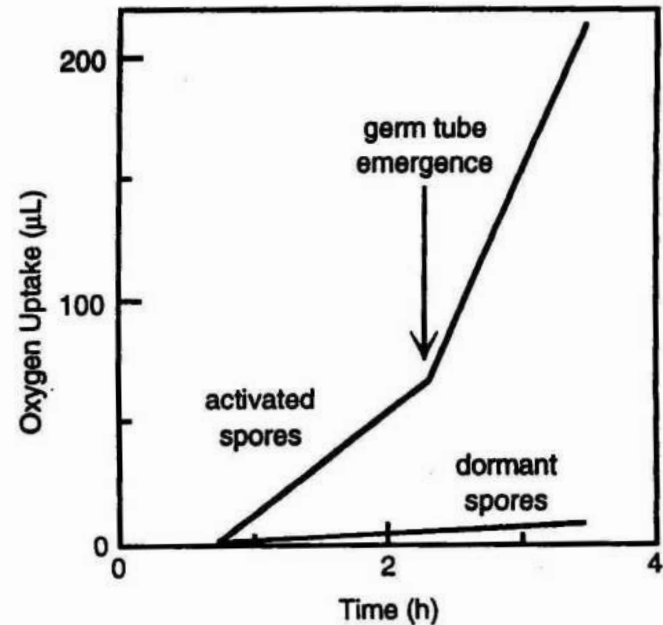


Figure 6. Oxygen consumption of activated and dormant ascospores of *Neurospora tetrasperma*. Drawn from the data of Goddard and Smith [74].

“Sharp transition” – sugere mudanças conformacionais (proteínas, lipídeos)

I. INÍCIO DA GERMINAÇÃO

Alterações Metabólicas

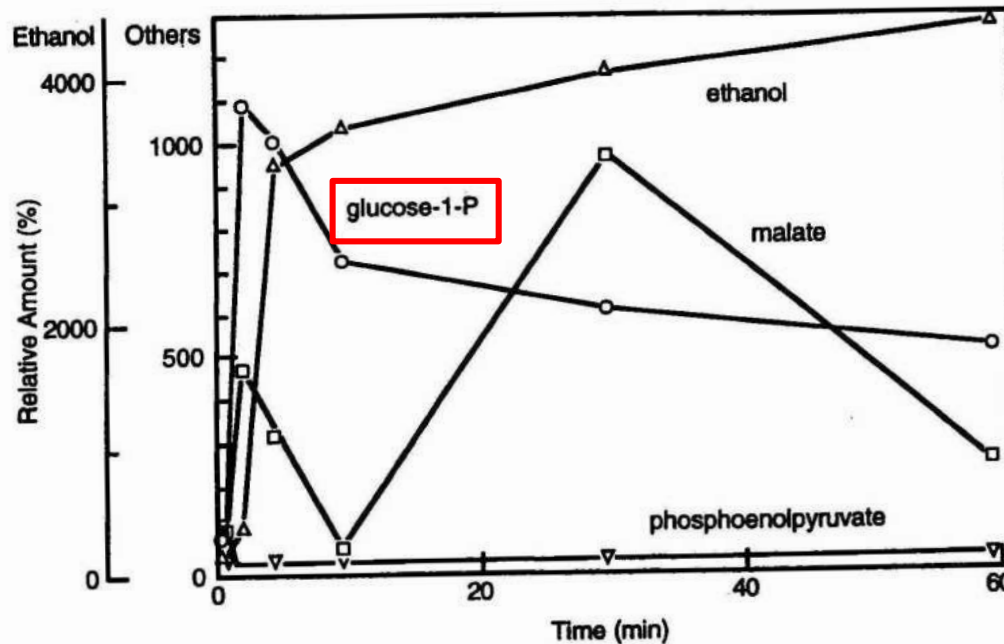


Figure 5. Concentrations of representative metabolites after activation of the ascospores of *Neurospora crassa*, based on those in unactivated, dormant spores equal to 100%. Drawn from the data of Eilers [73].

II. Crescimento esférico

“Esporos do tipo conídio e esporangiósporo iniciam o desenvolvimento vegetativo pelo crescimento em grandes células esféricas”

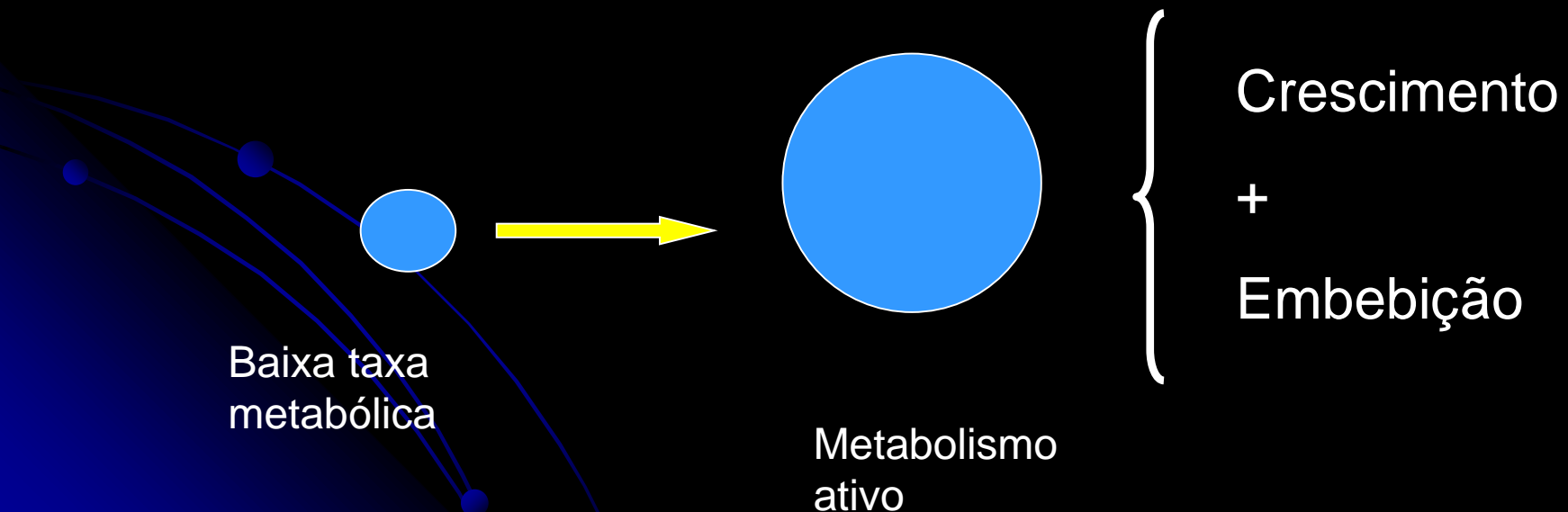


Table 2. Water Contents of Some Fungal Spores [14–16]

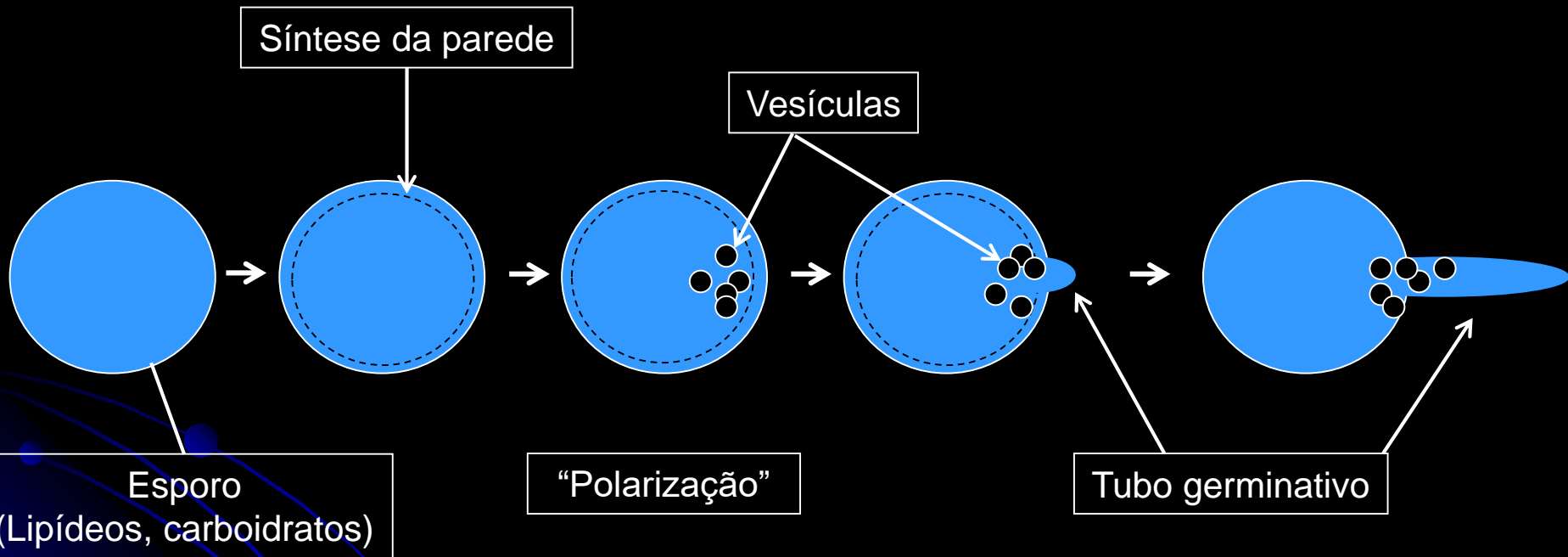
Fungus	Water Content (% Fresh Weight)
<i>Erysiphe polygoni</i>	72
<i>E. graminis</i> grown in room air	31
<i>E. graminis</i> grown in humid air	69
<i>E. cichoracearum</i> grown in room air	35
<i>E. cichoracearum</i> grown in humid air	66
<i>Penicillium expansum</i>	65
<i>P. italicum</i>	65
<i>P. digitatum</i>	6
<i>Aspergillus niger</i>	13
<i>A. niger</i> (different investigator)	55
<i>Alternaria tenuis</i>	86
<i>Botrytis allii</i>	82
<i>B. fabae</i>	88
<i>B. cinerea</i>	17
<i>Monilinia fructicola</i>	25
<i>Neurospora crassa</i>	74
<i>Venturia inaequalis</i>	82
<i>Peronospora destructor</i>	17
<i>Rhizopus nigricans</i>	47
<i>Uromyces phaseoli</i>	12

Dry spores – 6% a
25% water content

Wet spores – 52% a
75% water content

Água no esporo na forma de “água higroscópica” (não atuando como solvente)

III. Emergência do tubo germinativo



"Processo irreversível"

Electron micrographs of thin sections of germinating spores

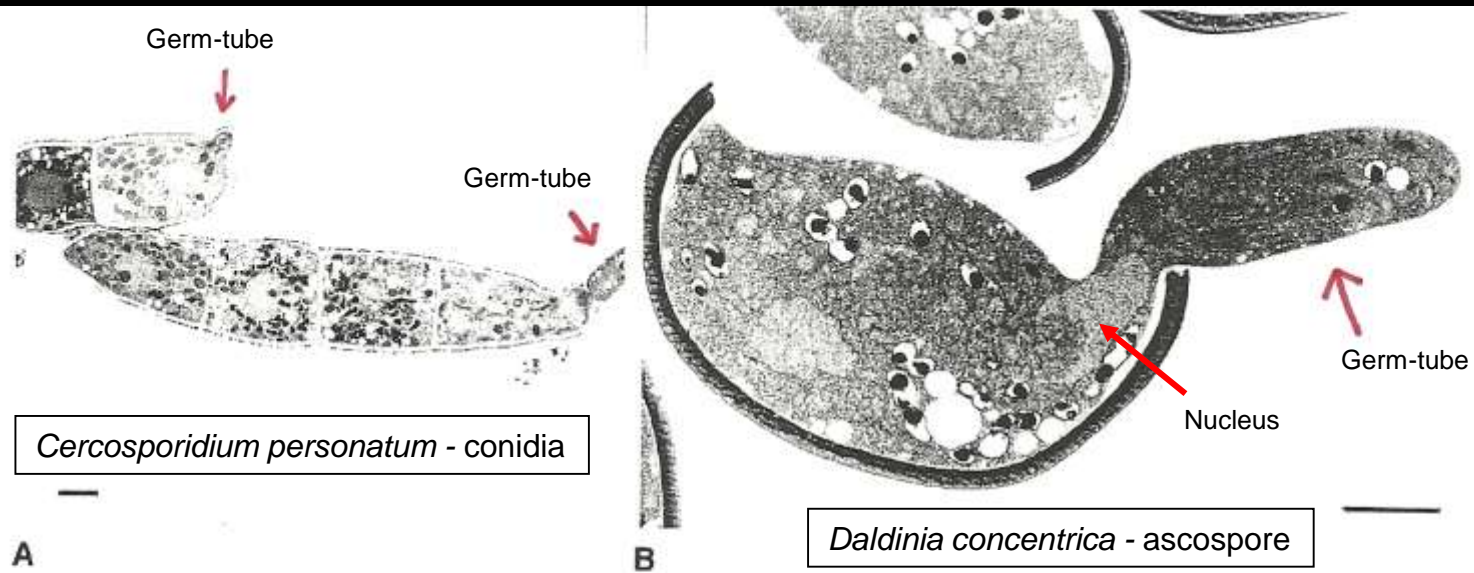


Figure 4.19 Electron micrographs of thin sections of germinating spores. **A.** Germinating multisepate conidia of the mitosporic fungus *Cercosporidium personatum*, with germ-tubes emerging from ends of spores, with their walls continuous with those of the parent spores. **B.** Germinating ascospores of *Daldinia concentrica*, with their thin cell walls continuous with the innermost layer of the complex spore wall. The densely stained thick outer wall has been ruptured during germination. Note the nucleus migrating into the germ-tube. In both species, note the numerous translucent lipid droplets. (A from C. W. Mims; B from A. Beckett.) Scale bars represent 2 μm.

Diagrammatic representation of the organization of wall growth at the hyphal tip

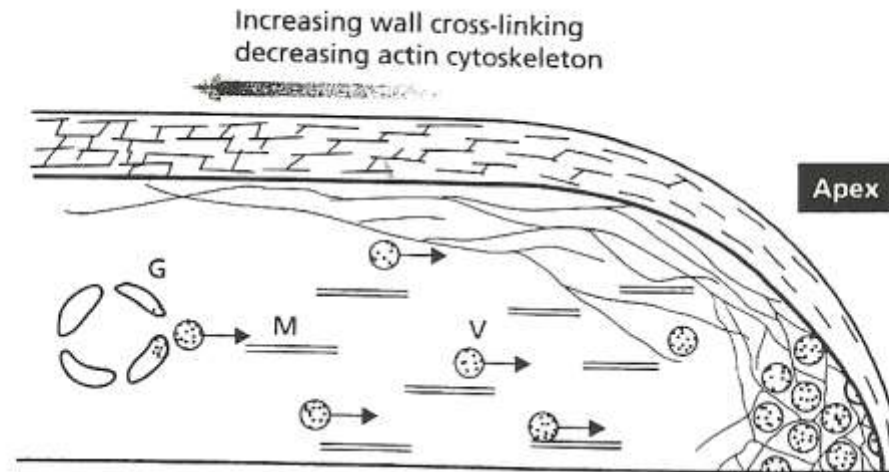


Fig. 3.3 Diagrammatic representation of the organization of wall growth at the hyphal tip. Only half of the tip is shown. Vesicles (V) derived from a Golgi body (G) are transported to the apex, perhaps by microtubule (M) mediated systems. The actin meshwork at the apex is

thought to provide structural support where the wall is thinnest and where there is little or no cross-linking of wall polymers. Behind the extreme tip the wall is progressively rigidified by cross-linking of wall polymers.

Hypothetical representation of unit wall synthesis in hyphal tip growth

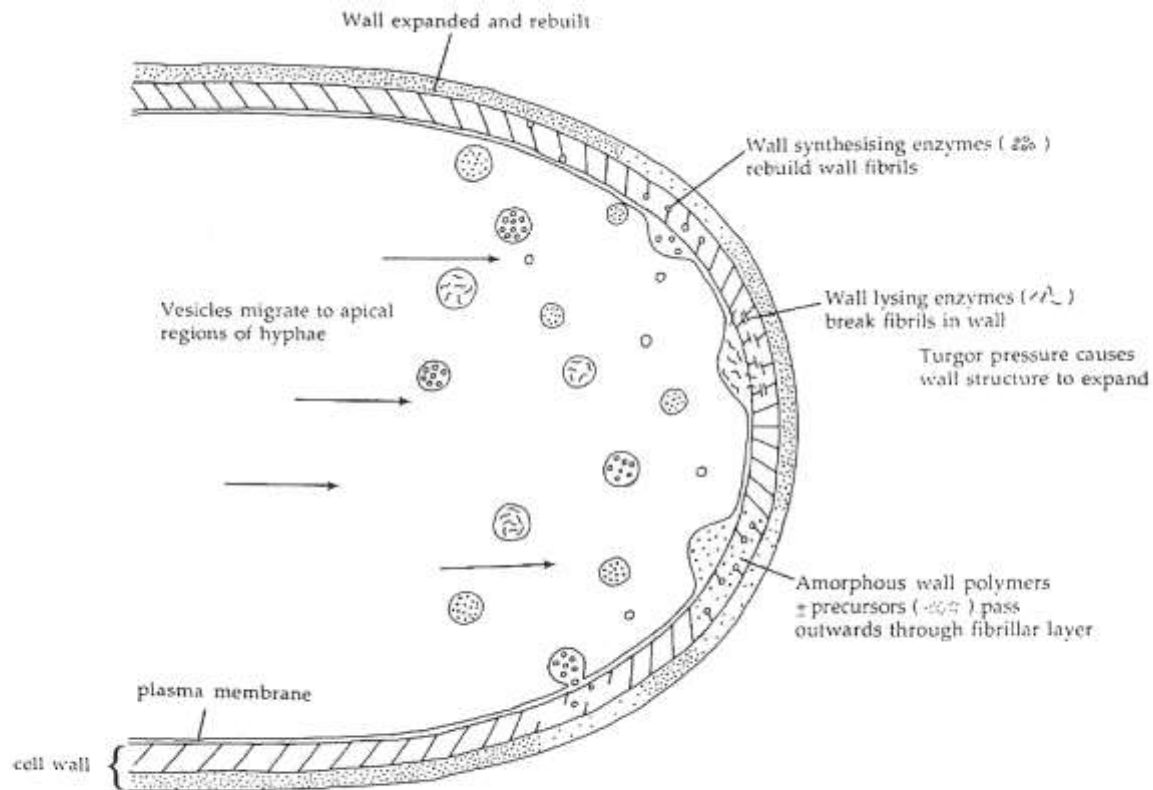
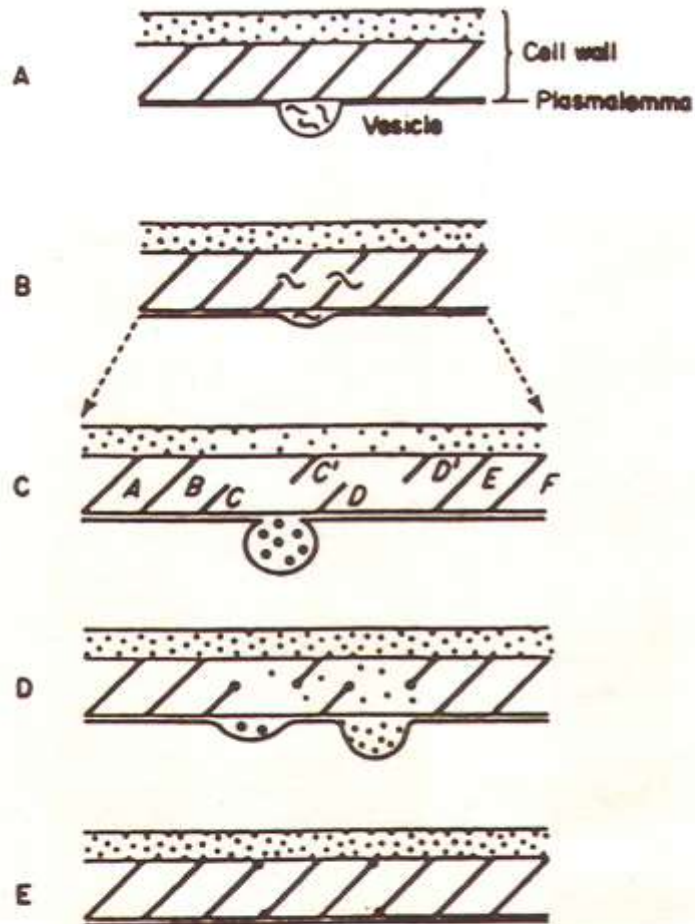


Fig. 1.6 Hypothetical representation of unit wall synthesis in hyphal tip growth. Vesicles transport wall polymers, precursor compounds and lytic enzymes to the apical dome where wall synthesis occurs (adapted from Bartnicki-Garcia, 1973).



∴∴ Amorphous component ≈ Wall-lysing enzymes
 // Microfibrils •• Wall-synthesizing enzymes

Fig. 1.8 Hypothetical representation of the events leading up to a unit of cell wall growth. (After Bartnicki-Garcia, 1973)

Vídeo

Crescimento hifa

<https://www.youtube.com/watch?v=i9T727tz7FA>

Botrytis cinerea – germinação do esporo

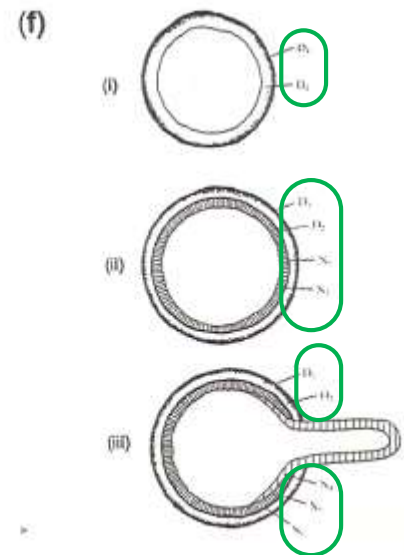
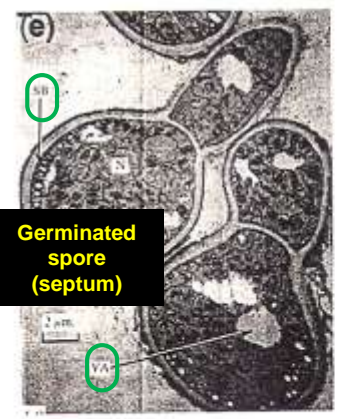
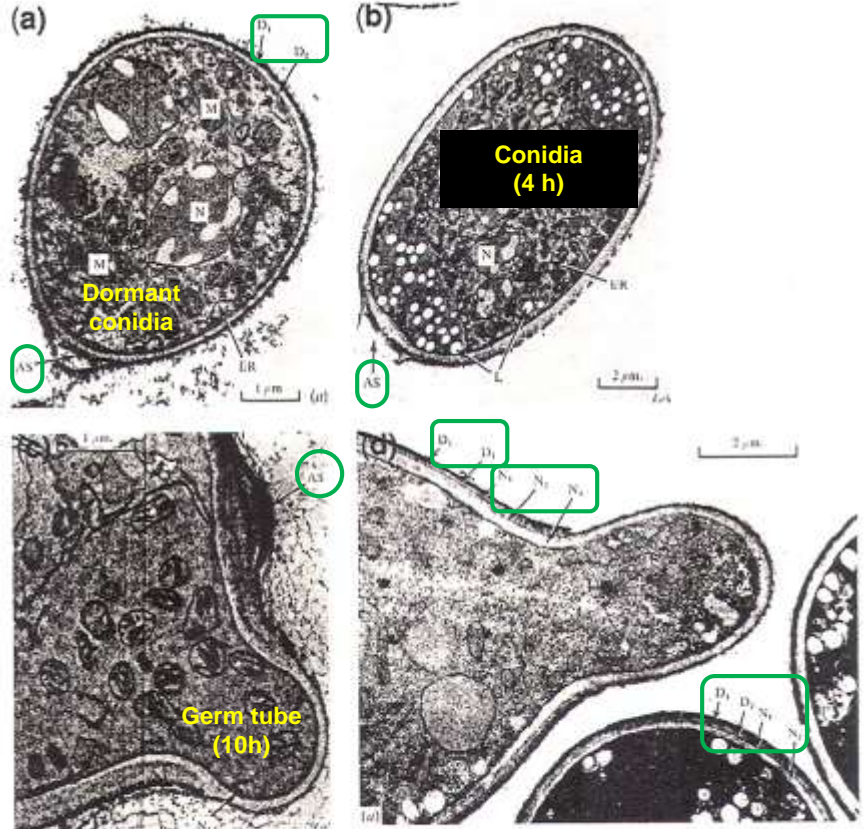


Figure 13.3: Electron micrographs, together with a diagram (all by kind permission of K. Gull and A.P.J. Trinci), showing the course of spore germination in *Botrytis cinerea*. (a) Dormant conidium showing two-layered spore wall. (b) Conidium after 4 h incubation in nutrient medium; a new layer is seen near the abscission scar. (c) Near median section of an incipient germ tube after 10 h in the nutrient medium showing new wall layers. (d) Newly emerged germ tube showing new wall layers, which can also be seen in the other spores. (e) Two fully germinated spores showing the septum at the base of the germ tube and the vacuolation of the spore and germ tube. (f) Schematic representation of the changes in wall structure during germination: (i) dormant conidium; (ii) swollen conidium 4–6 h after inoculation; (iii) conidium with germ tube approximately 10 h after inoculation. (a), (b), (c) and (e) KMnO₄-fixation; (d) glutaraldehyde-fixation. AS, abscission scar; D1, D2, layers of the spore wall; N1, N2, N3, new wall formed during germination; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; SB, storage body; VA, vacuole.

Hidrofobinas

- Proteínas secretadas pelos fungos (1996) – produto de genes altamente expressos durante a diferenciação

- Possuem em torno de 100 a.a. (sendo 8 resíduos cisteína – padrão permite dobras para gerar “hydrophobic domains”)

- Solúveis em água

- Auxiliam na adesão da hifa

- Hifas aéreas – contrapor a tensão superficial

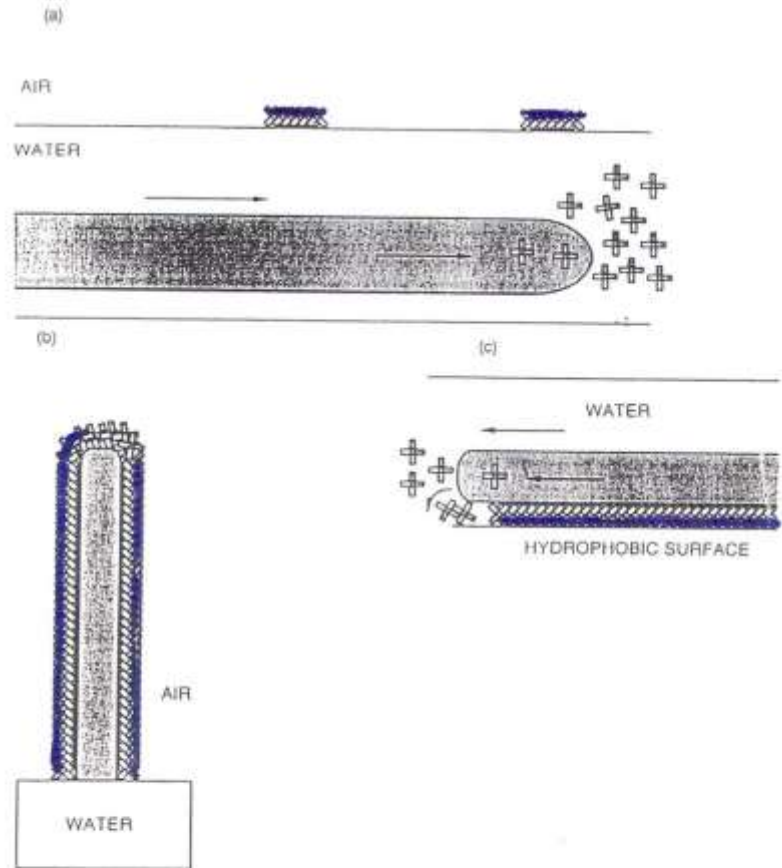
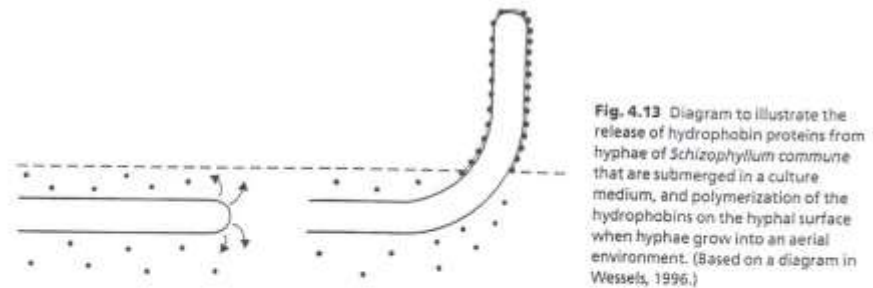
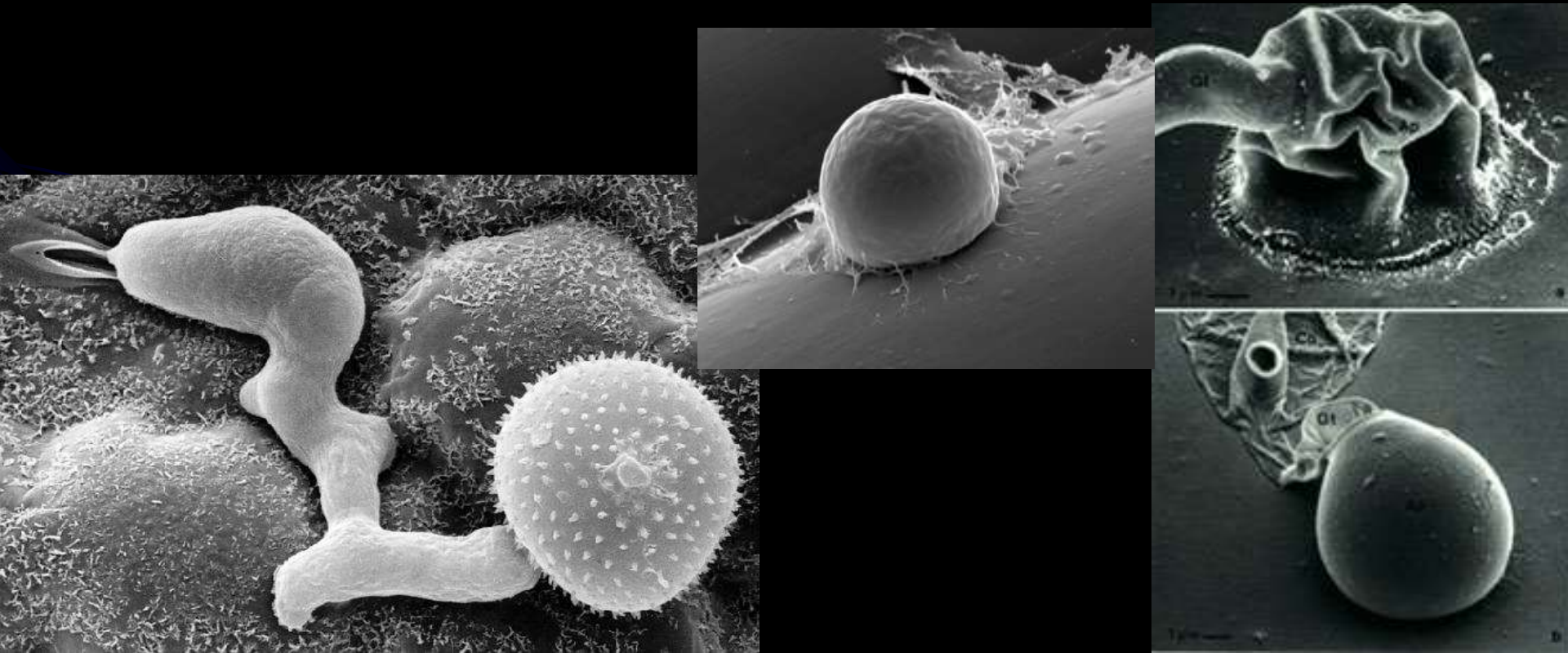


Figure 1.3: Schematic representation of the hydrophobin self-assembly process. Hydrophobin monomers are shown with four external loops represented as the arms of a cross. The proteins fold such that one side is predominantly hydrophobic (shaded) and the other hydrophilic (white). The amphipathic monomers are secreted into the surrounding medium at the apex when it is submerged. (a) Self-assembly occurs when the fungus reaches an air-water interface (b) or grows on a hydrophobic surface. (c) Reproduced from ref. 1 with permission from Academic Press.

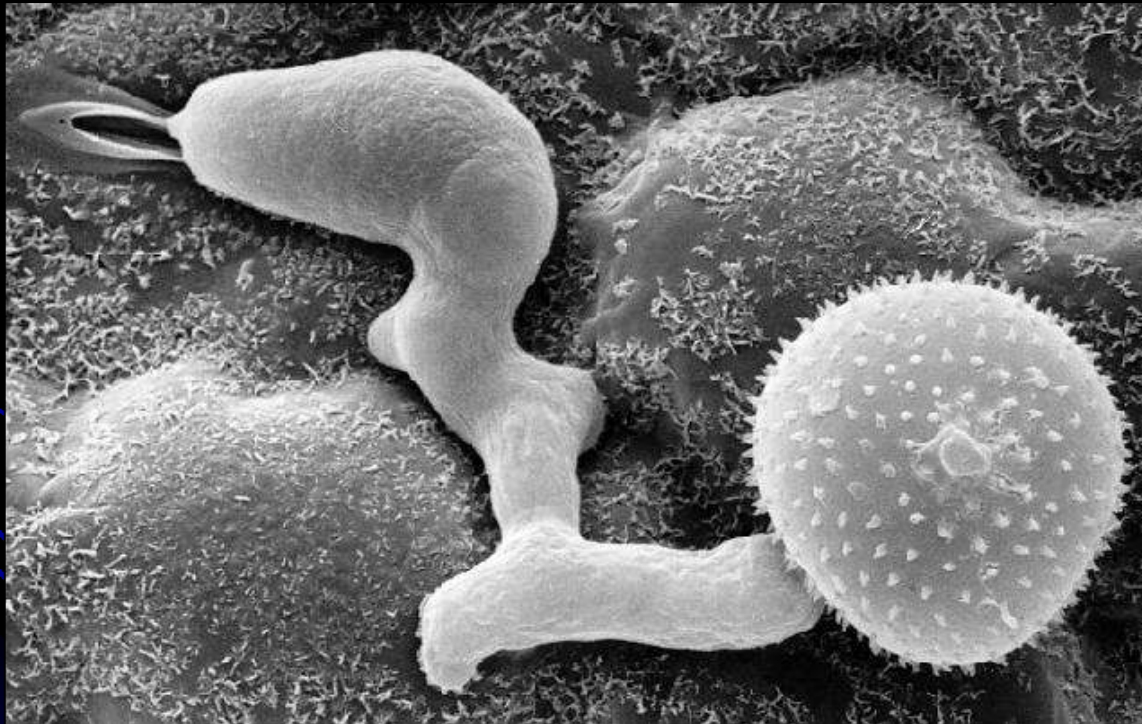
Formação do apressório

“Apressório – extremidade globosa (intumescida) de uma hifa ou tubo germinativo, a qual facilita a adesão e a penetração do hospedeiro pelo fungo”



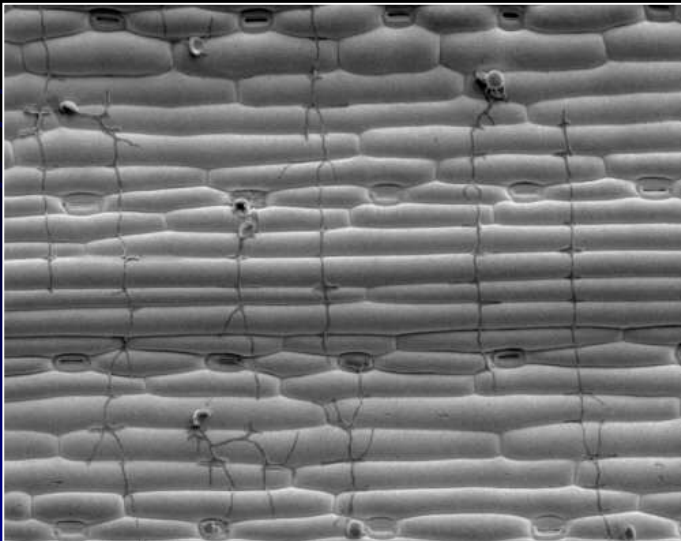
Formação de apressório

Quimiodiferenciação – diferenciação da estrutura mediada por compostos químicos.



Tigmodiferenciação

Diferenciação da estrutura mediada pela topografia do substrato



Puccinia hordei em cevada

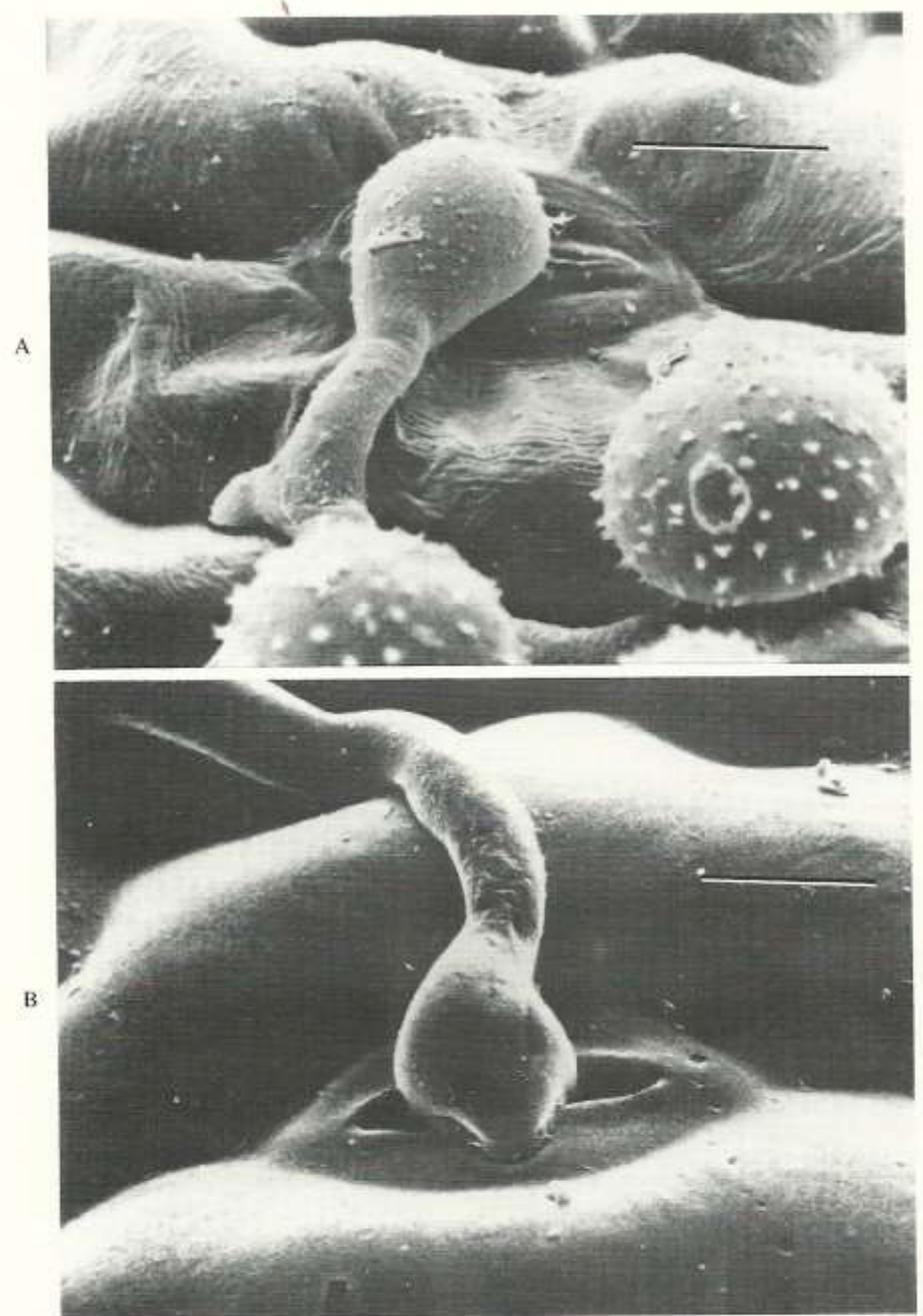


Fig. 1-19. Scanning electron micrographs of appressoria produced by *Uromyces phaseoli* over (top) closed stoma of pinto bean leaf lower surface and (bottom) open stoma on a replica of a pinto bean leaf lower surface (after Wynn, 317). Bar = 10 μ m.

Formação de apressório em Magnaporthe grisea

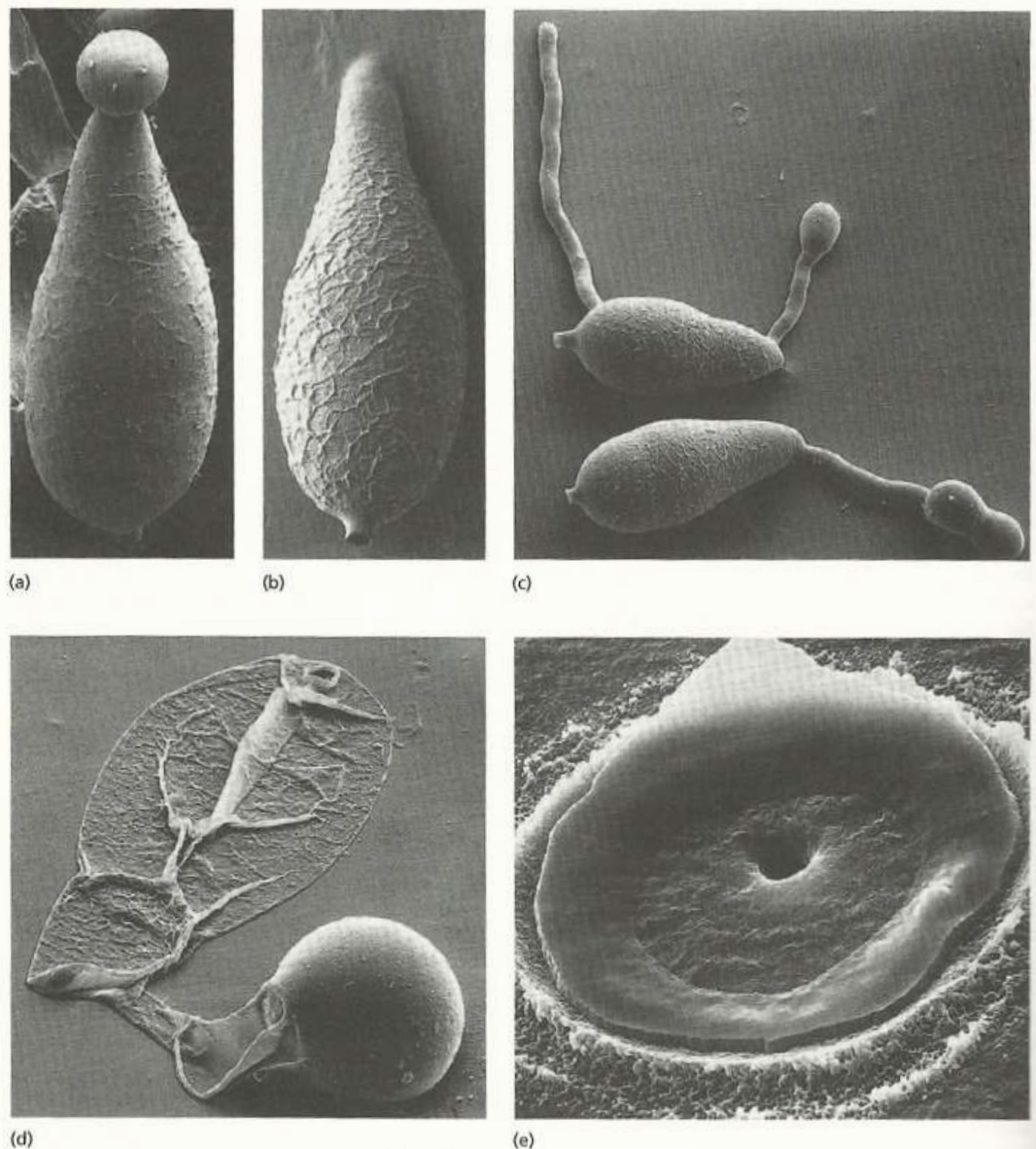


Fig. 6.3 Early infection stages of the rice blast fungus, *Magnaporthe grisea*. (a) Conidium with apical droplet of spore tip mucilage ($\times 2900$). (b) Conidium attached to substrate by adhesion of spore tip mucilage ($\times 2900$). (c) Germination of conidia and early stages of appressorium development, seen as swelling of germ tube apex (centre and lower right) ($\times 1100$). (d) Mature, globose, turgid appressorium attached to collapsed germ tube and conidium. A septum separates the appressorium from the

germ tube ($\times 3000$). (e) Remnants of appressorium attached to a polyethylene surface. The upper part of the cell has been removed by sonication. What remains is the appressorial pore, with the dent made by mechanical force of the penetrating peg clearly visible, and part of the smooth surrounding wall, composed of melanin. Note the halo of extracellular matrix material around the attachment site ($\times 15\,500$). (a, d & e, From Braun & Howard 1994; b & c, from Howard 1994.)

Formação de apressório em *Magnaporthe grisea*

X

Aumento da pressão intracelular

A parede celular é melanizada e rígida

Lipídeos / carboidratos metabolizados no esporo para produzir 3,2 M de glicerol no interior do apressório

Alta pressão leva a entrada de água (pressão hidrostática de 8 MPa = 40 x a pressão de um pneu de carro)

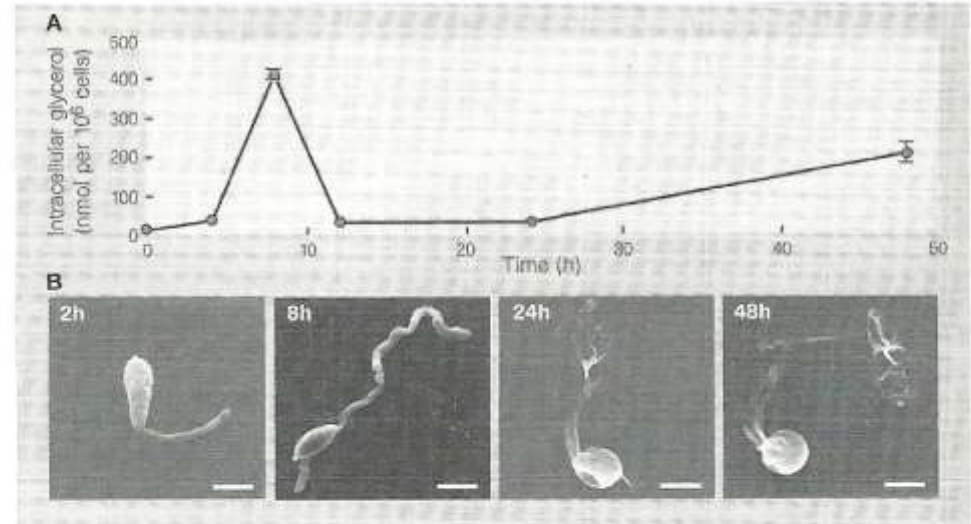


Figure 7.6 The formation of appressoria in *Magnaporthe grisea* accompanied by an increase in intracellular pressure. The internal pressure that drives infection hyphae of the rice blast fungus *M. grisea* through plant cell cuticles results from high osmotic pressure, generated metabolically in appressoria with strong walls. In this experiment conidia were allowed to germinate on a hydrophobic surface in water drops. During the first 8–10 h following germination glycerol was synthesized rapidly. From 10–20 h the glycerol was metabolized, decreasing the internal osmotic potential, and the conidium and germ-tubes collapsed. From 25 h appressoria began to form, separated from the hyphae by septa. Their walls became melanized and impermeable to glycerol, and glycerol level again rose. At 48 h the intracellular glycerol concentration, and the resulting pressure, were at their highest, because the glycerol was contained in a smaller volume within the appressoria, the rest of the hyphae having collapsed. Scale bars, 20 μ m. (Reproduced with permission from de Jong, J. C., McCormack, B. J., Smirnoff, N. & Talbot, N. J. (1997) *Nature* 389, 244–245, MacMillan Magazines Ltd.)

Vídeo

Germinação e formação de apressório – *Magnaporthe grisea*

<https://www.youtube.com/watch?v=v7da6bC-euQ>

PENETRAÇÃO

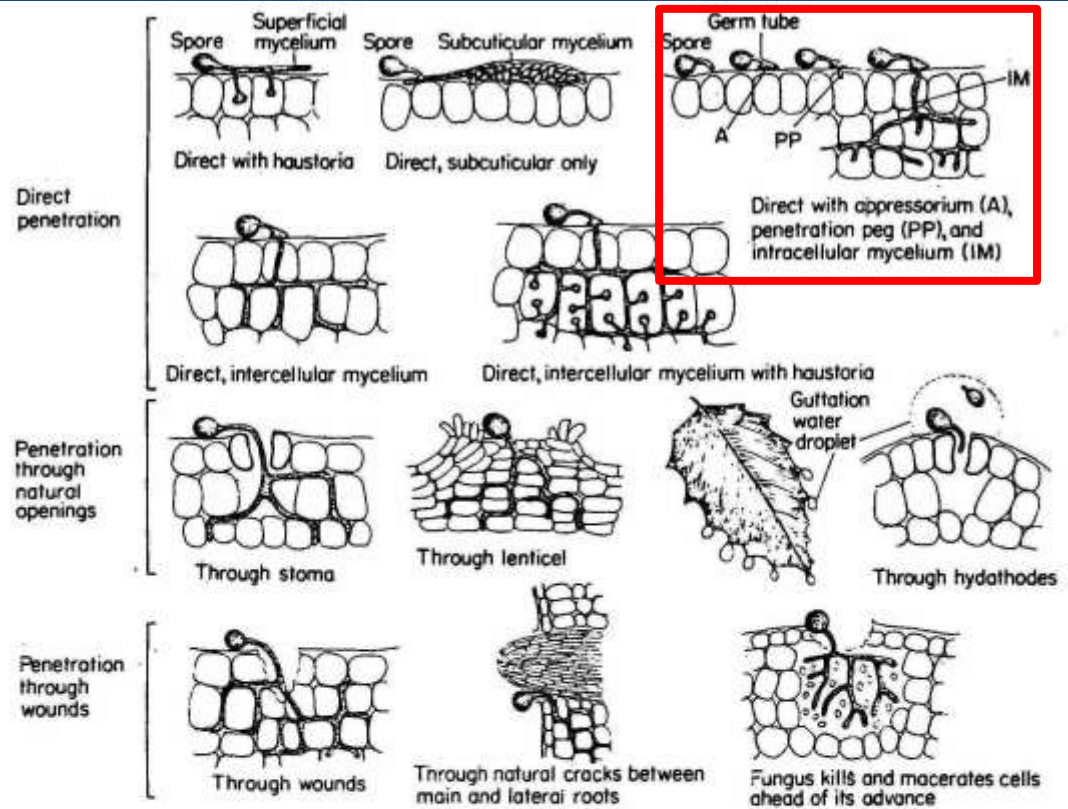


FIGURE 2-4 Methods of penetration and invasion by fungi.

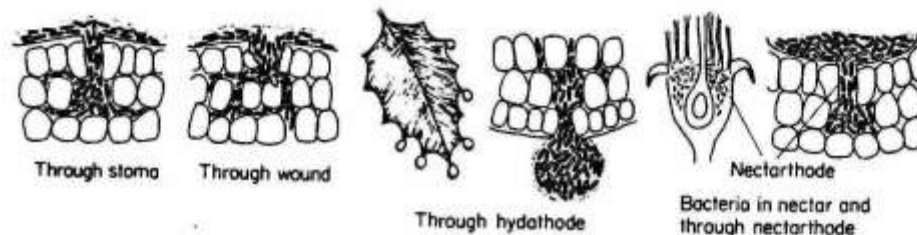


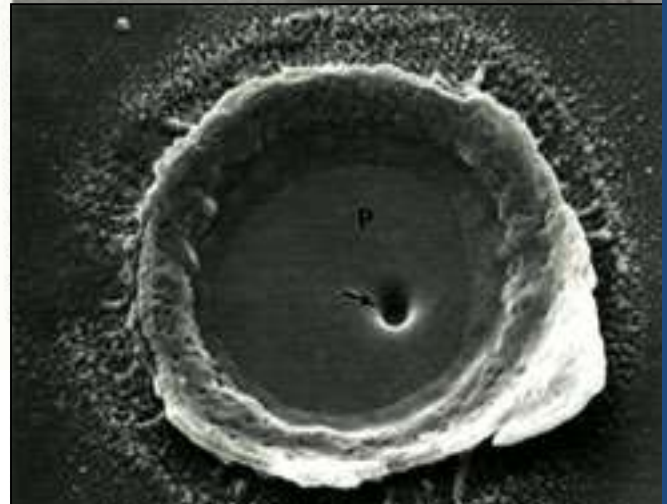
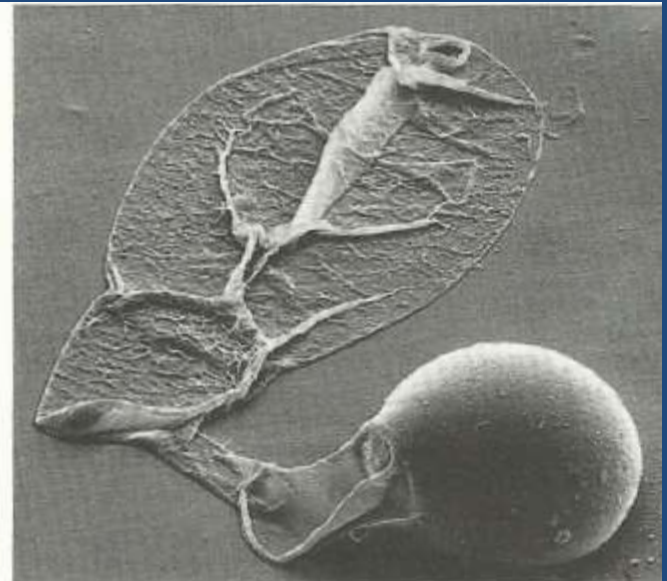
FIGURE 2-5 Methods of penetration and invasion by bacteria.

PENETRAÇÃO

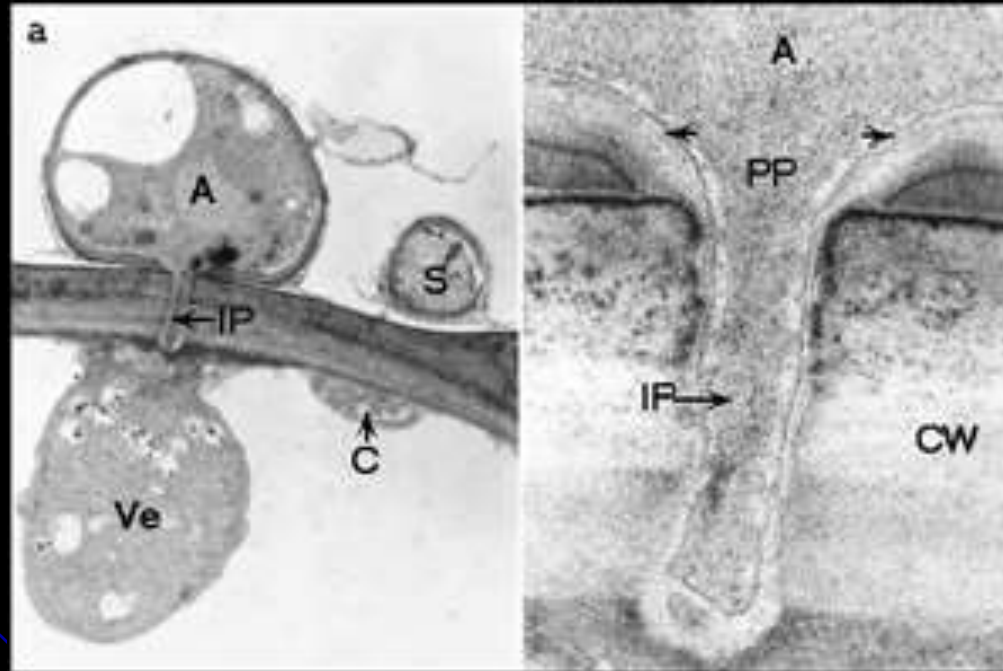
➤ Força mecânica

➤ Processo químico (enzimático)

Figure 13.1 Scanning electron microscopy of the germination and development of a conidium (spore) of the fungal rice pathogen *Magnaporthe grisea* on an artificial membrane of Mylar. (a) The conidium (C) has germinated to give rise to a germ tube (GT) and an appressorium (A). The conidium and the germ tube have collapsed. The appressorium, a specialized penetration structure, is highly turgid, single-celled and is completely separated from the germ tube by a septum (R.J. Howard and N.D. Read, unpublished micrograph). (b) The appressorium has been ruptured by sonication near the plane of attachment to the Mylar membrane. The upper and lower portions of the appressorium have separated revealing the underlying surface. Driven by an extremely high turgor pressure, the penetration peg, which arose from the appressorium, has created a hole (arrow) in the Mylar substratum. (Reprinted with permission from Howard *et al.*, 1991.)



Peg de penetração



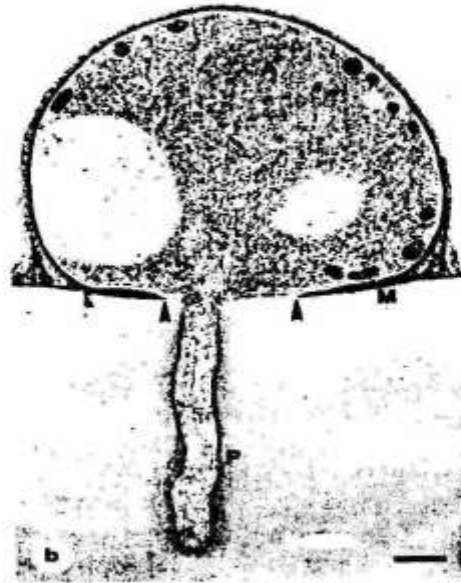
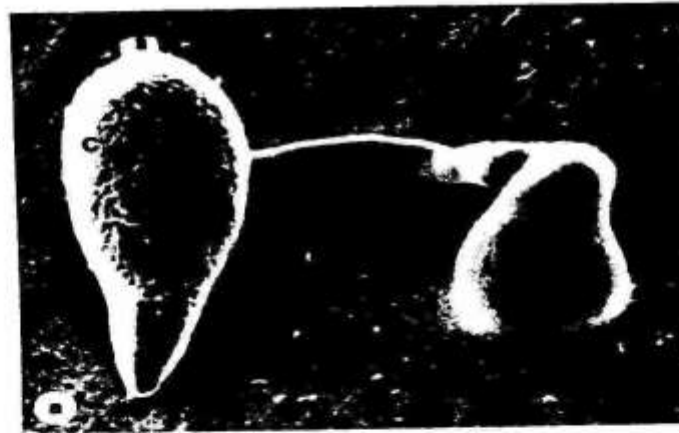


Fig. 3.3 Electron micrographs of the ascomycete *Magnaporthe grisea* which causes rice blast disease. (a) Scanning electron micrograph showing a mature appressorium (A) and conidium (C). Bar represents 5 μm . (b) Transmission electron micrograph (*in vitro*) showing penetration peg (P) extending into cellophane membrane substrate. Appressorium (A) closely adhered to the substrate by spore tip mucilage (M). Penetration peg has arisen from new inner wall layers overlying the appressorium pore (arrowed); probing with wheat germ agglutinin-colloidal gold showed the chitinous wall was not uniformly labelled. Bar represents 1 μm . Reproduced from Howard *et al.* (1990), courtesy of Prof. R. J. Howard and Springer-Verlag.

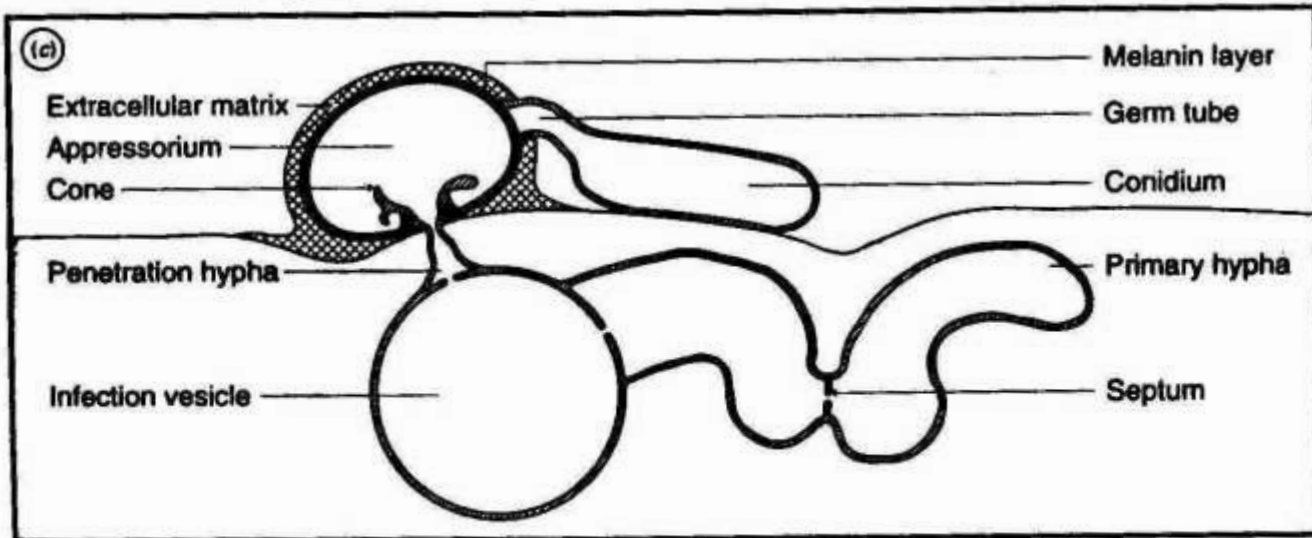
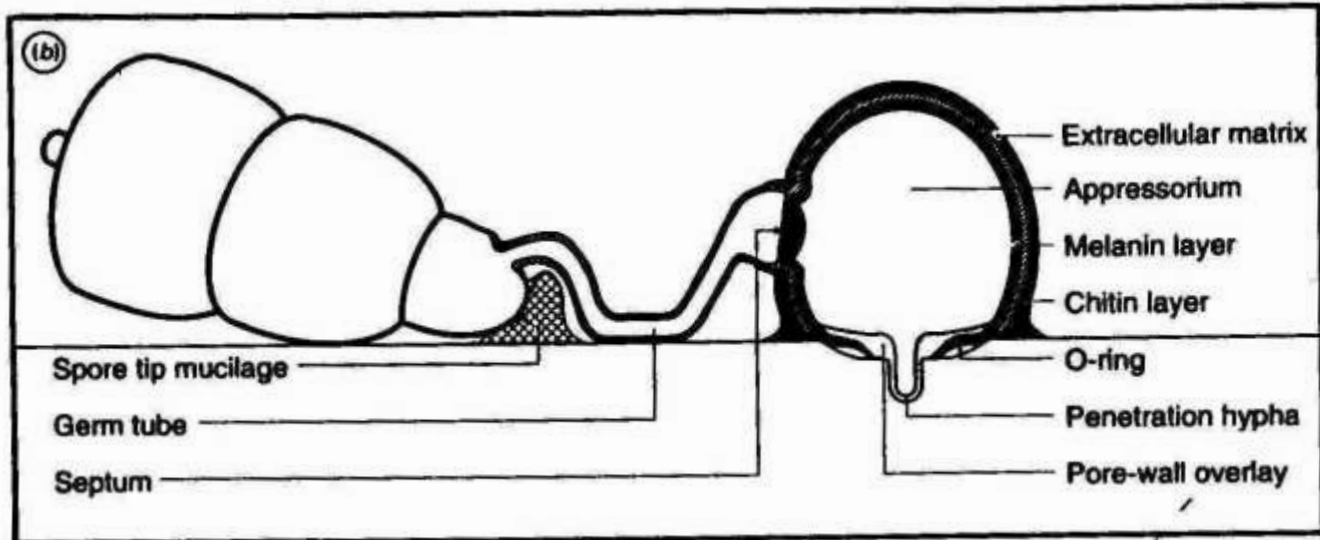
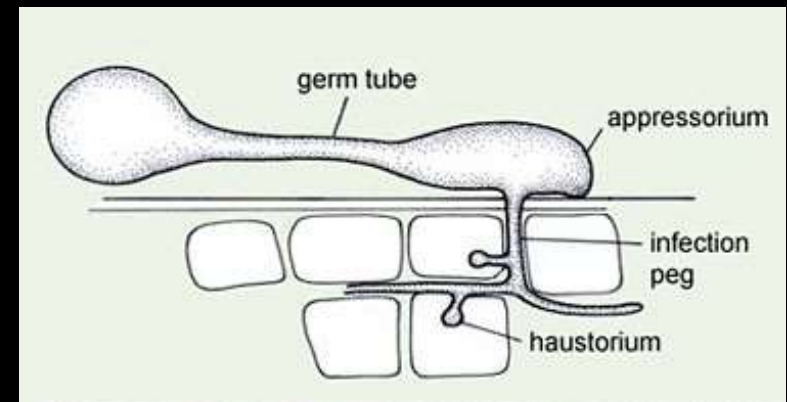
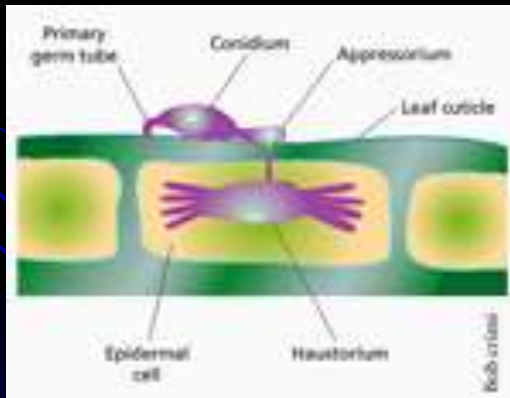


Figure 1. (a) Infection structures of *Uromyces viciae-fabae*. (b) Infection structures of *Magnaporthe grisea* (courtesy Dr R. J. Howard). (c) Infection structures of *Colletotrichum lindemuthianum* (courtesy Dr R. J. O'Connell). (The drawings are not to scale).

Haustório

“Estrutura ramificada ou não, especializada na absorção de nutrientes a partir do citoplasma da célula do hospedeiro”



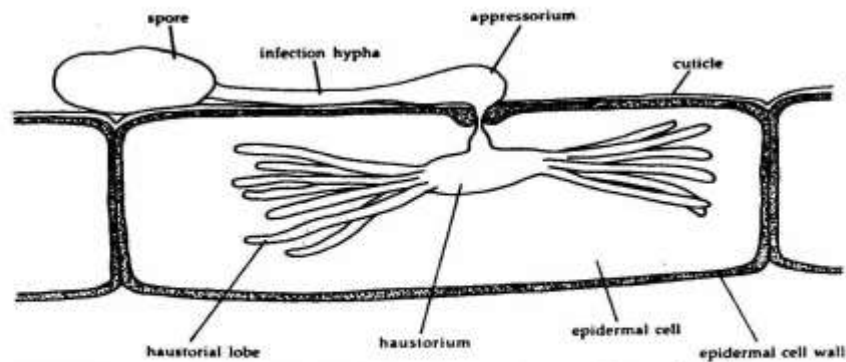


Fig. 3.10 Diagram to show *Erysiphe* haustorium in an epidermal cell.

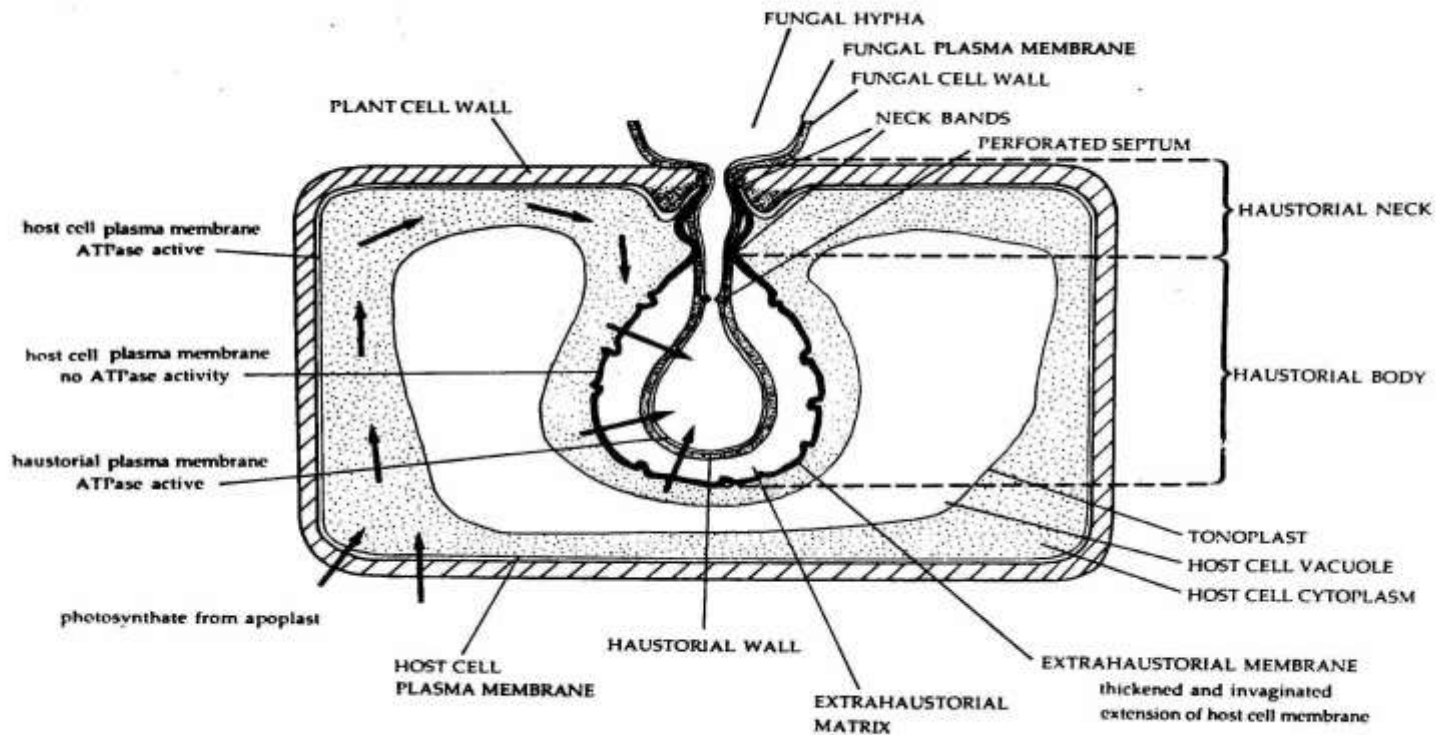


Fig. 3.13 Diagram of *Erysiphe* haustorium in an epidermal cell to show the major structural features and to indicate the probable route of solute transport into the fungal hypha (adapted from an unpublished drawing by Dr G. N. Greenhalgh, with permission).

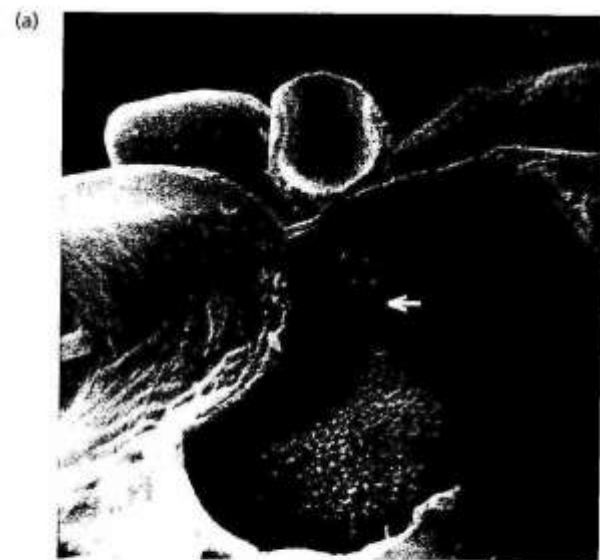
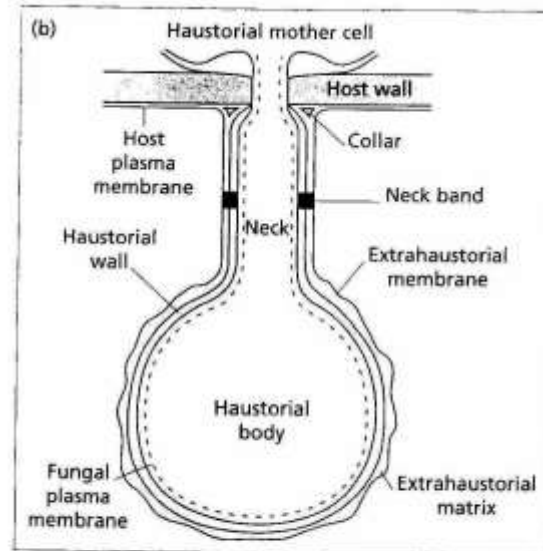


Fig. 6.8 The structure of haustoria. (a) Scanning electron micrograph of coffee leaf tissue infected by rust, *Hemileia vastatrix*. The tissue has been frozen and fractured to reveal a haustorium (H) within a mesophyll cell. Note a slight swelling (arrow) in the haustorial neck at the position of the



neck band, and the haustorial mother cell (HMC) external to the penetrated host cell ($\times 5000$). (Courtesy of Rosemarie Honneger.) (b) Diagrammatic interpretation of haustorial structure, showing the main interfacial components.

Haustório

