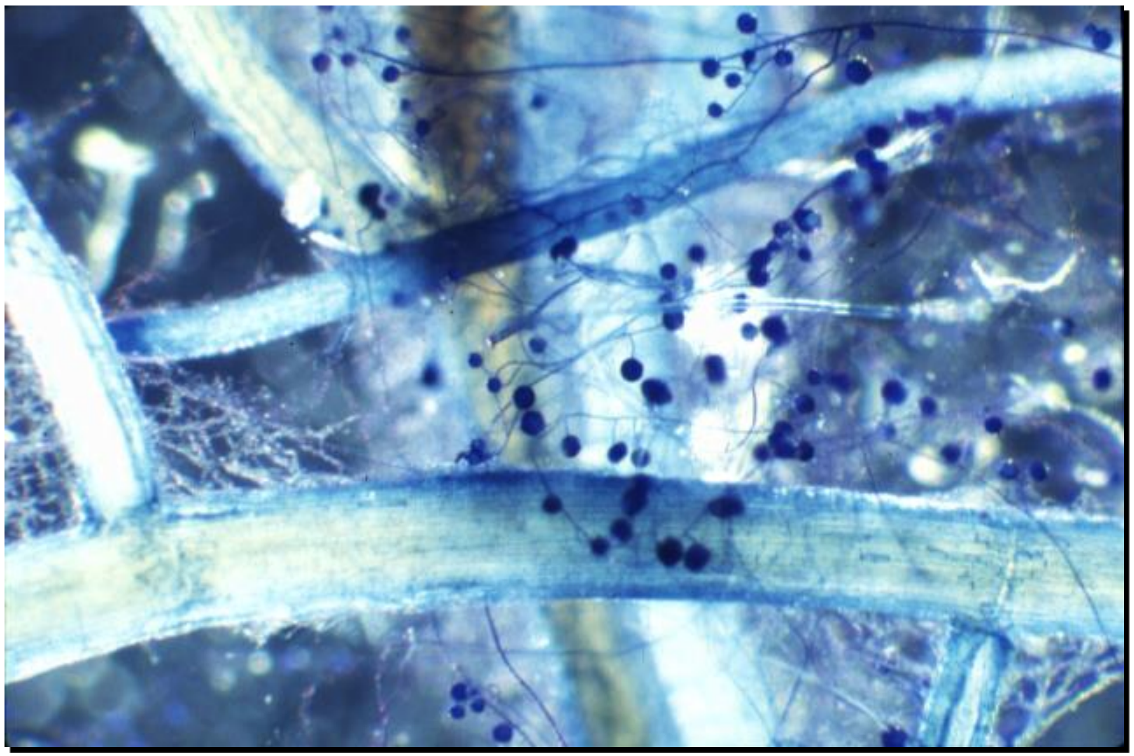
Mycorrhyzes in vitro:

De la recherche fondamental jusqu'à l'application à grande échelle



Dr. Alberto Bago
Científico Titular, CSIC
Co-promotor, MYCOVITRO S.L.

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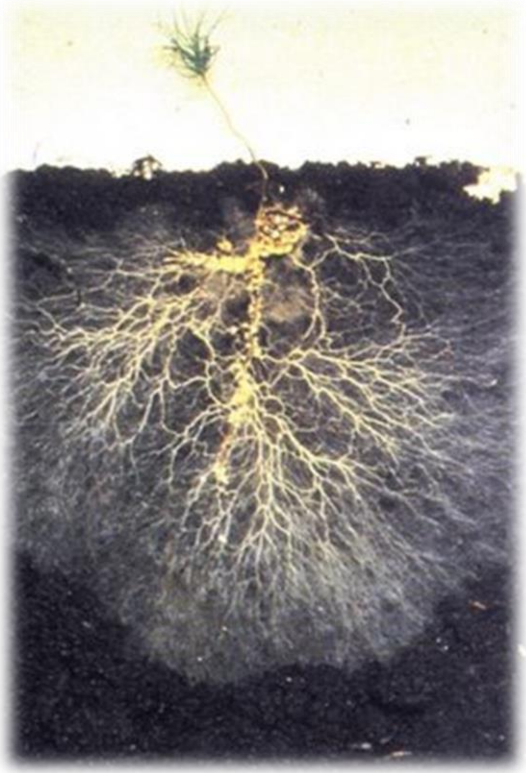
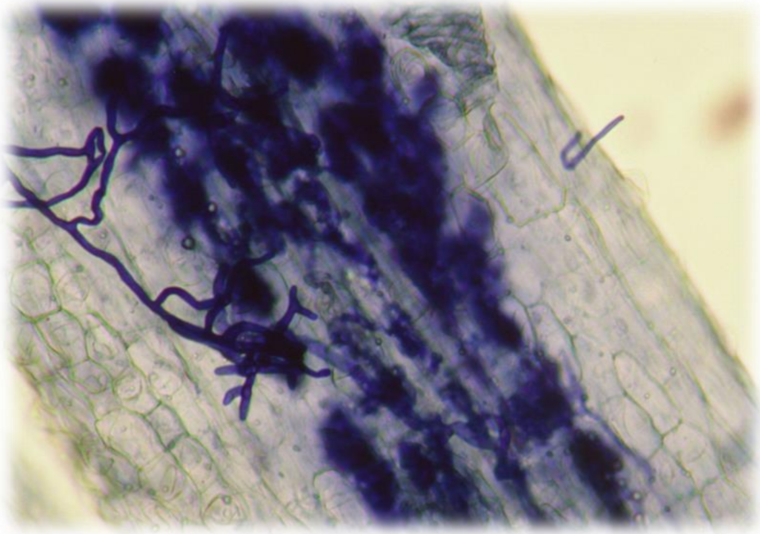
mycovitro
biotecnología ecológica

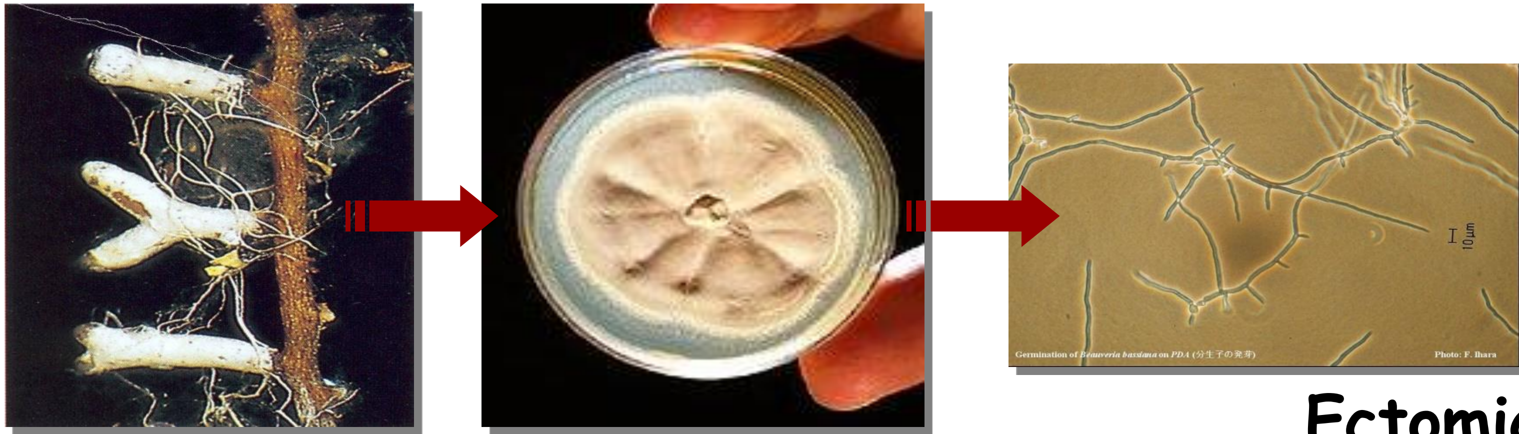
Mycorrhizas are mutualistic symbiosis established between 97% of the land plants and some types of soilborne fungi, named "mycorrhizal fungi"



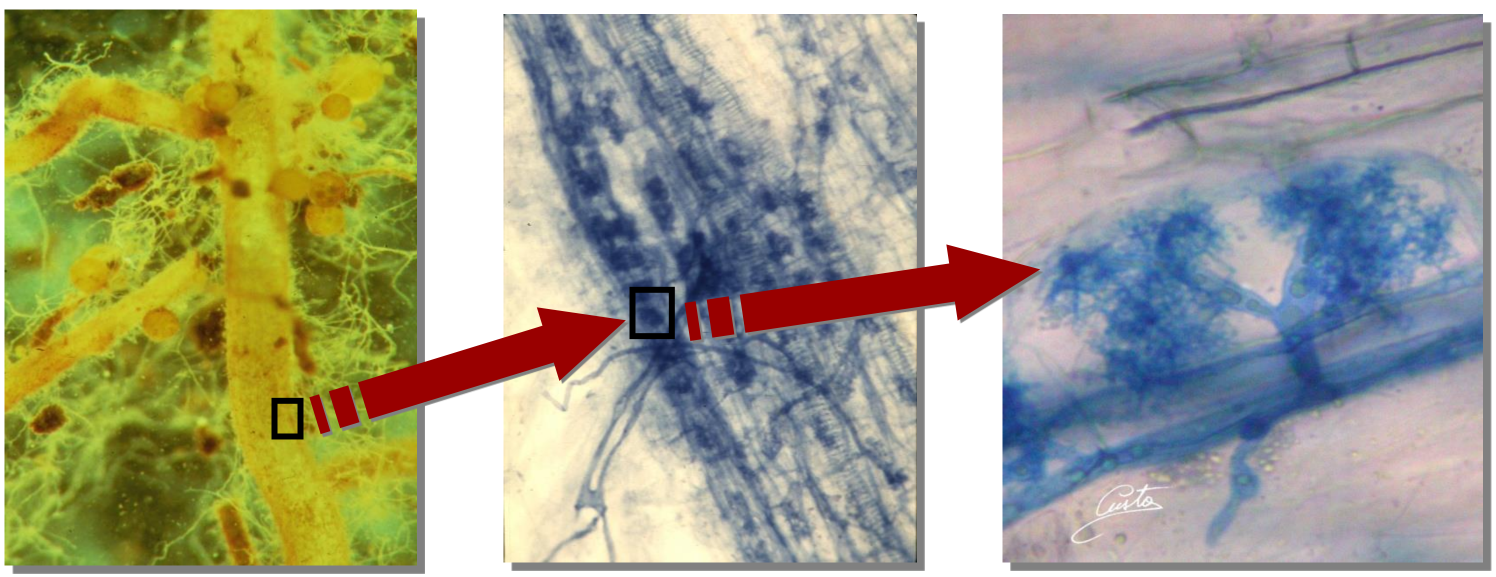


MYCORRHIZAS ESTABLISH THE PRIMARY NETWORK FOR ALL THE OTHER MICROBIAL SOIL COMMUNITIES

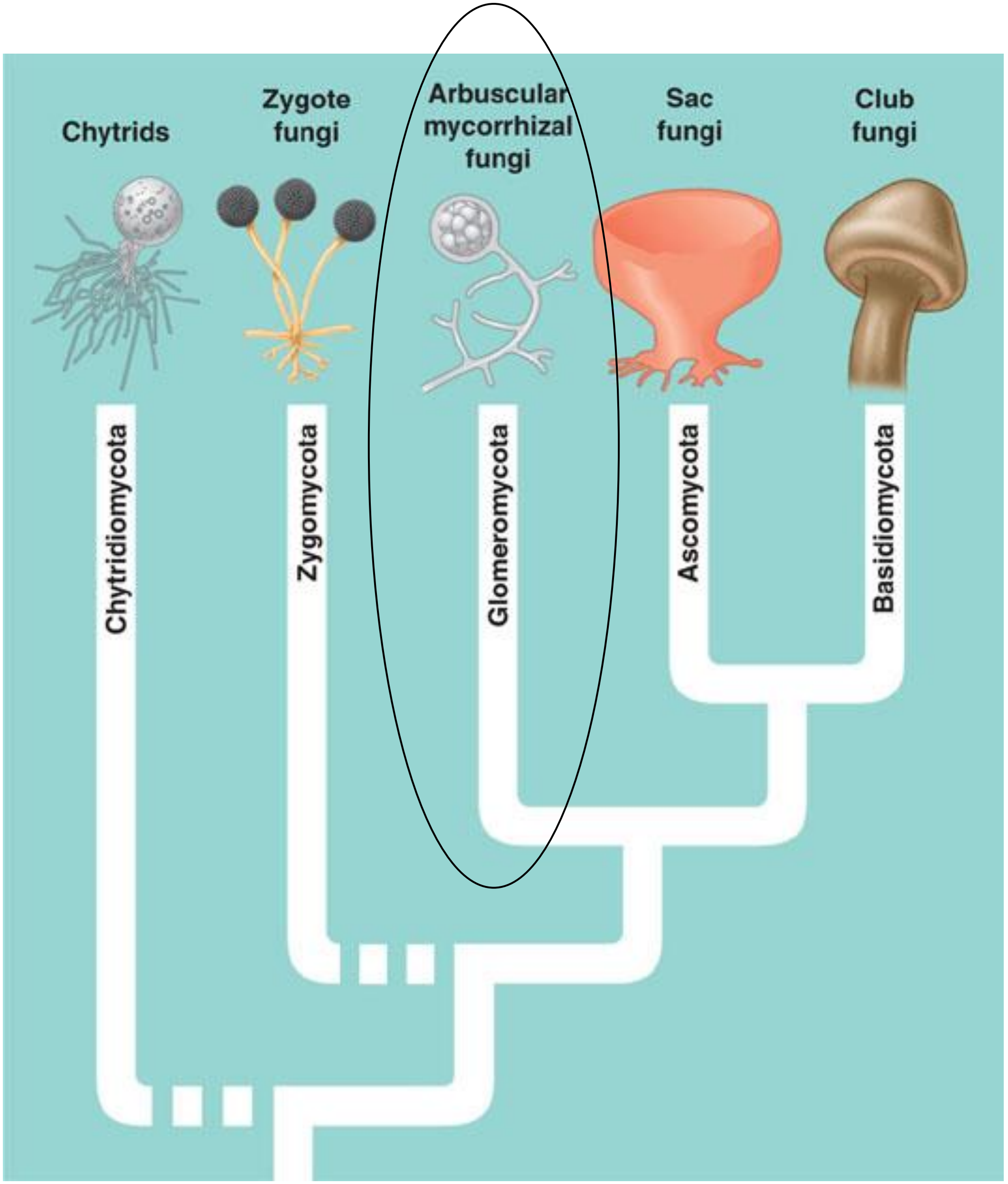
Five types of mycorrhizas, two more abundant



Ectomicorrizas



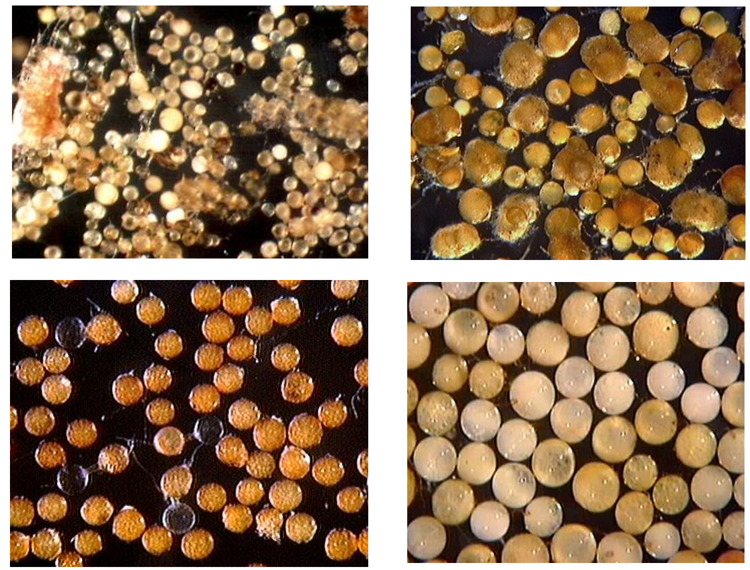
Arbuscular mycorrhizas



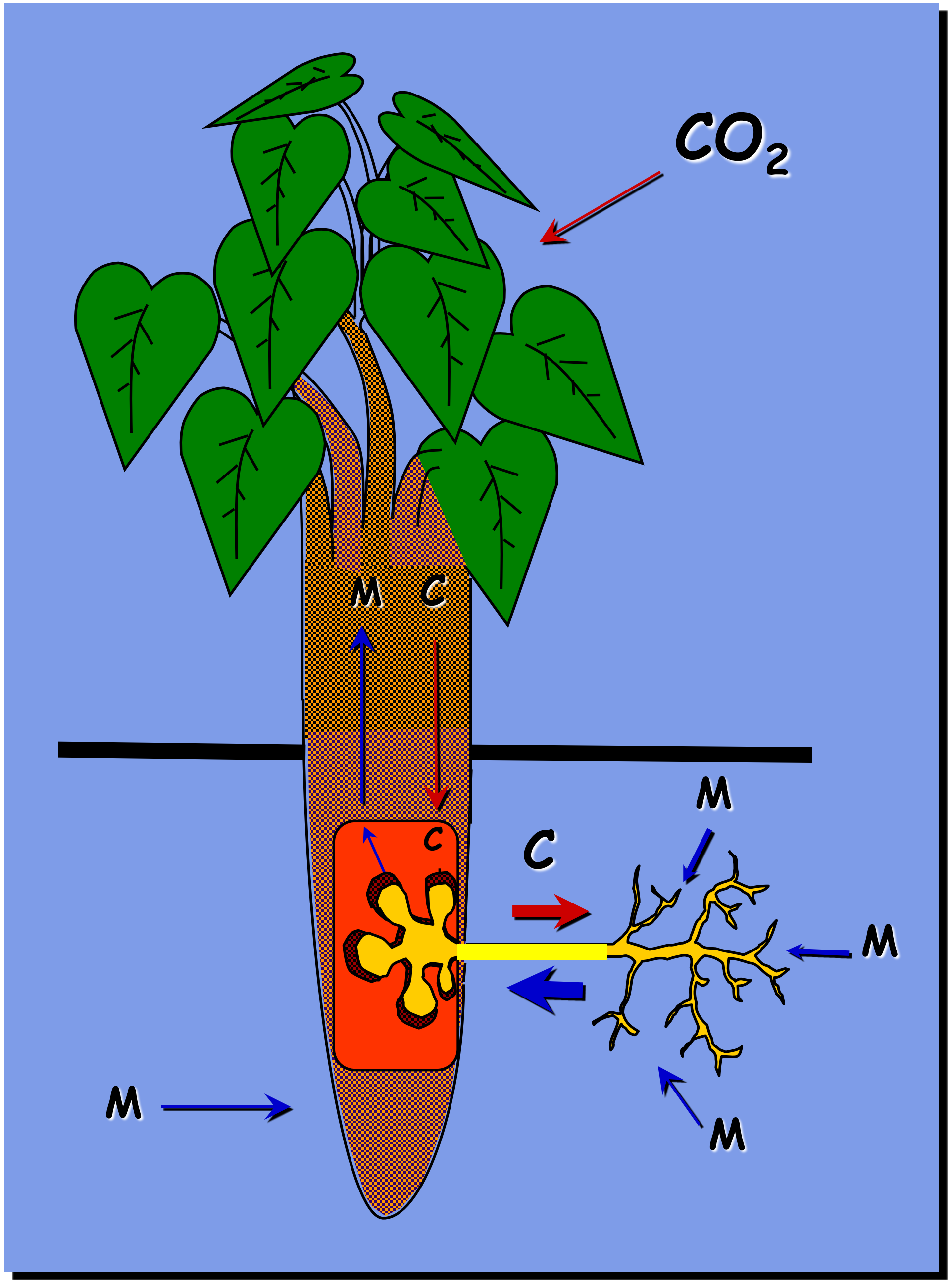
Kingdom Fungi

Arbuscular mycorrhizal fungi

- Monophyletic group, prior to Asco- and Basidiomycota



- Minimal differencies between spores; quite recent molecular tools



MYCORRHIZAS:

"...a symbiosis in which an external mycelium of a fungus supplies soil derived nutrients to a plant root."

(Smith and Read, 1997)

Mycorrhiza formation usually increases plant biomass

↓

Consequently, plant increases its photosynthetic rate in a 4 to20%

↓

...meaning that over five billion tons of C per year are fixed by mycorrhizal plants!!!!!

BENEFITS OF APPLYING MYCORRHIZA TO SOILS

✓ **Increases plant root area:**
Better efficiency in water and nutrients uptake.

✓ **Optimizes Plant Nutrition**

✓ **Absorption of less available nutrients, specially PHOSPHORUS**

✓ **Stimulates plant growth and survival during stress conditions such as** drought, salinity, poor soils or extreme pH, etc

✓ **Balances other elements absorption:** copper, clhorine,…

✓ **Better resistance against pathogens: Better nutritional status, cell wall thickening, activation of defense pathways, competition for space**

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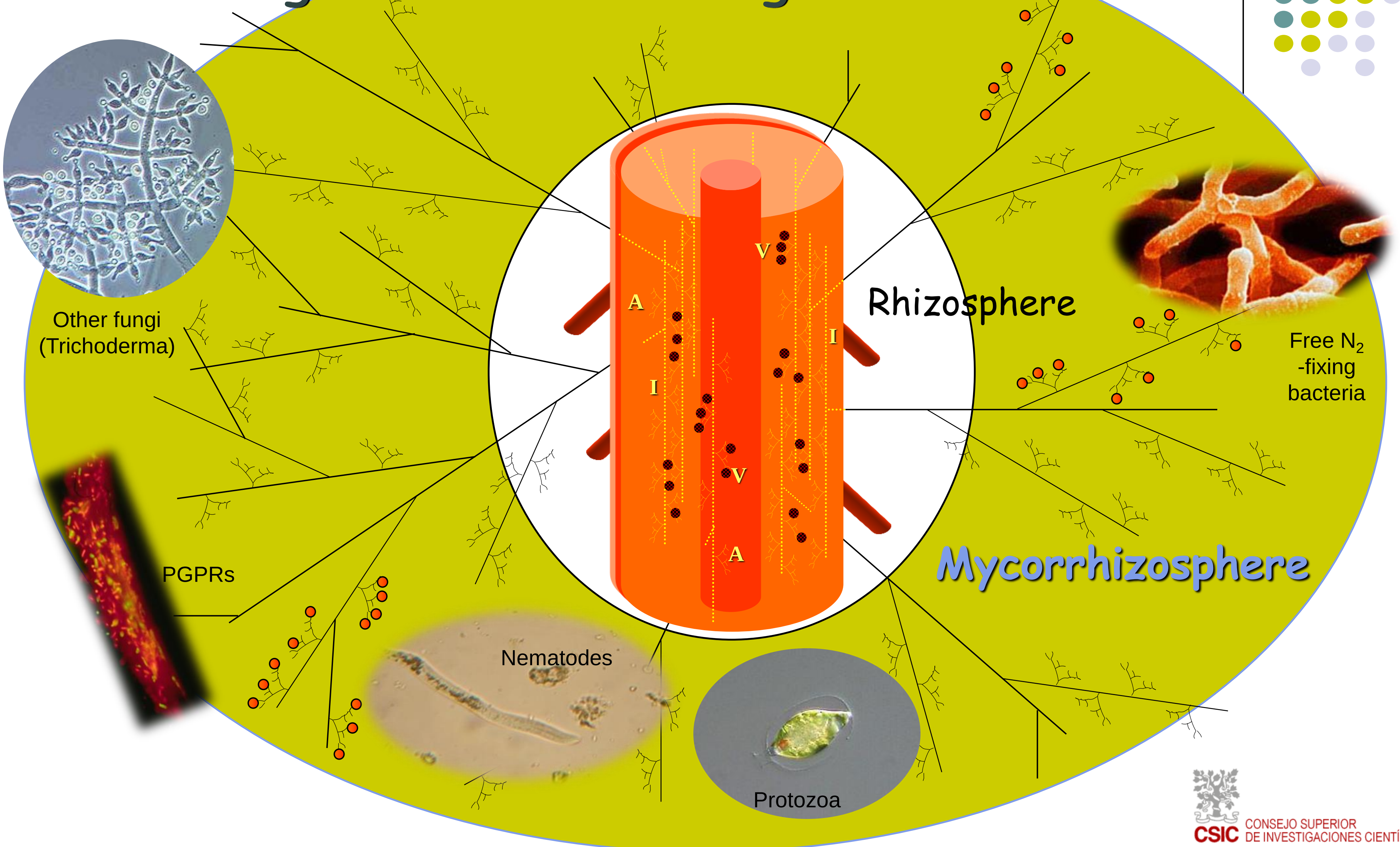
Arbuscular mycorrhizal fungi: mycelial networking and soil microorganism consortia



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If Mycorrhizae are so good, why we have not used them so far?

Because we didn't know them good enough to produce and use them correctly

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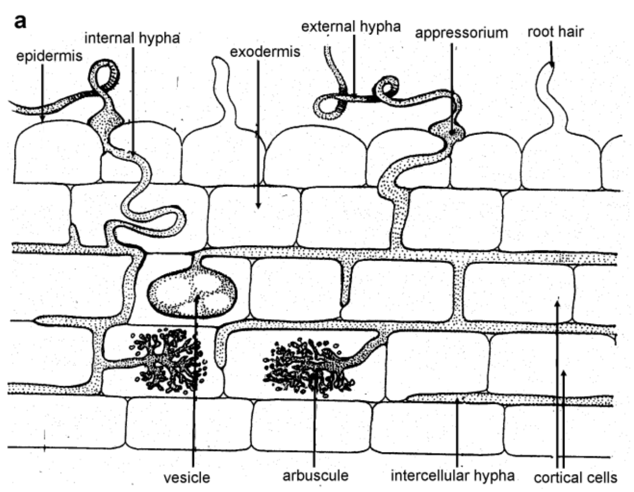
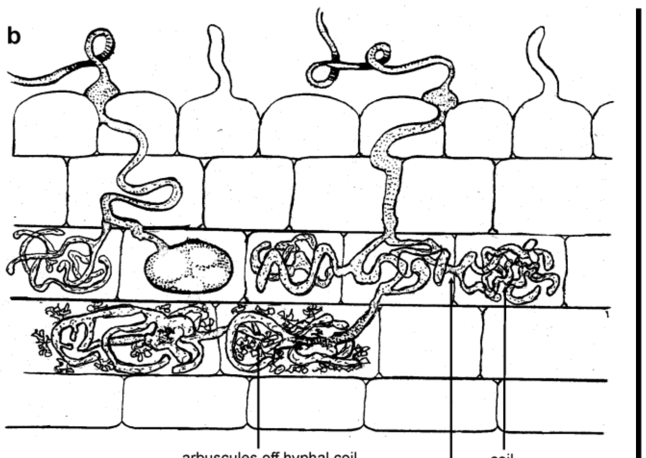
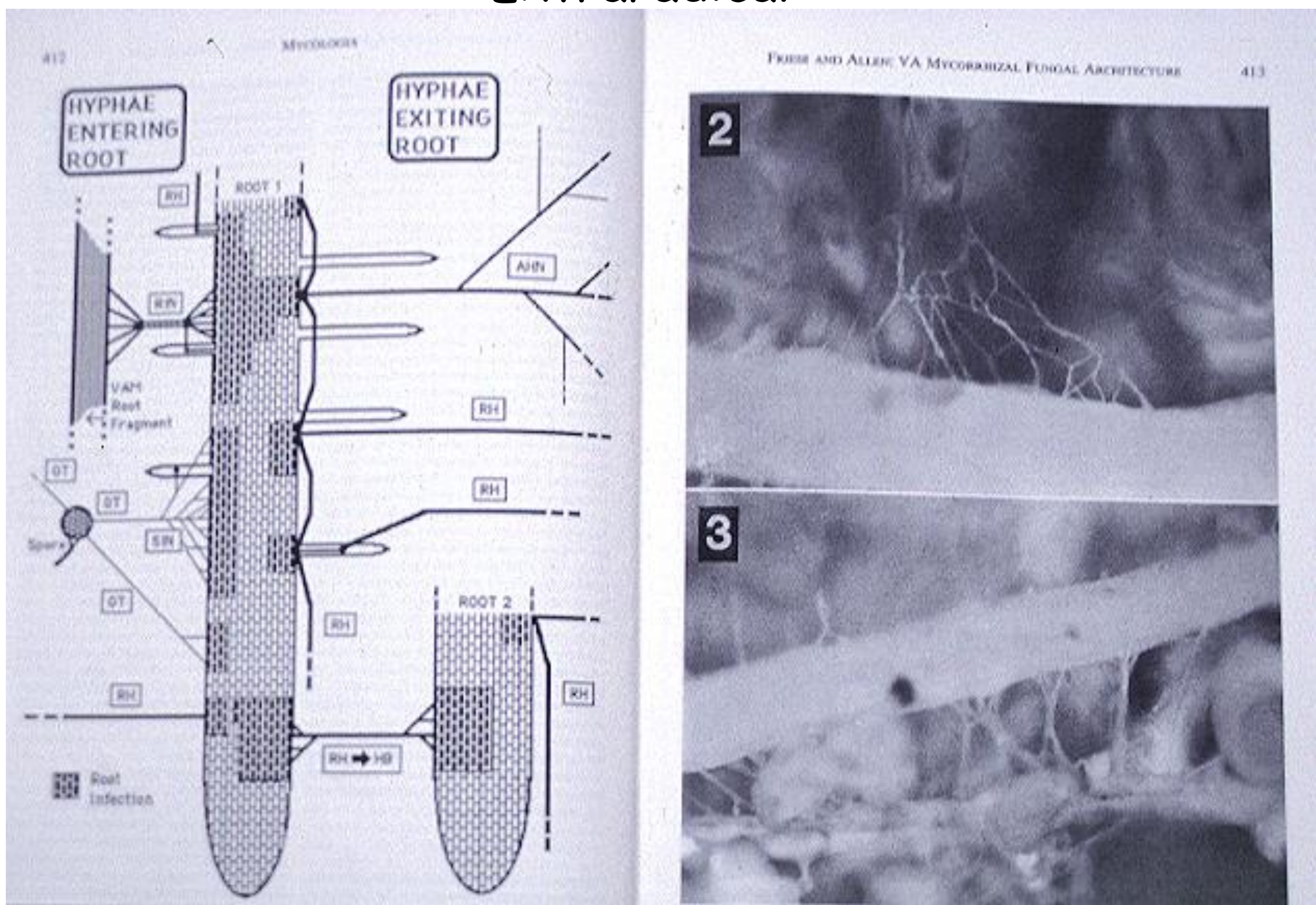
The fungal colony of arbuscular mycorrhizas as we knew it by 1990's



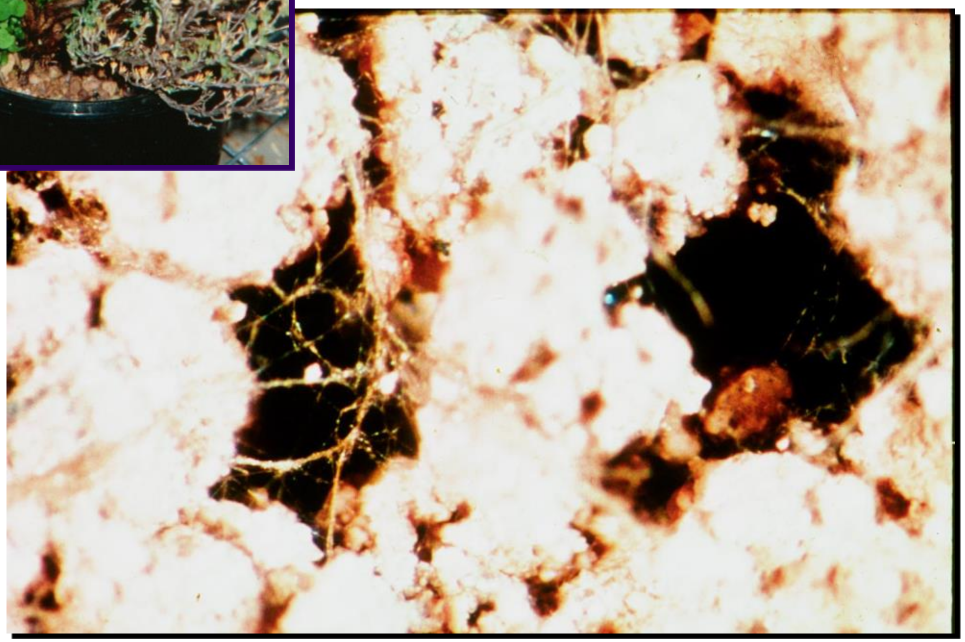


Intraradical

Extraradical



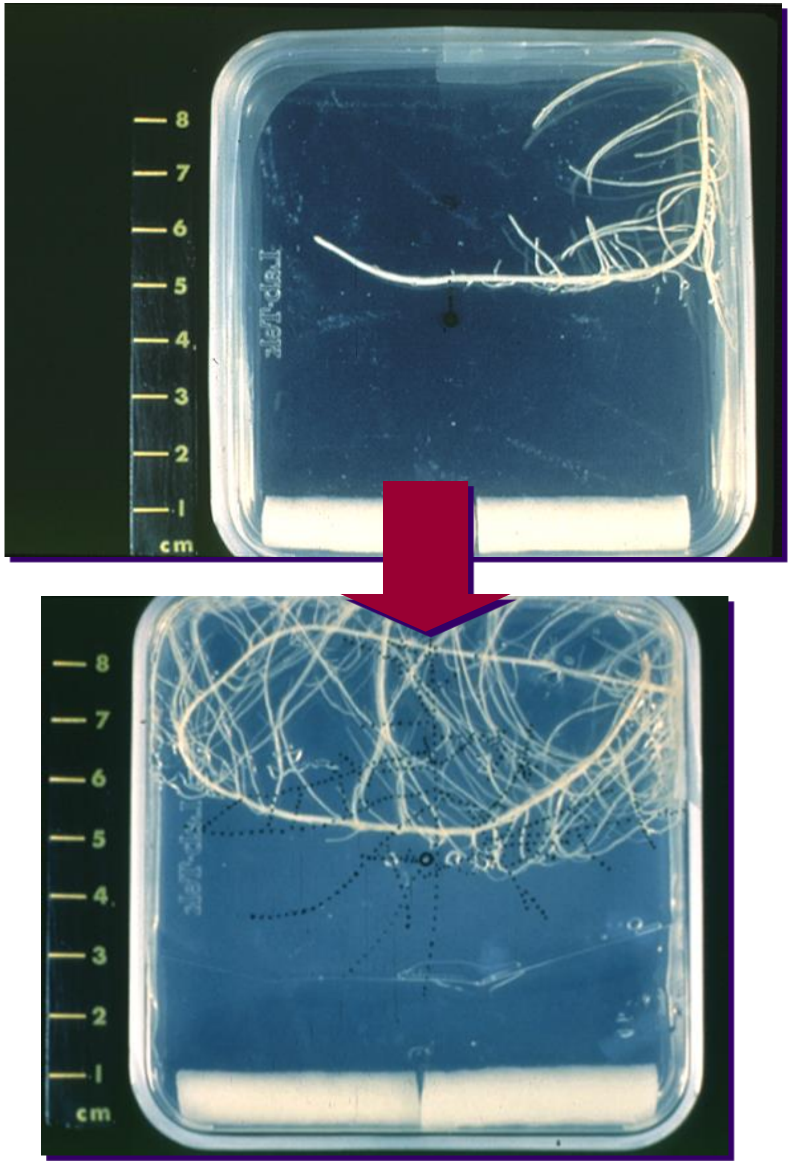
Different AMF culture systems



I. Soil systems:

- Difficult to follow on-site
- Extraction of hyphae is usually destructive
- Most of fine structures cannot be visualized

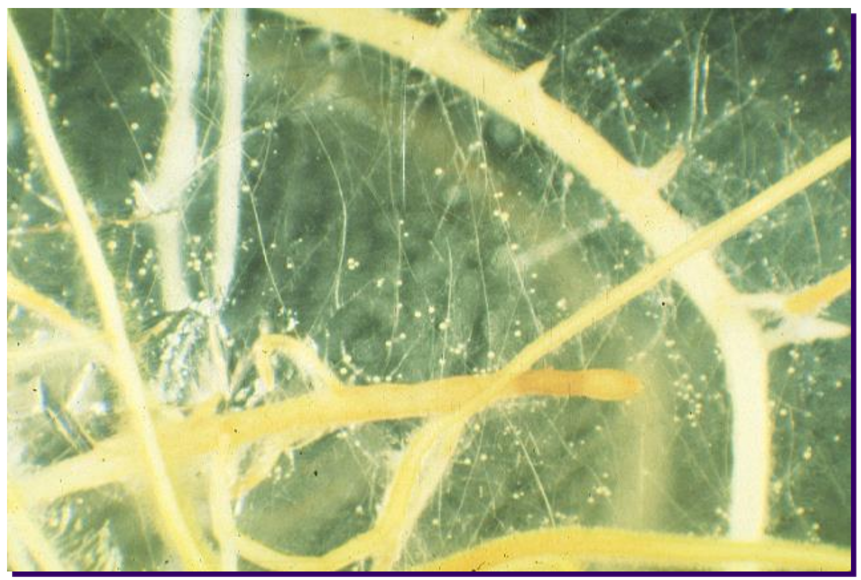
Different AMF culture systems



Bécard & Fortin (1988)

II. AM monoxenic cultures:

- Allow continuous, non-destructive measurements and observations
- Compatible with high resolution techniques and *in vivo* microscopy



Monoxenic culture of arbuscular mycorrhizas: historical milestones

1962 Mosse's first report of mycorrhiza under "aseptic" conditions

1975 Mosse and Hepper describe the first successful monoxenic culture

1986 Strullu and Romand use intraradical vesicles as starting material

1987 Mugnier & Mosse use ROC as host for mycorrhiza "monoxenic" culture

1988 Bécard and Fortin establish the first successful, continuous, aseptic mycorrhizal monoxenic culture

1996 St-Arnaud et al. develop the two-compartment system. First reports on physiological applications (Bago et al., Villegas et al.)

1998 Use of monoxenic cultures for studies on morphogenesis, biochemistry and genetics widens. Mycorrhizal "gurus" still reluctant.

2001 The Glomeromycota In vitro Collection (Canada, Belgium) is inagurated as the first bank of in vitro AM germoplasm.

2000's: The dawn of the "mycorrhizal monoxenic age"…

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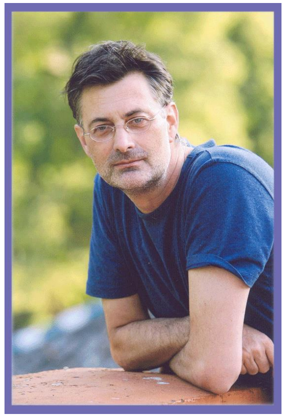
Research on MYCORRHIZAS at Université Laval

A. Fortin

G. Bécard

Y. Piché

L. Simon



H. Vierheilig

Monoxenic culture of mycorrhizas:
contribution to basic reserach

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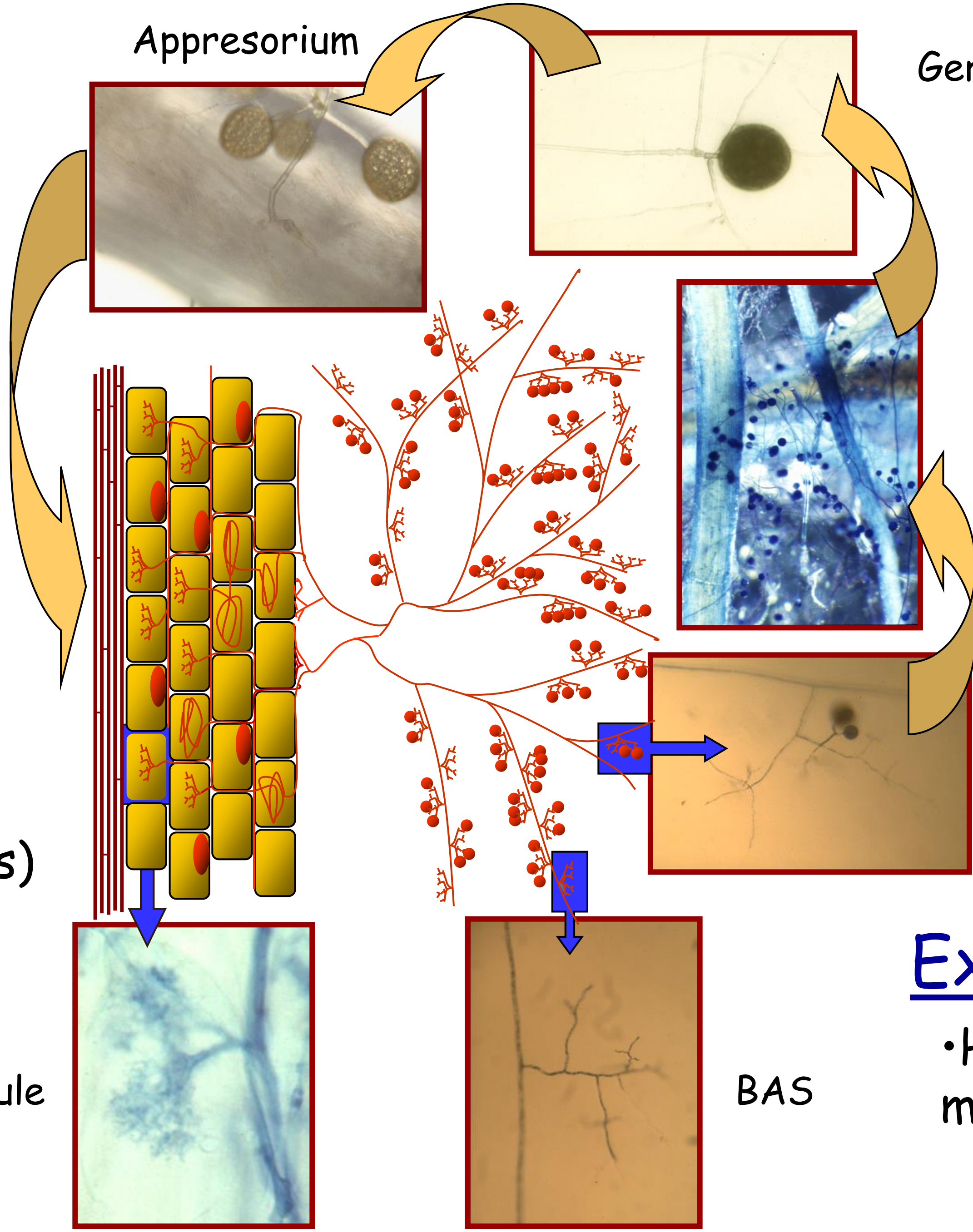
Life cycle



Intraradical

•Homogeneous medium (root cells)

Asymbiotic

•Spore sucessive regerminations until symbiosis establishment

Extraradical

•Heterogeneous medium (soil)

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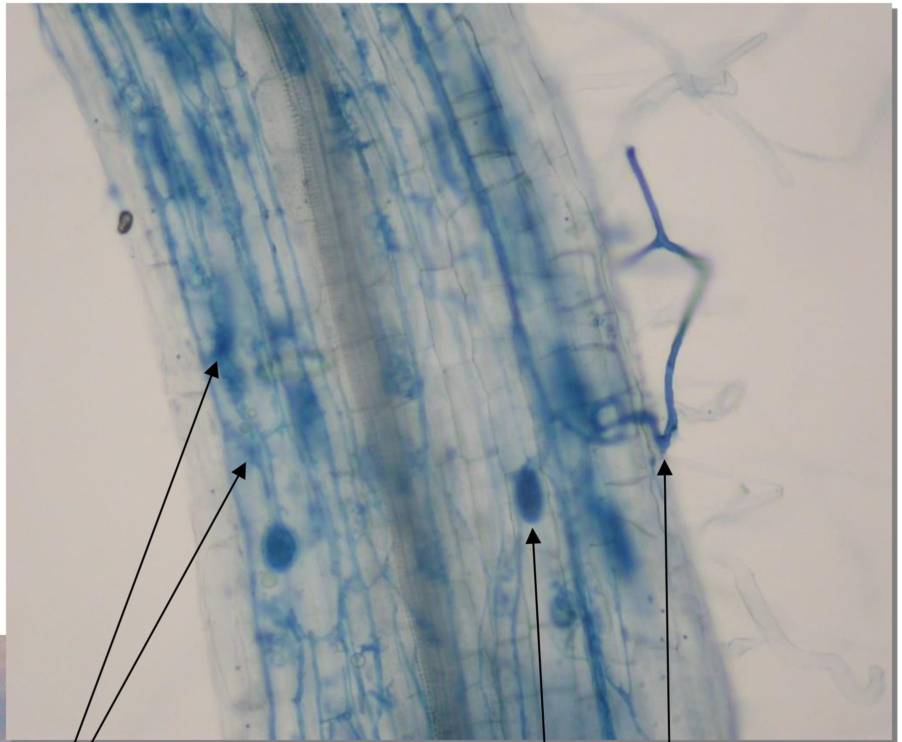
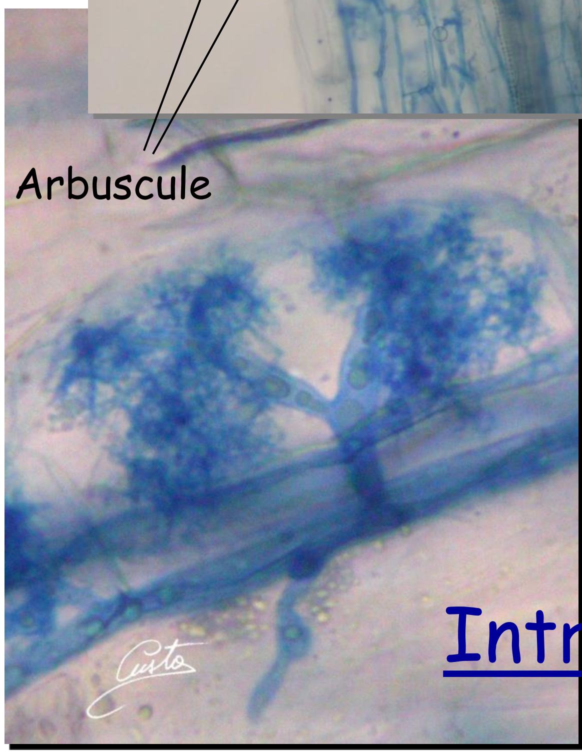
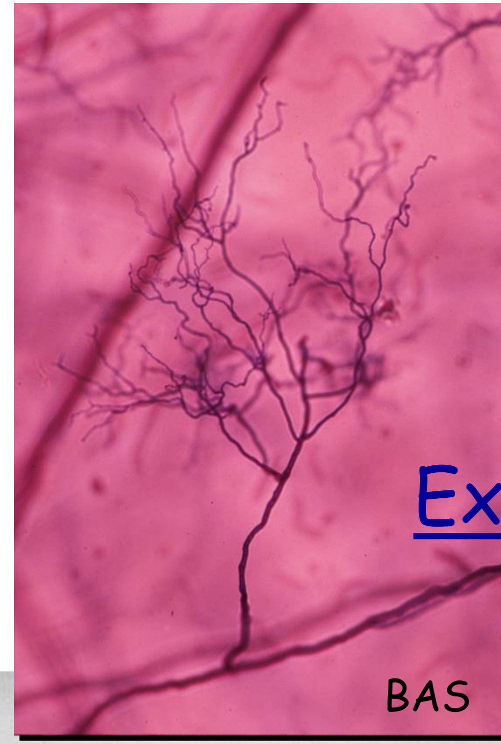
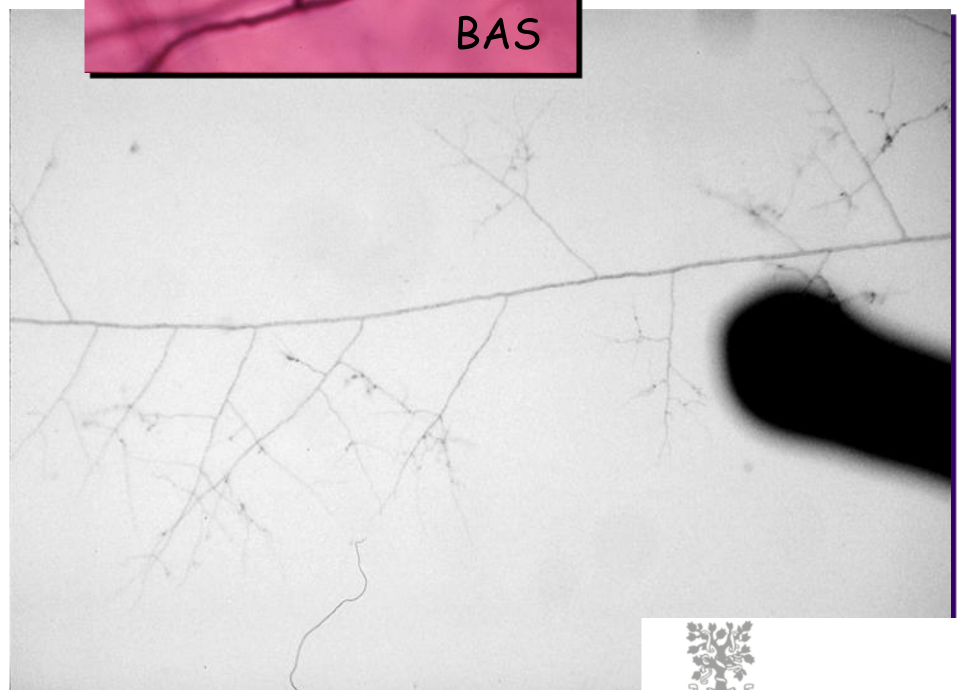




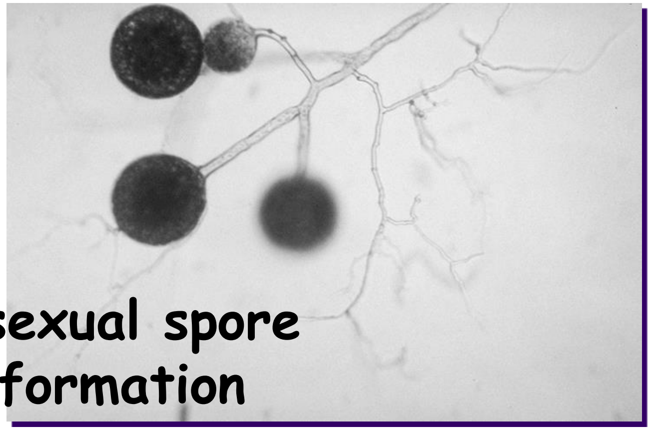
Intraradical

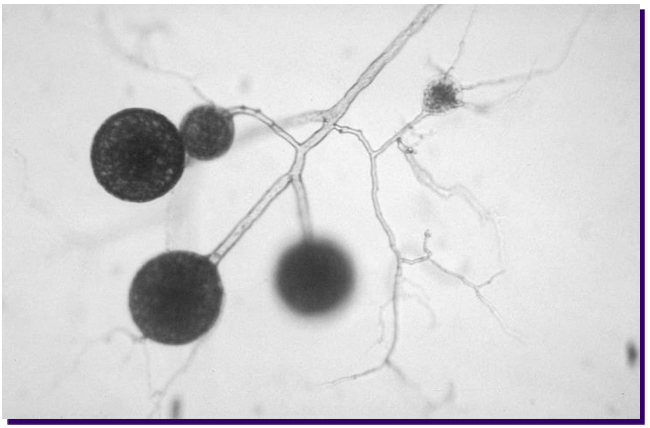


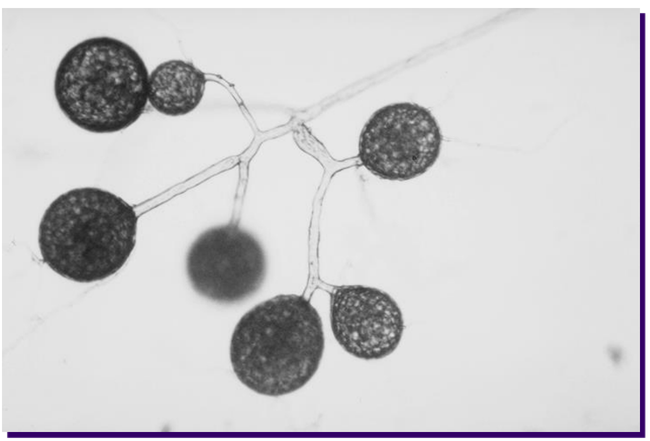
Extraradical



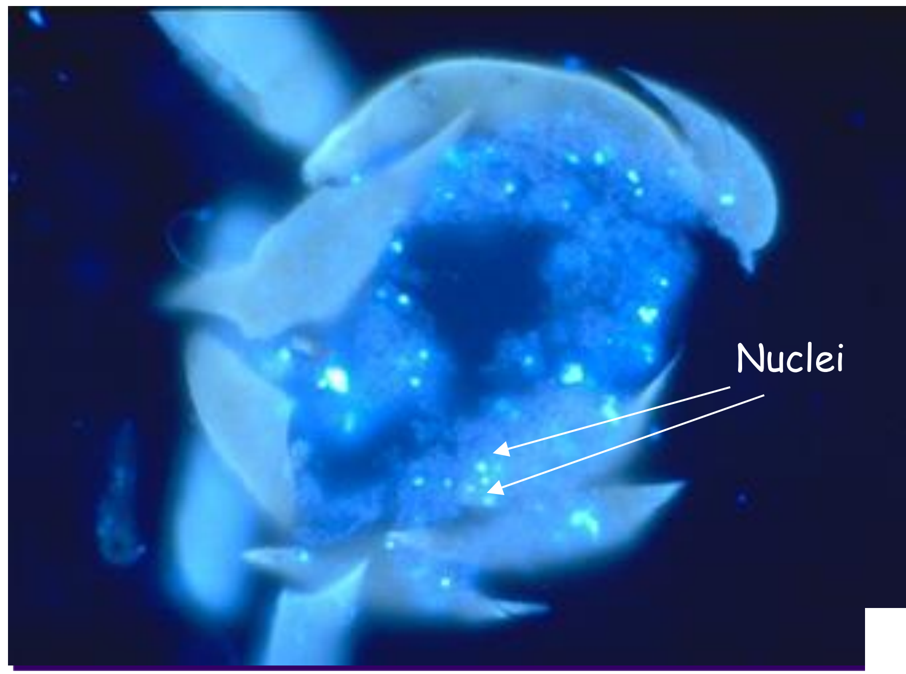
Asexual spore formation







Crushed spore



DAPI staining

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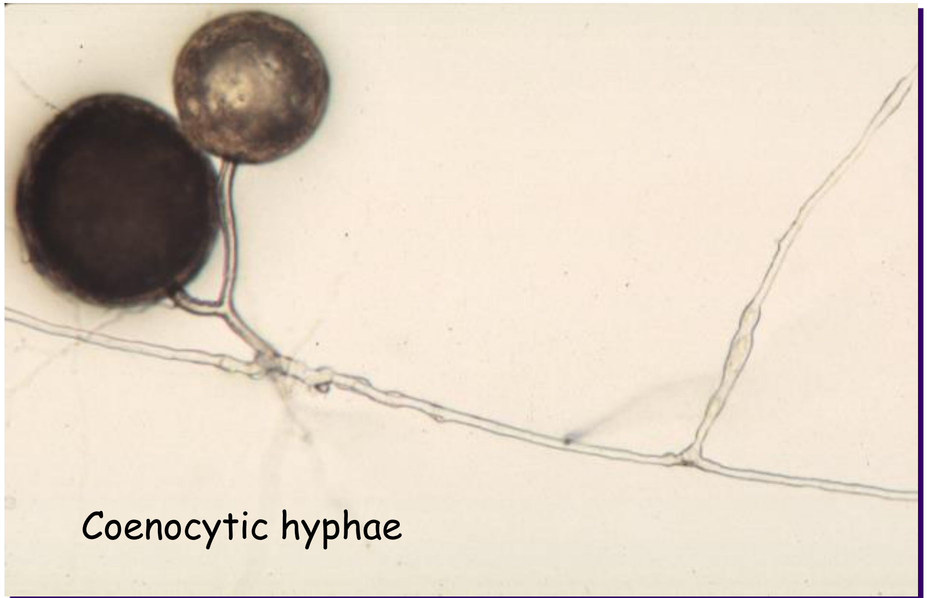
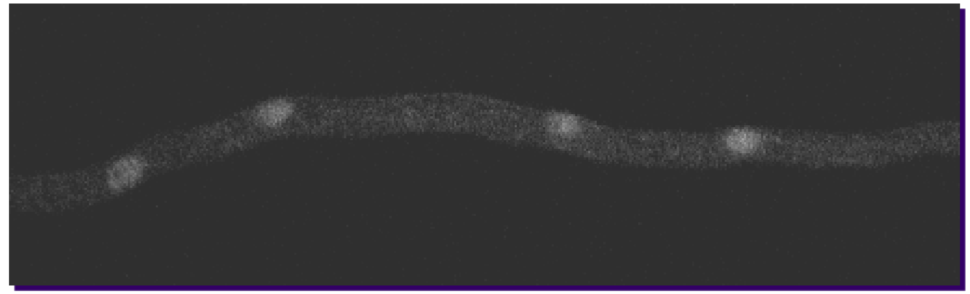
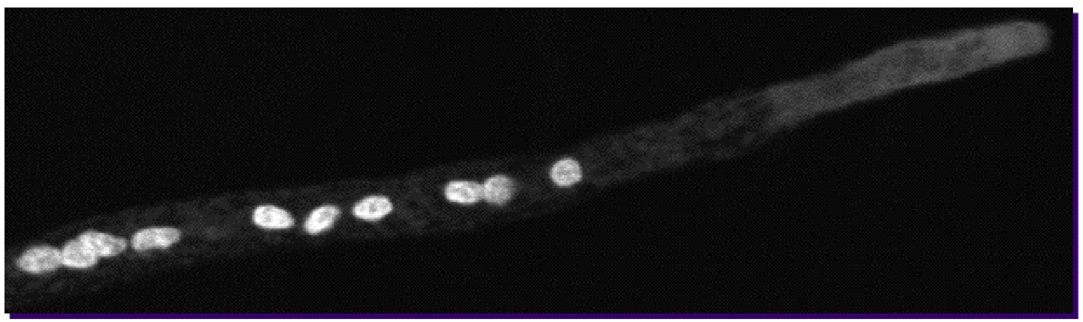
Arbuscular mycorrhizal fungi: coenocytic and multinucleated



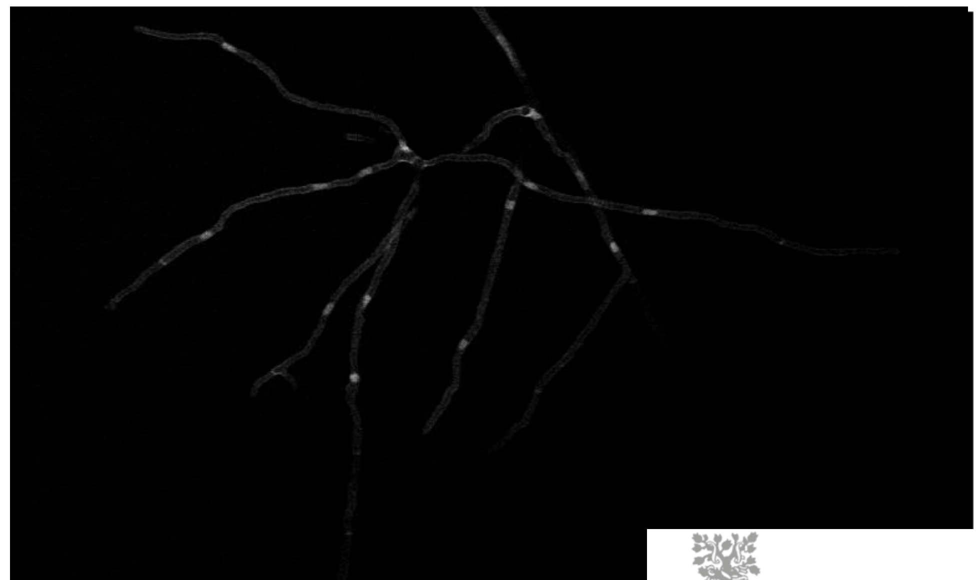
Coenocytic hyphae



Lateral nuclei controlling hyphae

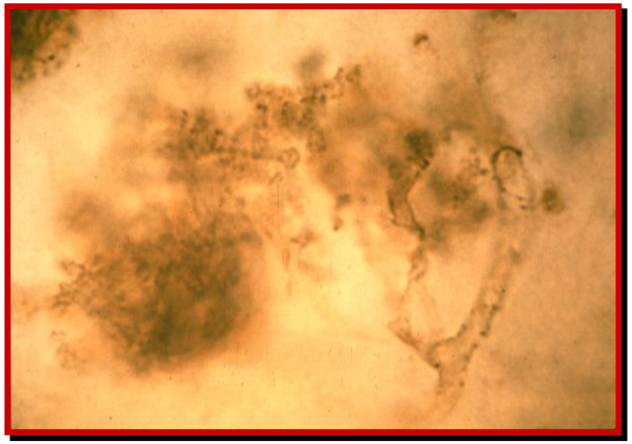


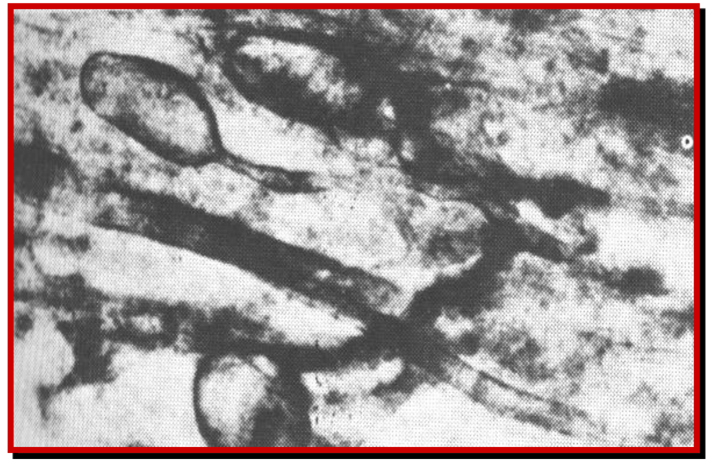
Migrating nuclei towards hyphal apex



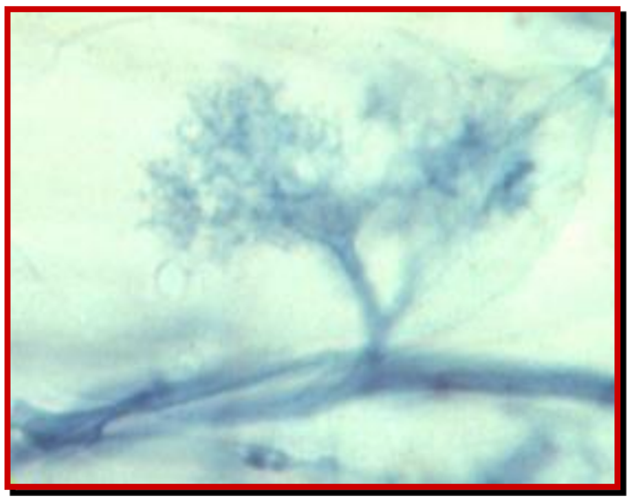
Nuclei in BAS

Co-evolution of Glomeromycota with land plants:



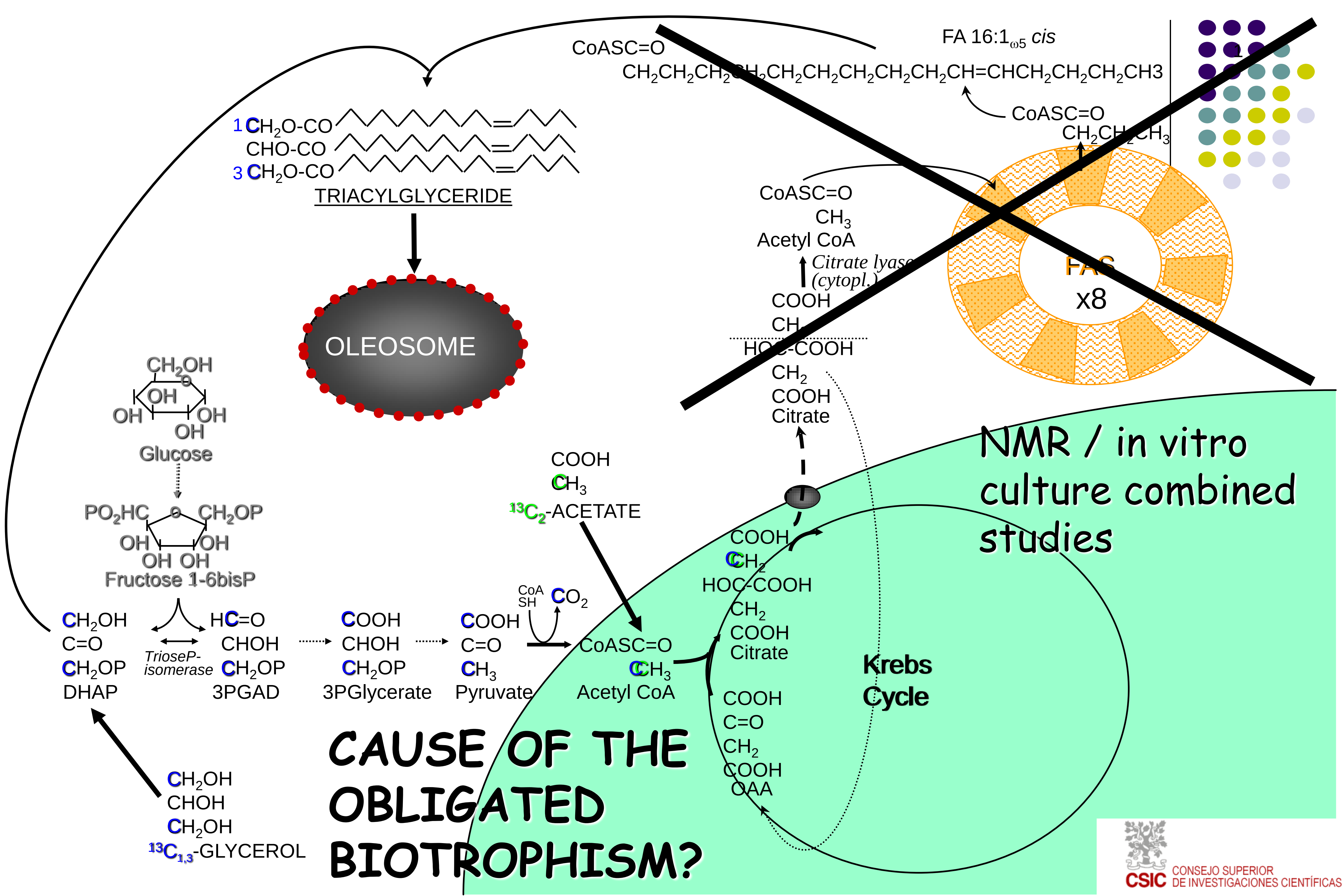


Fossil mycrrhiza c. 400 M year



Contemporary mycorrhiza

OBLIGATE SYMBIONTS


FA 16:1ω5 cis
CoASC=O
CH2CH2CH2CH2CH2CH2CH2CH2CH2CH=CHCH2CH2CH2CH3
CoASC=O
CH2CH2CH3
1 CH2O-CO
CHO-CO
3 CH2O-CO
TRIACYLGLYCERIDE
OLEOSOME
CoASC=O
CH3
Acetyl CoA
Citrate lyase
(cytopl.)
COOH
CH2
HOC-COOH
CH2
COOH
Citrate
FAS
x8
NMR / in vitro culture combined studies
CH2OH
OH
OH
OH
OH
Glucose
PO2HC
CH2OP
OH
OH
OH OH
Fructose 1-6bisP
COOH
CH3
13C2-ACETATE
CH2OH
C=O
CH2OP
DHAP
TrioseP-isomerase
HC=O
CHOH
CH2OP
3PGAD
COOH
CHOH
CH2OP
3PGlycerate
CoA SH
CO2
COOH
C=O
CH3
Pyruvate
CoASC=O
CH3
Acetyl CoA
COOH
CH2
HOC-COOH
CH2
COOH
Citrate
COOH
C=O
CH2
COOH
OAA
Krebs Cycle
CH2OH
CHOH
CH2OH
13C1,3-GLYCEROL
CAUSE OF THE OBLIGATED BIOTROPHISM?
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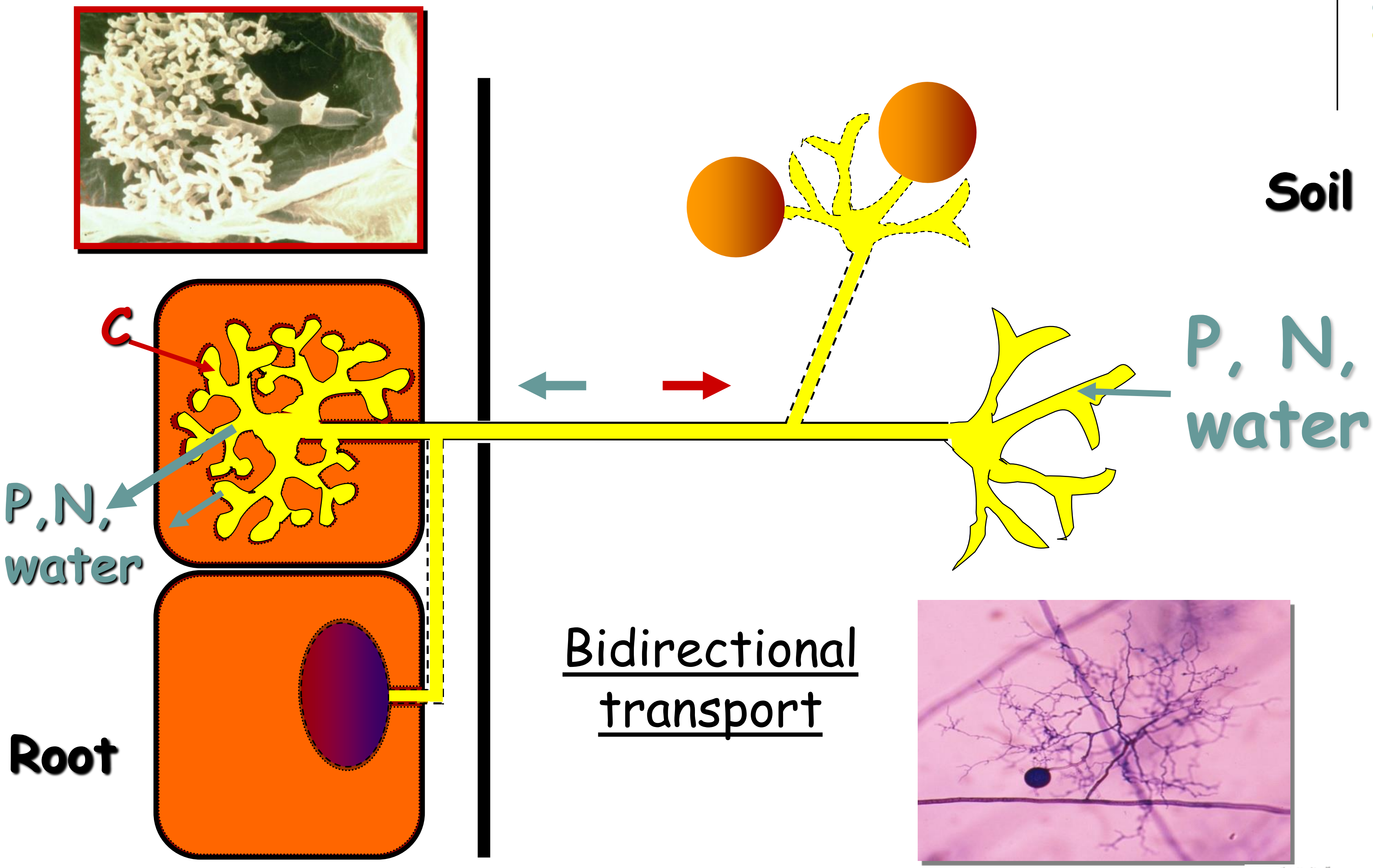
Arbuscular mycorrhizas physiology



Bago (2000) Plant & Soil

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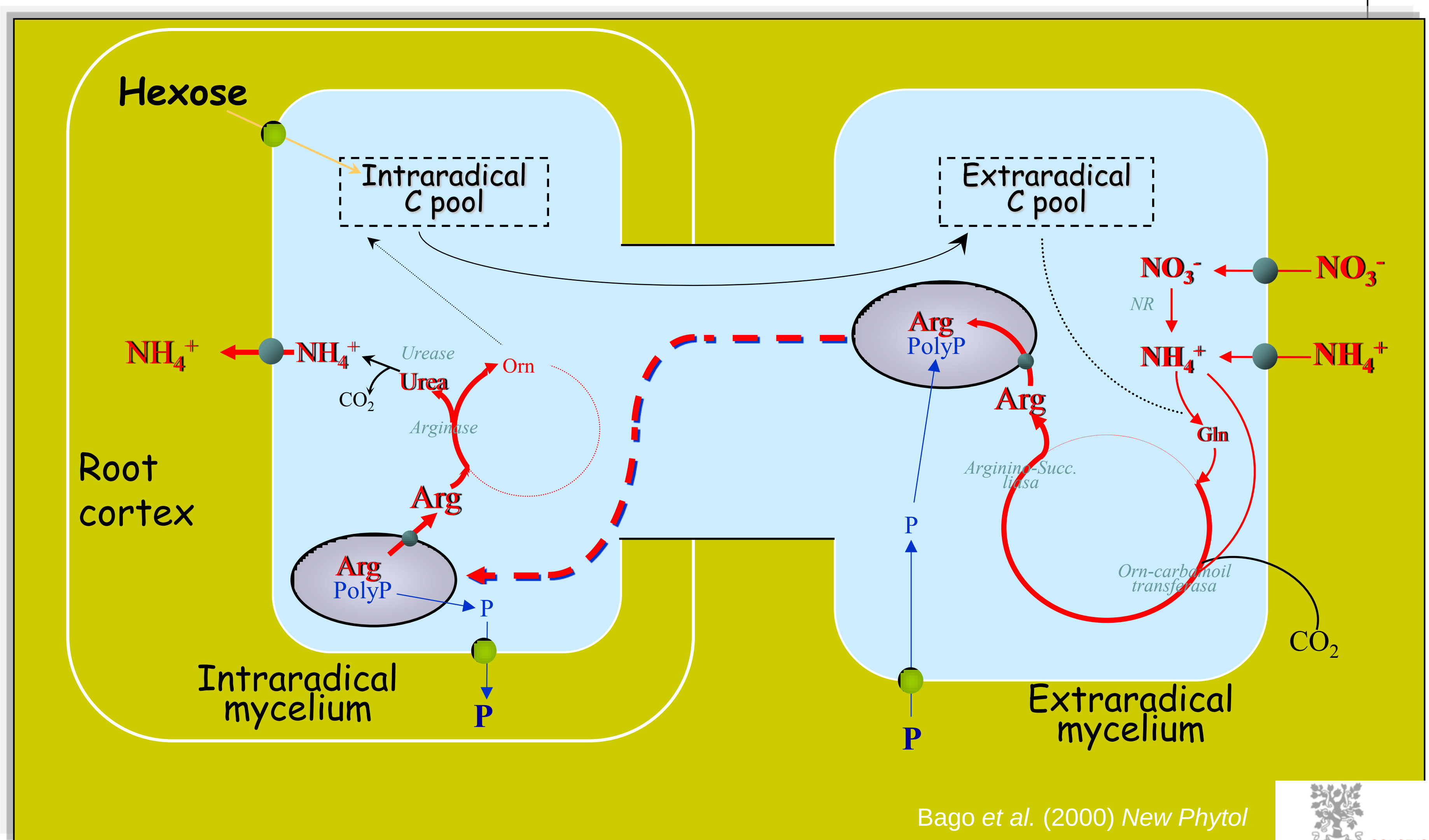
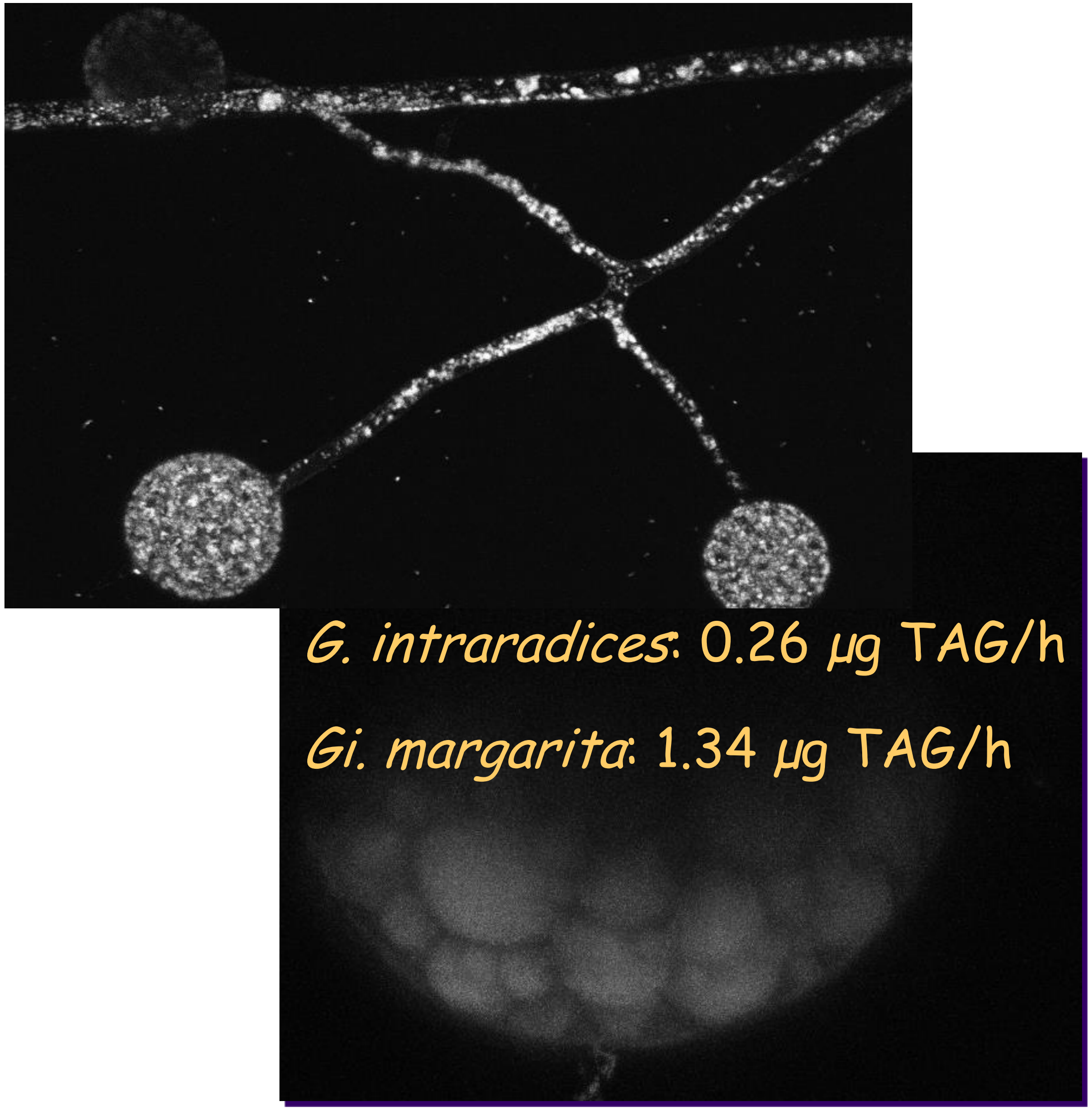
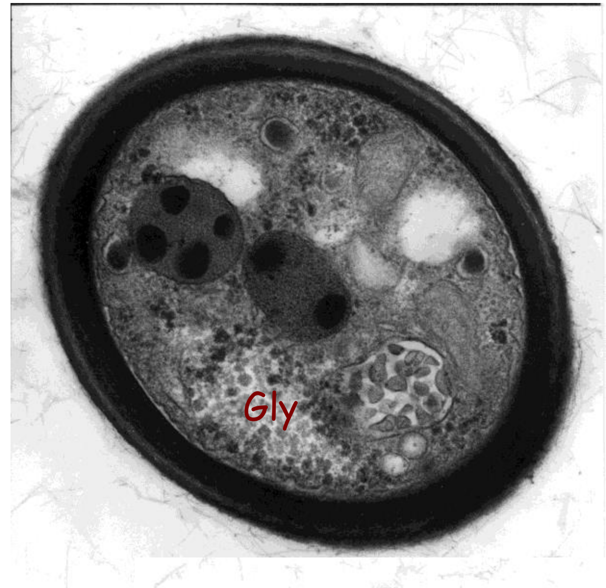
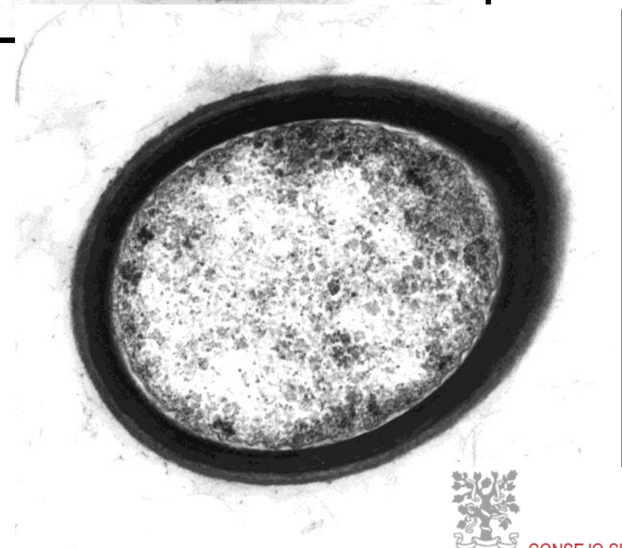
Carbon, nitrogen and phosphorus combined fluxes in AMF: an integrative proposal



Lipid and glycogen fluxes at the origin of massive C intra- to extraradical exportation in AMF







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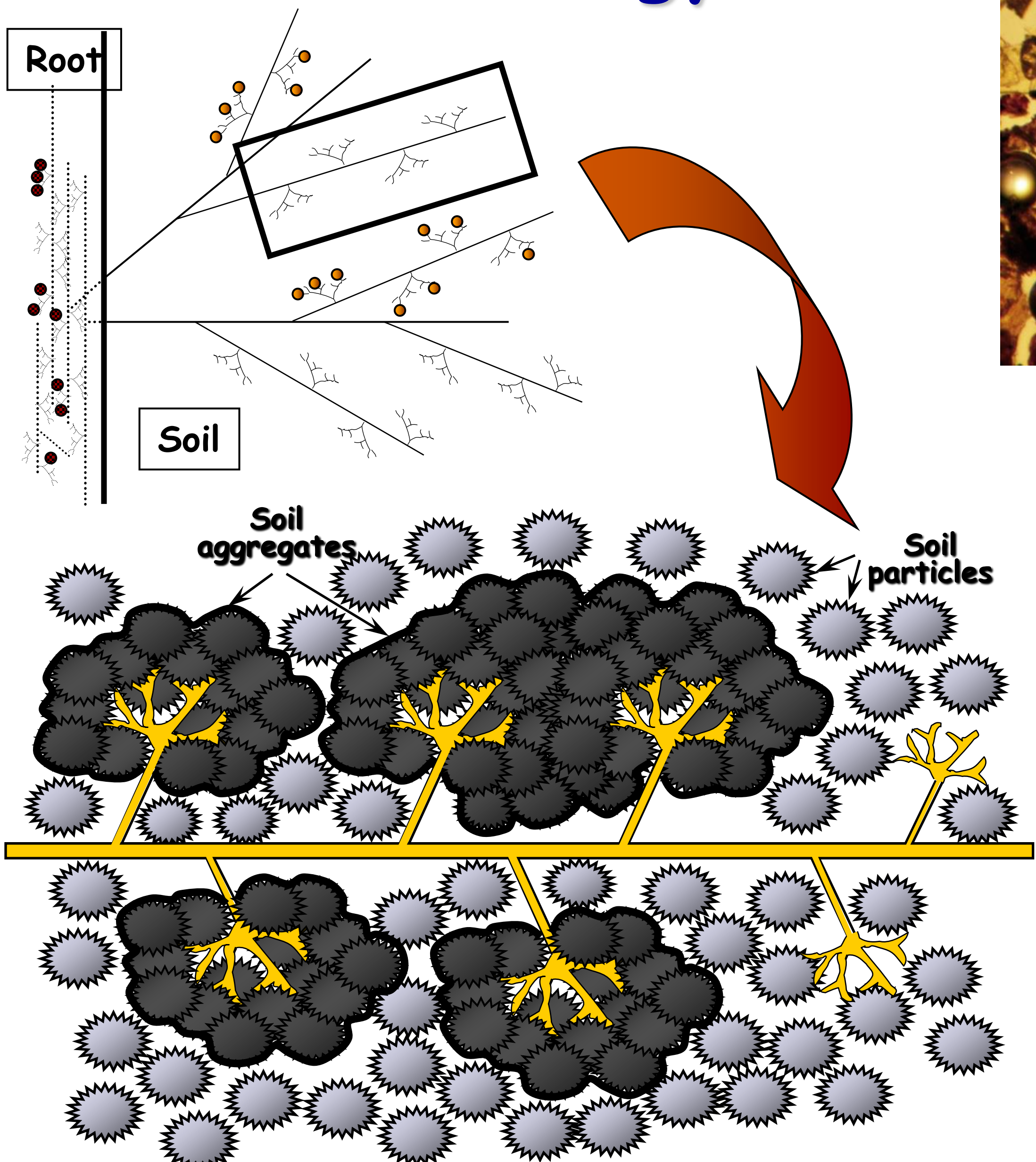
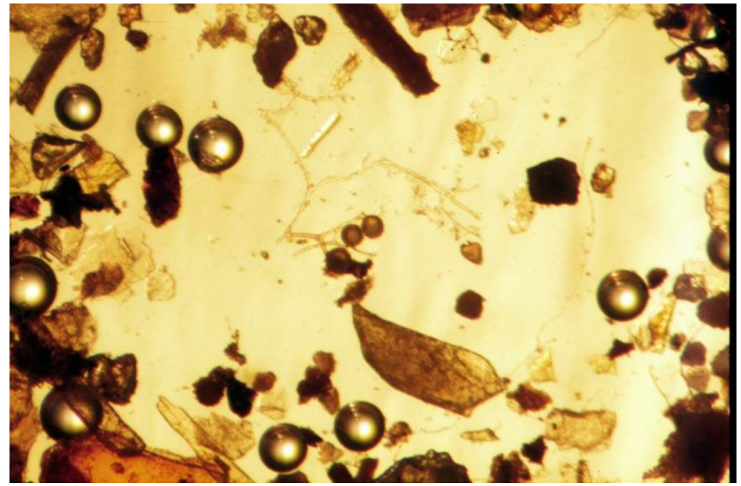
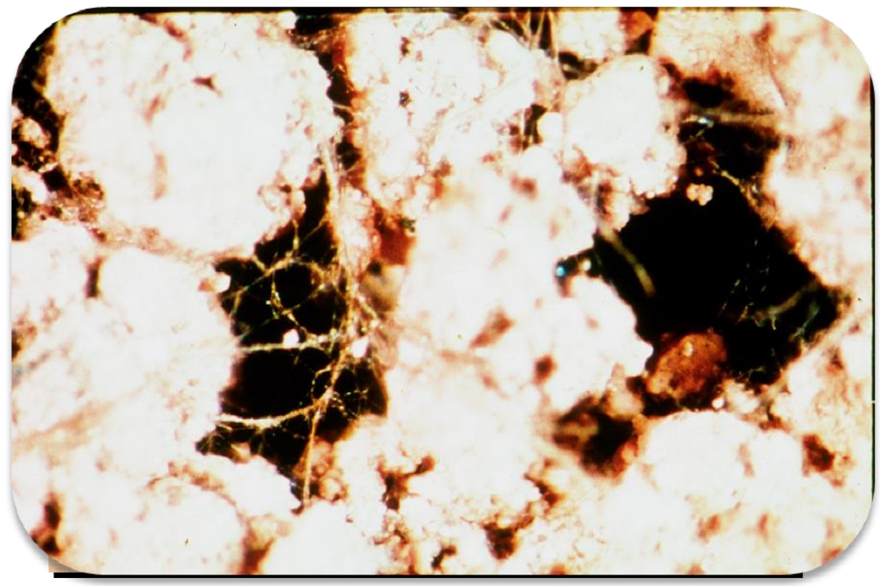
AM ecology



Bago et al. (2000)





Stable aggregate formation

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Monoxenic culture of mycorrhizas:

contribution to inoculant production and large scale use

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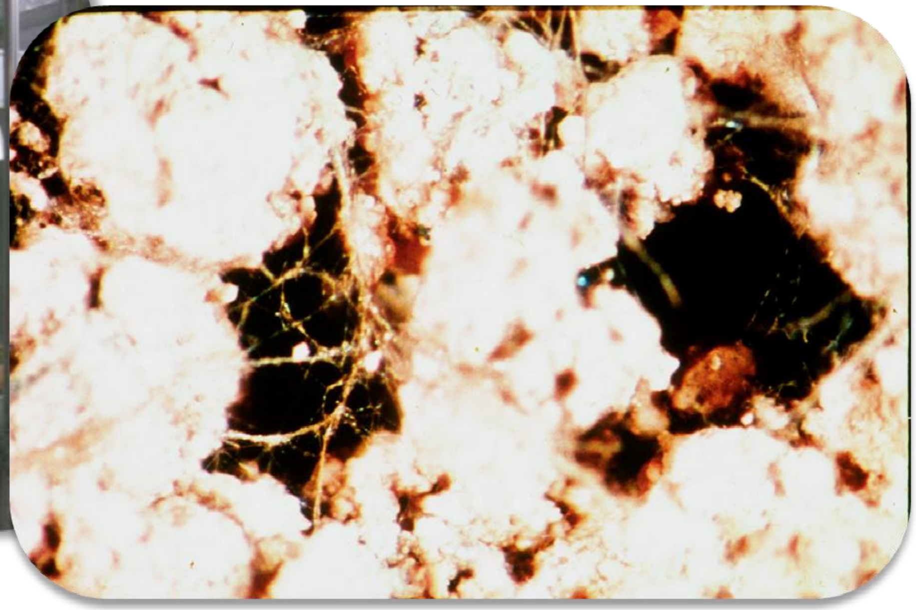
Cultivation of Mycorrhiza in SOIL or SOLID substrates (conventional methods)



- **Being solid:**
  - ➢ Loss of viable structures of colonization
  - ➢ Presence of non-soluble substrates
  - ➢ Difficult application by irrigation

- **Not being a sterile production process:**
  - ➢ Presence of other microorganisms

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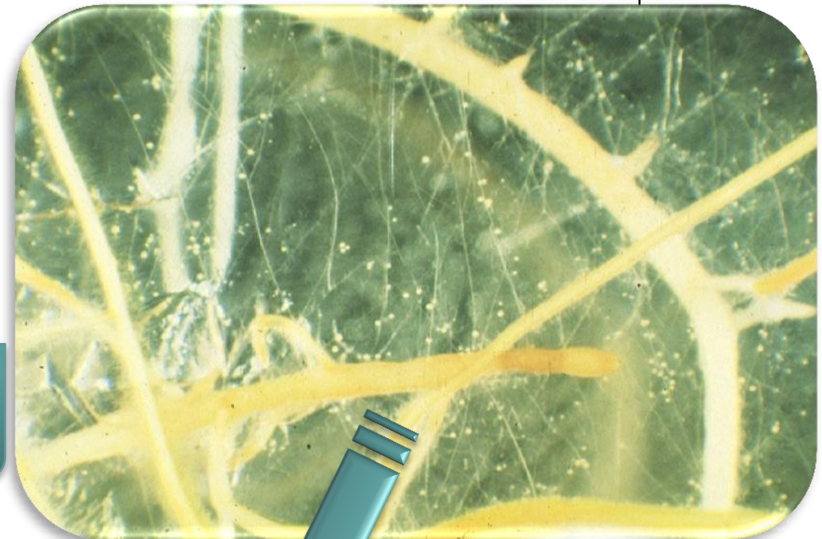
Optimizing mycorrhiza cultivation“IN VITRO” first…



…then making it <u>available</u>!!

CSIC Patent WO/2007/014974:

“Mycorrhizal aseptic inoculant and procedures of application in in-vitro and ex-vitro conditions”

Inventors: Custodia Cano Romero, Alberto Bago Pastor

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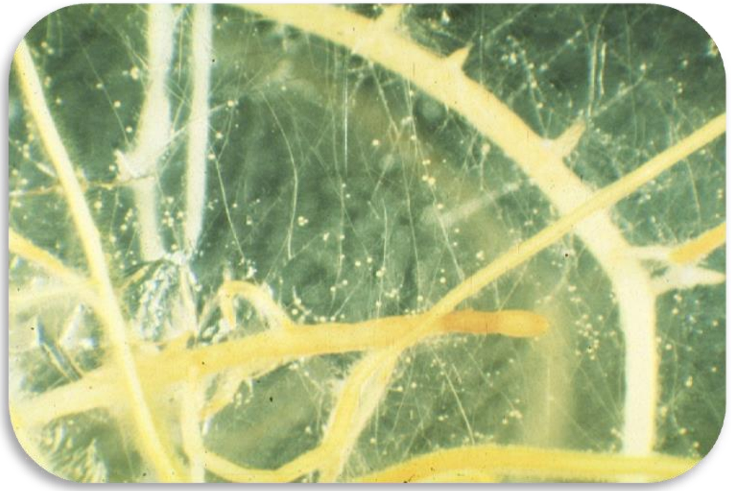
Ultrapure, autochthonous mycorrhiza gel-type inoculants



- **Since it is a GEL…**
  - Perfect application by irrigation
  - No insoluble substrates
  - Keeps active all ways of mycorrhization

- **Since it is sterile-produced ("in vitro")…**
  - Absence of other microorganisms

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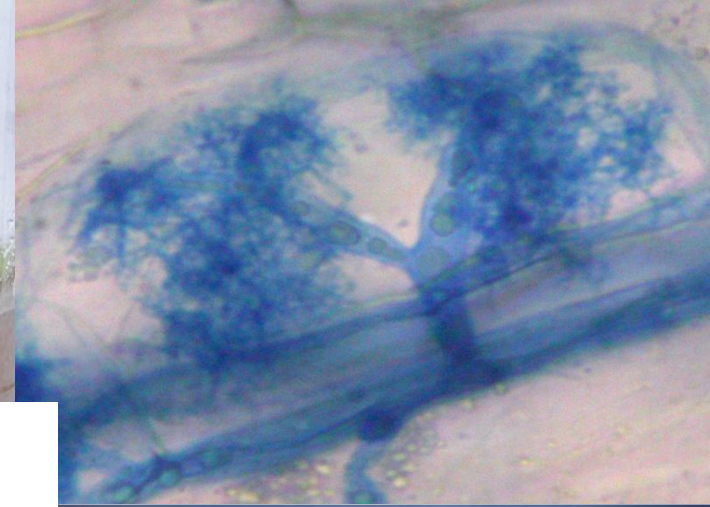


mycovitro

biotecnología ecológica

Empresa spin-off del **CSIC**
Consejo Superior de Investigaciones Científicas

vs. OTHER MYCORRHIZAL PRODUCTS

mycovitro

agrocode

The ONLY mycorrhizae gel product in the world!

CSIC CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

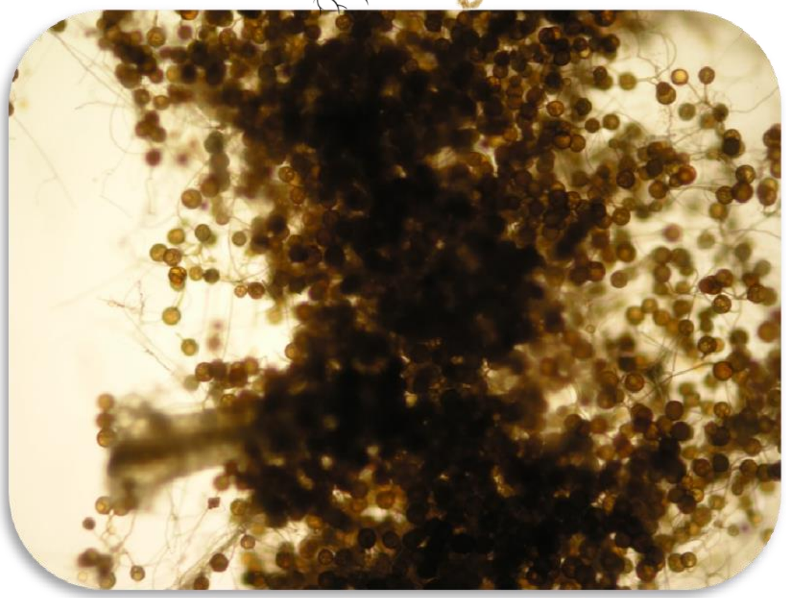
	MYCOGEL	OTHER
Application by Irrigation (Do not contain clay rests or other non-soluble substances due to its production process)	✓	✗

mycogel

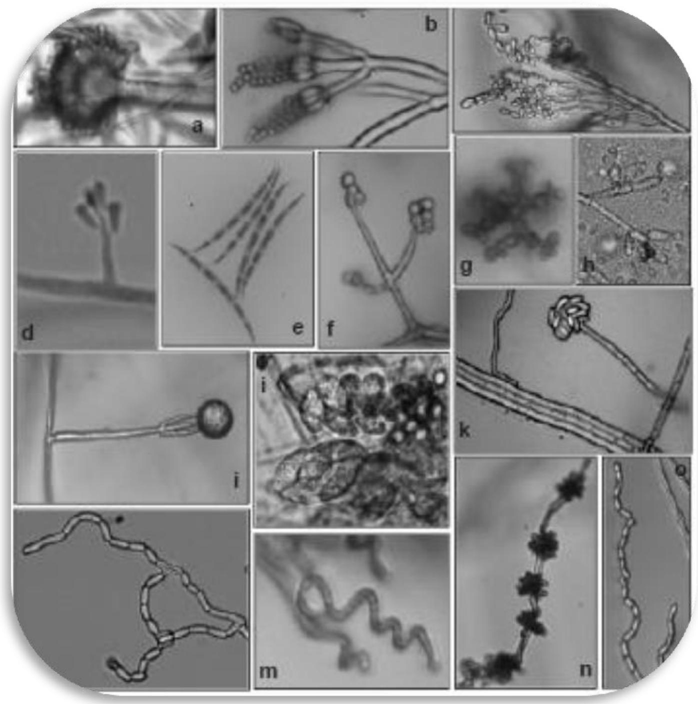
Totally soluble ***semi-liquid gel*** vs. competitor products with clay and other non solubles substances

	MYCOGEL	OTHER
Certificate of purity (Due to "in vitro" cultivation, we certify that the product is free of other microorganisms)	✓	✗

mycogel



VS.

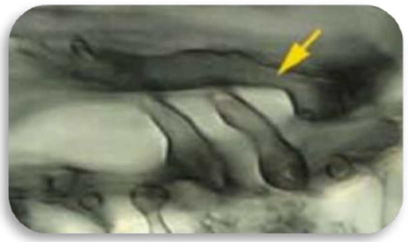


Sterile conditions of production from culture to botteling. Solid, conventional formulations don´t allow this process.

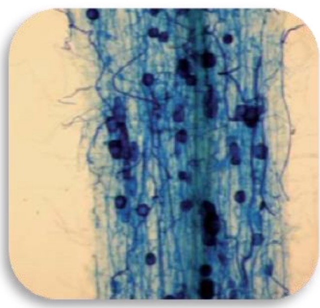
	MYCOGEL	OTHER
Colonization time (3 different propagation ways are active in the gel, accelerating fungus colonization time)	2 weeks	4 weeks

- **3different propagule types:**

\+ Colonization Speed -

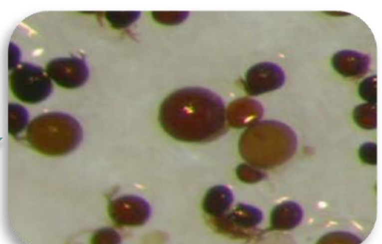


HYPHAE



ROOTS FRAGMENTS

- **Faster colonization**
- **Only survive in a gel medium**



SPORES: Long lasting, but slow colonization speed

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MYCOGEL

OTHER

Autochthonous fungi by crop/country

Autochthonous Fungi:

- Better **adaption** to soil conditions (physical, chemical and microbiological).
- Better **efficacy** on plants
- Promote **Biodiversity** and microbiological recovering/sustainability of soil ("calling effect")

Non Autochthonous Fungi:

- Selection of **infective**, agressive fungi
- Substitution of authochtonous species **(lost of biodiversity)**

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Isolation of autochthonous arbuscular mycorrhizal fungi (c. 1 year each!)

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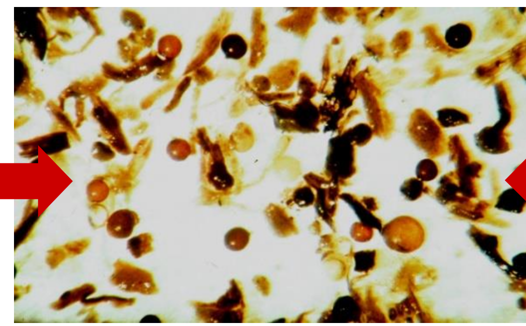
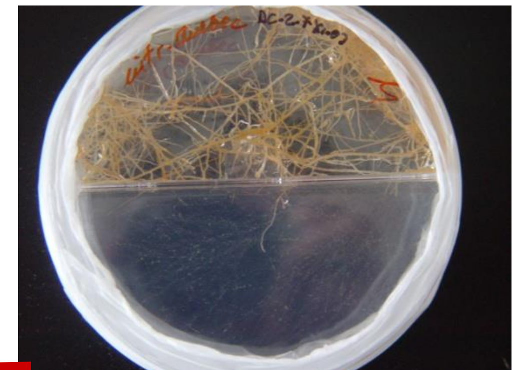
1) Soil prospection

2) Mycorrhizal “traps” to multiply fungi



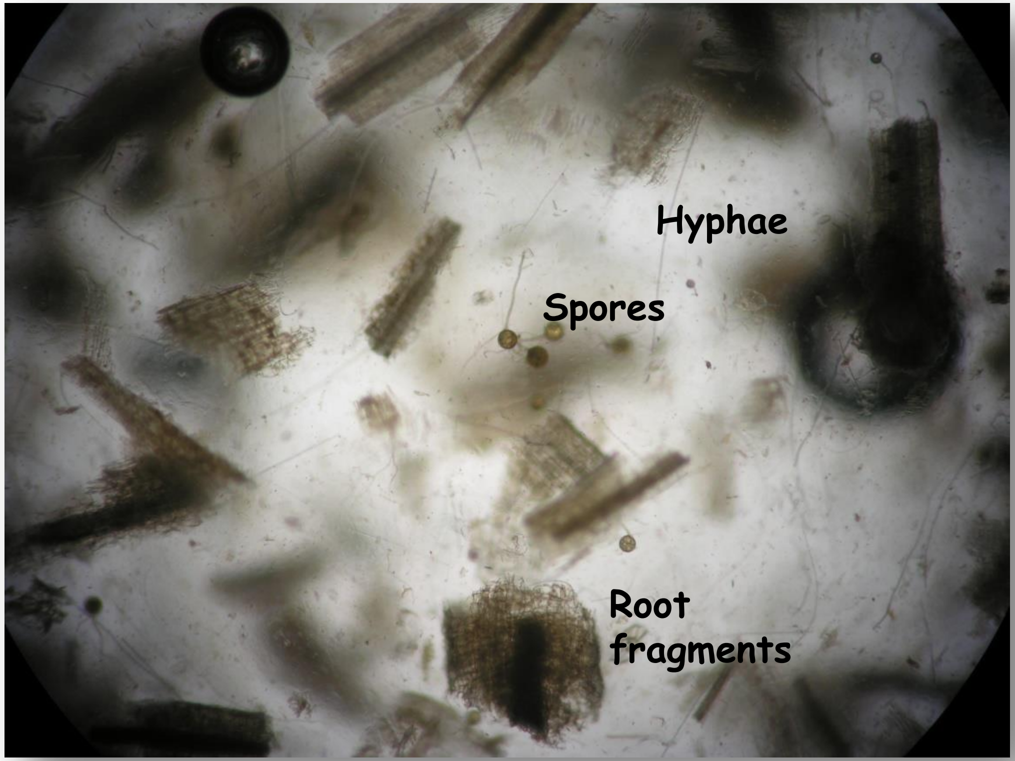
3) Isolation and purification of arbuscular myorrhizal fungi



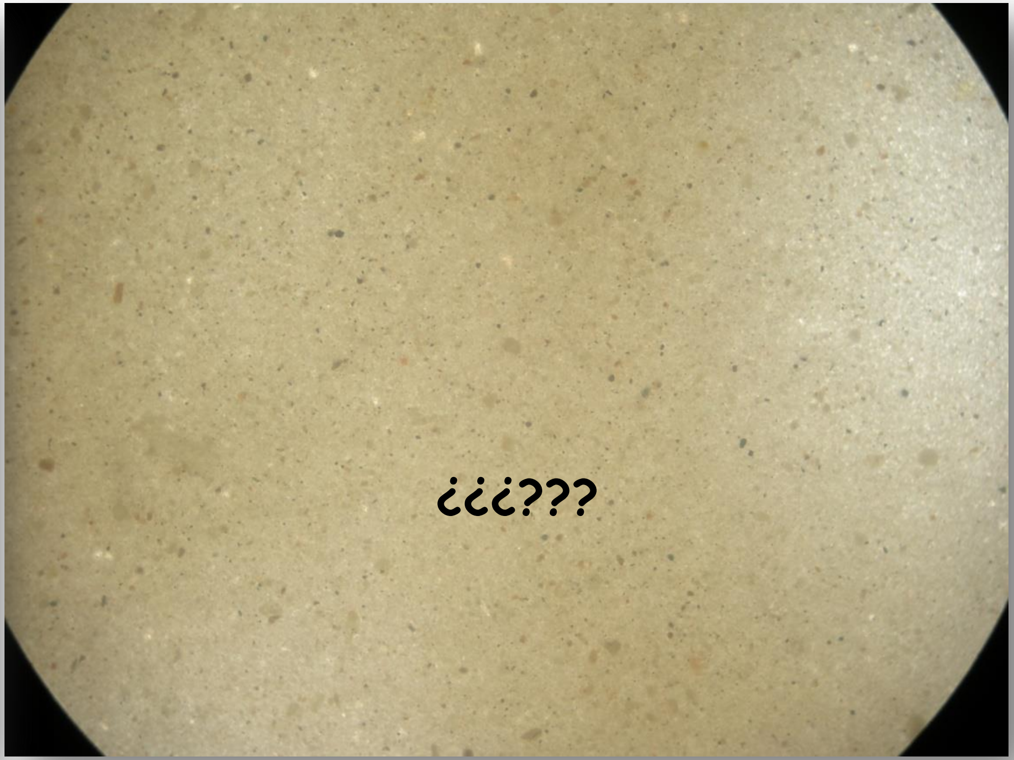
4a) In vitro culture

4b) Ex vitro culture

	MYCOGEL	OTHER
Propagules concentration (Our patented "in vitro" production process allows obtaining ultra-concentrated propagules formulation)	50.000.000 cfu/l	100 spores/g 100.000 cfu/l



mycogel



OTHERS

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Effects of applying MYCOGEL in crops

✓Higher Plant **Vigor**

✓Higher **Root Development**

✓**Earlier and Better Yield**

✓Better **Fruit Quality** (flavour, shelf life)

✓Increases Plant **Resistance** to drought and pathogens

✓**HEALTHIER PRODUCTION**

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Trials and Results

CSIC CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

mycovitro biotecnología ecológica

agrocode

TRAIL RESULTS

LETTUCE (Granada - SPAIN)

GARLIC (Granada - SPAIN)

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TRAIL RESULTS

TOMATO	Campaign 09/10	Campaign 10/11
Surface (Ha)	0,6	0,6
Number of plants	3.500	3.500
Variety	Torry	Torry
Production (Kg)	40.000	60.000
Fertilization cost N	29 €	--
Fertilization cost P	100 €	--
MYCOGEL cost	--	40 €

EGGPLANT	Campaign 09/10	Campaign 10/11
Surface (Ha)	0,4	0,4
Number of plants	2.200	2.200
Variety	Cristal	Cristal
Production (Kg)	29.400	32.000
Fertilization cost N	29 €	--
Fertilization cost P	90 €	--
MYCOGEL cost	--	20 €

Data obtained under agronomic conditions. El Ejido (Almería, Spain)

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TRAIL RESULTS

TON/Ha	1ª	2ª	3ª	1ª+2ª	TOTAL
Control 0Kg/Ha	3,3	2,3	0,8	5,6	6,4
MYCOGEL® 0Kg/Ha	4,1	1,7	0,7	5,8	6,5
Control 50Kg/Ha	6,1	2,5	1,4	8,6	10,0
MYCOGEL® 50Kg/Ha	8,6	3,1	0,9	11,7	12,6
Control 130Kg/Ha	9,1	2,6	1,1	11,7	12,8
MYCOGEL® 130Kg/Ha	10,4	3,3	0,8	13,7	14,5

POTATO

Trial carried out by Universidad Nacional (COLOMBIA)

TRIAL RESULTS

mycogel

Oil Palm Tree

0 P + MYCOGEL®

50% P Control

Trial carried out by Universidad Nacional (COLOMBIA)

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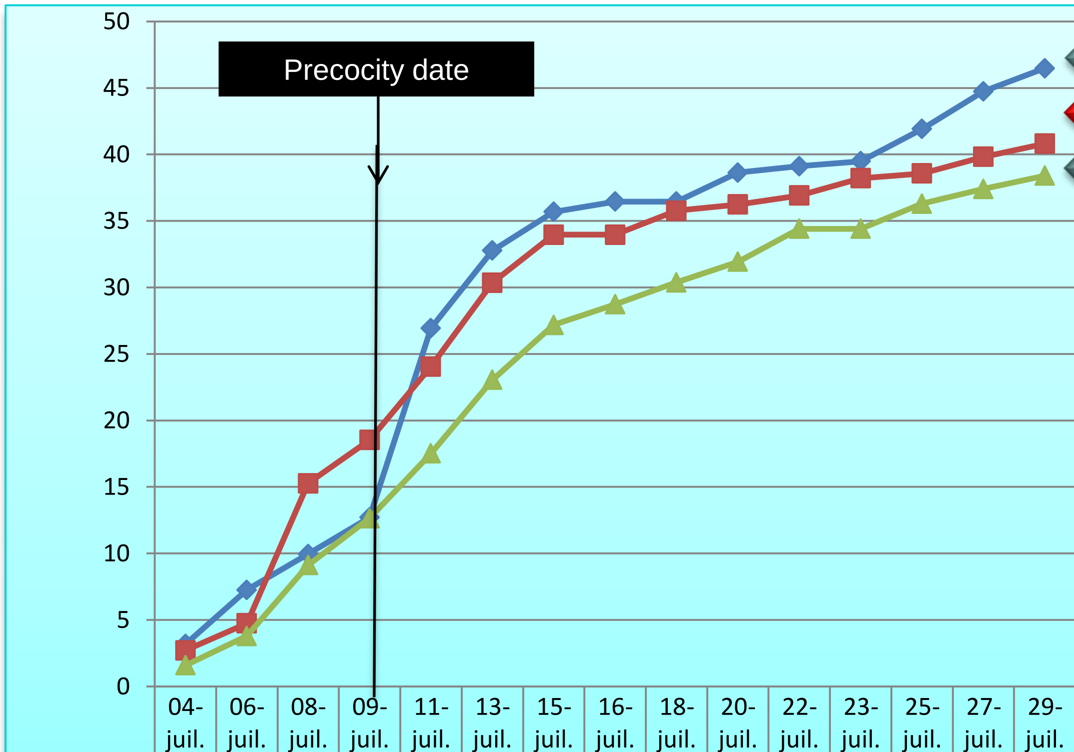
TRIAL RESULTS

Melon



	04-juil.	06-juil.	08-juil.	09-juil.	11-juil.	13-juil.	15-juil.	16-juil.	18-juil.	20-juil.	22-juil.	23-juil.	25-juil.	27-juil.	29-juil.
H	3,2	7,3	9,9	12,7	26,9	32,8	35,7	36,5	36,5	38,6	39,1	39,5	41,9	44,7	46,5
H+	2,7	4,7	15,3	18,5	24,1	30,4	34,0	34,0	35,8	36,2	36,9	38,2	38,6	39,8	40,8
T	1,6	3,8	9,1	12,7	17,5	23,1	27,2	28,7	30,4	31,9	34,4	34,4	36,3	37,4	38,4

MYCOGEL®
MYCOGEL® + Humic acids
CONTROL -

	Total Weight (Ton/Ha)	Nº fruits / line
Control	**38,4**	**7,8**
MYCOGEL®	**46,5**	**9,3**
MYCOGEL® + HUmic	**40,8**	**8,3**

Trial carried out by Independent consultant Valorhiz, (FRANCE)

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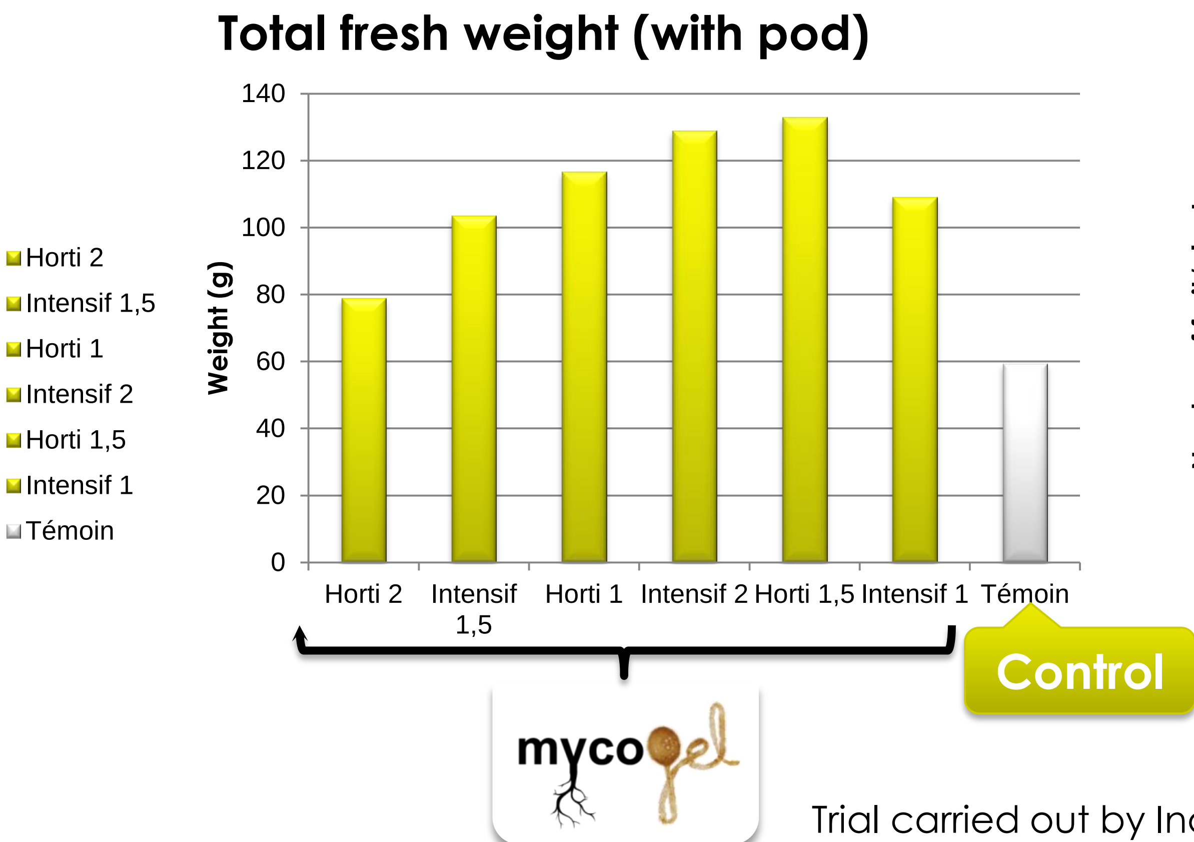
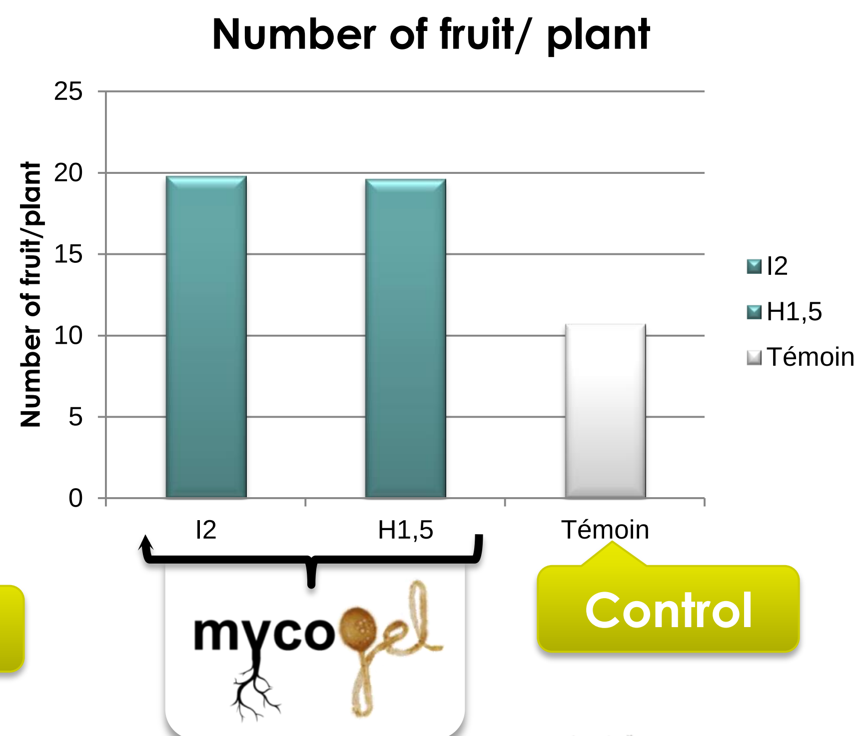
TRIAL RESULTS

Green Beans





Trial carried out by Independent consultant Valorhiz, (FRANCE)

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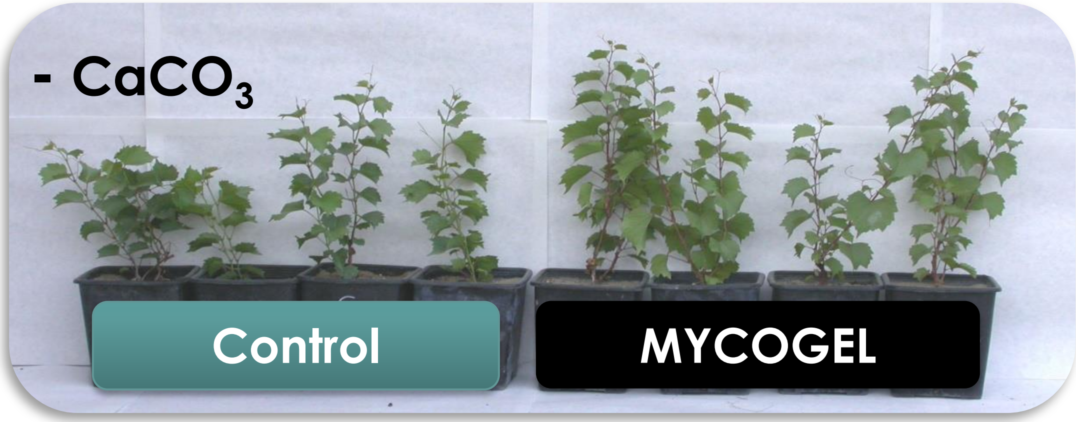
TRIAL RESULTS

Vineyard



Olive tree

Trial carried out by CSIC (Granada, SPAIN)

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TRIAL RESULTS

Banana

Trial carried out by ICIA (Canary Islands, SPAIN)

Avocado

Trial carried out by IFAPA (Malaga, SPAIN)

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Mycorrhyzes in vitro:

De la recherche fondamental jusqu'à l'application à grande échelle

MERCI!

¡GRACIAS!

Dr. Alberto Bago
Científico Titular, CSIC
Co-promotor, MYCOVITRO S.L.

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