Resistência é regra

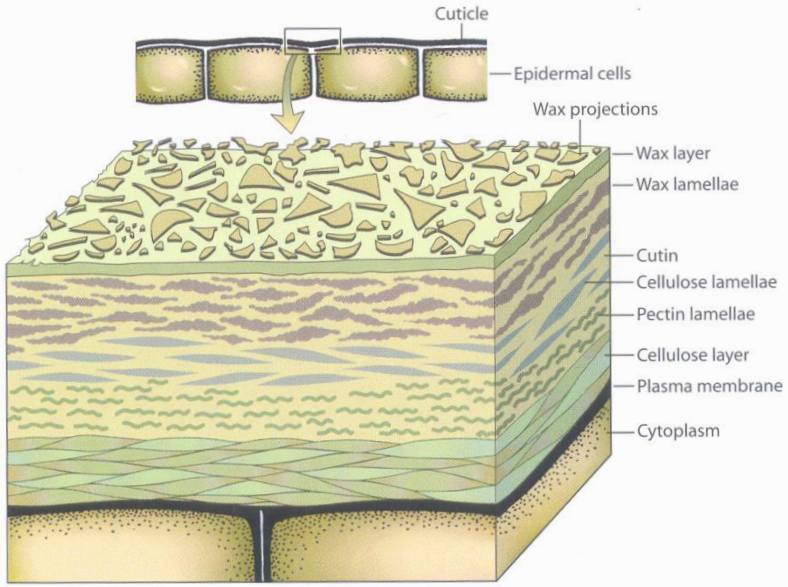
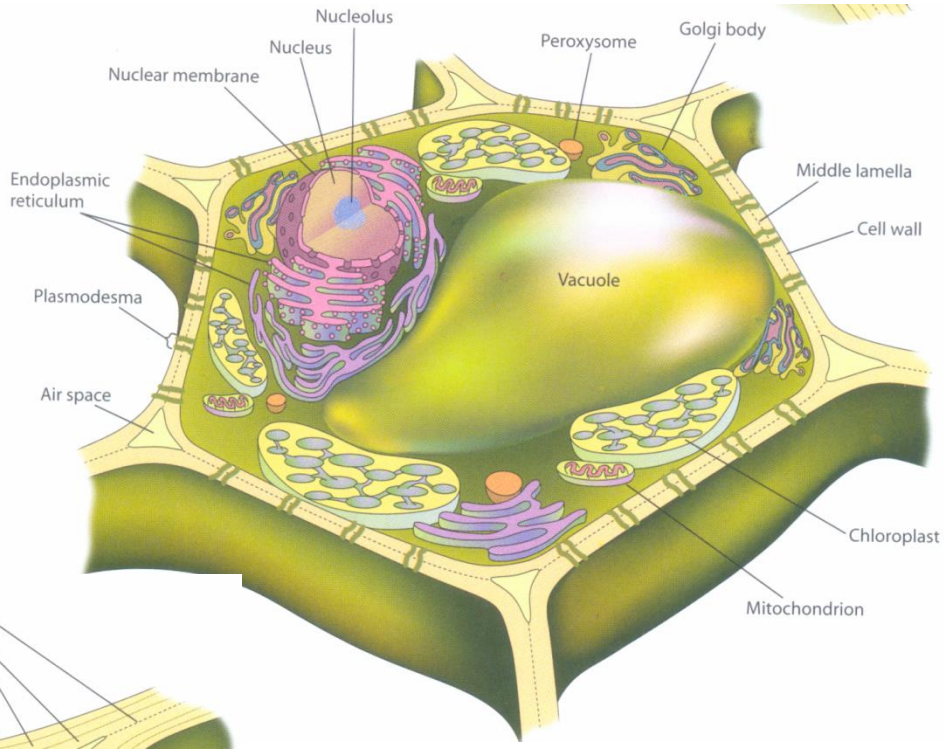
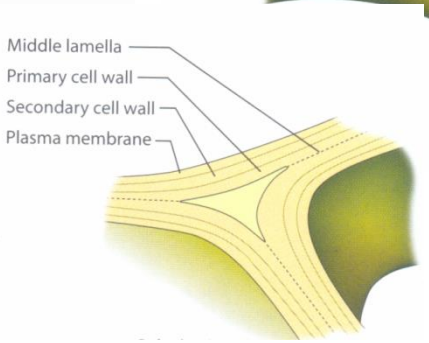


FIGURE 5-3 Schematic representation of the structure and composition of the cuticle and cell wall of foliar epidermal cells. [Adapted from Goodman *et al.* (1967).]





Imagens de Agrios (2005)

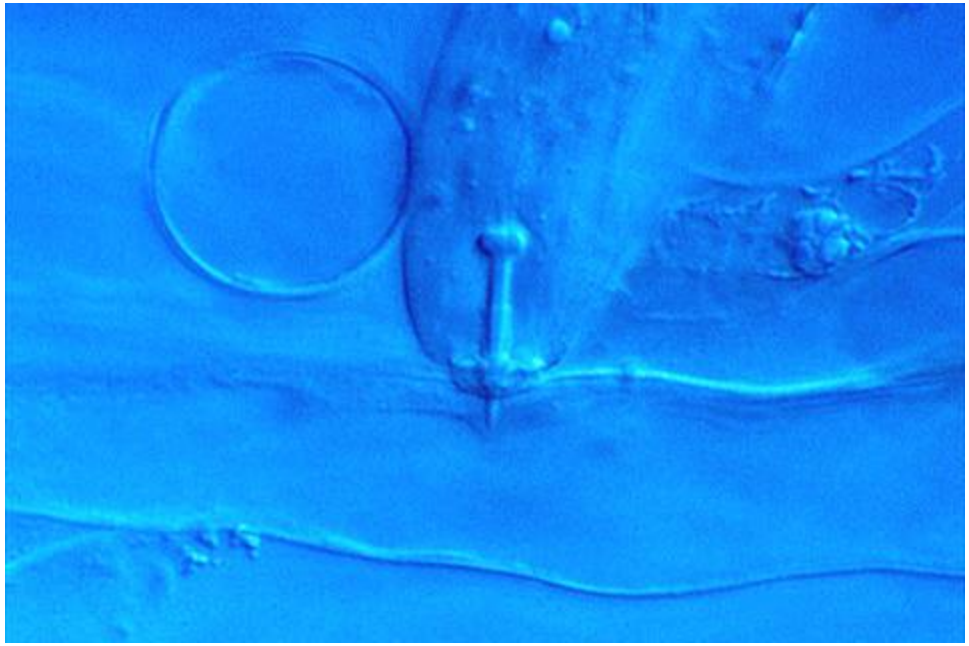
Como patógenos atacam plantas?

Forças **mecânicas**
bioquímicas

- Enzimas
  - cutícula e parede
- Toxinas
  - não-específicas
  - específicas
- Reguladores de crescimento
- Polissacarídeos
  - Doenças vasculares
- Outros

Como patógenos atacam plantas?

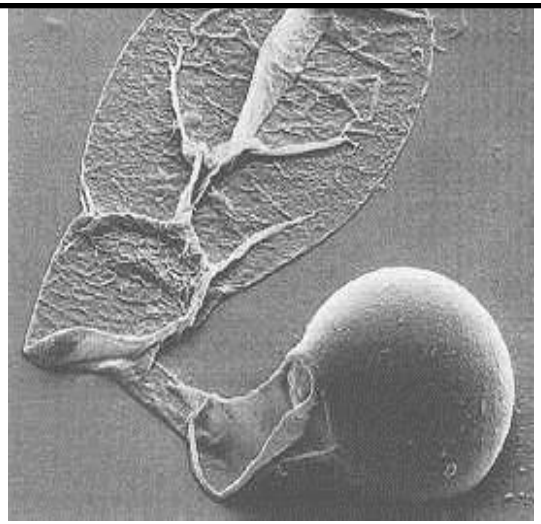
Forças mecânicas



Estilete de um nematoide penetrando a raiz do hospedeiro.

(Courtesy U. Zunke, NemaPix) Davis & MacGuidwin (2005) Lesion nematode disease http://www.apsnet.org/edcenter/intropp/lessons/Nematodes/Pages/LesionNematode.aspx

Apressório de *Magnaporthe grisea* preso ao conídio em colapso



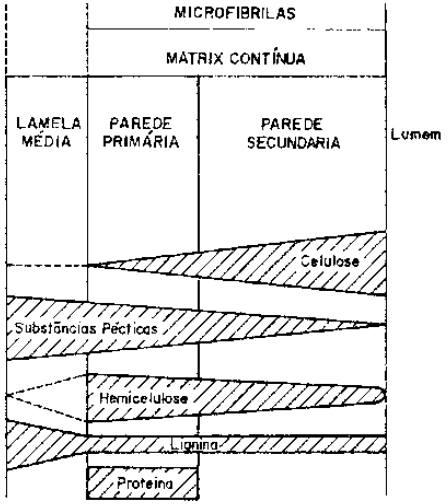
Efeito do peg de penetração em superfície de polietileno, após retirada do apressório

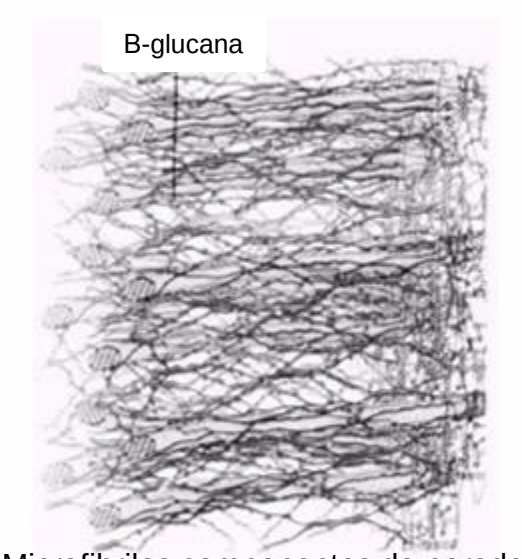
Como patógenos atacam plantas?

Forças bioquímicas

- Enzimas
  - cutícula e parede
- Toxinas
  - não-específicas
  - específicas
- Reguladores de crescimento
- Polissacarídeos
  - Doenças vasculares
- Outros

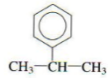
Enzimas extracelulares





Microfibrilas componentes da parede celular de gramíneas

Cutinase	Cutina poliéster de ácido graxo
Enzimas Pécticas	polissacarídeo ác. galacturônico + ramnose
pectina esterases	Pectina
poligalacturonases	Pectato
pectato liases	Pectato
Hemicelulases	polímeros de xilose, arabinose, galactose...
xilanase	Xilana
arabanase	Arabana
Celulase	Celulose polissacarídeo de glicose
Ligninase	Lignina polímero de fenilpropano
Protease	Proteína
Fosfolipase	Fosfolipídeos
Amilase	Amido polissacarídeo de glicose



Cutina - cutinases

Fungos e *Streptomyces scabies*

Cutina

Poliester insolúvel (polímero lipídico)

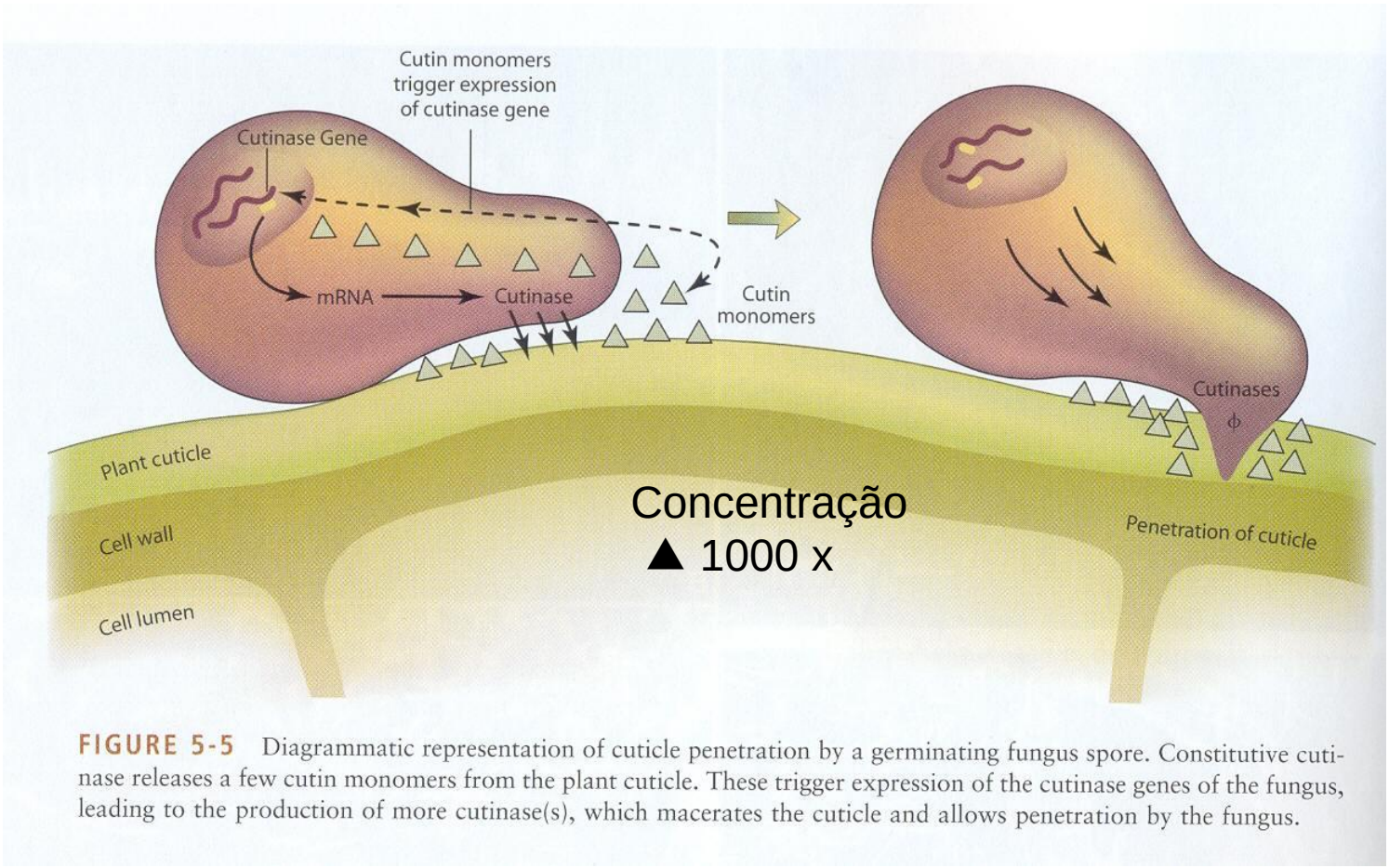
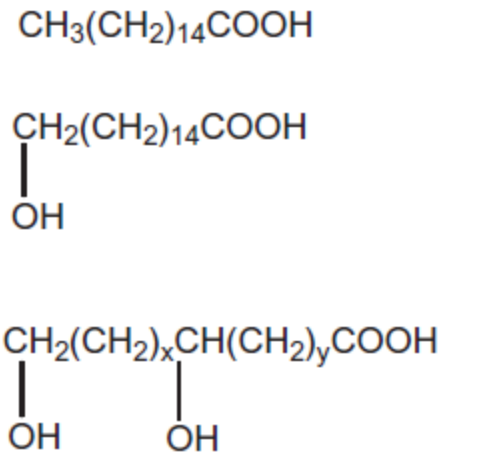


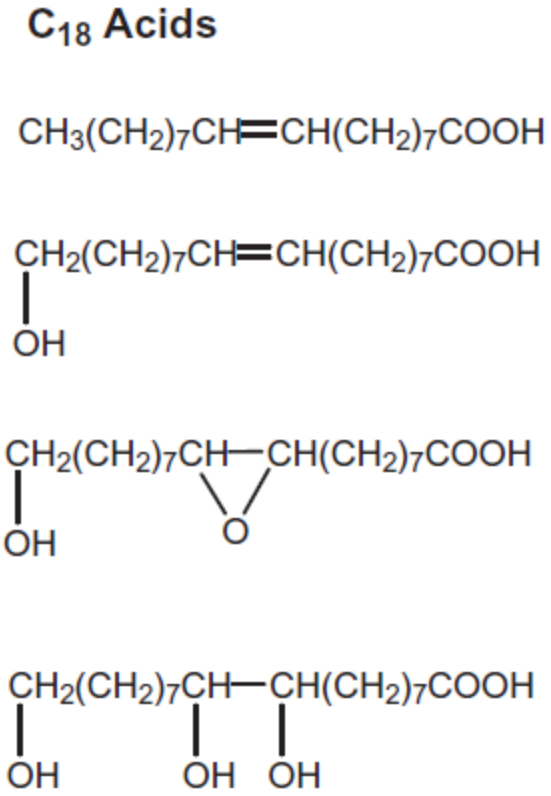
FIGURE 5-5 Diagrammatic representation of cuticle penetration by a germinating fungus spore. Constitutive cutinase releases a few cutin monomers from the plant cuticle. These trigger expression of the cutinase genes of the fungus, leading to the production of more cutinase(s), which macerates the cuticle and allows penetration by the fungus.

Ácidos graxos - monômeros cutínicos

C_{16} Acids



C_{18} Acids



C_{16} origina-se do ácido palmítico enquanto que C_{18} origina-se do ácido oléico ou linoléico

Cutina - cutinases

Fusarium solani f. sp. *pisi* - ervilha

Isolado T-8 com múltiplos genes produtores de cutinase

Isolado T-30 com baixa produção de cutinase

Mutante PNB-1 com 1 gene que produz 10 to 20% da cutinase produzida por T-8

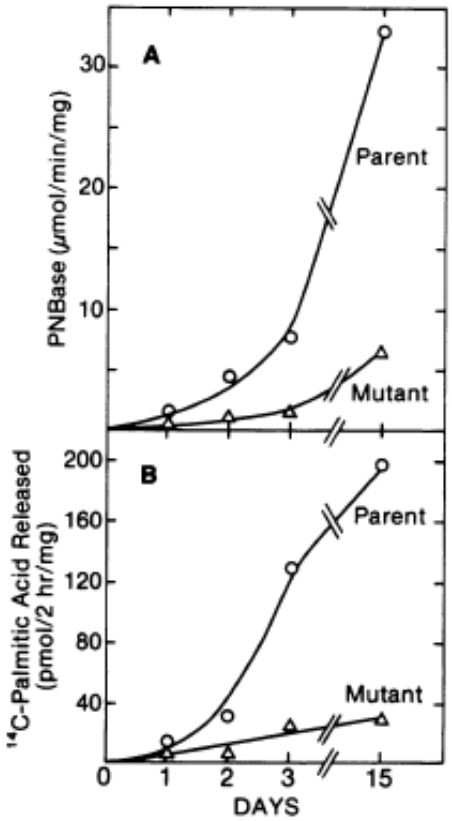


FIG. 3. Comparison of specific activity of cutinase produced by parent and mutant strains grown on cutin-containing medium. The parental strain T-8 and the mutant strain PNB-1 were grown on medium containing 200 mg of cutin. Enzyme activity was measured by PNBase (A) or by the hydrolysis of radioactively labeled natural substrate, [^{14}C]cutin (B).

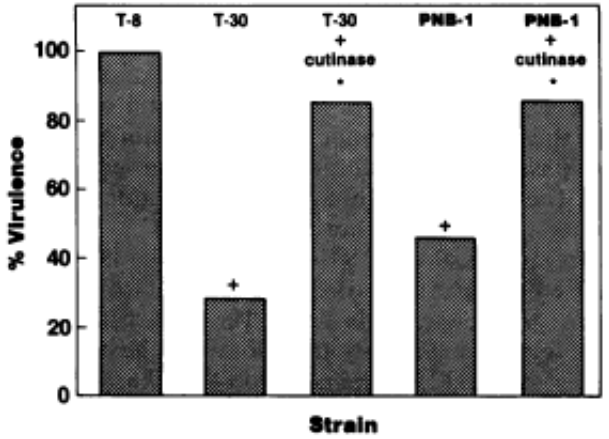


FIG. 7. Effect of cutinase addition on the virulence of the strains. The pea stem bioassay was used to evalute the virulence of the strains (see Materials and Methods); where indicated, purified cutinase was added to inoculum at a final concentration of 1 mg/ml. The data were analyzed by a chi-square test. The symbol + indicates that the data were significantly different from the T-8 strain ($P < 0.05$). The symbol * indicates that the data were not significantly different from the T-8 strain ($P > 0.5$).

Dantzig et al., 1986

Cutina - cutinases

Fusarium solani f. sp. *pisi* - ervilha

Isolado T-8 com múltiplos genes para cutinase

isolado 77-2-3 com 1 gene - produz 10 to 20% da cutinase de T-8

Mutante 77-102 do isolado 77-2-3 não produz cutinase

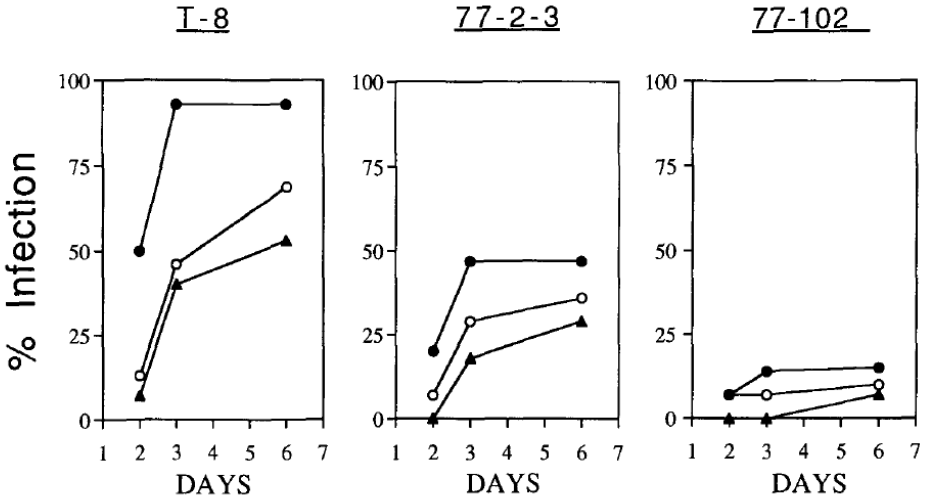
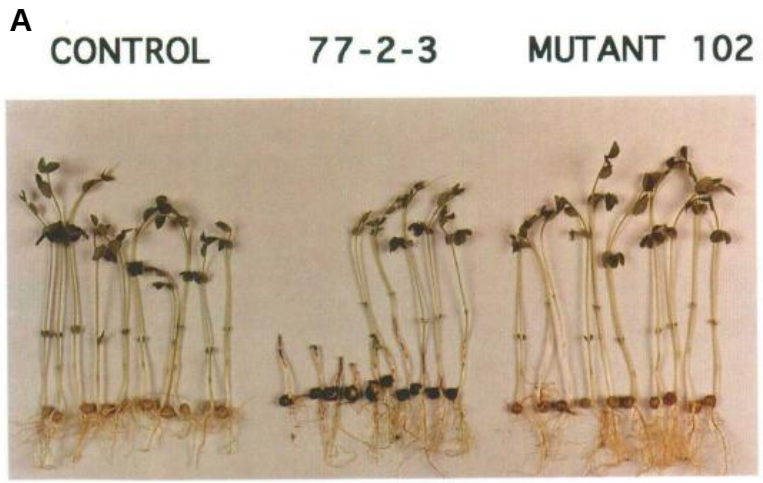


Figure 3. Time Course of Lesion Development in Pea Stem Bioassays.

Shown is the time course of lesion development when spore suspensions of *F. s. pisi* isolates T-8 and 77-2-3 and the cutinase gene–disrupted mutant were inoculated on pea stem segments for the periods indicated. Spores (10^5 [●], 10^4 [○], or 10^3 [▲]) in 5-μL droplets were placed on each of 15 stems used for each data point.



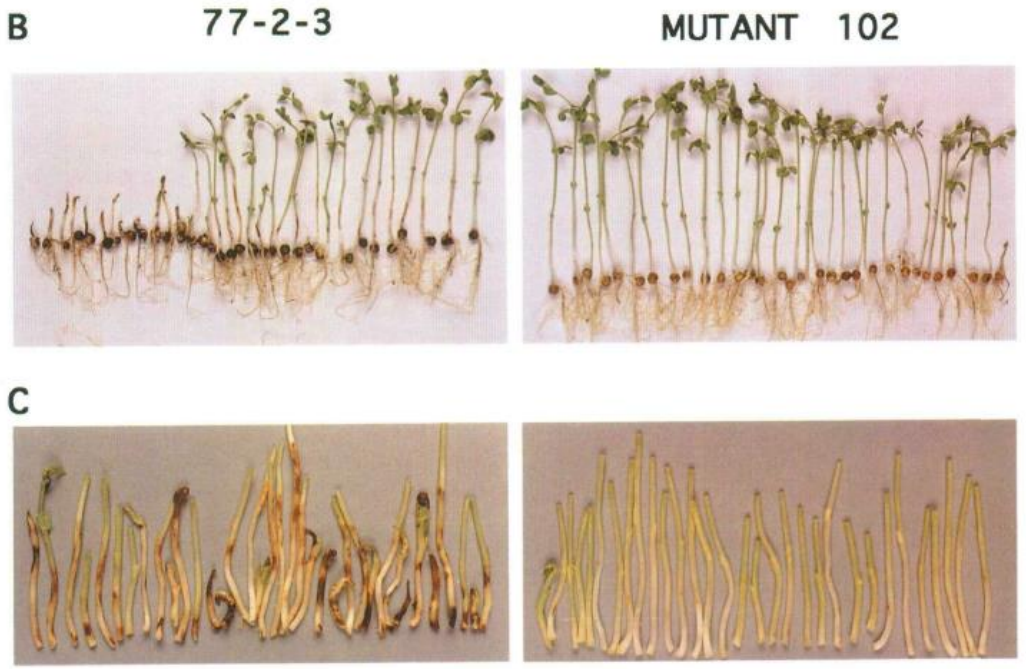


Figure 6. Infection of Pea Seedlings by *F. s. pisi* 77-2-3 and Cutinase Gene–Disrupted Mutant 77-102.

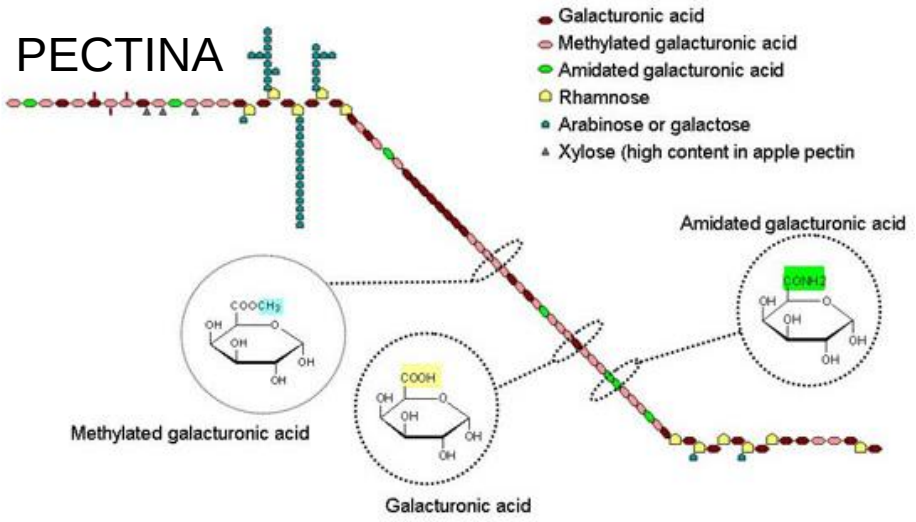
Rogers et al., 1994

Substâncias pécticas – enzimas pectolíticas

Lamela média e parede celular
Galacturona + Rhamnose

Erwinia em couve

PECTINA



Monilia em pêssego

Pectinases - Patógenos causadores de podridão mole

Substâncias pécticas – enzimas pectolíticas

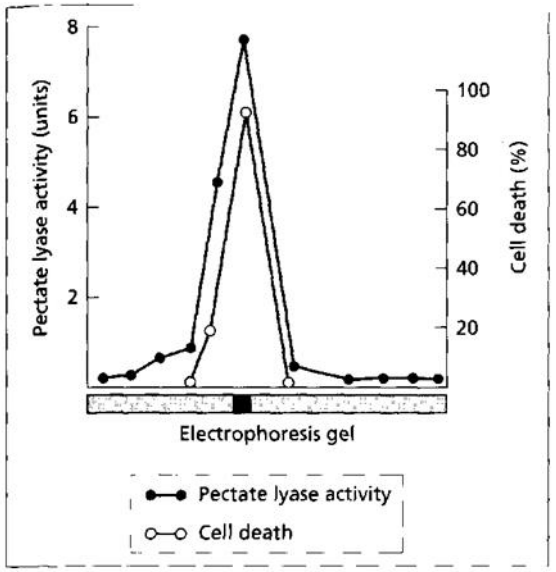
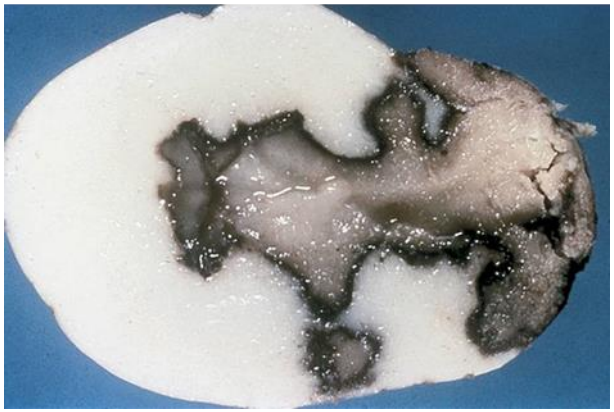


Fig. 8.3 Separation of a pectolytic enzyme (endopectate lyase) from *Erwinia* by acrylamide gel electrophoresis. The enzyme has migrated in the gel as a single band. Note that high enzyme activity coincides with greatest lethal activity towards potato cells. (Data from Basham & Bateman 1975.)



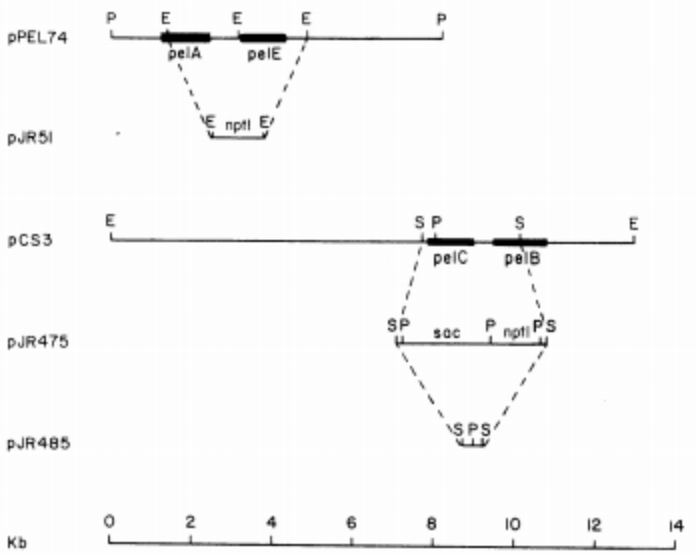


Fig. 1. Restriction map of the deletions in cloned *pel* genes used to construct *Erwinia chrysanthemi* Pel⁻ mutants. The cloned region of the *pel*-containing plasmids, including the location of specific *pel* genes and the relevant restriction sites, are shown for pPEL74 and pCS3. Dashed lines denote the replacement of sequences. These deletion derivatives were used to mutate AC4150 to UM1005 via a series of gene replacements: pJR475 (AC4150 to UM1002), pJR485 (UM1002 to UM1003), pJR51 (UM1003 to UM1005). Abbreviations: sac, *sacB sacR*; P, *Pst*I; E, *Eco*RI; S, *Sau*3A. The 28-bp fragment located in the place of the deleted *pelB* and *pelC* sequences in pJR485 is not drawn to scale.

Table 3. Maceration of potato tuber tissues by *Erwinia chrysanthemi* wild-type and Pel⁻ strains

Strains	Wet weight (g) of macerated tissue per inoculation site	
	Tuber slices[a]	Whole tubers[b]
AC4150	1.26 ± 0.44	0.196 ± 0.068
UM1005	0.27 ± 0.14	0.003 ± 0.006

[a] Bacteria were stabbed 4 mm deep into tuber slices with a toothpick. Macerated tissue was gently scraped out and weighed after 28-hr incubation. Values represent mean and SD of six slices.

[b] Bacterial suspensions containing 7.5×10^8 (AC4150) and 5.8×10^8 (UM1005) colony forming units in 25 μl were injected into whole potato tubers. Macerated tissue was weighed after 68-hr anaerobic incubation. Values represent mean and SD of 11 inoculation sites.

(Ried & Collmer, 1988)

Enzimas extracelulares

Quorum sensing

Table 1 Extracellular enzymes produced by *Erwinia chrysanthemi*

Name	Activity[a]	Strain[b]	Secretion pathway	Length amino acids[c]	Mr[d] kd	pI[d]	Substrate[e]	Products	pHopt	References
PelA	endo-Pectate lyase	EC16 3937	Out	361 + 31	44	4.2–4.6	PGA	predominant oligomers: di-to dodecamers	8.6	9, 43, 102, 130
PelB	endo-Pectate lyase	EC16 3937[f]	Out	353 + 22	39	8.8	PGA	predominant oligomers: tri-tetramers	8.9–9.5	9, 66, 71, 102
PelC	endo-Pectate lyase	EC16	Out	353 + 22	39	9	PGA	predominant oligomers: tri-tetramers	8.8–9.5	9, 120, 130
PelD	endo-Pectate lyase	B374	Out	360 + 31[g] 355 + 30	43	>10	PGA	n.d.	9	136
PelE	endo-Pectate lyase	B374 3937 EC16	Out	363 + 41[g]	45	>10	PGA	predominant oligomers: dimers	9	9, 71, 111, 136
ExoPeh	exo-Polygalacturonase	EC16	Out	577 + 27	67	8.3	PGA	dimers		53
EGY	Cellulase	3937	?[h]	309 + 22	35	8.8	CMC	—	5.5	50
EGZ	Cellulase	3937	Out	383 + 42	43	4.3	CMC	cellobiose	6.2–7.5	8, 20, 51
Pem	Pectin methylesterase	B374 3937	Out	342 + 24	37	9.6–9.9	Pectin	Pectate	5–9	76, 99, 100
PrtA	Protease	B374 EC16	Prt	454 + 18	50		Gelatin Azocasein			34, 47
PrtB	Protease	B374	Prt	465 + 16	53		Gelatin Azocasein			36
PrtC	Protease	B374	Prt	462 + 17	55		Gelatin Azocasein			36a
PrtG	Protease	B374	Prt	460 + 15	52		Gelatin			48
PlcA	Phospholipase	EC16	?[h]	358	39		Lecithin	phosphatidyl choline		70

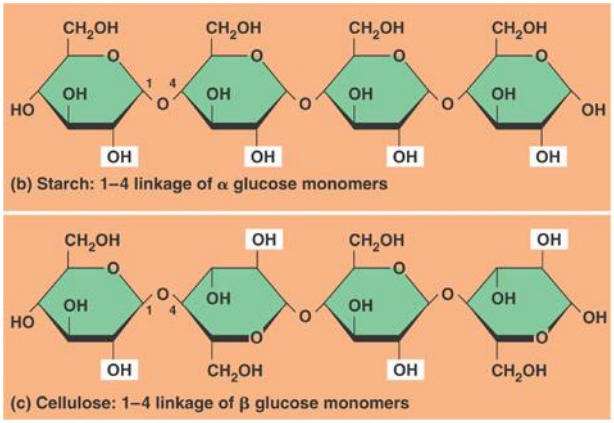
Table 2 Extracellular enzymes produced by *Erwinia carotovora*

Name	Activity	Strain[a]	Secretion pathway	Length amino acids[b]	Mr[c] kd	pI[c]	Substrate[d]	pHopt	References
PelA	endo-Pectate lyase	EC	Out	352 + 22	44	9.4	PGA	8.5	80
PelB	endo-Pectate lyase	EC	Out	352 + 22	44	9.4	PGA	8.3	78
PelC	endo-Pectate lyase	SCRI193	Out	358 + 16	42	10.3	PGA		63
Peh	Polygalacturonase	SCRI193 SCC3193 EC[e]	Out	376 + 26	42	>10	PGA	5.5	61, 79, 120
CelS	Cellulase	SCC3193	Out	232 + 32	27	5.5	CMC	6.8	121
CelV	Cellulase	SCRI193	Out	505 + 32	50	4.5	CMC MUC	7	32
Prt1	Protease	EC14	?	347	38	4.8	Gelatin		74
PnlA	Pectin lyase	DB71[f]	?	270	37		Pectin		24

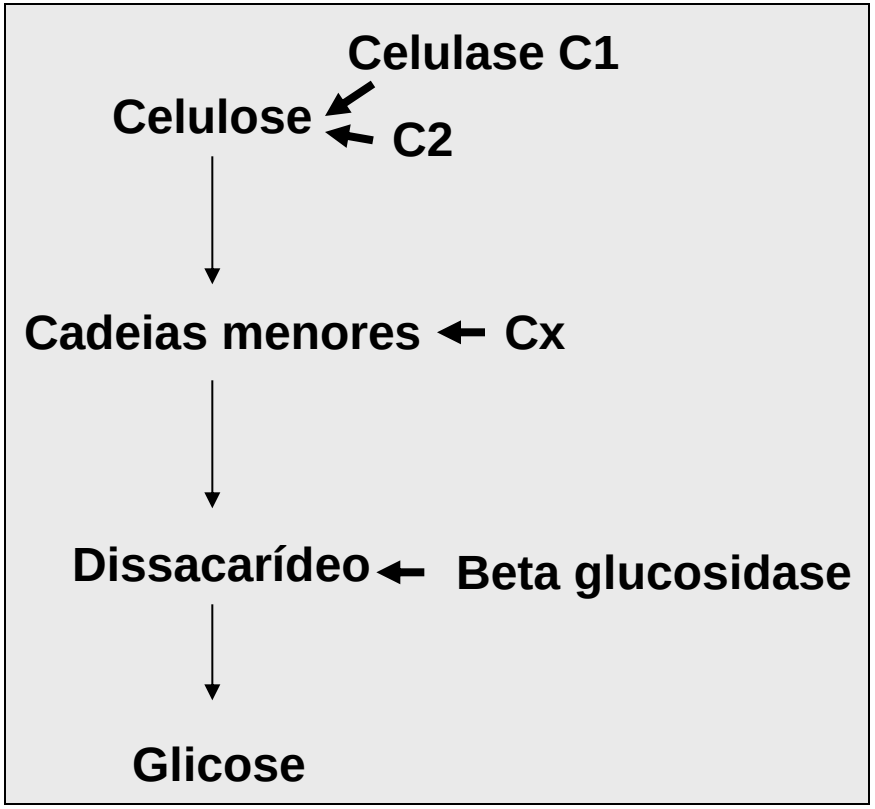
Barras et al., (1994)

Celulose - celulases

Parede – fibrilas
Polissacarídeo de glucose



Fungos, bactérias, nematóides e plantas parasitas



Fusarium em milho

Enzimas extracelulares

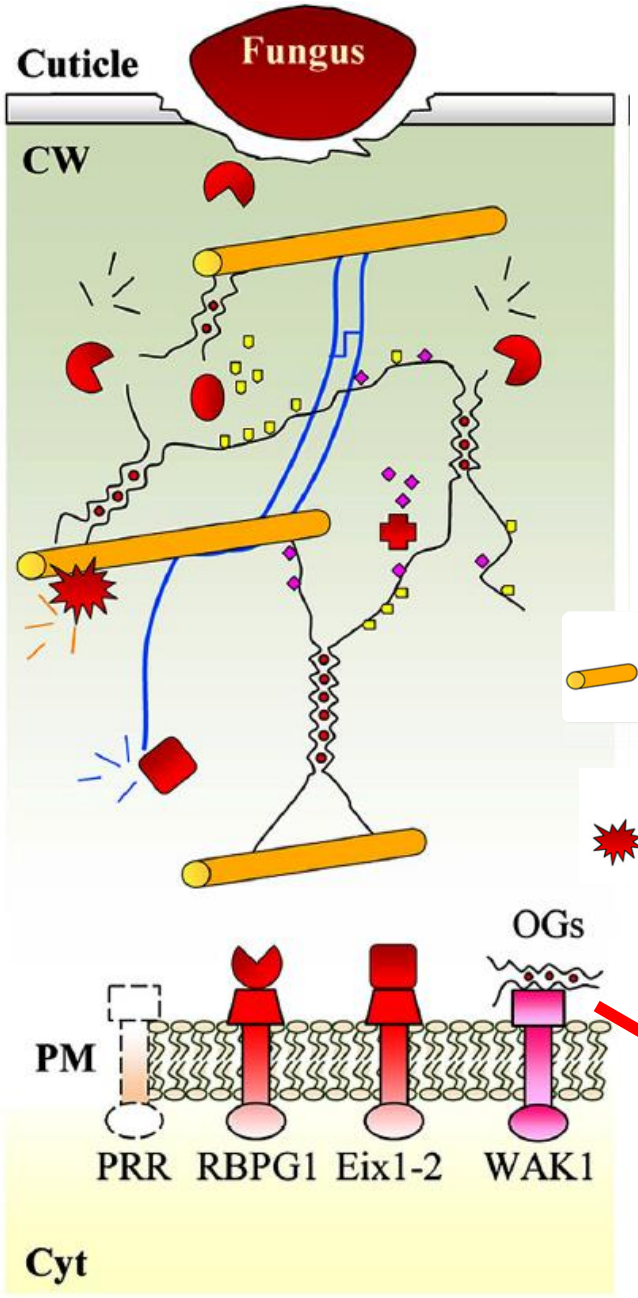
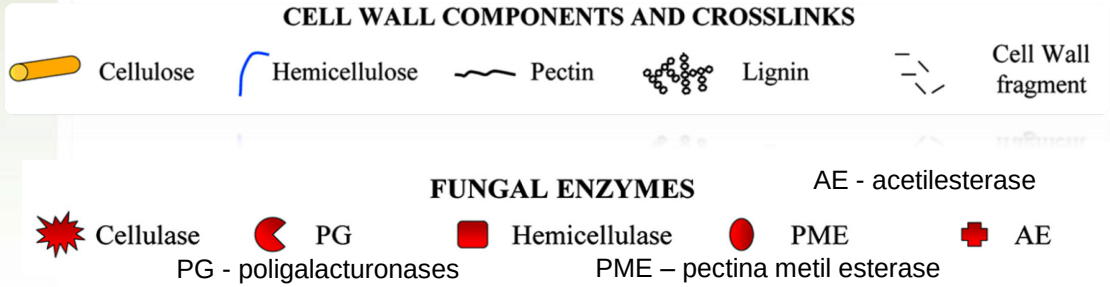


FIGURE 1 | Cell wall dynamics during necrotrophs invasion. (A) Necrotrophic fungi secrete a large arsenal of cell wall degrading enzymes (CWDEs) like PGs, hemicellulases and cellulases, assisted by PMEs and Aes in the apoplastic space to degrade cell wall polymers and facilitate the availability of nutrients.

(Bellincampi et al., 2014)



Receptores na membrana do hospedeiro

Toxinas

Sintomas

- produtos de microrganismos patogênicos
- causam danos nos tecidos
- envolvidos na patogênese
- baixo peso molecular
- ativas em conc. fisiológicas

Protoplasto

NÃO

- características enzimáticas
- características hormonais
- caracteristicas de ácido nucléico

Toxinas

Não seletivas (inespecíficas)

Componentes secundários de patogenicidade
incrementam severidade

Seletivas (específicas)

Componentes primários de patogenicidade
produz sintomas característicos da doença

Toxinas

Não seletivas (inespecíficas)

Tabtoxina

Pseudomonas syringae pv. *tabaci*

Dipeptídeo tóxico após hidrólise na planta

Atua nas tilacóides - necrose com halo

Faseolotoxina

Pseudomonas syringae pv. *phaseolicola*

Tripepetídeo tóxico após hidrólise

Crestamento e halo

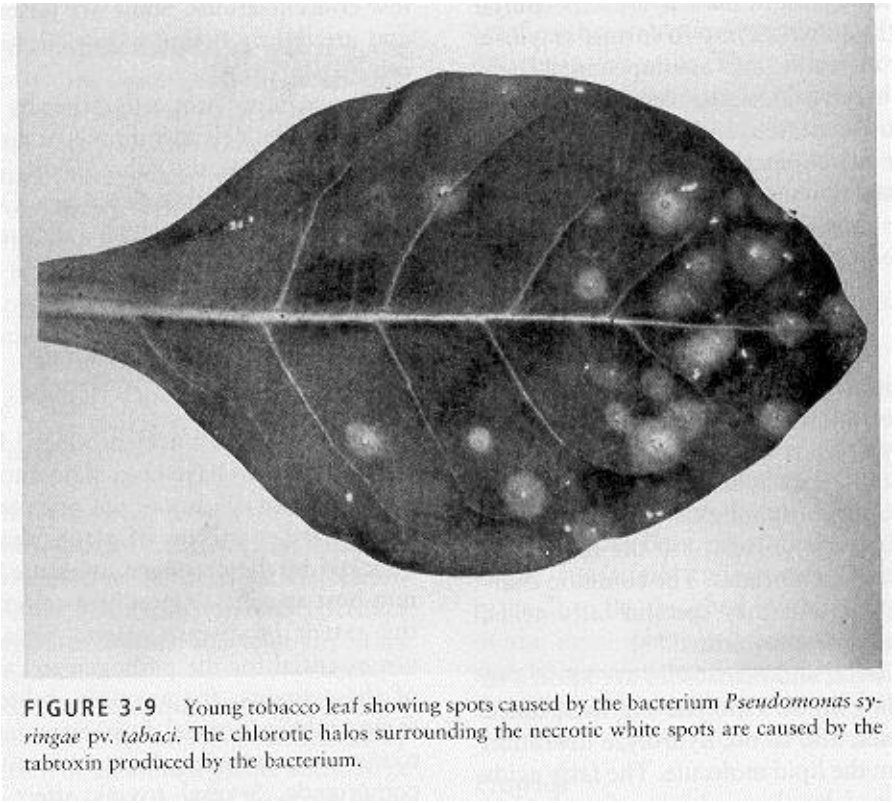
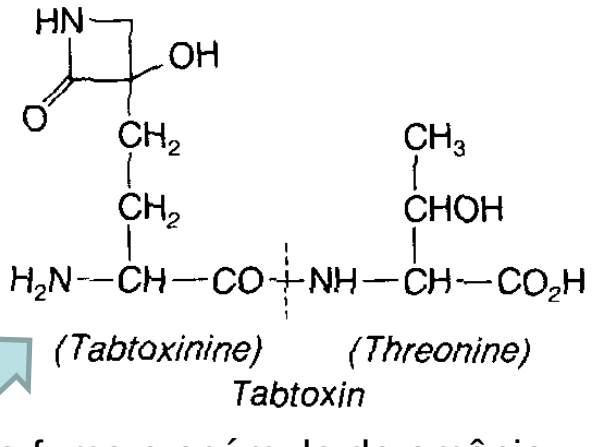


FIGURE 3-9 Young tobacco leaf showing spots caused by the bacterium *Pseudomonas syringae* pv. *tabaci*. The chlorotic halos surrounding the necrotic white spots are caused by the tabtoxin produced by the bacterium.



Inibe enzimas no fumo e acúmulo de amônia

Toxinas

tabtoxina

faseolina

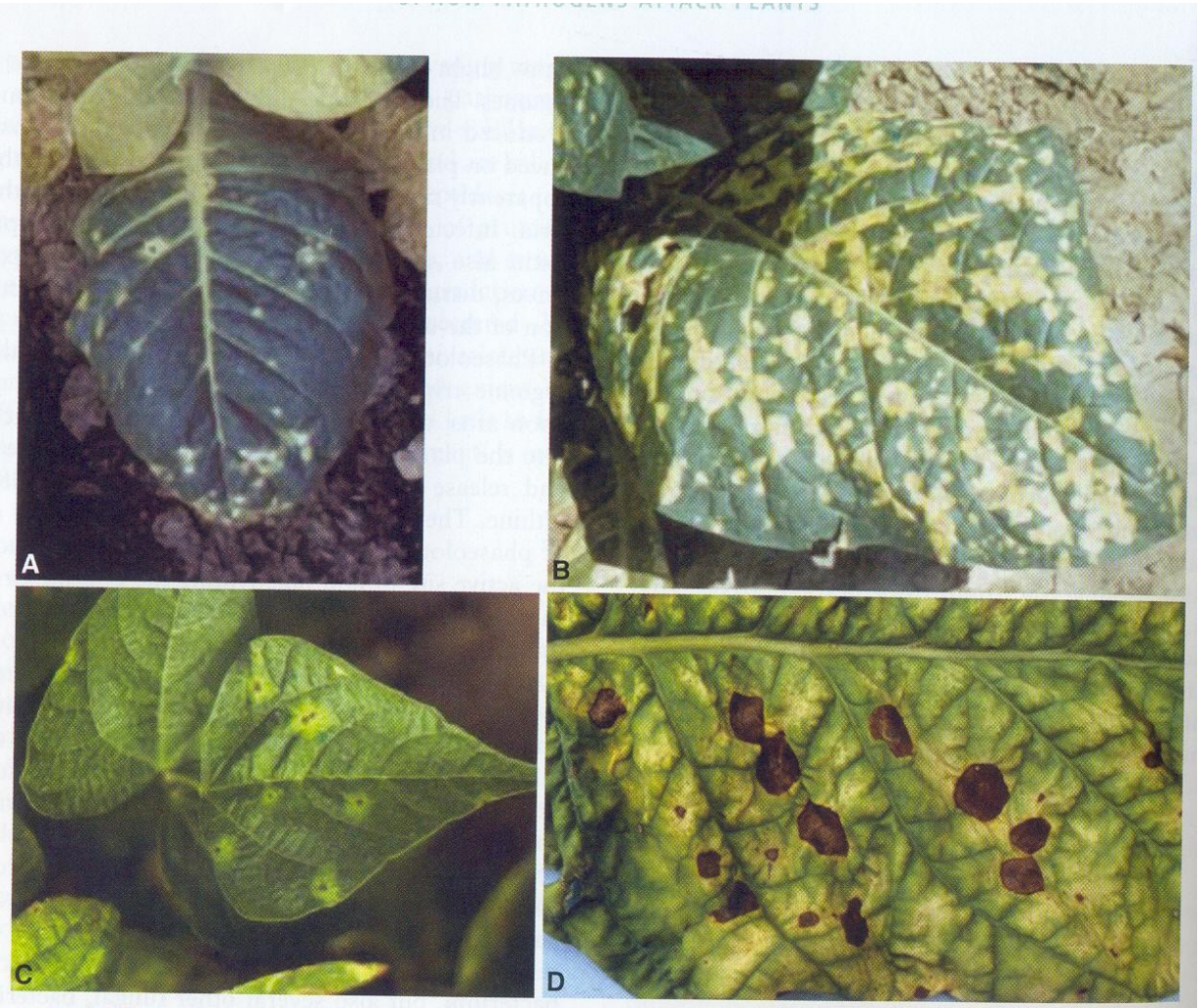


FIGURE 5-13 Symptoms caused by nonhost-selective toxins. Early (A) and semiadvanced (B) symptoms of young tobacco leaves showing spots caused by the bacterium *Pseudomonas syringae* pv. *tabaci*. The chlorotic halos surrounding the necrotic white spots are caused by the tabtoxin produced by the bacterium. (C) Leaf spots and halos caused by the toxin phaseolotoxin produced by the bacterium *Pseudomonas phaseolicola*, the cause of halo blight of bean. (D) Leaf spots and chlorosis caused by the *Alternaria alternata* toxin. [Photographs courtesy of (A, B, and D) Reynolds Tobacco Co. and (C) Plant Pathology Department, University of Florida.]

Toxinas

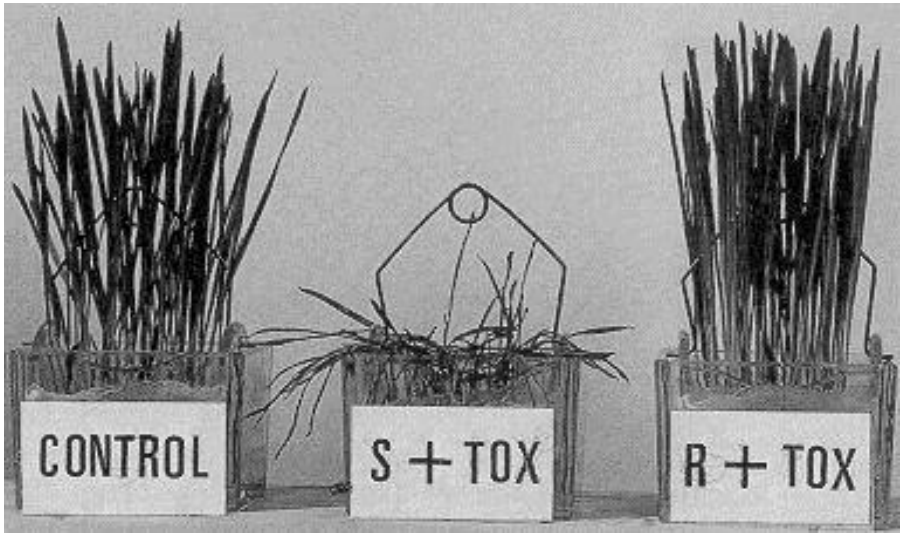
Seletivas (específicas)

Toxina HV (victorina)

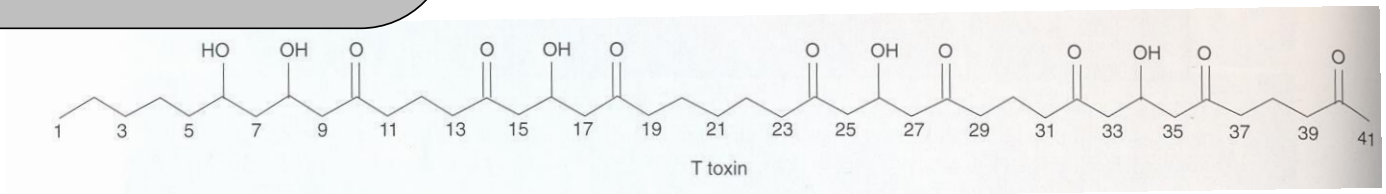
Helminthosporium victoriae
Cochliobolus victorae
específica de aveia com gene Vb (resistência à ferrugem)
Permeabilidade das membranas
morte da planta

Toxina HmT ou T

Helminthosporium maydis
Cochliobolus heterotrophus
específica de milho com macho esterilidade citoplasmática
membrana mitocondrial



Victorina adicionada à solução nutritiva de seedlings, 3 dias antes da foto. Controle = cv. victoria sem toxina, S+tox = cultivar victoria com toxina e R + tox = cv. resistente com toxina.



Toxinas

Mancha marrom de Alternaria em tangerina

Alternaria spp. - ampla gama de toxinas seletivas e não seletivas.

A. alternata – toxinas seletivas
ACRL – Limão Cravo (Rangpur lime)
ACT - Tangerinas

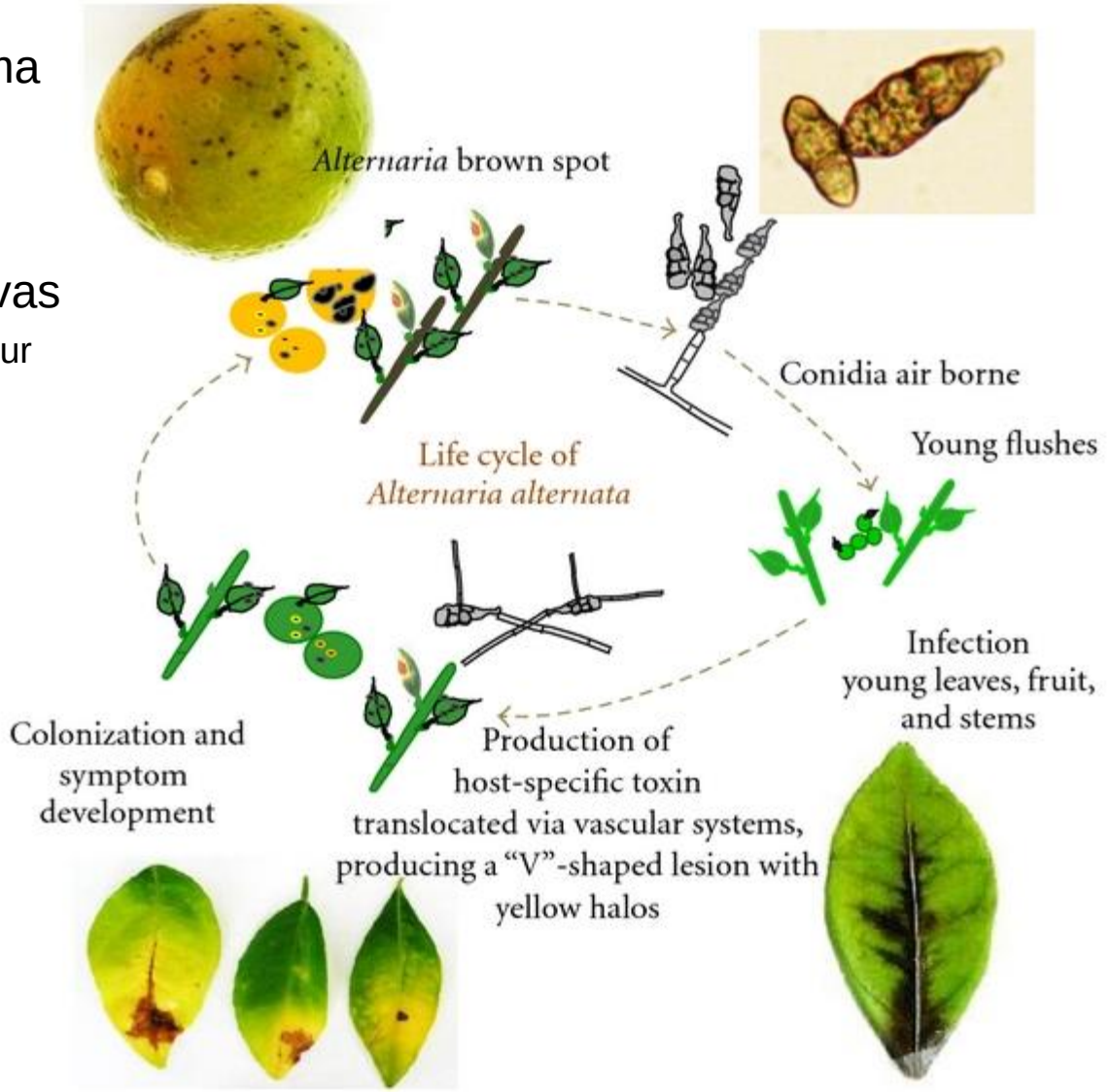
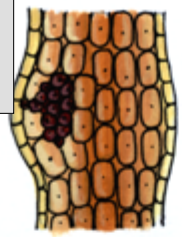


Figure 1: Life cycle of *Alternaria alternata*, the causal agent of citrus brown spot. ACT toxin produced by the tangerine pathotype of *A. alternata* is transported via the vascular system and formation of necrotic lesions on a detached calamondin leaf (bottom right).

Chung (2012)

Hormônios

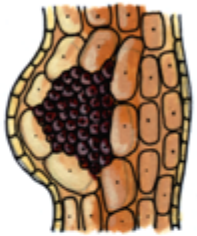


Colonização do tecido radicular

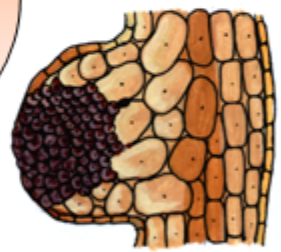
Bactéria + célula vegetal

DNA da bactéria se unindo ao da célula vegetal

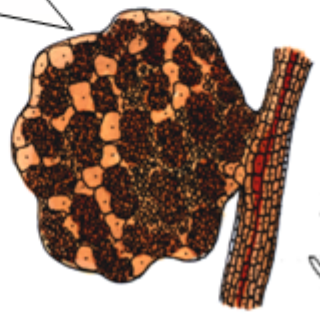
DNA da bactéria unido ao DNA da célula vegetal



Início da hiperplasia



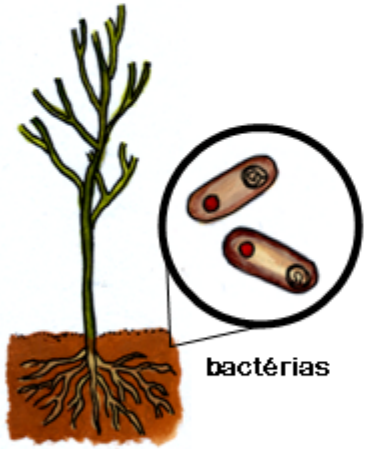
Hipertrofia e hiperplasia de células com desenvolvimento da galha



Galha na superfície da raiz

Sintoma de galha no sistema radicular

Multiplicação das bactérias no solo



bactérias

Presença de bactérias no solo

Penetração através de ferimento

Hormônios

Agrobacterium tumefaciens

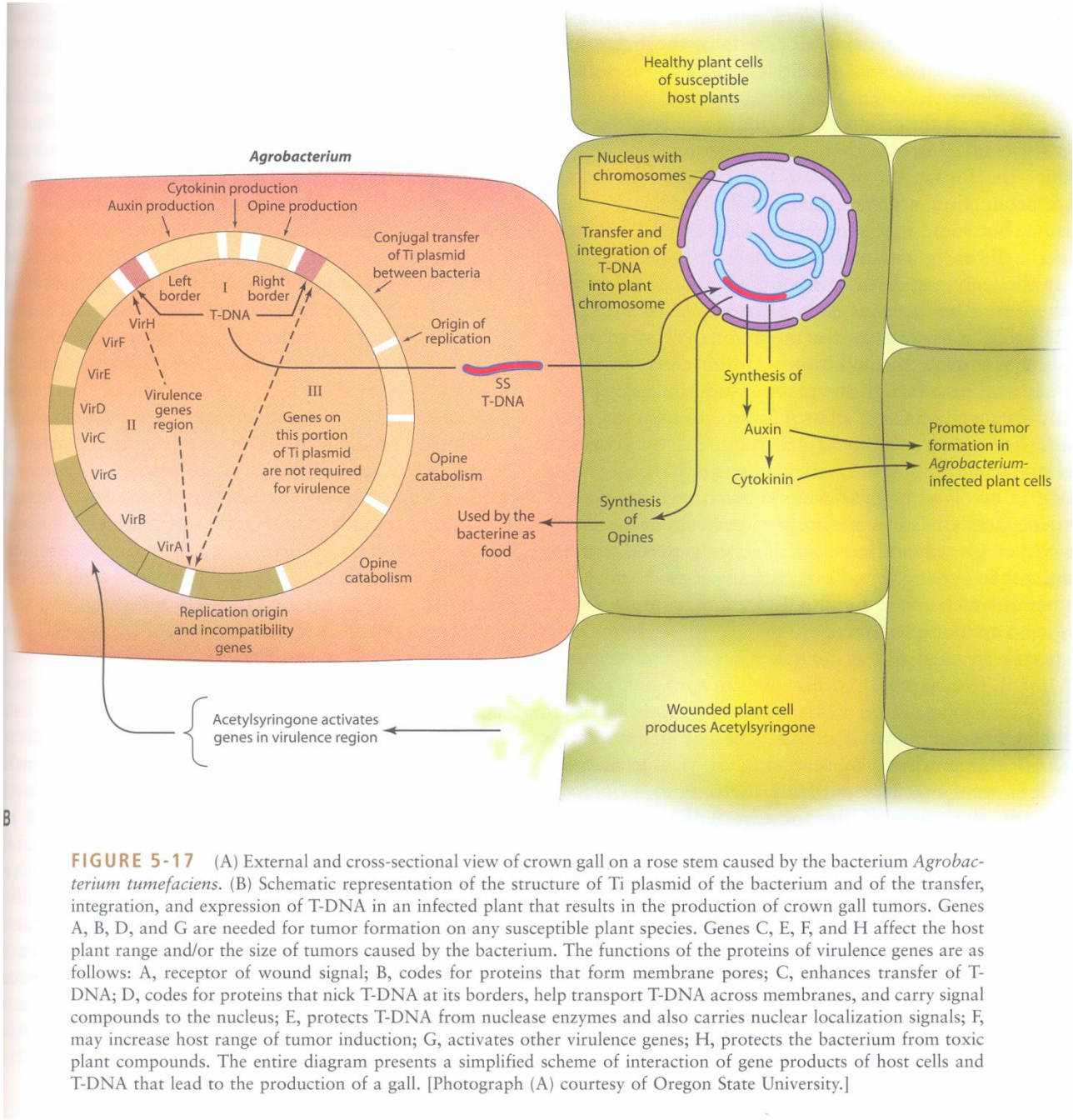


FIGURE 5-17 (A) External and cross-sectional view of crown gall on a rose stem caused by the bacterium *Agrobacterium tumefaciens*. (B) Schematic representation of the structure of Ti plasmid of the bacterium and of the transfer, integration, and expression of T-DNA in an infected plant that results in the production of crown gall tumors. Genes A, B, D, and G are needed for tumor formation on any susceptible plant species. Genes C, E, F, and H affect the host plant range and/or the size of tumors caused by the bacterium. The functions of the proteins of virulence genes are as follows: A, receptor of wound signal; B, codes for proteins that form membrane pores; C, enhances transfer of T-DNA; D, codes for proteins that nick T-DNA at its borders, help transport T-DNA across membranes, and carry signal compounds to the nucleus; E, protects T-DNA from nuclease enzymes and also carries nuclear localization signals; F, may increase host range of tumor induction; G, activates other virulence genes; H, protects the bacterium from toxic plant compounds. The entire diagram presents a simplified scheme of interaction of gene products of host cells and T-DNA that lead to the production of a gall. [Photograph (A) courtesy of Oregon State University.]

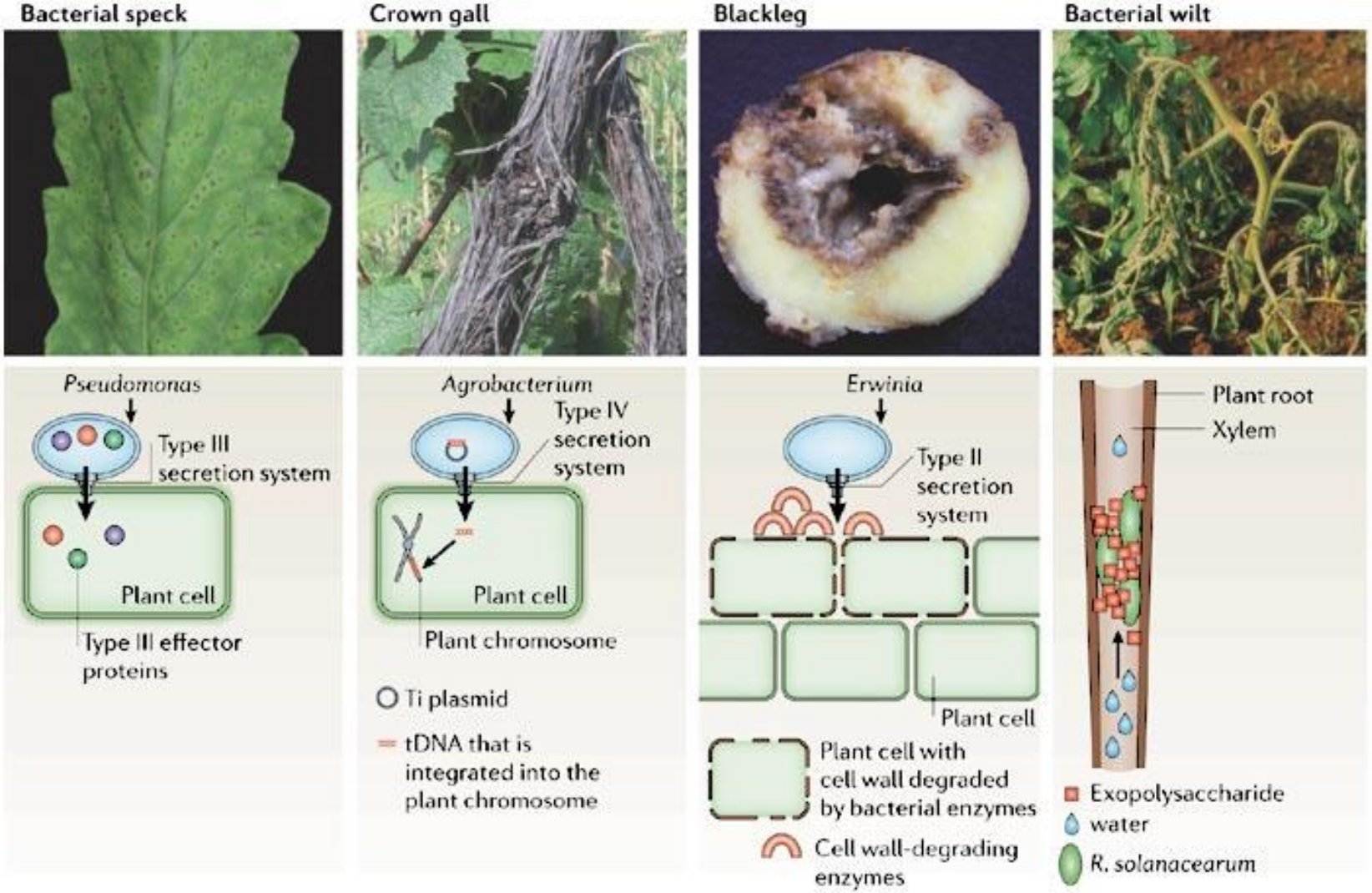


Figure 1 | **Disease symptoms caused by some bacterial pathogens of plants and representative virulence mechanisms used by these pathogens.** P. syringae pv. tomato enters the leaf apoplastic space through stomata or wounds, and uses a type III secretion system to inject a large number of virulence (effector) proteins into the plant cell. Agrobacterium tumefaciens uses a type IV secretion system to inject a tumour-inducing transfer DNA (tDNA) into the plant cell cytoplasm. Erwinia carotovora subspecies atroseptica uses a type II secretion system to deliver cell wall-degrading enzymes (for example, cellulases and pectinases) to the plant cell wall. Ralstonia solanacearum enters plant roots through wounds and multiplies in the xylem vessels in which it produces exopolysaccharides that are believed both to interfere with recognition and to inhibit water transport through the vascular system. Each of these four pathogens also uses other virulence mechanisms