The Use of Biological Method for Plant Disease Control

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Introduction

Disease Biological Control Definition

Broad Sense: Use of any organism to control a pathogen, including the resistance of the host plant itself as a natural and effective form of biological control.

Common Sense: biological control limited to antagonistic organisms (generally microorganisms) used to reduce attacks of crops by pathogens.

Introduction

<u>* Antagonistic effects:</u>

- direct or indirect, and

- due to introduced organisms or manipulation of existing organisms.

*** Definition also extended to any <u>natural products</u> extracted from living organisms or from other sources such as soil.**

Disease Biological Control

(1) Hypovirulence

Introduced antagonists may be hypovirulent (or sometimes avirulent) strains of the same pathogen → they spread and cover the host plants → reduction of the infection by virulent strains.

Disease Biological Control

Hypovirulence

Ex: Case of chestnut blight (*Cryphonectria parasitica*) in Europe: introduced hypovirulent strains → reduce infection by virulent strains.

Disease Biological Control

Hypovirulence

How: Hypovirulent strains carry in their cytoplasm virus-like double-stranded RNA → limit the pathogenicity of the virulent strains.

Double-stranded RNAs pass through mycelial anastomoses from hypovirulent to virulent strains → the latter become hypovirulent → the disease development decreases or stops.

Disease Biological Control

(2) Competition

On plant surfaces:

- Host-supplied nutrients: exudates, leachates and senesced tissue, +

- Nutrients from waste products of other organisms such as insects (e.g. aphid honeydew)

Disease Biological Control

Competition

Competition between pathogens and non-pathogens: Non-pathogens may develop and spread on host plant surface → covering the plant surface prevents pathogen establishment → limiting disease incidence and severity.

Disease Biological Control

Competition

For soilborne pathogens: non-pathogenic plant-associated micro-organisms → protect plants by rapid non-pathogenic colonization → exhausting the limited available substrates → no place for pathogens to start infection.

Disease Biological Control

(3) Siderophores

- Low molecular weight compounds in bacteria with high affinity to iron.
- They search and mop up all available iron in the immediate environment.
- Numerous pathogens need iron → essential mineral nutrient for growth and sometimes even for virulence.

Disease Biological Control

Siderophores

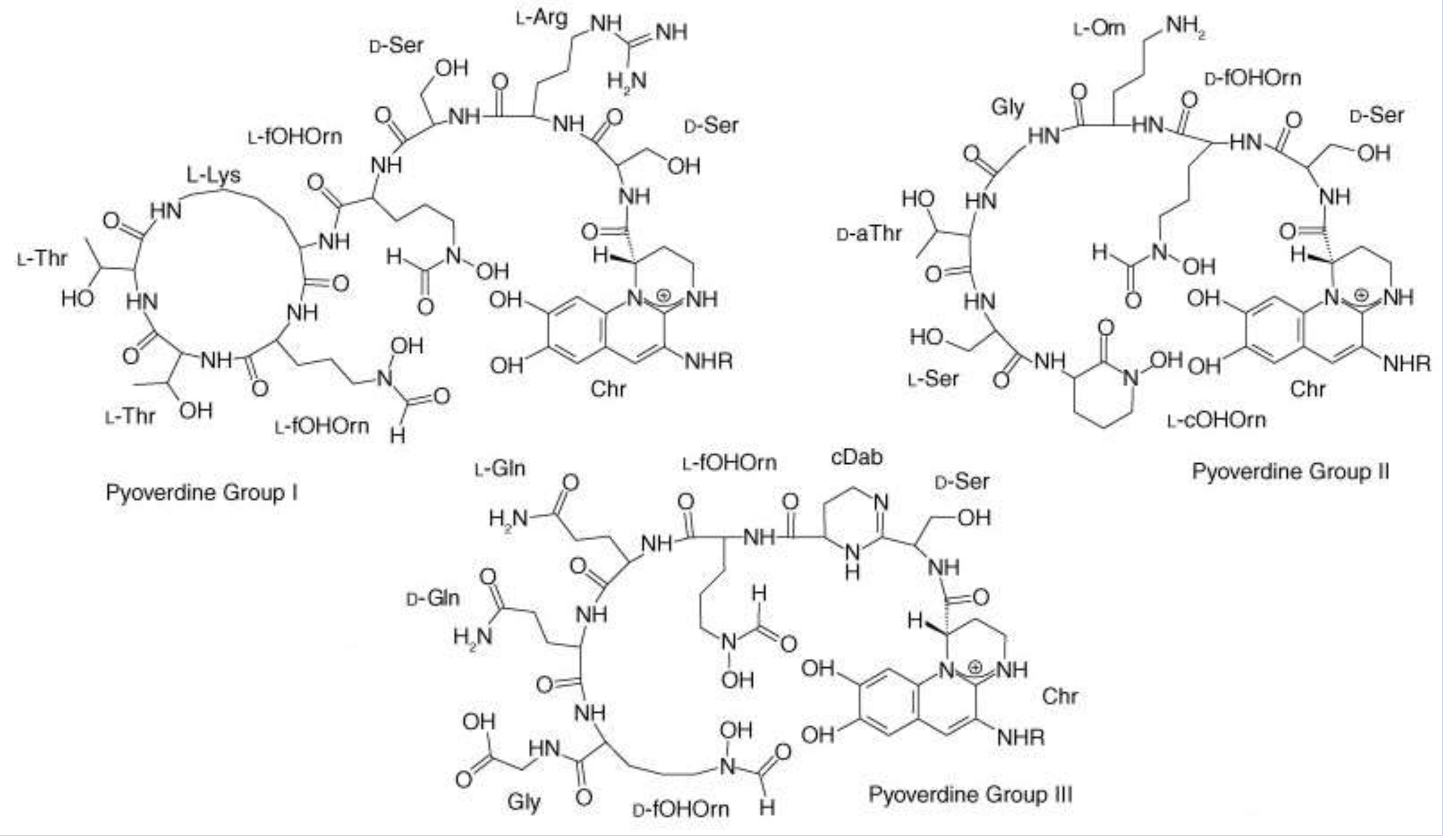
- Some fluorescent pseudomonads can control pathogens through production of siderophores (Ex: Pyoverdin, pyochelin) that sequester all available iron,

- Pathogens cannot develop and live with unavailable iron → they die.

Disease Biological Control

Siderophores

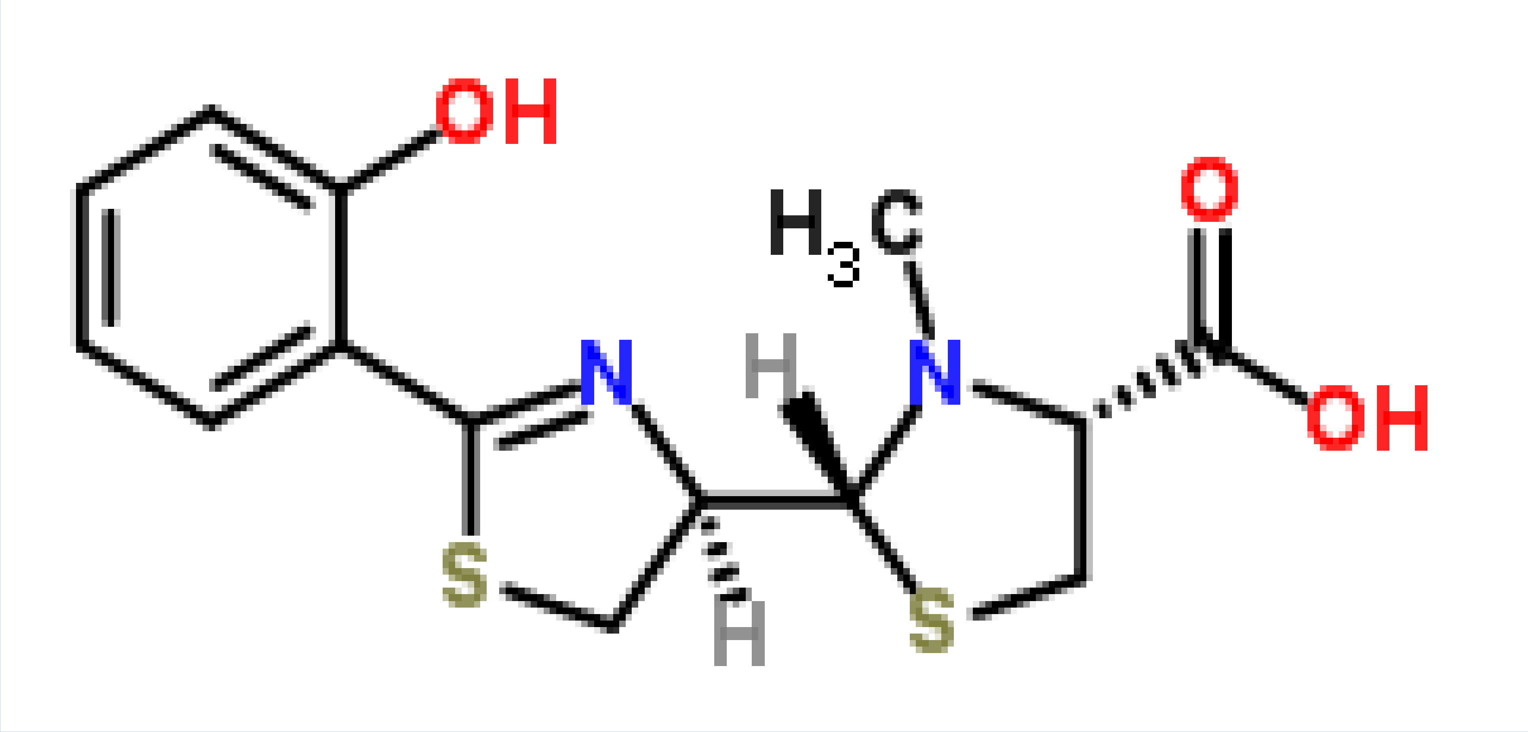
Ex: Pyoverdin



Disease Biological Control

Siderophores

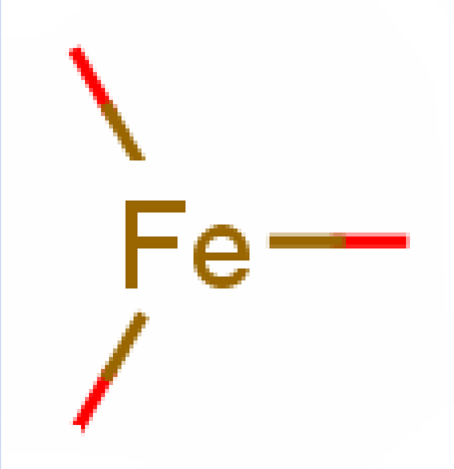
Ex: Pyochelin



Disease Biological Control

Siderophores

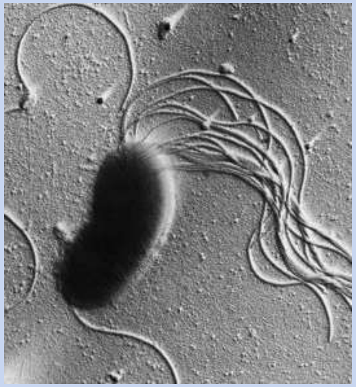
- In the rhizosphere of highly oxidized and aerated soil → iron is present in ferric form (Fe^{3+}), insoluble in water, available at low pH, exists at very low concentration.



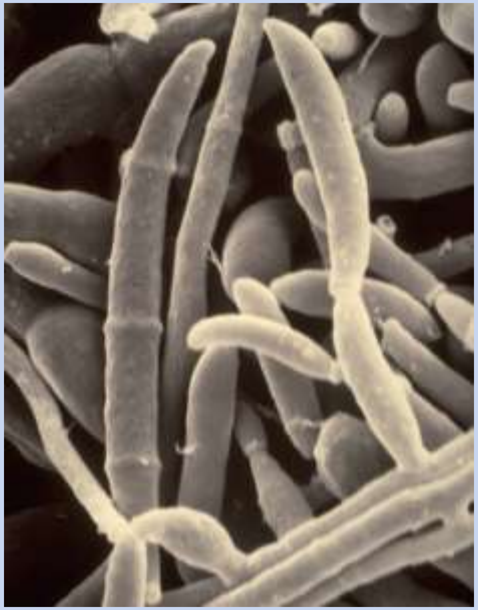
Disease Biological Control

Siderophores

Ex: With siderophore, *Pseudomonas fluorescens* uptakes whole limited available iron → inhibits germination of chlamydospores of *Fusarium oxysporum*.



Ferric iron



Disease Biological Control

Siderophores

Ex: **The same situation: *Pseudomonas aeruginosa*** → ***Pythium*.**

Ferric iron

Disease Biological Control

(4) Lytic enzymes

*** Many microorganisms release lytic enzymes → can hydrolyze a wide variety of polymeric compounds, including <u>chitin</u>, <u>proteins</u>, <u>cellulose</u>, <u>hemicellulose</u>, and <u>DNA</u> → can result in the suppression of plant pathogen activities.**

Disease Biological Control

Lytic enzymes

Ex: Biocontrol of *Pythium ultimum* in the rhizosphere of sugar beet by *Stenotrophomonas maltophilia*: due to the production of extracellular protease.

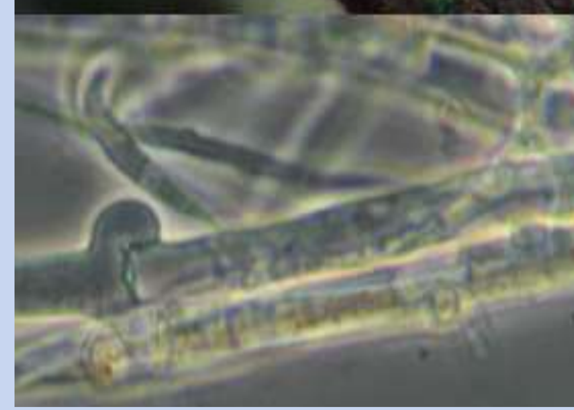
Lytic enzymes

Disease Biological Control

Lytic enzymes

Ex: ***Serratia marcescens*** **with chitinase production → can control the pathogen** ***Sclerotium rolfsii.***

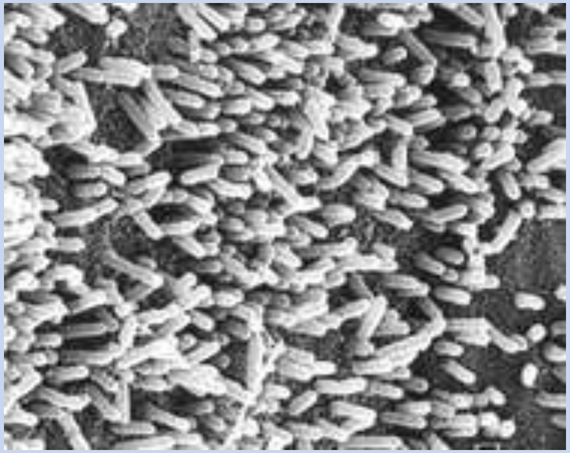
Lytic enzymes →



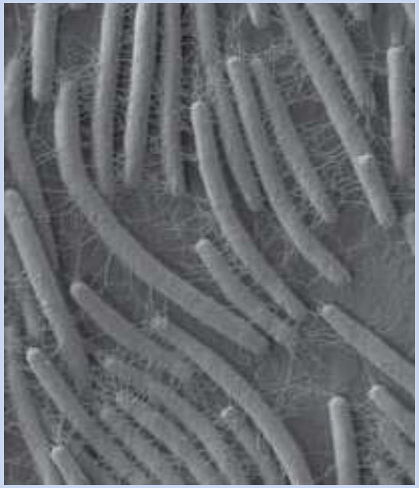
Disease Biological Control

Lytic enzymes

Ex: *Lysobacter* and *Myxobacteria* produce lytic enzymes → suppressing fungal plant pathogens.



***Lysobacter* colonizing tomato root**

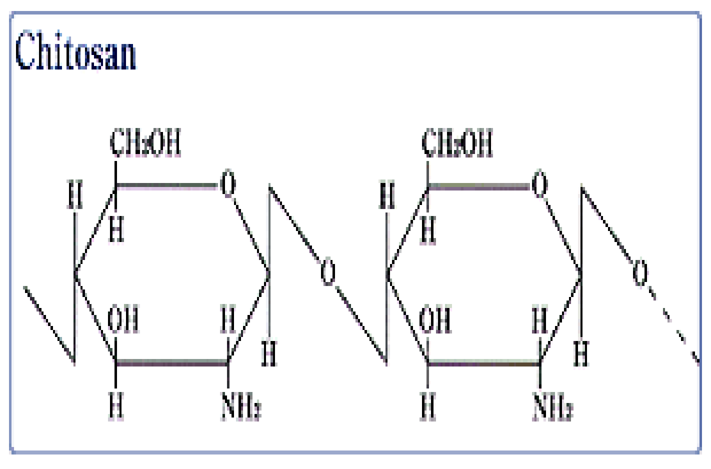


Myxobacteria

Disease Biological Control

Lytic enzymes

Ex: **Addition of chitosan can stimulate microbial chitosanase → acting against *Fusarium oxysporum* f. sp. *radicis-lycopersici***



microbial chitosanase

Disease Biological Control

Lytic enzymes

Ex: Production of hydrogen peroxide in the rhizosphere, catalyzed by glucose oxidase from *Talaromyces flavus* → responsible for the biocontrol of eggplant wilt caused by *Verticillium dahliae*.

Glucose oxidase reduces the growth of *V. dahliae* only in the presence of eggplant roots → supply of glucose from the roots was of major importance.

Disease Biological Control

Lytic enzymes

Talaromyces flavus

Eggplant roots

Glucose

Glucose oxidase

Rhizosphere

H_2O_2

Verticillium dahliae

Disease Biological Control

(5) Other microbial products

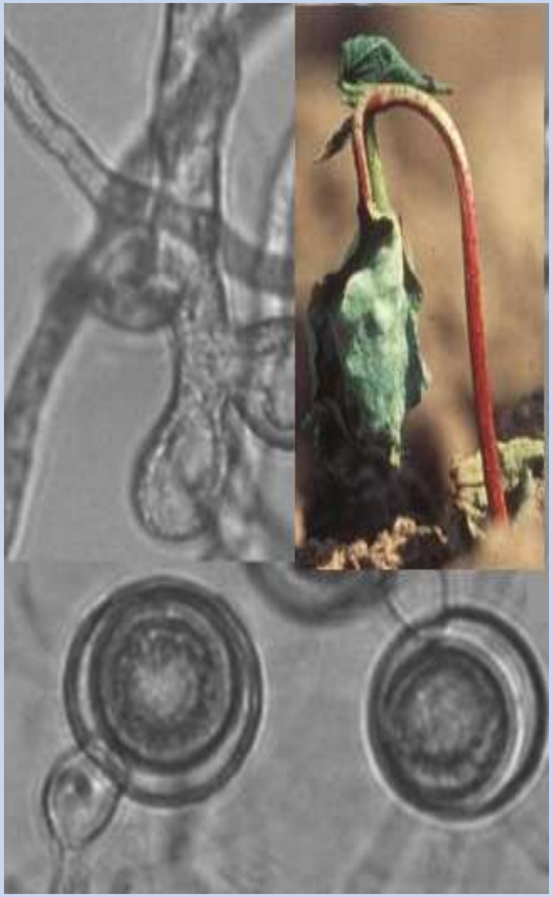
* **Hydrogen cyanide (HCN) blocks cytochrome oxidase pathway and is highly toxic to all aerobic microorganisms: *Pseudomonas fluorescens* produces HCN → suppression of black rot of tobacco caused by *Thielaviopsis basicola*.**

Disease Biological Control

Other microbial products

<u>Ammonia</u> (NH_3) produced by *Enterobacter cloacae* → involved in the suppression of *Pythium ultimum* causal agent of the damping-off of cotton.

NH_3



Disease Biological Control

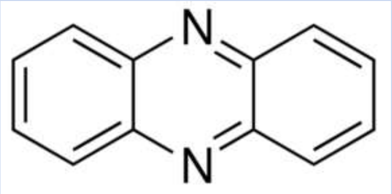
(6) Antibiotics

*** Antibiotics: toxic substances produced by antagonists at low concentrations that poison or kill pathogens → crucial role in biological control.**

Ex: *Pseudomonas fluorescens* active against *Gaeumonnomyces graminis* by producing the antibiotic phenazine.

Disease Biological Control

Antibiotics

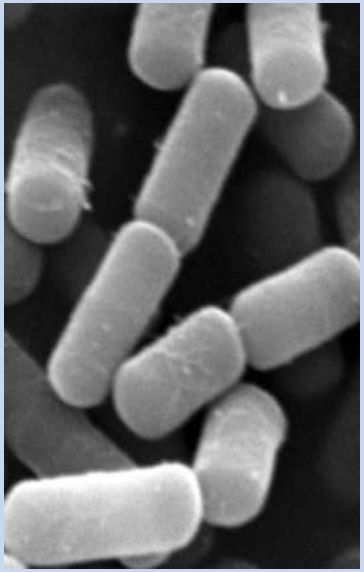


Phenazine

Non-producing antibiotic mutant gives poor control → more than 50% of the control is due to production of this antibiotic.

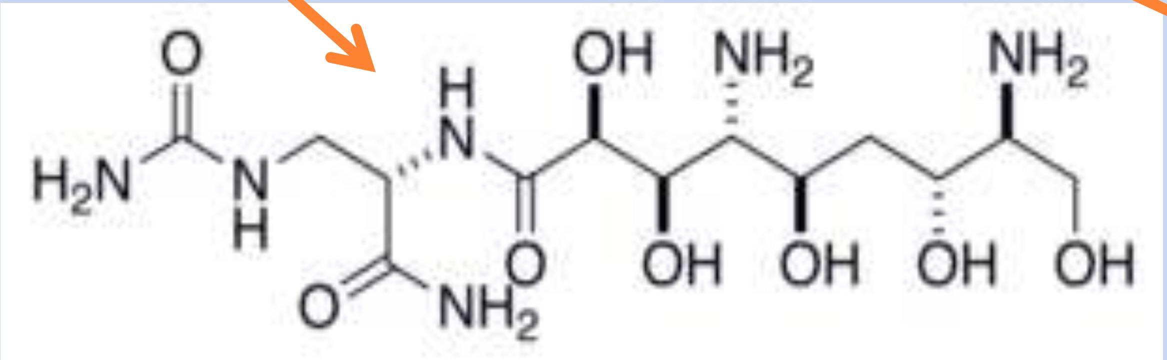
Disease Biological Control

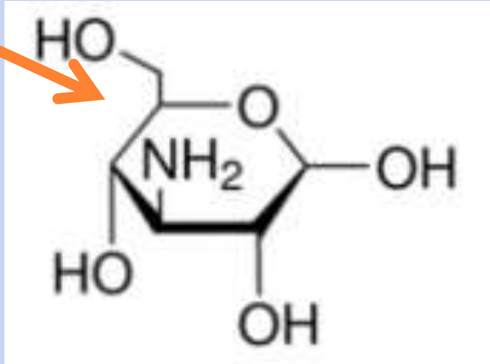
Antibiotics



- Different strains produce multiple antibiotics → can suppress one or more pathogens.

- **Ex:** One strain of *Bacillus cereus* produces both kanosamine and zwittermycin.

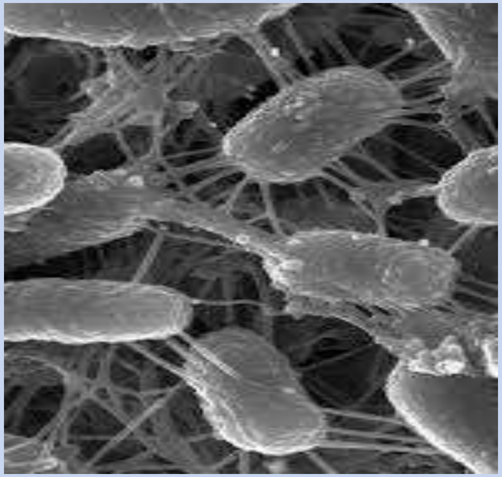


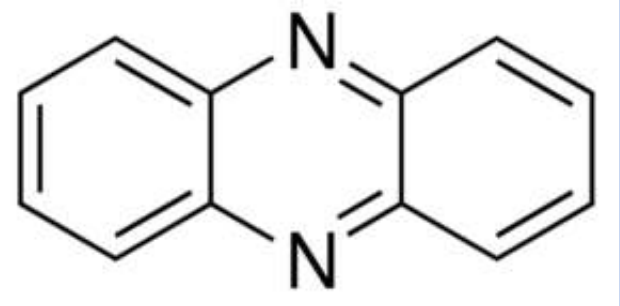


Disease Biological Control

Antibiotics

- Genetically engineered strains of *Pseudomonas putida* to produce phenazine → improves capacities to suppress plant diseases in field-grown wheat.

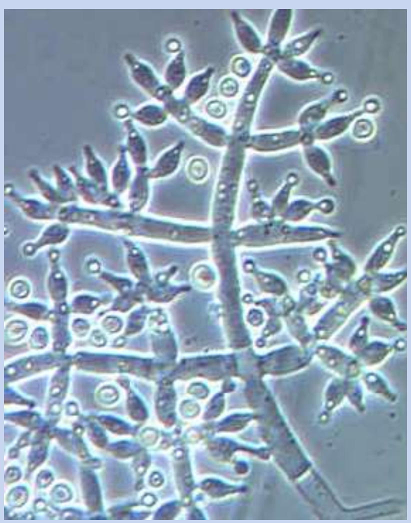


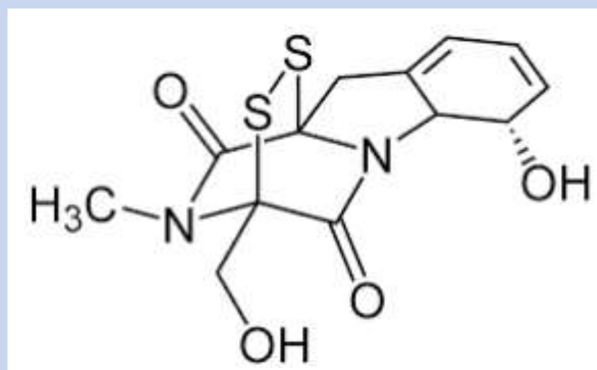


Disease Biological Control

Antibiotics

- **Gliotoxin production by *Trichoderma* → responsible for cytoplasmic leakage from *Rhizoctonia solani* cell membranes → biocontrol.**

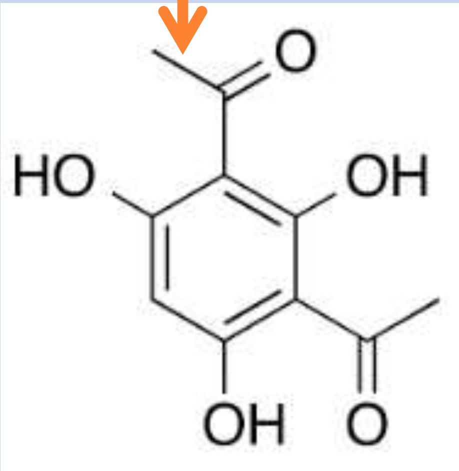


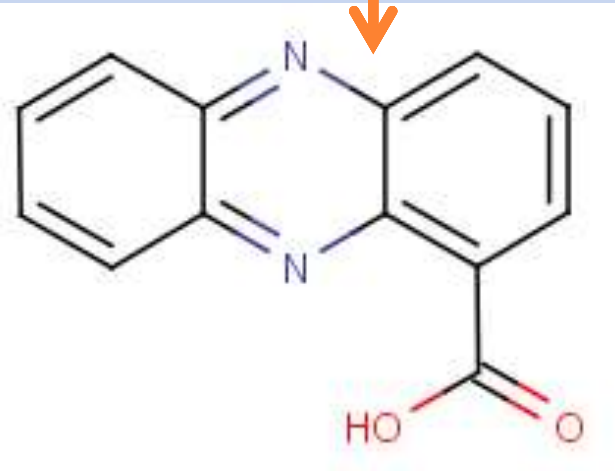


Disease Biological Control

Antibiotics

- Some pseudomonads produce 2,4-diacetylphloroglucinol and phenazine-1-carboxylic acid controlling wheat root diseases.

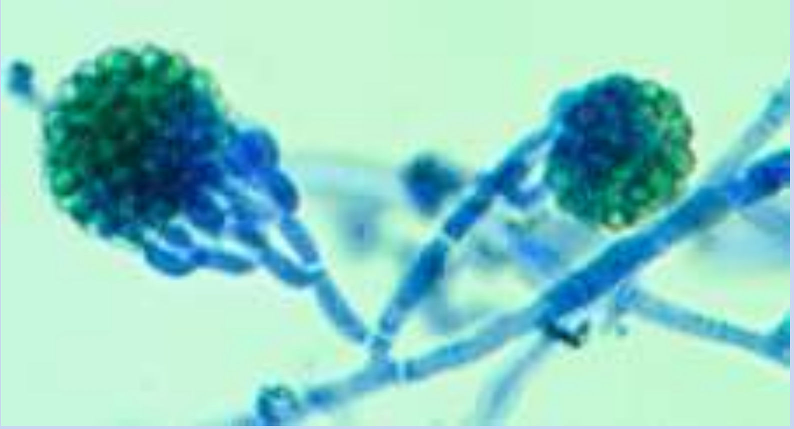




Disease Biological Control

(7) Parasitisme

- Viability of spores and survival structures of pathogens may be reduced by direct parasitism. Since 1930's, it was noted that *Trichoderma* and *Gliocladium* can control plant pathogenic fungi by parasitism.



Disease Biological Control

Parasitisme

* **Most important one:** ***Sporidesmium sclerotivorum*, obligate parasite of sclerotia of many fungal pathogens: *Sclerotinia*, *Sclerotium*, *Botrytis*,…**

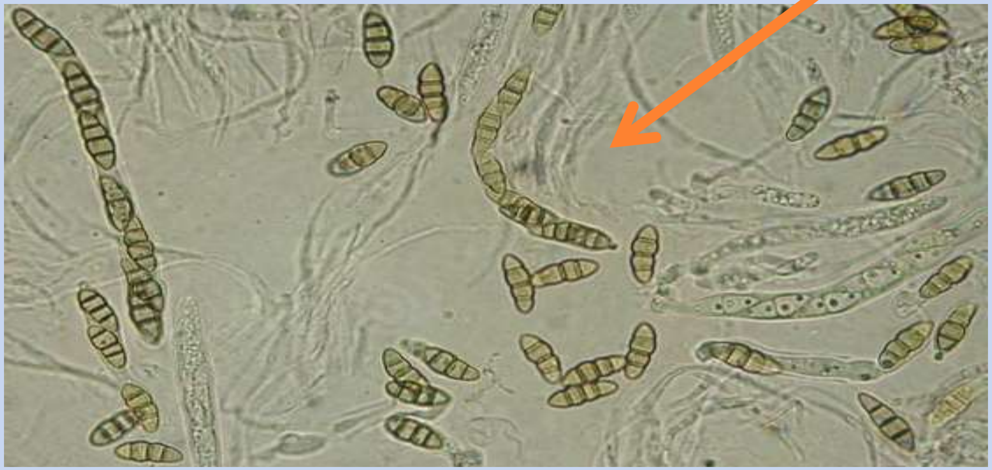
Sclerotia diffuse chemicals → stimulate germination of nearby conidia of the antagonist → infects pathogen sclerotia causing lysis.

Disease Biological Control

Parasitisme

- This parasitisme (*Sporidesmium sclerotivorum*) → can control lettuce disease caused by *Sclerotinia minor*.

- Another sclerotial parasite: *Coniothyrium minitans*.



Disease Biological Control

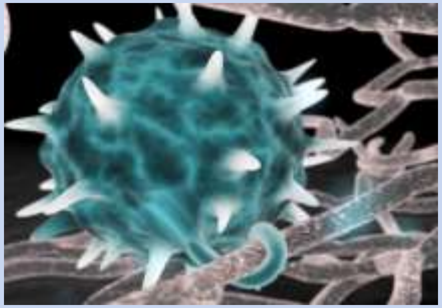
Parasitisme

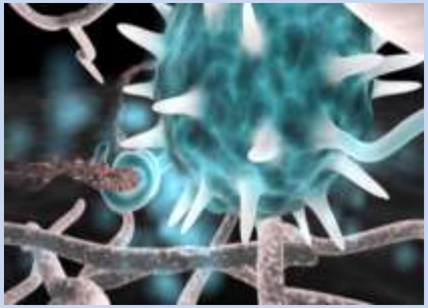
*** When antagonists are fungi infecting other pathogenic fungi → <u>Mycoparasites</u>. They may vary from <u>necrotrophic</u> to <u>biotrophic</u> species.**

Disease Biological Control

Parasitisme

* Some antagonistic fungi are even close relatives to pathogenic fungi: *Pythium oligandrum* the mycoparasite of *Pythium ultinum*, causal agent of seedling damping-off of sugar beet, cress and carrot.





Parasitisme

Disease Biological Control

Parasitisme

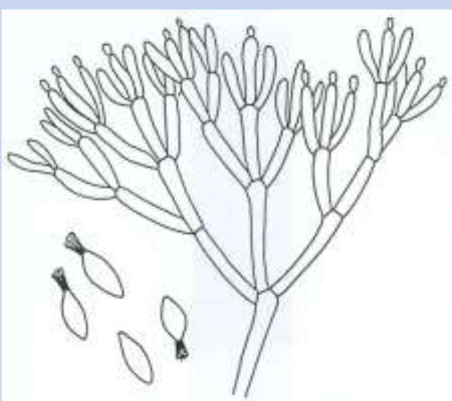
- Mycoparasitisme is generally accompanied by release from antagonist of extracellular enzymes such as β-1,3 glucanases, chitinases, cellulases and proteases.

- Degradation products of the cell wall → act as inducers of these enzymes in the host-parasite system.

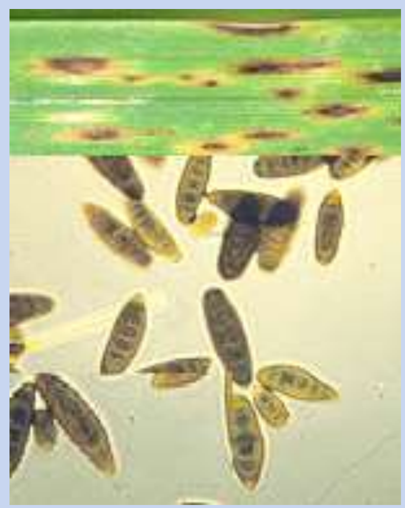
Disease Biological Control

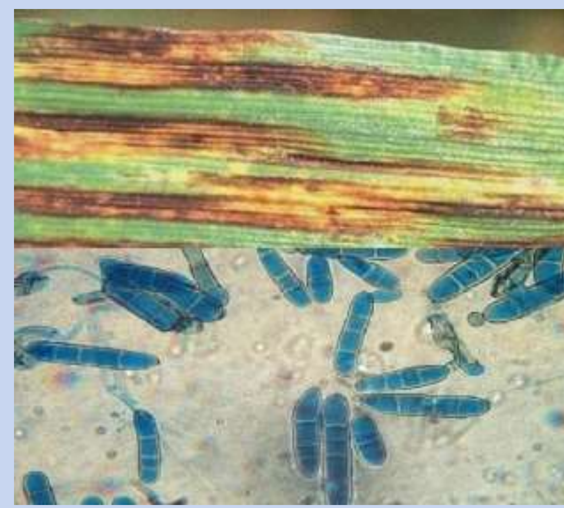
Parasitisme

* *Myrothecium verrucaria*, can parasitize hyphae of *Cochliobolus sativus* and reduce infection of barley by *Drechslera teres* (with seed treatment).



Parasitisme

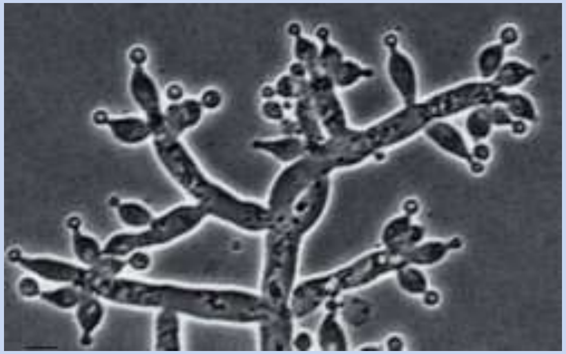




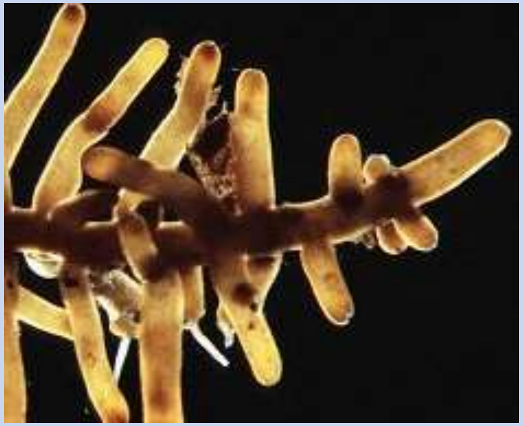
Disease Biological Control

Parasitisme

*** Mycoparasites may also attack beneficial fungi such as those forming mycorrhizae. For example, *Trichoderma harzianum* → *Glomus intraradices*.**



Parasitisme



Disease Biological Control

(8) Suppressive Soils

*** In suppressive soils, soilborne pathogens develop much less and cause much milder diseases than in conducive soils.**

Disease Biological Control

Suppressive Soils

*** Examples of reduced activity pathogens in suppressive soils:**

- *Fusarium oxysporum* (vascular wilts),

- *Gaeumannomyces graminis* (cereal take-all),

- *Phytophthora cinnamomi* (tree root rots),

- *Pythium* spp. (seedling damping-off).

Disease Biological Control

Suppressive Soils

* **The cause of pathogen suppression in the soil: the presence of antagonistic microorganisms → they act through production of antibiotics or lytic enzymes, competition for food, or direct parasitism of pathogens.**

Disease Biological Control

Suppressive Soils

*** Those antagonists may be fungi (*Trichoderma, Penicillium, Sporidesmium*) or bacteria (*Pseudomonas, Burkholderia, Bacillus, Streptomyces*).**

*** Soil suppressiveness totally disappears when the soil is sterilized: Biological property.**

Disease Biological Control

(9) Practical Aspect

- Biological control method is still now limited, particularly in field conditions.

- Unlike in the laboratory, in the greenhouse and in the storage depot (or any other confined spaces), results in the field are not usually successful.

Disease Biological Control

Practical Aspect

- The major problems:

(1) introduced microorganisms generally fail to compete with the existing microflora or

(2) soil amendments are not too selective to increase only the antagonist populations.

Disease Biological Control

(10) Use/Soil pathogens

*** Most common: *Trichoderma harzianum* and *Gliocladium virens* effective against:**

Pythium, Phytophthora, Rhizoctonia, Heterobasidion, Sclerotinia, Sclerotium, Fusarium.

Disease Biological Control

Use/Soil pathogens

- *Sporodesmium sclerotivorum* and *Coniothyrum minitans* effective against:

Sclerotinia sclerotiorum.

- *Talaromyces flavus* antagonist to:

Verticillium species and *Rhizoctonia solani*.

Disease Biological Control

Use/Soil pathogens

- Bacteria: *Bacillus*, *Enterobacter*, *Pseudomonas*, *Burkholderia* effective against:

Sclerotium cepivorum, *Gaeumannomyces graminis*, *Phytophthora* sp., *Pythium* sp.

- Nematode *Aphelenchus avenae*: antagonist to *Rhizoctonia* and *Fusarium*.

Disease Biological Control

(11) Use/Aerial pathogens

- **Many antagonists are proved to protect host plants from aerial pathogens (in research).**
- **The yeast *Pichia gulliermondii* effective against:**

↓

***Botrytis* and *Penicillium*.**

Disease Biological Control

Use/Aerial pathogens

- *Chaetomium* **sp. and** ***Athelia bombacina*** **control:** ⟹ ***Venturia inaequalis.***

- ***Botrytis cinerea*** **suppressed by**

↑

Penicillium **sp. or** ***Trichoderma*** **sp.**

Disease Biological Control

(12) Use/Postharvest pathogens

- Many yeasts, such as *Candida oleophila*, protect fruits against

***Botrytis*, *Penicillium*, and *Rhizoctonia* rots.**

Disease Biological Control

Use/Postharvest pathogens

- Green mold caused by *Penicillium digitatum* and *Botrytis* rot are controlled by

Trichoderma viride.

Disease Biological Control

(13) Mycorrhiza Case

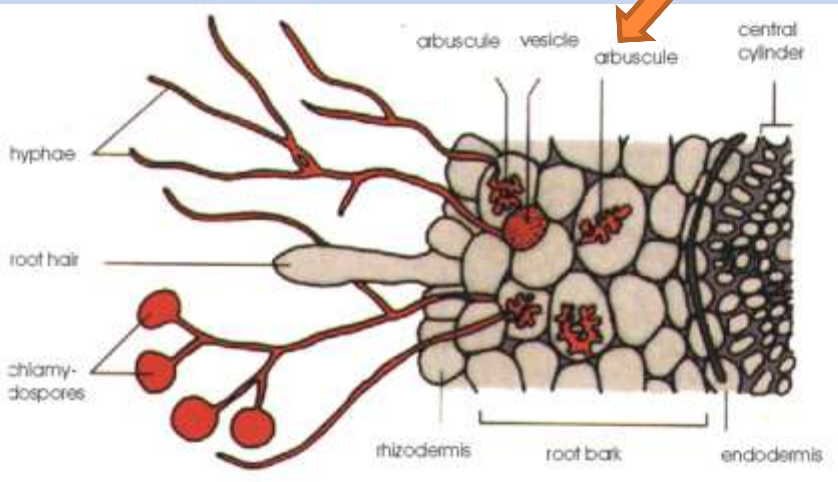
- **Mycorrhizae form as result of mutualistic root symbiosis between certain fungi and many plants (roots).**

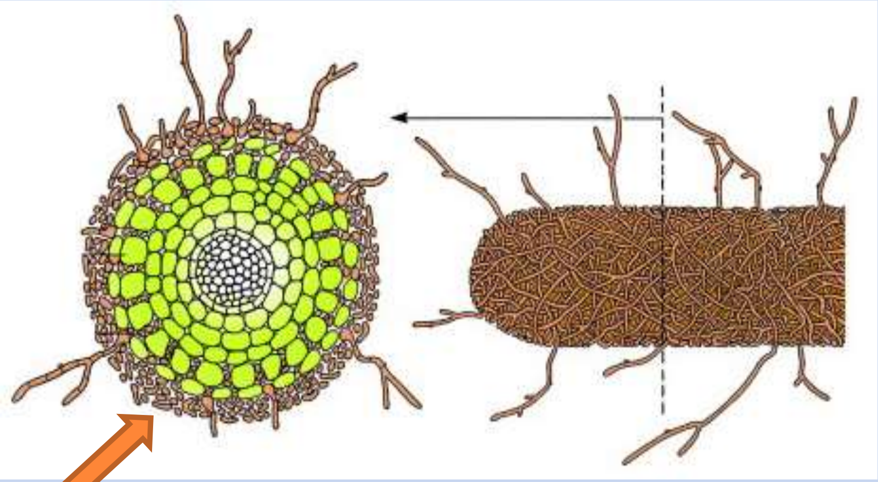
- **Mycorrhizal fungi can prevent root infections by reducing the pathogen access sites and by stimulating host defense.**

Disease Biological Control

Mycorrhiza Case

* Some are endomycorrhizal fungi.

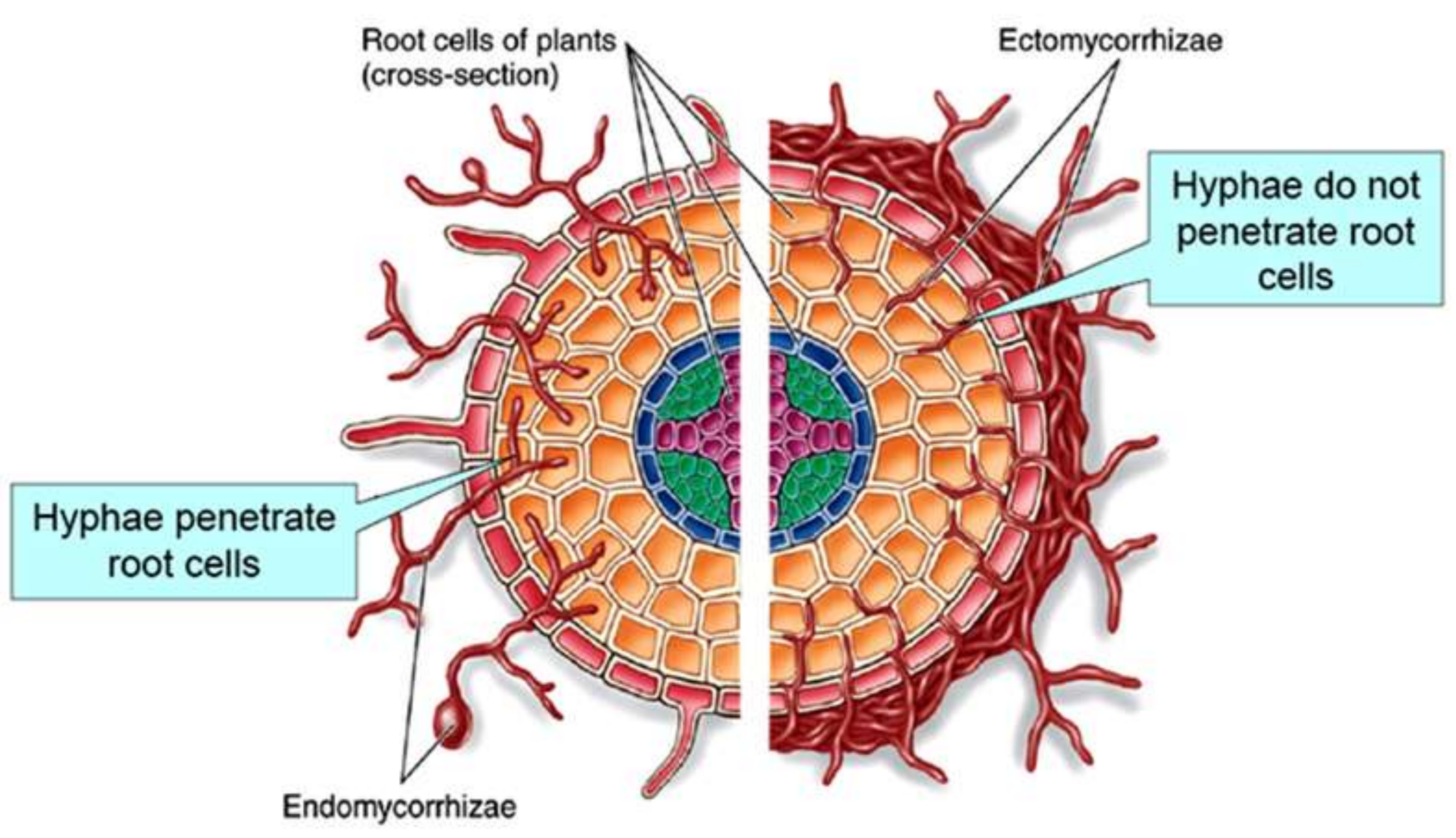




* Others are ectomycorrhizal fungi.

Disease Biological Control

Mycorrhiza Case



Disease Biological Control

Mycorrhiza Case

- Endomycorrhizal fungi can reduce the incidence of root-knot nematode, increase plant stress tolerance and block pathogen infections.

Disease Biological Control

Mycorrhiza Case

- The damage due to *Pseudomonas syringae* on tomato is reduced when the plants are well colonized by these endomycorrhizae → actions include physical protection and chemical interaction.

Disease Biological Control

Mycorrhiza Case

- Endomycorrhizal fungi can act also indirectly to suppress plant pathogens → enhancing nutrition to plants, increasing lignification of roots, changing chemical composition of plant tissues like antifungal chitinase, isoflavonoids,…

Disease Biological Control

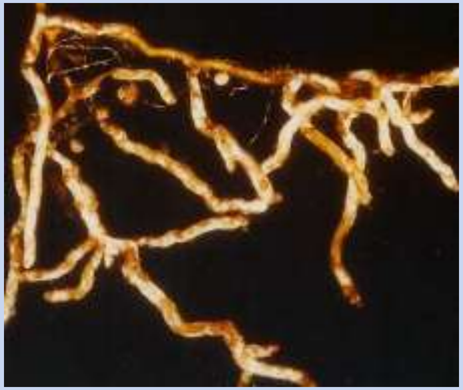
Mycorrhiza Case

- Ectomycorrhizal fungi proliferate outside the root surface and form a hyphal sheath around roots.
- Ectomycorrhizal fungi act by multiple mechanisms including antibiosis, fungistatic compounds, physical barrier,…

Disease Biological Control

Mycorrhiza Case

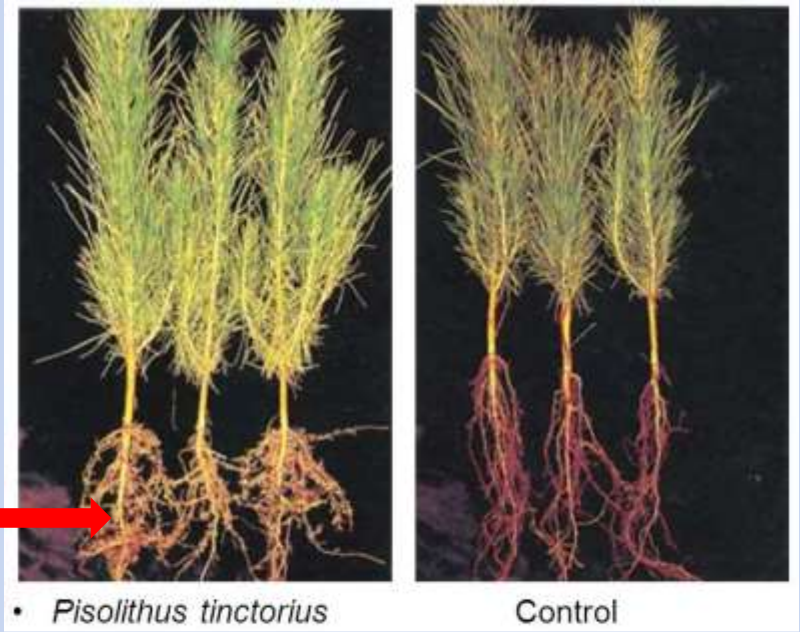
- **Ex:** ***Paxillus involutus*** **ectomycorrhiza controls root rot caused by *Fusarium oxysporum* and *Fusarium moniliforme* in red pine.**



Disease Biological Control

Mycorrhiza Case

- *Pisolithus tinctorius*, another ectomycorrhizal fungus, reduces sand pine disease caused by *Phytophthora cinnamomi*.



Disease Biological Control

(14) Host Resistance Induction

- In addition to direct and indirect actions of antagonists (on pathogens), they can stimulate the host resistance.

- Hence, host inoculation with plant-growth-promoting rhizobacteria (PGPR) is effective in controlling many diseases: anthracnose (*Colletotrichum lagenarium*), angular leaf spot (*Pseudomonas syringae* pv. *lachrymans*), bacterial wilt (*Erwinia tracheiphila*).

Disease Biological Control

Host Resistance Induction

- Resistance is induced by chemical elicitors produced by PGPR (salicylic acid, siderophores, lipopolysaccharides,…).

- PGPR act also by increasing plant growth, for example, by associative N_2 fixation, solubilizing nutrients such as P, promoting mycorrhizal function, regulating ethylene production in roots, releasing phytohormones, decreasing heavy metal toxicity.

Disease Biological Control

Host Resistance Induction

- In addition to PGPR, other endophytic (and even epiphitic) bacteria can induce resistance in host plant.

- Advantage of the endophytic bacteria is to be relatively protected from the competitive organisms and the environmental stress.

Disease Biological Control

(15) Biocontrol Agents Combination

- Improving the biological method in the rhizosphere may be to add mixtures of biocontrol agents, (exhibiting different or complementary modes).

- Ex: Application of a combination of three PGPR, *Bacillus pumilus*, *Bacillus subtilis* and *Curtobacterium flaccumfaciens* provided greater control of several pathogens on cucumber than when inoculated singly.

Disease Biological Control

Biocontrol Agents Combination

- **Ex.: Combination of fungi and bacteria:** ***Trichoderma koningii*** **+** ***Pseudomonas chlororaphis*** **or** ***P. fluorescens*** **provided greater suppression of take-all of wheat than** ***T. koningii*** **alone.**

- **Important: No member of the combination should be inhibitory to another.**

Disease Biological Control

(16) Selection of Antagonists

- Selection starts with screening of large number of microorganisms.

- Selected antagonists must be able to colonize the habitats, to occupy specific niches and to interfere with the growth and survival of the target pathogen.

- Hence, the best place to look for potential antagonists: is the specific environment in which they will be used.

Disease Biological Control

(17) Production of Antagonists

- **Ex:** Against pathogens infecting plant roots: look for antagonists in the rhizosphere.
- Antagonists need to be harvested, packaged, and delivered in a viable form.
- Once applied, antagonists must grow and persist in the environment for sufficient time to exert effective control against pathogens.

Disease Biological Control

Production of Antagonists

- Different antagonist formulations: freeze-drying cultures, mixture with inert carriers (clay, talk), encapsulation in alginate polymer,...

- Application methods: as liquid spray or drench, seed-dressing, powder, or pellets.

Disease Biological Control

(18) Commercialized Antagonists

*** Fungi**

- *Trichoderma harzianum* (F-Stop) for control of soilborne fungal pathogens,

- *Gliocladium virens* (GlioGard) for control of seedling diseases,

- *Trichoderma harzianum/T. polysporum* (BINAB T) for control of wood decays,

- *Candida oleophila* (Aspire) for control of postharvest decay in citrus and apples.

Disease Biological Control

Commercialized Antagonists

*** Bacteria**

- *Pseudomonas fluorescens* (Dagger G) for control of *Rhizoctonia* and *Pythium* damping-off of cotton,

- *Bacillus subtilis* (Kodiak) as a seed treatment.

Disease Biological Control

(19) Natural Products Use

*** From soil:**

- Copper sulfate (blue color): $CuSO_4$
- Sulfur (yellow color): S

*** From living organisms:**

- Essential oils from plants

Disease Biological Control

Bio-fungicides in Tunisia

* **Biological fungicides (antagonists and substances) are encouraged:**

↓

Bio-fungicide License Fee = 50% of Chemical Fungicide License Fee

END

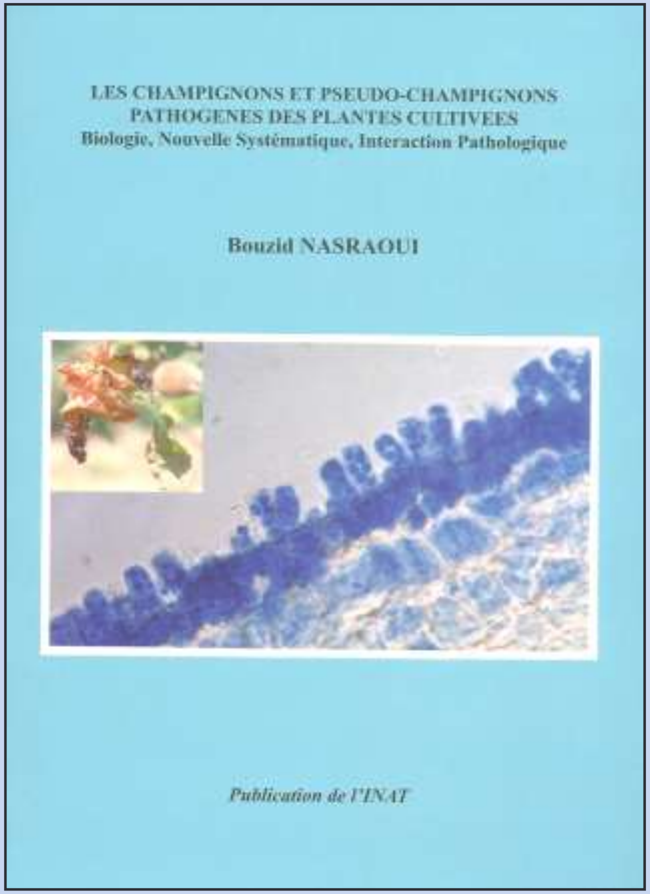
* Some presentations:

- The Reform of the Pesticide Registration Procedure in Tunisia (2009 - 2012) [2015]

- Main Cereal Rusts [2016]

- The Main Quarantine Bioaggressors Threatening Strategic Crops in Maghreb Countries [2017]

* My last book: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Biologie, Nouvelle Systématique, Interaction Pathologique
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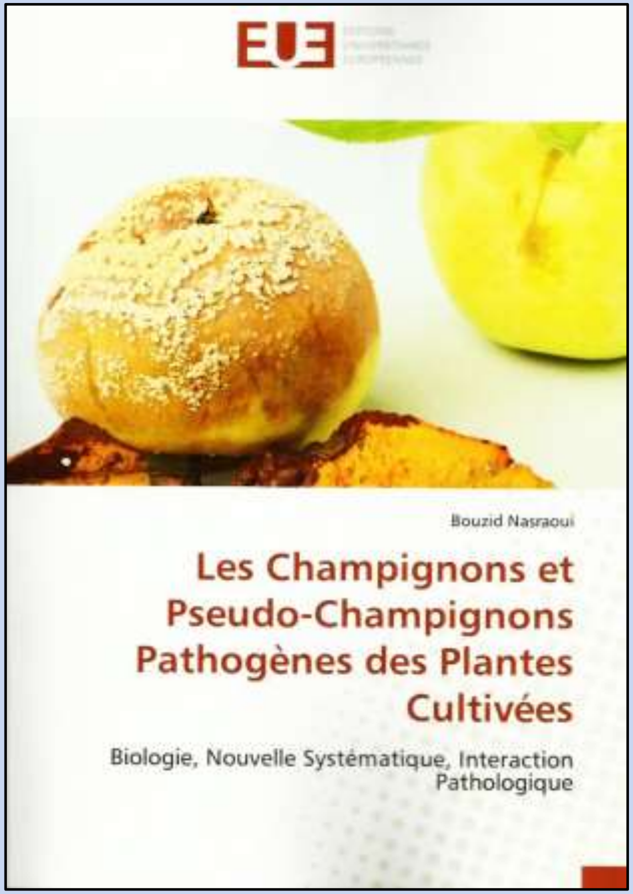
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*** Presentations and book are freely available on my personal website:**

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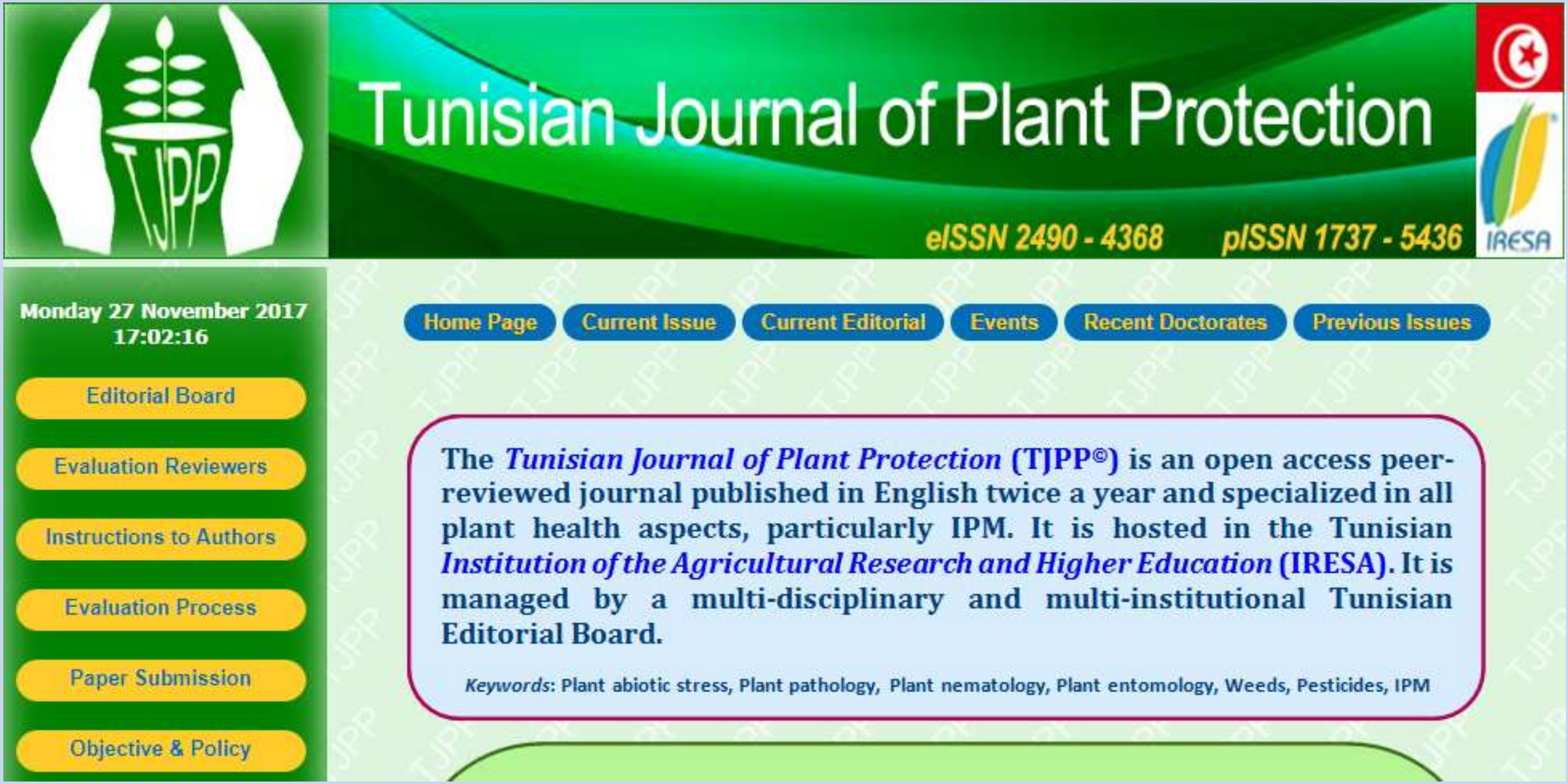
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Thank you for your attention