Arbuscular Mycorrhiza in Agronomic Crops Taxonomy, Ecology, Practical Aspects

Agricultural Seminar at Aleksandras Stulginskis University
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by

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Overview

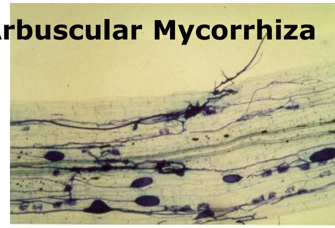
- What is Mycorrhiza
  - Ectomycorrhiza – Endomycorrhiza (Arbuscular Mycorrhiza)
  - Some basic taxonomy of fungi forming Arbuscular Mycorrhiza
  - What does Arbuscular Mycorrhiza do – how does it work
- Ecology of Arbuscular Mycorrhiza (AM) and AM Fungi
  - Methods of investigation
  - Mycorrhiza in Agroecosystems – natural occurrence
  - What is good – what is bad for mycorrhiza
- Practical Aspects: can we positively manage mycorrhiza in agronomic crops?
  - Inoculation technology
  - Use of biostimulants

Beneficial Symbiotic Soil Fungi form MYCORRHIZA with roots

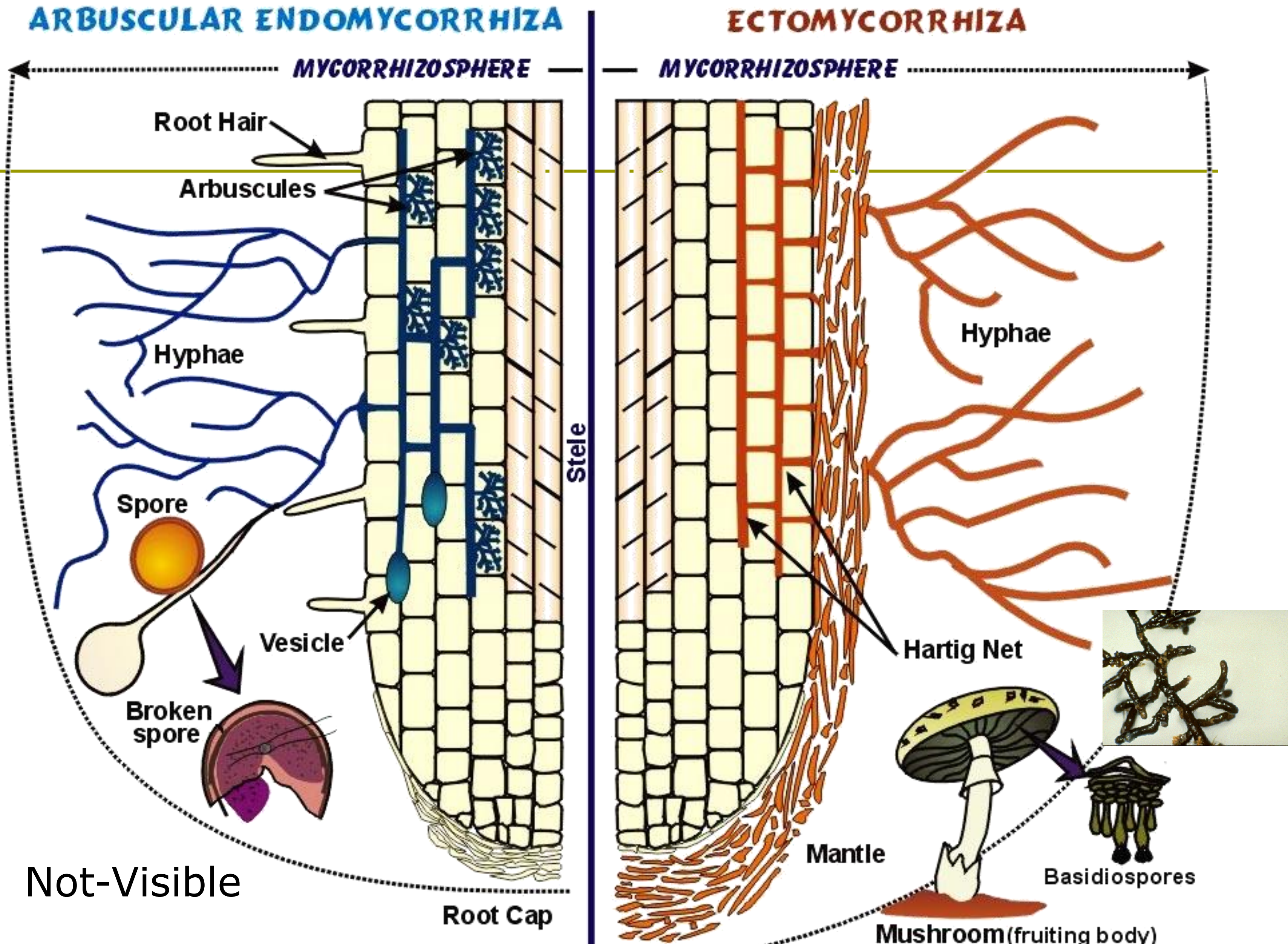
Greek: **Mycor- (fungus) + rhizos (root)**

Mycorrhiza is the mutualistic symbiosis between roots of higher plants and soil borne fungi

- Some 2000 soil fungi of the Ascomycetes, Zygo-mycetes and Basidiomycetes form **ecto-mycorrhiza** with roots of some 3000 species of trees, some shrubs (fungi edible, poison, truffles)
- Special mycorrhiza of Ericaceae and Orchidiae
- Soil fungi of the Glomeromycota (about 250 spp.) form **arbuscular mycorrhiza (endo-mycorrhiza)** with roots of ca. 70% of plant kingdom
- Some plants never form mycorrhiza (incl. some crops like Brassicaceae, Chenopodiaceae, Lupinus)



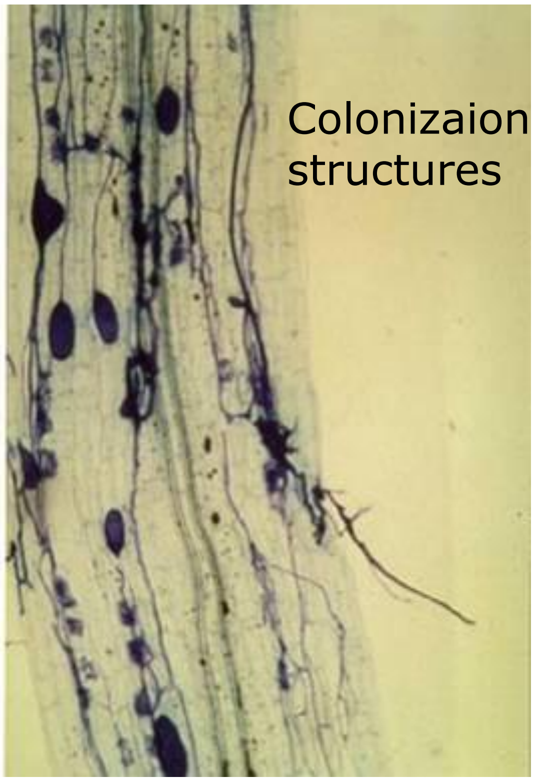
Ectomycorrhiza of pine tree

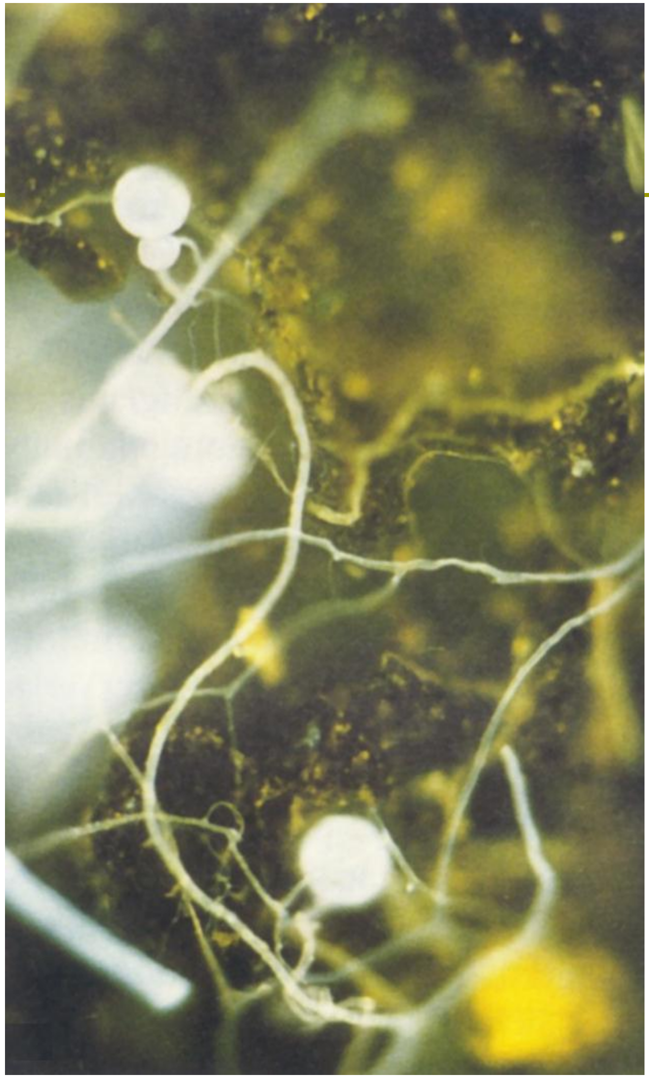


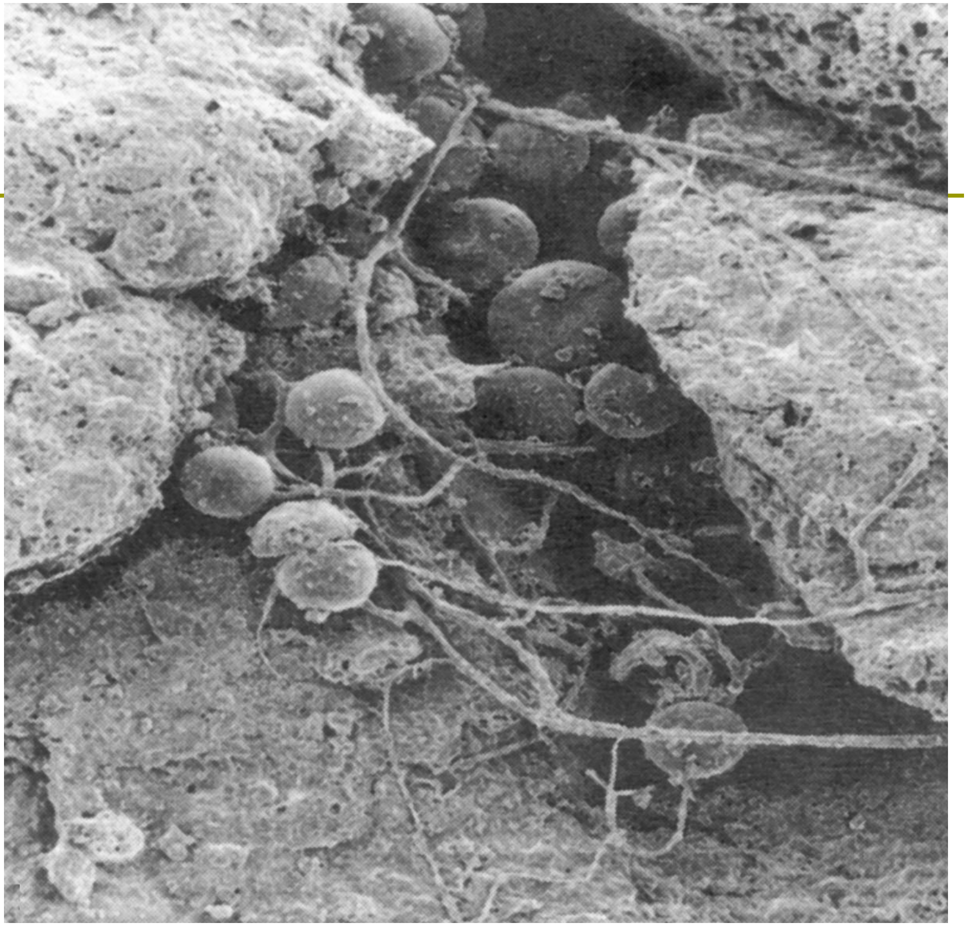
Not-Visible

Visible

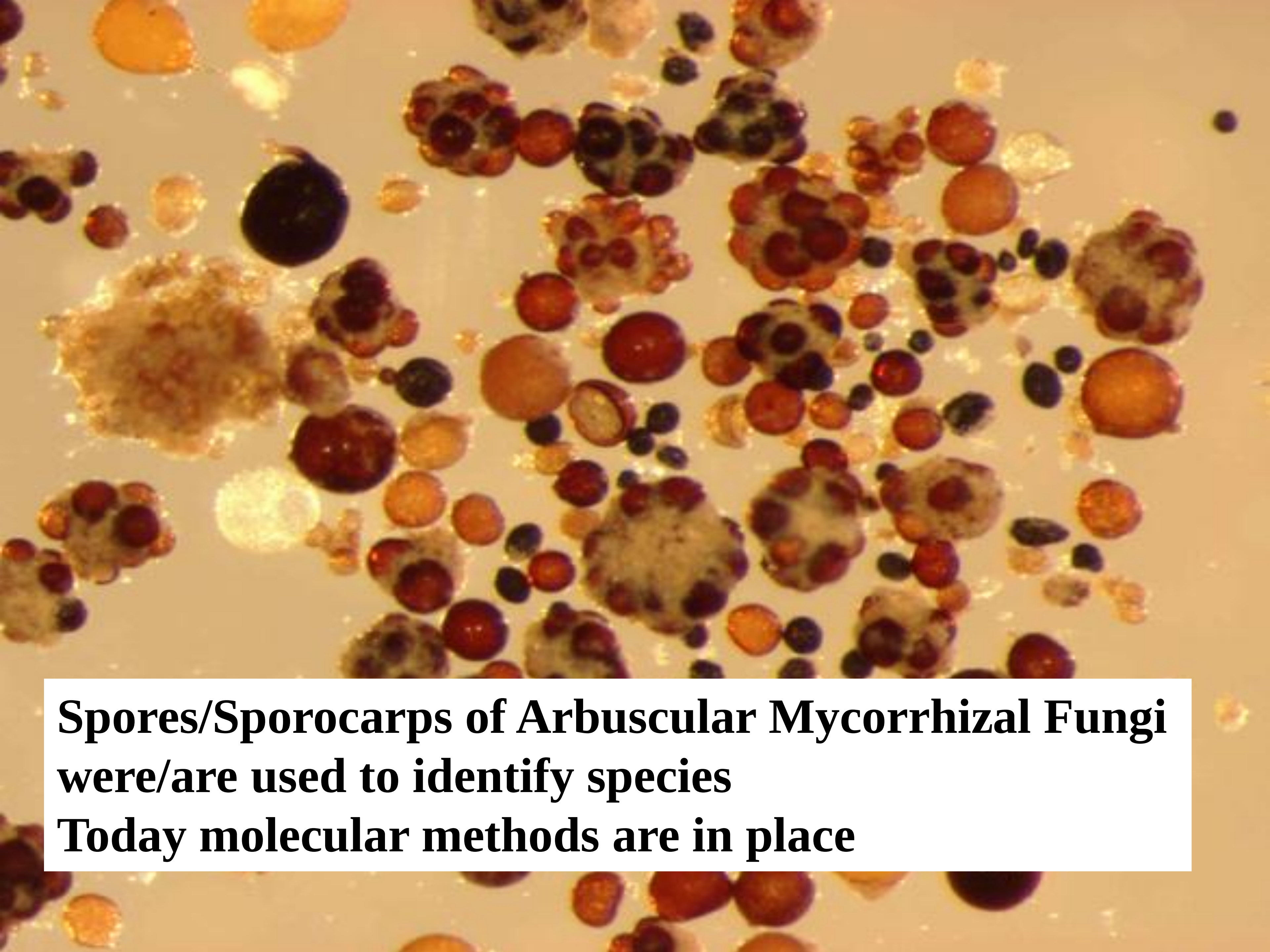
Endomycorrhiza = arbuscular mycorrhiza & vesicular arbuscular mycorrhiza







Arbuscular mycorrhiza fungi form spores in soil (resting spores), and some also in roots



Spores/Sporocarps of Arbuscular Mycorrhizal Fungi were/are used to identify species
Today molecular methods are in place

The first description - 1844

GIORNALE

BOTANICO ITALIANO

COMPILATO

PER CURA DELLA SEZIONE BOTANICA DEI CONGRESSI SCIENTIFICI ITALIANI

DA

FILIPPO PARLATORE

Professore di Botanica e di Fisiologia vegetale e Direttore dell'erbario centrale italiano nell' I. e R. Museo di Fisica e Storia Naturale di Firenze, Socio di varie Accademie italiane e straniere ec. ec.

ANNO 1.° PARTE I.ma TOMO 2.°

FIRENZE

PER LA SOCIETA' TIPOGRAFICA

1844

from: Tulasne LR, Tulasne C (1844)
Fungi nonnulli hypogaei, novi v. minus cogniti auct.
Giorn. Bot. Ital. 2 (1): 55-63

(63)

GLOMUS †

Endogone Link, Diss. 1., 33 (verisimillime) — Fries S. M. II., 297.

Peridium leve byssaceum album, stratum efficiens gossypinum inseparabile tenue continuum clausum indehiscens, pariete interno floccos minutissimos emittens. Moles seu substantia interior solida uniformis unicolor venis cellulisve distituta, sporangiisque sphaericis crassis levibus, pulpa quadam granulosa repletis, creberrimis nec non et flocculis intermixtis minutis parcis rigidulis composita.

Fungilli globosi irregulares et etiam amorphi subinodori, nunc plane hypogaei, nunc ad schidia lignea foliave decidua terra vix obruta in opacis sylvarum subepigaei addicti, arrhizi.

Genus Linkianum pro synonymo hujusce habemus, nihilominus *sporidia minuta globosa* hactenus in sporangiorum utero frustra quaesivimus.

1. *Glomus microcarpus* †

Globosus sat regularis candidus intus solidus dilute lutescens, homogeneus. — Pisi magnitudine.

In Turonia et agro Parisiensi (*Boulogne*, *Vincennes*) infrequens, Augusto-Octobre.

2. *Glomus macrocarpus* †

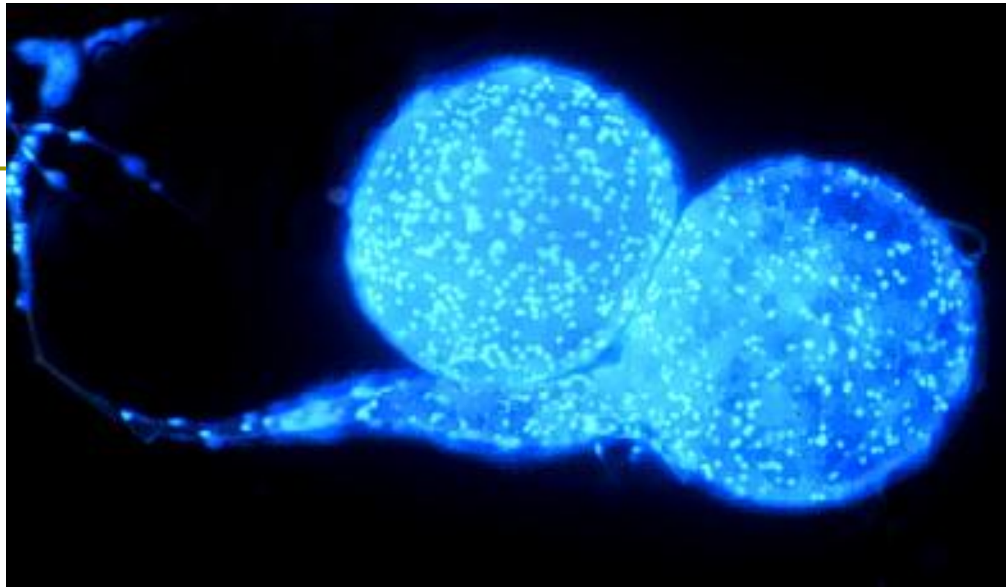
Subamorphus, sordide griseus, peridio tenuissimo vel obsoleto; sporangiis crassissimis.

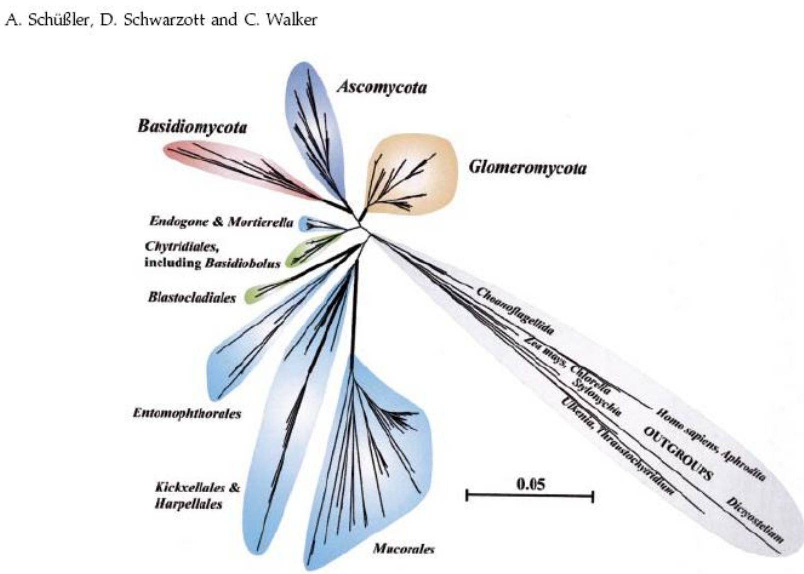
Cum praecedente sed frequentior prope Parisios, Aestate Autunno.

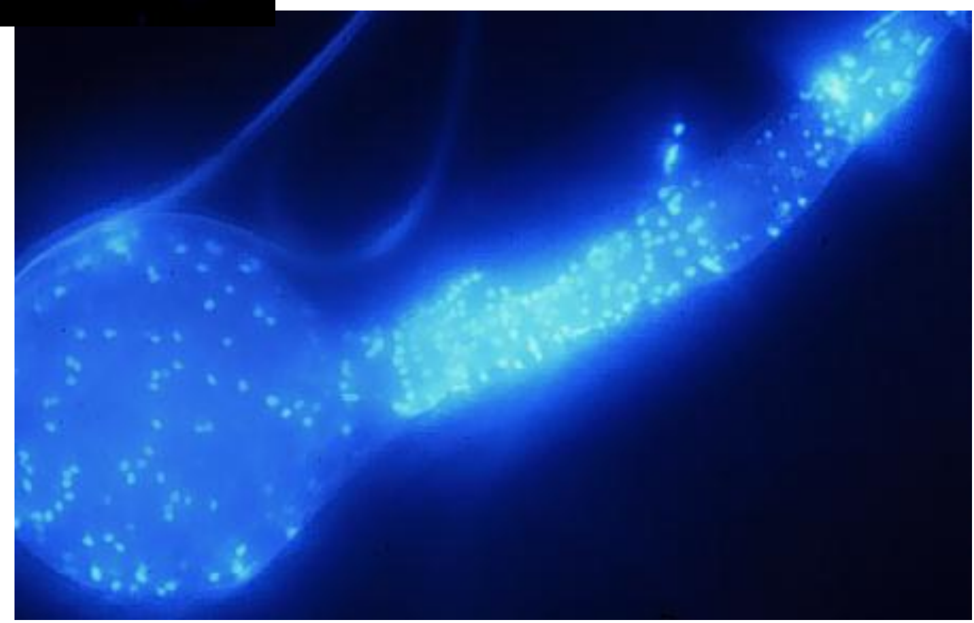
First species described had spores formed in sporocarps - all with glomoid spore formation

Multinucleoide Organismens

Exist on earth since 400 Mill. years







Schüßler et al. 2001 used molecular methods and separated Glomeromycota from other fungal classes

Glomeromycota have 3 classes

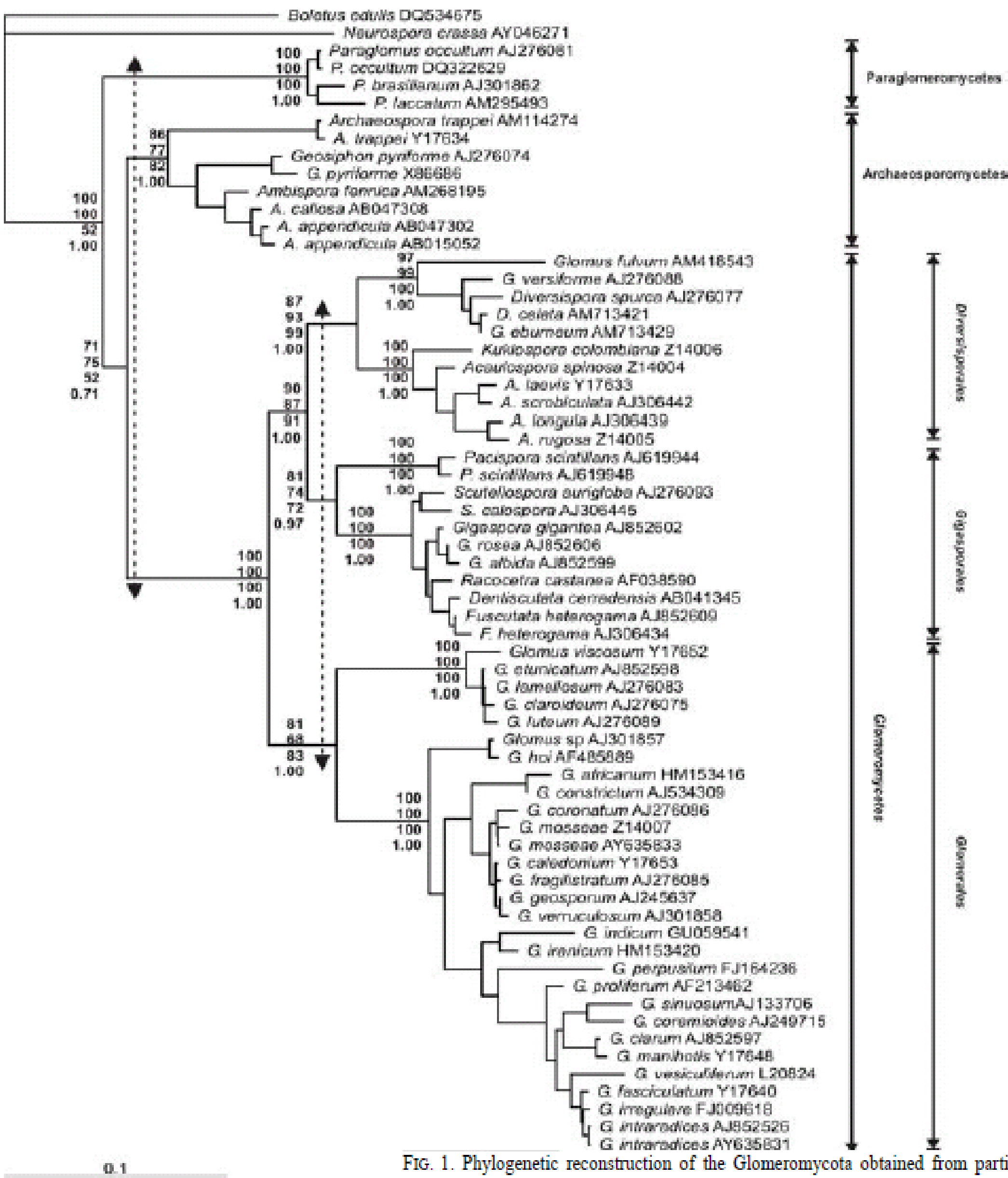


FIG. 1. Phylogenetic reconstruction of the Glomeromycota obtained from partial SSU rDNA sequences (~1800 bp). The NJ, ML and bayesian analyses were performed with GTR+G+I substitution model. Sequences are labeled with their database accession numbers. Support values are from neighbor-joining (NJ), maximum parsimony (MP), maximum likelihood (ML) and Bayesian analyses, respectively. Only topologies with bootstrap values of at least 50% are shown. (Consistency Index = 0.47; Retention Index = 0.81).

Paraglomeromycetes

Archaeosporomycetes

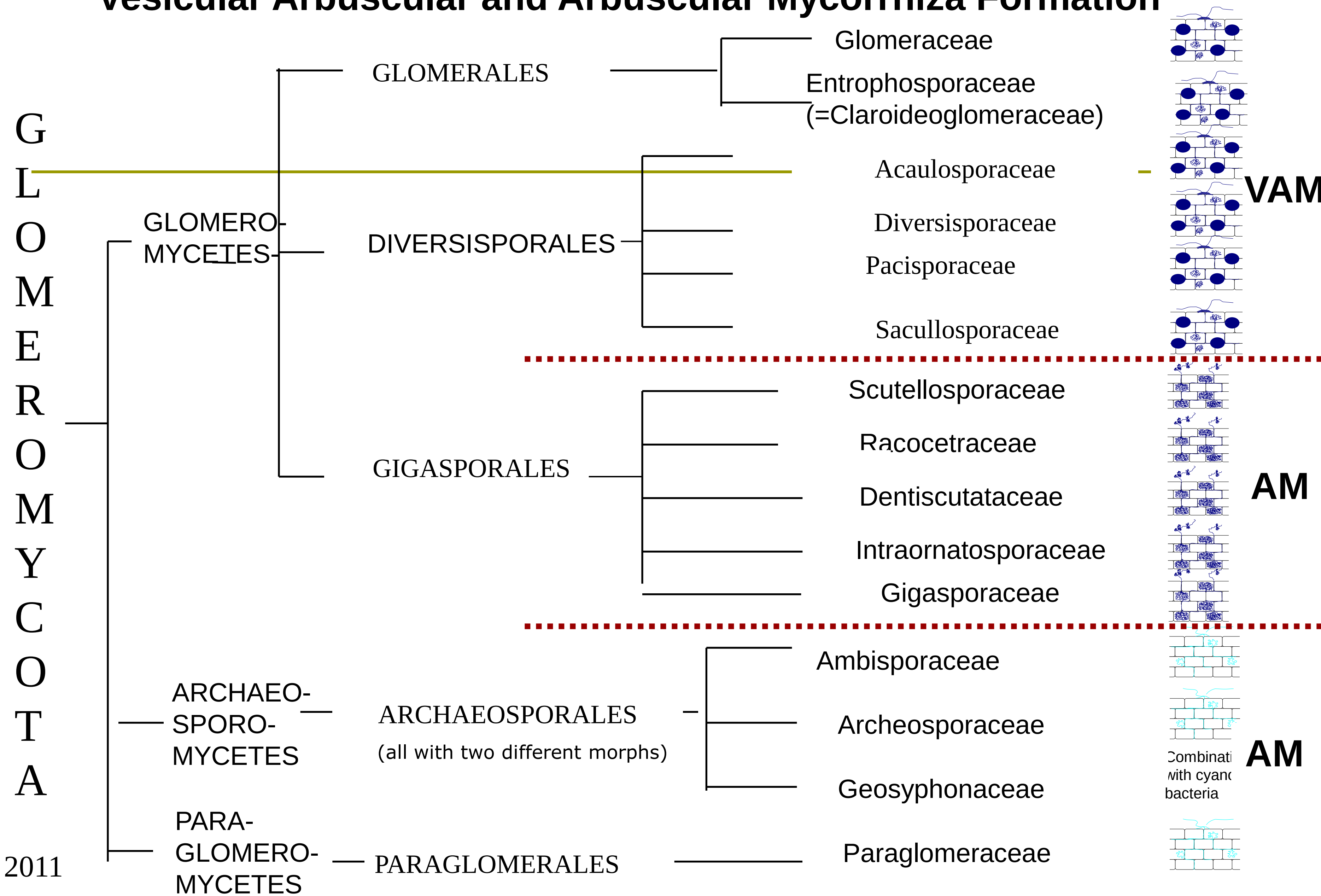
Glomeromycetes
Diversisporales

Glomeromycetes
Gigasporales

Glomeromycetes
Glomerales

Oehl et al. 2011

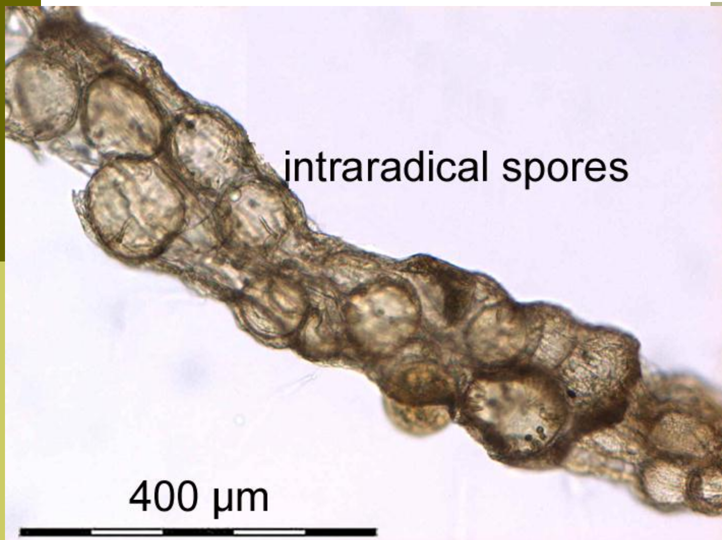
Vesicular Arbuscular and Arbuscular Mycorrhiza Formation

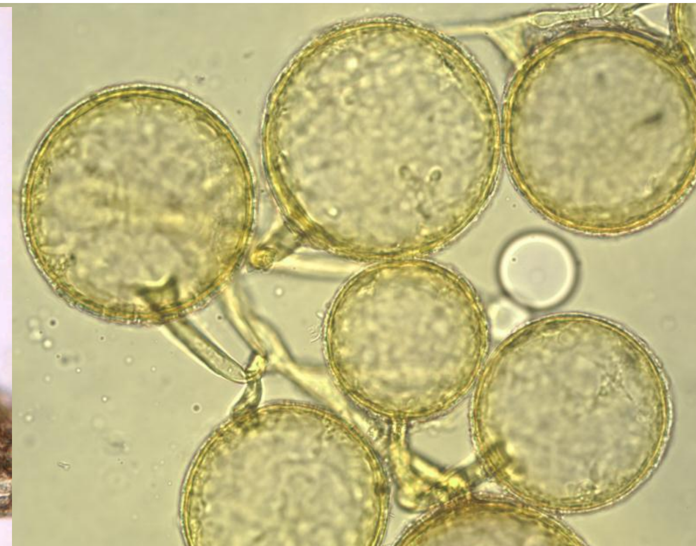


Oehl, Ineichen, Sieverding unpublished, but see IMAFUNGUS 2011

Most important in practice: GLOMERALES - GLOMERACEAE

Glomus intraradices group (Rhizophagus / Rhizoglomus)

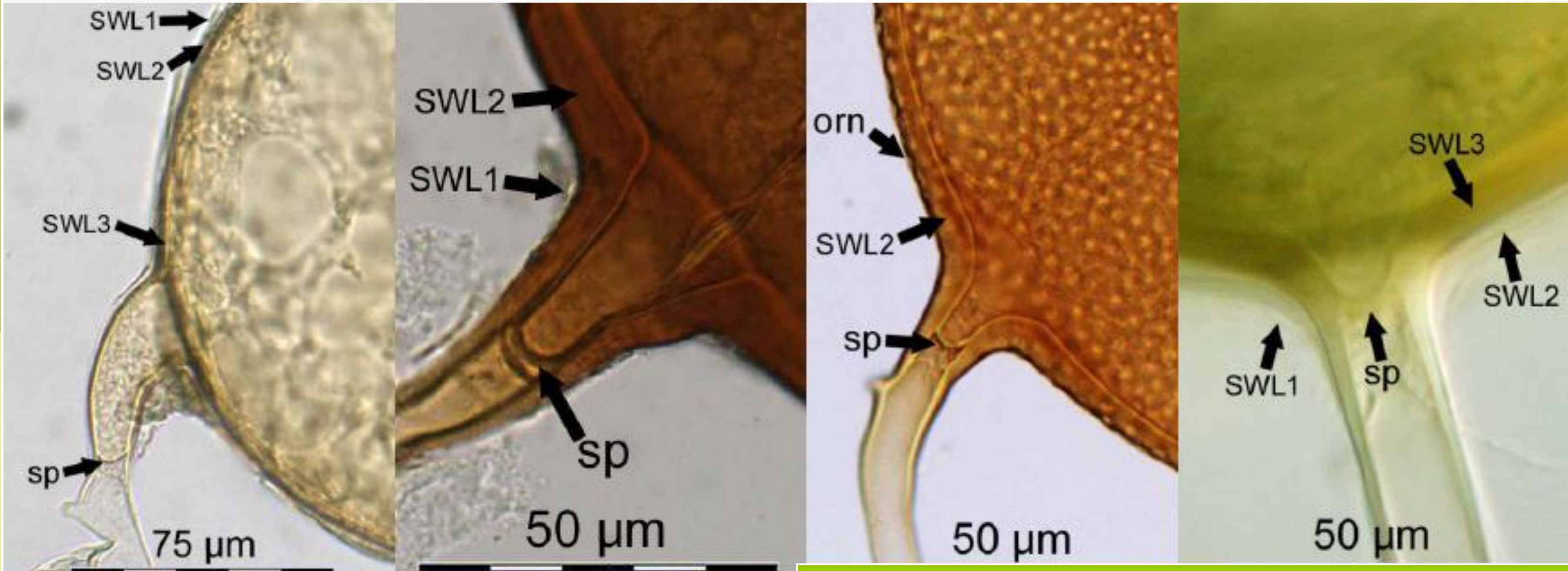


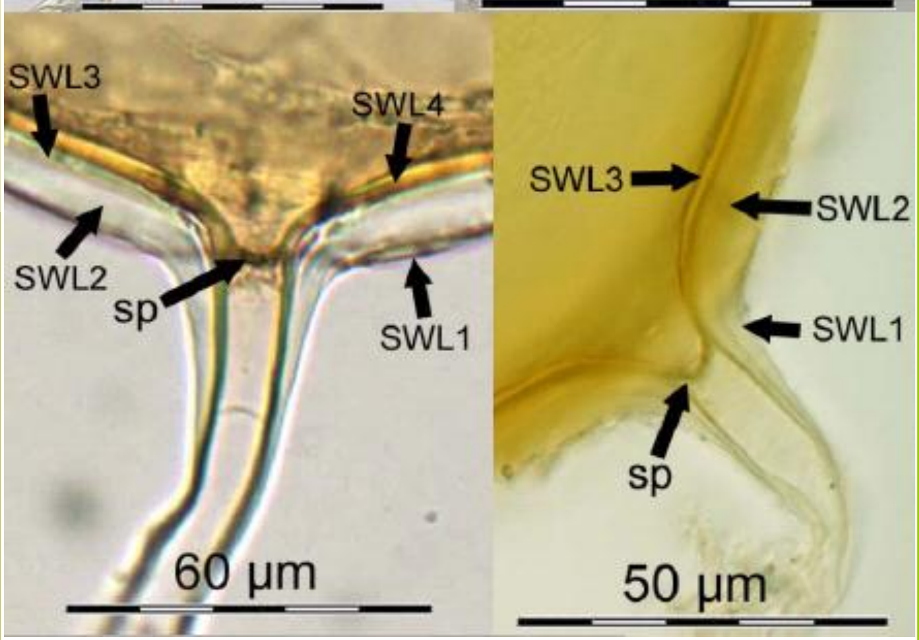


This is the species most used in studies and as a commercial inoculant

GLOMERALES - GLOMERACEAE

Funneliformis

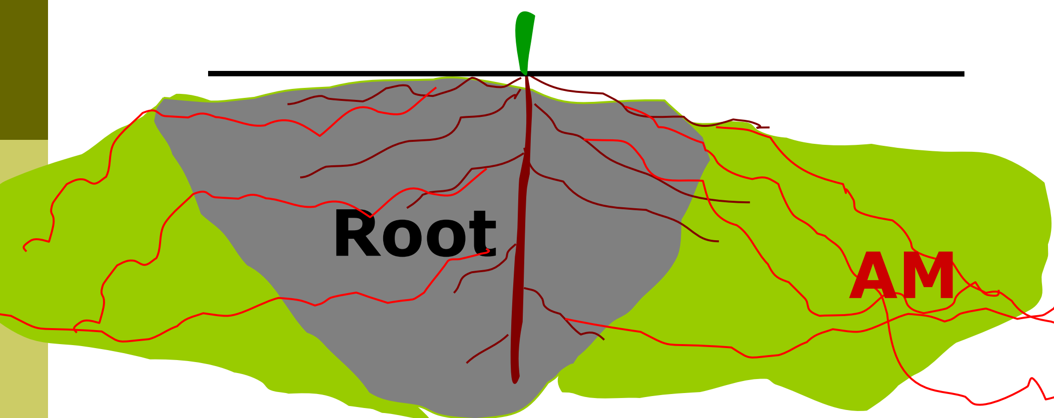




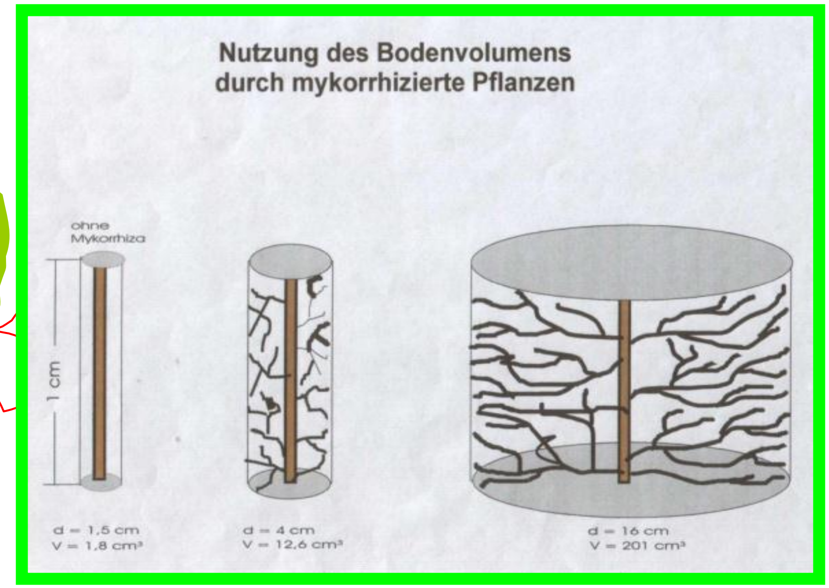
This genus is often found in agricultural soils and at pH 6-8 many species of this genus are highly effective

How does MYCORRHIZA work

Symbiosis of Fungus with Plants (Roots)



Increased soil volume explored by fungal mycelium for nutrients



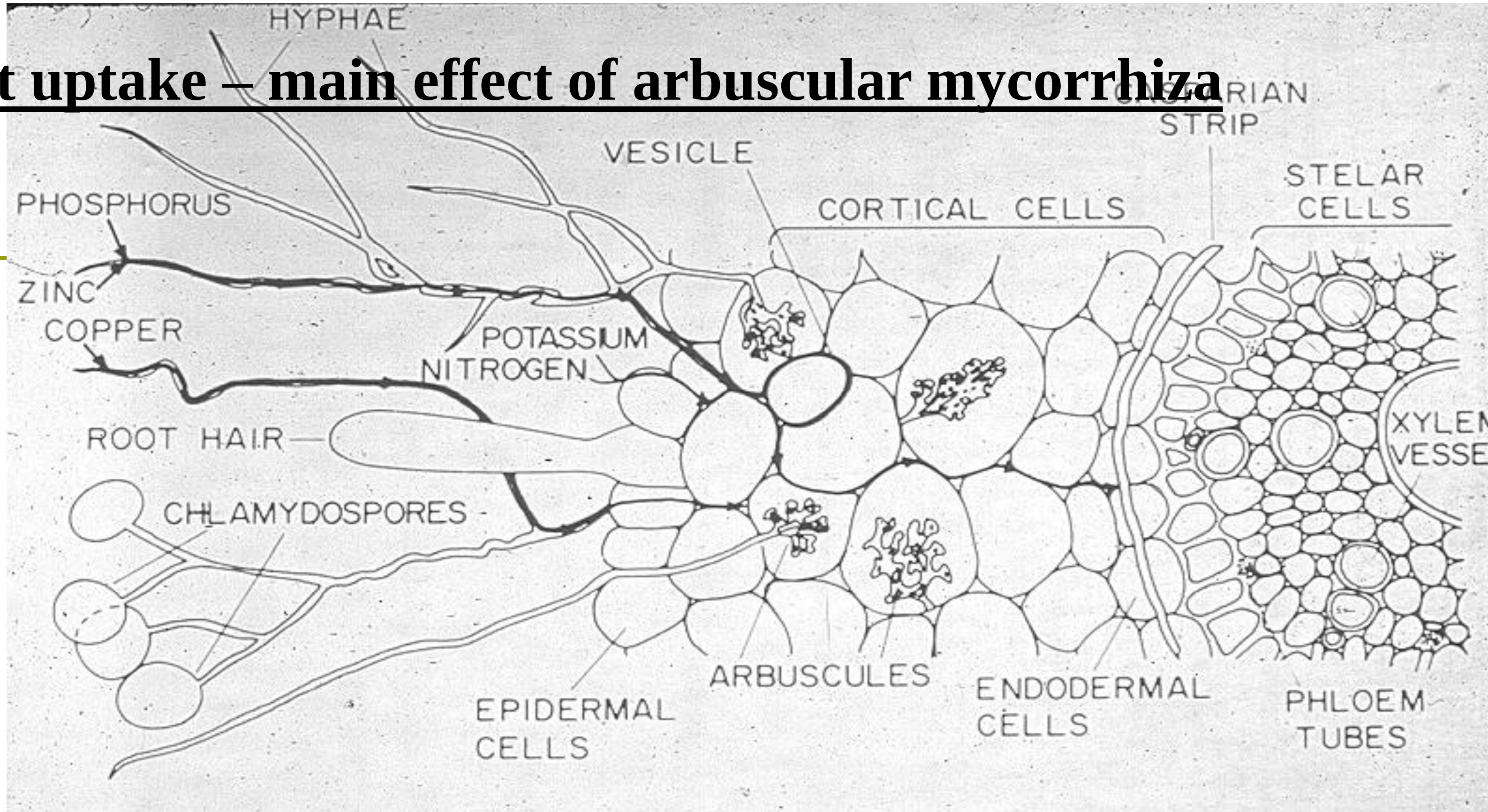
1.8 cm3 12.6 cm3 201 cm3

Exchange of nutrients and assimilates

- Fungus absorbs with root external hyphae nutrients from soil and transports to roots
- Plant gives photosynthates (assimilates in form of carbohydrates, sugars) to fungus

- Fungus needs less carbohydrates than roots for development – hence the benefit is great for the plant

Nutrient uptake – main effect of arbuscular mycorrhiza



Macro-Nutrients: direct absorption by fungus and transport to root by fungal mycelium:

P, NO_3^-, NH_4^+, Ca, Mg, (K – mass flow)

Micro-Nutrients: direct absorption and transport by mycelium:

Zn, Cu, S, B, Mo (essential nutrients)

Cd, Ni, Sr, Se (heavy metals)

Br, J (non-essential elements)

Indirect uptake? (higher concentrations found in mycorrhized plants):

Fe, Mn, Cl (also assumed for Na, Co, Si)

Mycorrhiza absorbs
Plant available phosphate
More efficient than the root

Phosphat is a growth limiting element in acidic soils, and is mainly needed at the beginning of season

Dependency of plants on mycorrhiza

Clusia minor

Effect of arbuscular mycorrhiza on drought stress

INOQ Turf | Kontrolle | Volldünger

je 7 g Mischsaat/Kiste

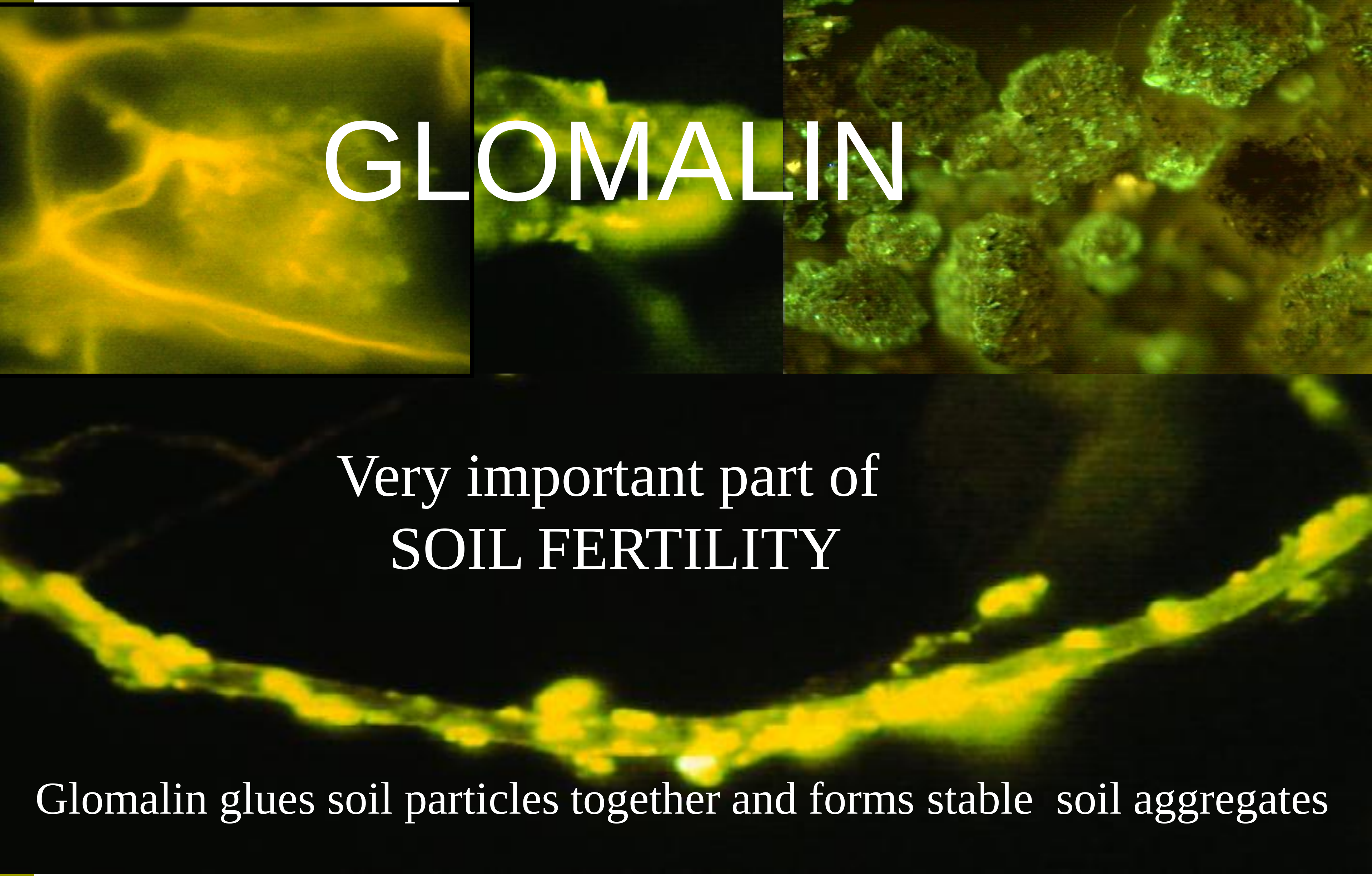
10% Deutsches Weidelgras *BELRAMO*
28 % Deutsches Weidelgras *TWINS (t)*
20 % Deutsches Weidelgras *STRATOS M*
20 % Wiesenschwingel *MIMER*
17 % Wiesenlieschgras *CLIMAX*
5 % Wiesenrispe *BALIN*

Aussaat 20.4.05
Foto 20.7.05

Inoq GmbH   Dr. Carolin Grotkass   www.inoq.de

Nematode control in vegetables by arbuscular mycorrhiza

Root must be colonized with arbuscular mycorrhiza before nematodes attack; combination with biostimulants is useful



GLOMALIN

Very important part of
SOIL FERTILITY

Glomalin glues soil particles together and forms stable soil aggregates

Glycoprotein - Potentially the most important benefit of mycorrhizal fungi for a sustainable agricultural production

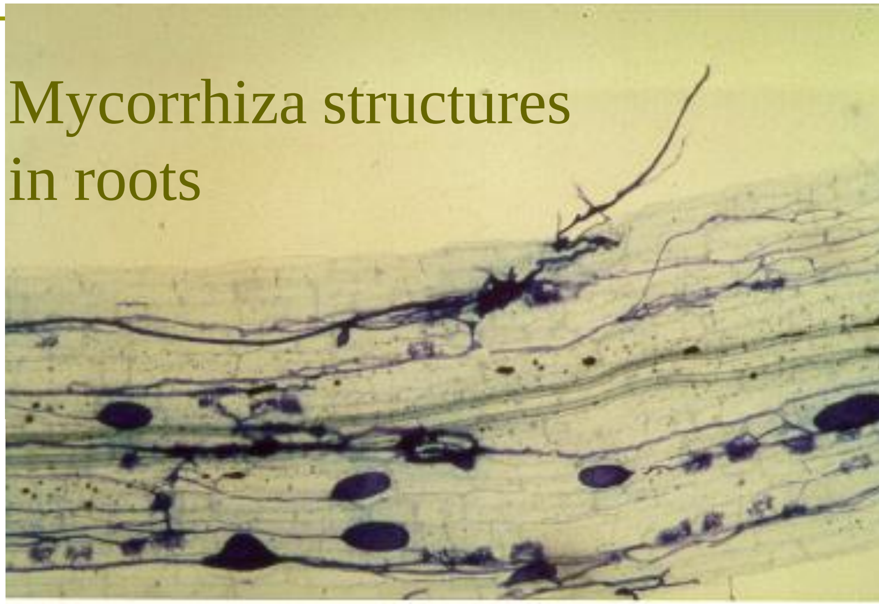
Benefits of arbuscular mycorrhiza

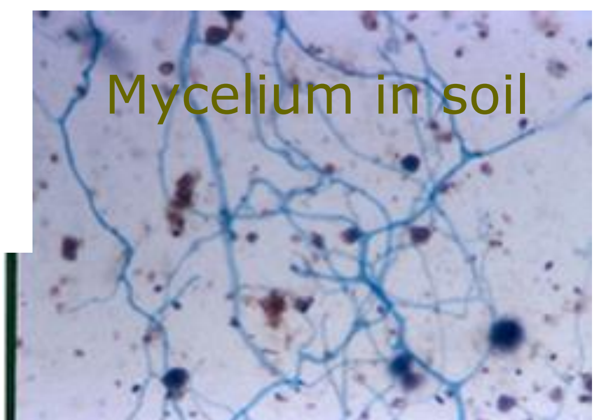
- Savings of 50% Phosphate fertilizer, if mycorrhiza is promoted
- Making more effective use of fertilized P fertilizers
- More balanced uptake of fertilizer nutrients (N, K, Mg, micro-nutrients) with better plant growth
- Better survival during short drought periods
- Significant better N-fixation by legumes via *Rhizobium*
- Plays role in soil health (nematode control) and in soil aggregation (soil fertility)

Where are the fungi: natural occurrence

- Ca. 250 fungal species (known) form arbuscular mycorrhiza with > 70% of all plants of the plant kingdom
- **Fungal species are naturally present in all terrestrial ecosystems, and also all agro-ecosystems**
- The arbuscular mycorrhiza is important for most agricultural, horticultural and ornamental crops: all cereals, pasture grasses & herbs, potato, maize, sunflower, legumes, vegetables – tomato-cucumber-lettuce-beans-herbs-etc, ornamentals, flowers, medicinal herbs, most shrubs and bushes, but also for many grass and broad-leaf weeds
- IMPORTANT: Some plant species NEVER form mycorrhiza: e.g. Brassicaceae (rape, cabagge, etc.), Chenopodiaceae (sugarbeet)
- Many crop plants are obligate mycotroph (do not survive without mycorrhiza, e.g. onions, maize, grasses, legumes), others are factultativ mycotroph (survive and grow under high nutrient fertilization conditions, e.g. cereals)

How to investigate arbuscular mycorrhiza – make them visible



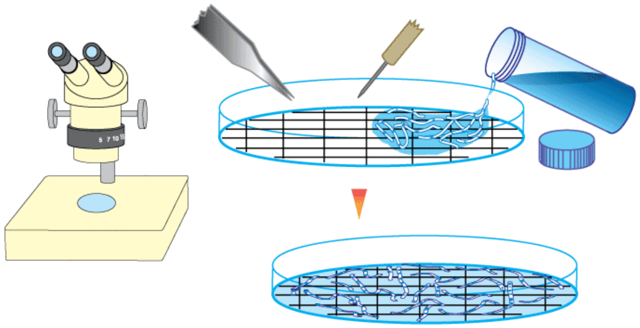


Arbuscular and Vesicular-Arbuscular Mycorrhiza

Arbuscular mycorrhiza cannot be isolated with common microbiological methods

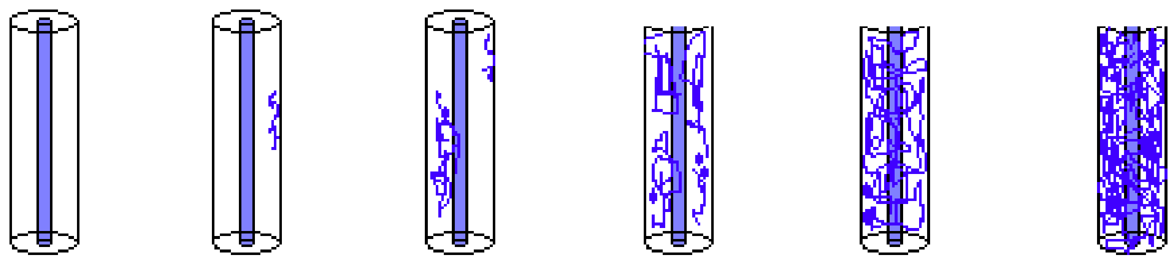
How to quantify structures i

Grid-line intersection method



Quantification of mycorrhizal root colonization

MICORRIZAS VA:



CATEGORIA	**0**	**1**	**2**	**3**	**4**	**5**
DENSIDAD VISUAL (%)	**0,0**	**1,0**	**2,5**	**15,5**	**35,5**	**47,5**

TROUVELOT ET AL.

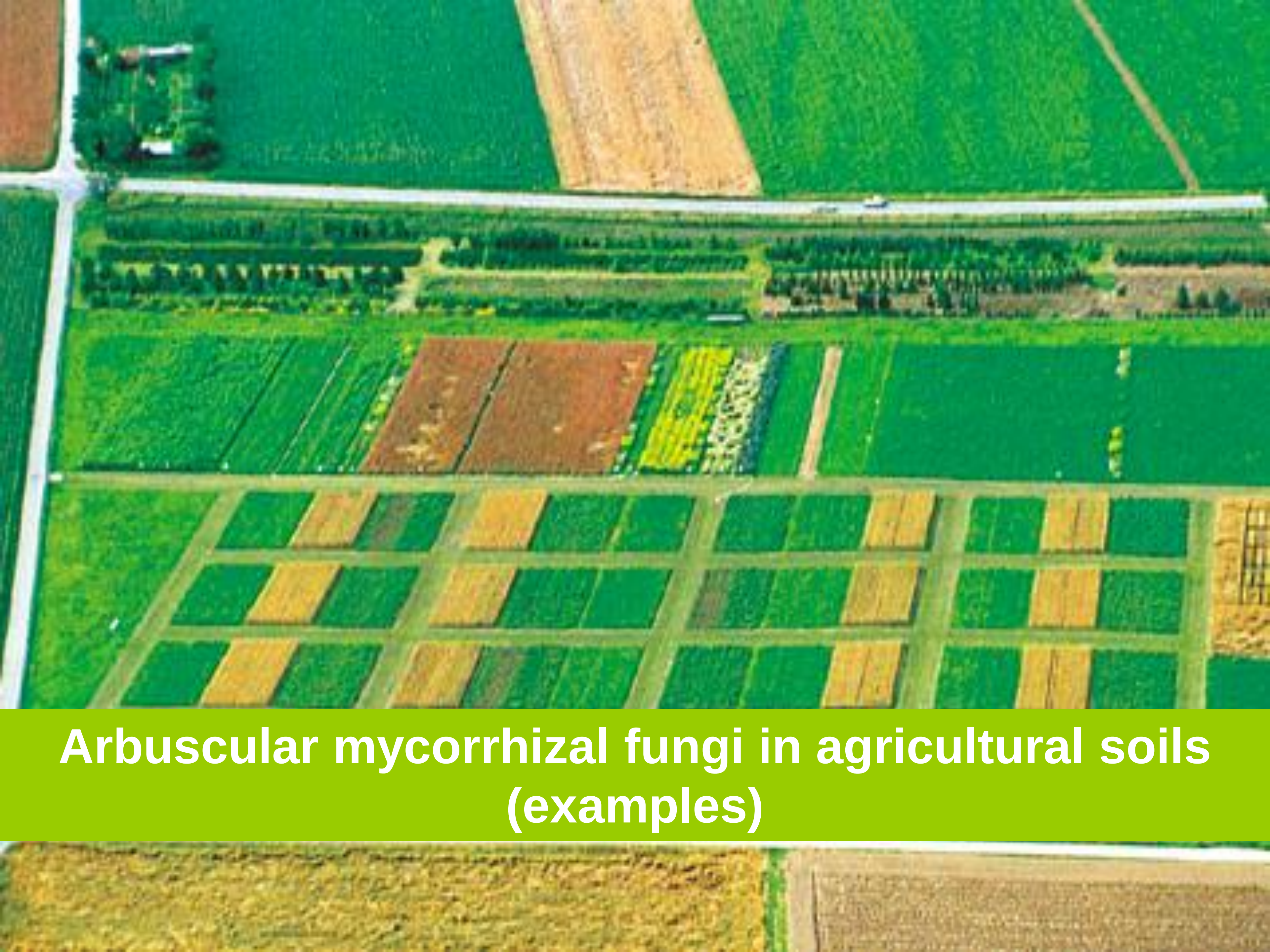
How to recognize the fungi: Spores/Sporocarps of Glomeromycota

Trap cultures

Take representative sample

Separation from soil

Isolation and quantification



Arbuscular mycorrhizal fungi in agricultural soils (examples)

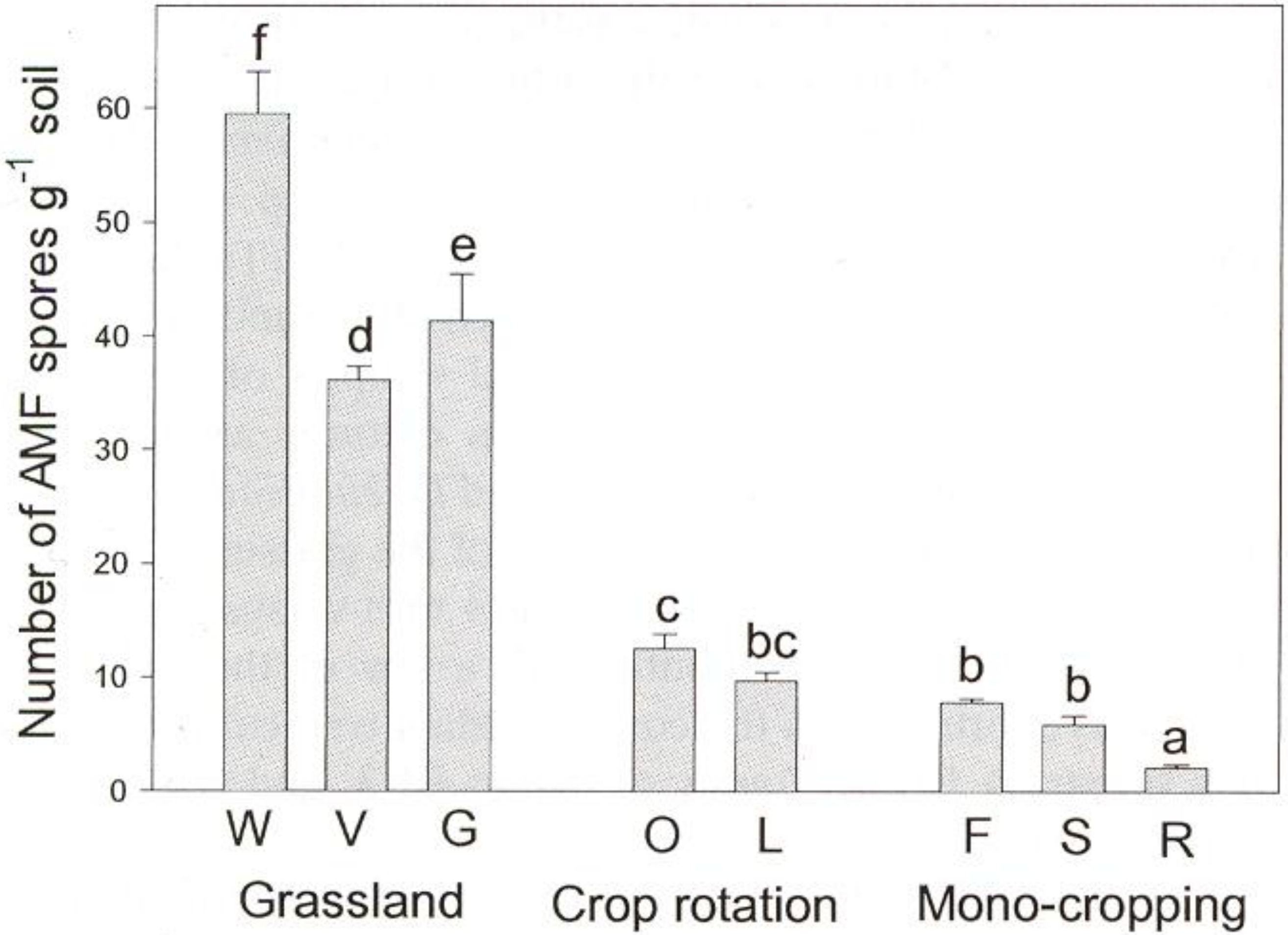


FIG. 1. AMF spore abundance at field sites (W, V, G, O, L, F, S, and R) with different cultivation practices. Input and management intensity increase from left to right. Data are reported as averages and standard deviations for four replicate plots per site. Nonsignificant differences between sites are indicated by identical letters above the bars and were determined by using Fisher's LSD test at the 5% level after a one-way ANOVA.

Source: Oehl et al., 2003

TABLE 3. Relative spore abundance of AMF species found at field sites and absolute numbers of spores identified

Glomales species or strain	% Spore abundance (no. of spores) at site							
	W	V	G	O	L	F	S	R
G. aggregatum						4.1 (12)	18.8 (34)	9.8 (8)
G. caledonium		0.1 (1)		1.6 (8)	3.6 (10)	7.9 (23)	9.9 (18)	
G. mosseae group[a]	1.3 (24)	3.0 (55)	1.9 (29)	5.9 (29)	17.3 (48)	10.7 (31)	31.5 (57)	54.9 (45)
G. geosporum	1.8 (34)	0.9 (16)	3.2 (47)	4.7 (23)	6.1 (17)	2.4 (7)	5.5 (10)	3.7 (3)
G. occultum group[b]	4.9 (90)	0.9 (17)	6.9 (103)	6.7 (33)	7.2 (20)	20.7 (60)	7.7 (14)	6.1 (5)
G. etunicatum	1.4 (26)	1.2 (22)	3.4 (50)	10.6 (52)	10.4 (29)	21.7 (63)	5.5 (10)	3.7 (3)
G. constrictum	1.5 (28)	3.7 (68)	2.8 (42)	6.8 (35)	6.8 (19)	1.4 (4)	2.8 (5)	1.2 (1)
G. diaphanum	0.3 (5)	0.1 (2)	4.5 (67)	6.3 (31)	15.2 (44)	17.6 (51)	14.4 (26)	14.6 (12)
S. calospora	0.2 (3)	5.5 (101)	1.1 (47)	6.3 (31)	4.2 (5)	5.2 (15)		6.1 (5)
G. fasciculatum group[c]	6.1 (112)	1.8 (34)	6.0 (89)	2.8 (14)	4.3 (12)	8.3 (24)	3.9 (7)	
Glomus sp. strain BR9	20.8 (384)	7.2 (132)	23.0 (342)	7.9 (39)	14.4 (40)			
G. invermaium	2.8 (51)	0.3 (6)	5.8 (86)	12.8 (63)	9.7 (27)			
G. dominikii	0.1 (1)		0.3 (5)	0.6 (3)	1.1 (3)			
A. laevis group[d]		0.1 (1)	2.0 (30)	8.1 (40)				
A. paulinae			2.1 (32)	13.0 (64)				
A. longula				0.8 (4)				
Acaulospora sp. strain BR1[e]				0.4 (2)				
S. pellucida				3.9 (19)				
G. heterosporum	8.2 (152)	9.1 (167)	5.7 (85)					
G. macrocarpum	0.8 (14)	14.6 (270)	0.2 (3)					
Glomus sp. strain BR2	10.5 (195)	2.0 (36)	11.1 (166)	0.4 (2)				
Glomus sp. strain BR3[f]	0.6 (11)	7.6 (140)	1.0 (15)					
E. infrequens	0.3 (5)	0.1 (1)	0.1 (1)					
Glomus sp. strain BR4	25.2 (466)	0.2 (5)	18.0 (268)					
Glomus sp. strain BR5	1.7 (31)	2.8 (51)			0.7 (2)			
G. microcarpum	0.7 (13)	1.0 (19)						
Glomus sp. strain BR6			0.8 (12)					
G. mortonii		18.1 (334)						
G. rubiforme		16.0 (295)						
Glomus sp. strain BR7		3.4 (63)						
Glomus sp. strain BR8		0.9 (16)						
G. globiferum		0.1 (2)						
G. ambisporum	5.1 (94)							
G. sinuosum	3.1 (57)							
G. versiforme	2.4 (44)							
G. tortuosum	0.3 (5)							
Archaeospora sp. strain BR10[g]	0.2 (3)							

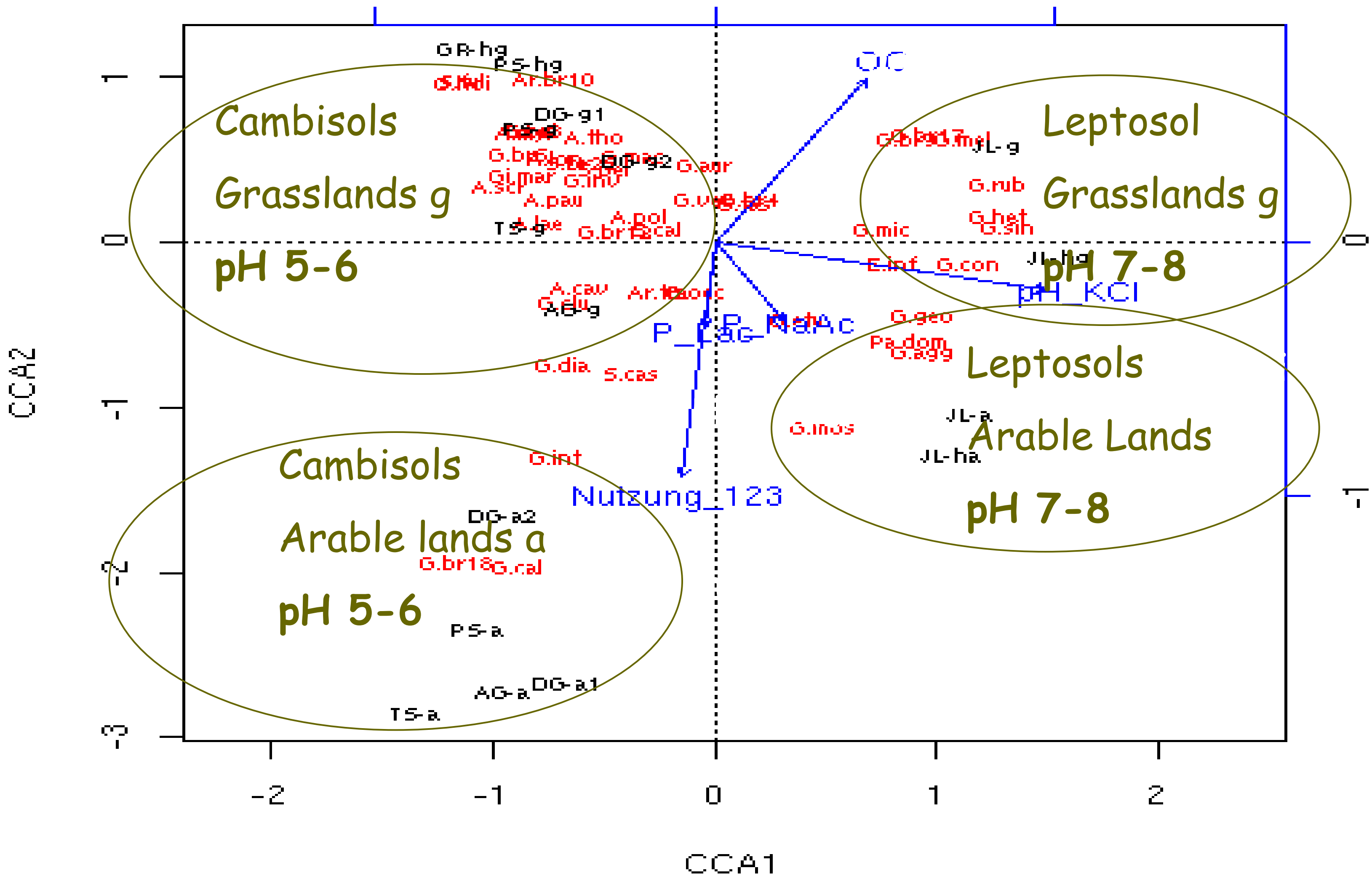
Generalist
Oehl et al. 2003
F. mosseae
F. geosporum
P. occultum
C. etunicatum
S. constrictum
G. diaphanum
G. fasciculatum
S. calospora

Relation to soil chemical characteristics, Europe

AMF species	r (linear regression)				
	pH (H_2O)	**SOM Organic carbon**	**Available P (E_1)**	**Available K**	**Number of weed species**
Glomus diaphanum	-0.26	-0.48*	**0.51***	0.42	0.26
G. caledonium	-0.36	-0.21	**0.56***	**0.63***	-0.36
G. etunicatum	0.19	0.09	-0.33	-0.36	0.34
G. fasciculatum	0.06	0.09	-0.16	-0.14	0.19
G. albidum & *Parag.occultum*	0.29	-0.19	-0.27	0.46	-0.25
G. constrictum	0.37	0.31	0.08	0.03	-0.03
G. invermaium	0.19	-0.03	-0.20	-0.3	-0.37
Pacispora dominikii	**0.62***	0.21	**-0.51***	-0.20	**0.61***
Scutellospora calospora	0.10	0.24	**-0.48***	**-0.55***	0.32
S. pellucida	-0.27	-0.28	**-0.48***	**-0.58***	**0.48***
Acaulospora paulinae	0.09	-0.14	**-0.62***	**-0.67***	0.40
A. thomii	0.13	-0.24	**-0.49***	**-0.55***	0.43
A. laevis	0.04	-0.15	**-0.53***	**-0.57***	0.38
A. longula	0.23	0.26	**-0.70***	**-0.58***	**0.56***
A. scrobiculata	0.21	-0.42	**-0.66***	0.57	0.39

Source: Oehl et al.)

Canonical correspondence analysis of spore counts and the explanatory variables pH, P_Lac, P_NaAc, soil organic matter and land use intensity



Two groups of fungal species: pH dependent

What does damage and what helps the native mycorrhiza?

Negative

- Soil sterilization
- Heavy fertilization with high amounts of N, P,
- NH_4 more than NO_3-N
- Cereals mono-culture
- Several years solo rape or sugarbeet
- Total weed control
- Intensive ploughing and soil disturbance

Often done in conventional agriculture

Positive

- Moderate fertilization with N, P, K, Mg
- Organic fertilizer
- Crop rotations & mixed cropping
- Mechanical weed control
- Zero or low tillage farming
- Integrated Pest Management (pesticides only if required)
- Use of mycorrhiza biostimulants

Often done in bio-farming

Why care about mycorrhiza in agriculture

- Mycorrhiza is important for nutrient uptake, mainly phosphates, water uptake, nematode control, soil errosion control
- Plant needs phosphates mainly at beginning of growth
- We can promote the mycorrhiza and save phosphates and other fertilizers
- Through most of conventionel agronomic practices (over-fertilization, mono-culture, etc.), the concentration and quality of native mycorrhiza decreased
- The problem is the low concentration of native mycorrhizal fungi under field conditions, or the un-known status of the quality of native mycorrhiza

Options to promote mycorrhiza on farmers level

- Inoculation technology (application of inoculum under seed or root)
- Use of biostimulants (natural products and micro-organisms or combinations to stimulate native mycorrhiza)

Myorrhiza Inoculation Technology

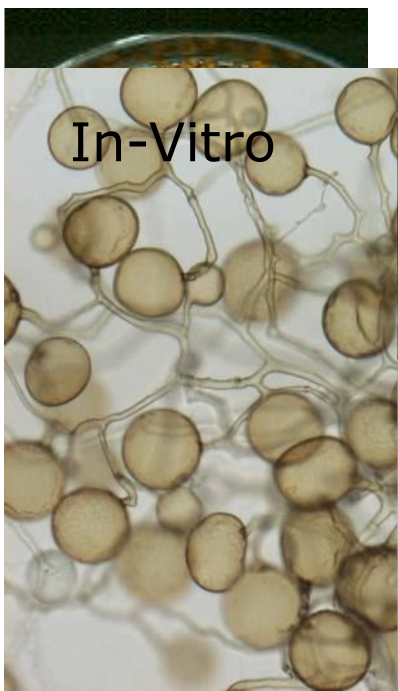
Inoculation technology means: The Introduction of selected mycorrhizal fungi to the crop or plant. Thus, this is active and direct management of the mycorrhiza in the plants rhizosphere

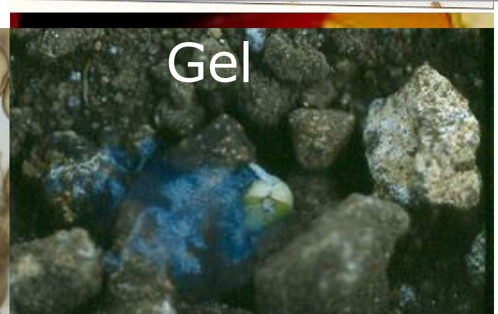
This technology requires:

- Selected fungal species
- Commercially available inoculum in huge quantities
- Practical field inoculation/application technologies
- Machinery for application

Currently this technology is only practical in nurseries and intensive vegetable and flower production. Inoculum costs between 300 and 1000 € / ha. Technology is not practical and not economical for broad acreage agriculture like wheat, barley, maize, sunflower or legume production. No practical application machinery available; seed treatment with mycorrhizal inoculum does not work!

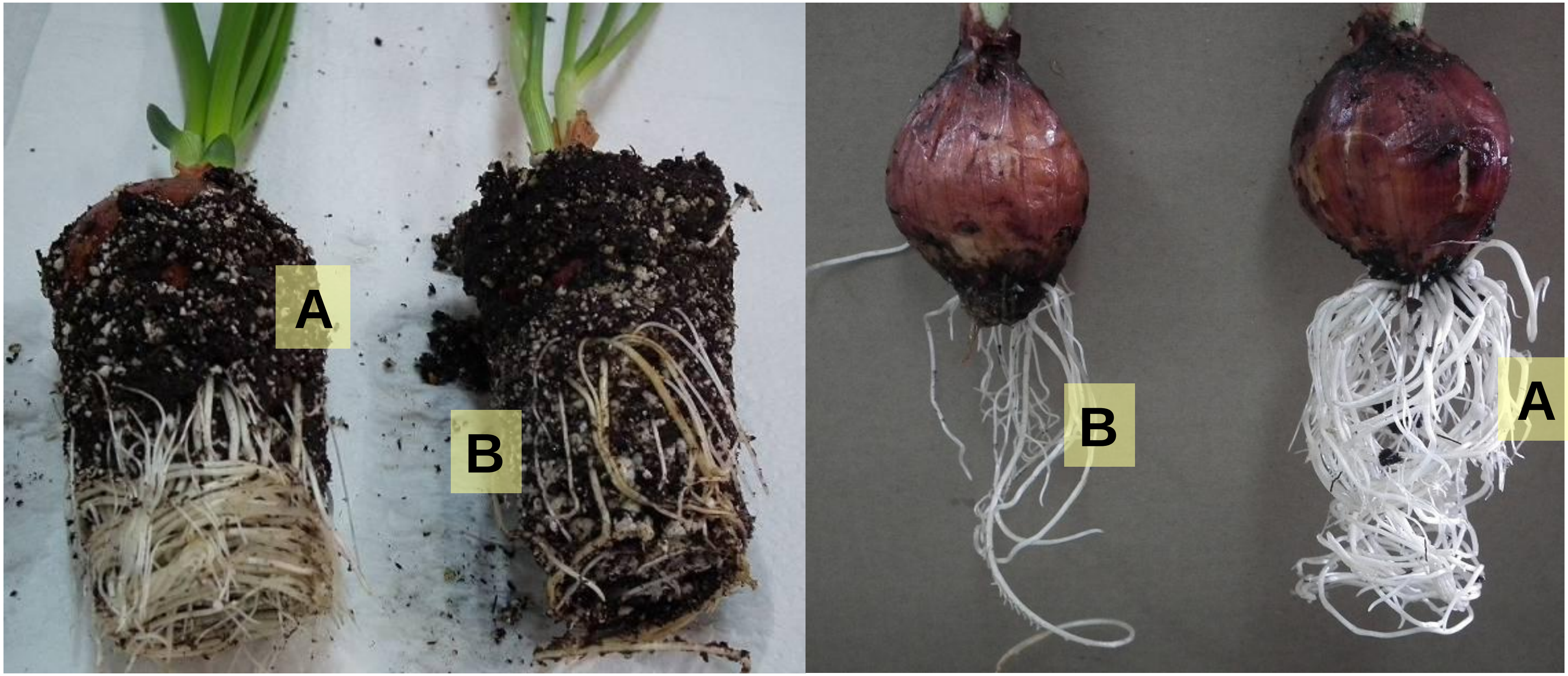
AM Inoculum – what is used as carrier





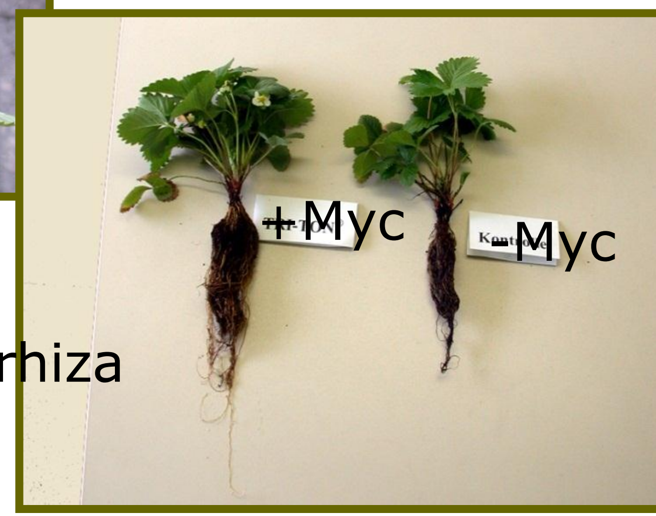
BACTOLiVE AM BASIC (carrier is expanded broken clay – Leca
Advantage: long survival time (>5 years)
Of AM fungus

Good success of mycorrhizal inoculations in vegetable and Nursery production (trees, flowers)



Onion bulbs grown in soilless mixture (peat/vermiculite/perlite) and treated (A) or not (B) treated with BACTOLiVE AM BASIC inoculum (after 3 weeks of inoculation)
Experiment conducted by Dr. Al Karaki, (2012)

Examples of growth responses Strawberries



With mycorrhiza

Inoculation of each plant with 10 ml BACTOLiVE AM BASIC in planting hole

Mechanical application technology not sufficiently developed

First intents failed

Inoculation technologies - summary

- Useful in nurseries
- Expensive (may range from 120 – 1000 Euros / ha in broad acreage crops)
- Seed treatment not successful (e.g. with in-vitro produced spores) – also expensive
- Not sufficient inoculum available
- Quality of inoculum highly variable
- Success of inoculation not guarantteed
- Lack of methods to define where inoculation is necessary – there are situations where inoculation is not needed

Alternatives to inoculation technology

- Use biostimulants to improve the native arbuscular mycorrhiza
  - Mycorrhizal fungi are everywhere but the concentration in soil and quality is often insufficient
  - Biostimulants can be applied with seed, e.g. cereals
  - Biostimulants are rather inexpensive (3–30 €/ ha) as compared to mycorrhizal inoculants (125-1000 €/ha)

Biostimulants investigated for seed treatment in wheat

- **Calcite**: a micronized calcite derived from natural mineral deposits of **calcite**. RHIZO-MIC CALCITE of company RHIZO-MIC UG, Germany). Dose in growth chamber experiment: 0.1 and 1 mg/seed
- **Formononetin**: potassium salt of 7-hidroxy, 4’-metoxy **isoflavone** (MYCONATE product of Plant Health Care USA). In growth chamber: 0.1 and 1 mg/seed; in field: 100 g/ha on seed
- **Silicate**: water soluble sodium silicate with mainly 36% **silicon** (QUICK SOL product of Beyond International Inc. Miami, USA). In growth chamber: 0.1 and 1 mg/seed; in field 3 L/ha in furrow diluted with water under seed)
- **Fosfobio**: **phosphate solubilizing bacteria**, *Bacillus megaterium* (product OikoBac Fosfobio of company Oiko Chile Ecological Resources Inc.). In growth chamber 0.1 and 1 mg/seed; in field: 100 ml/ha to seeds)
- **Nitrobio**: **nitrogen fixing bacteria**, Azotobacter spp. (OikoBac Nitrobio of company Oiko Chile Ecological Resources Inc.). In growth chamber 0.1 and 1 mg/seed)
- **BACTOLiVE SEED**: **beneficial soil bacteria (5 different Bacillus spp.) and a beneficial soil fungus, *Trichoderma harzianum*, on sea weeds extracts as carrier** (RHIZO-MIC SEED for cereals, product of RHIZO-MIC UG, Germany). In growth chamber: 0.1 and 1 mg/seed; in field: 10 g / 100 kg of seed after dilution in water.

Biostimulant effect on growth and mycorrhizal root colonization in wheat, 18 DAT

Growth chamber experiment, 5 reps

Treatment 0.1 mg/seed	Shoot dry weight (mg/plant)	Root dry weight (mg/plant)	Mycorrhizal root colonization (%)
Not treated	57 ab	30 b	54 b
Fosfobio	54 b	23 c	58 ab
Nitrobio	51 b	20 c	56 b
BACTOLiVE SEED	60 a	44 a	62 a

Promote the native mycorrhiza by biostimulants (natural products and MO)

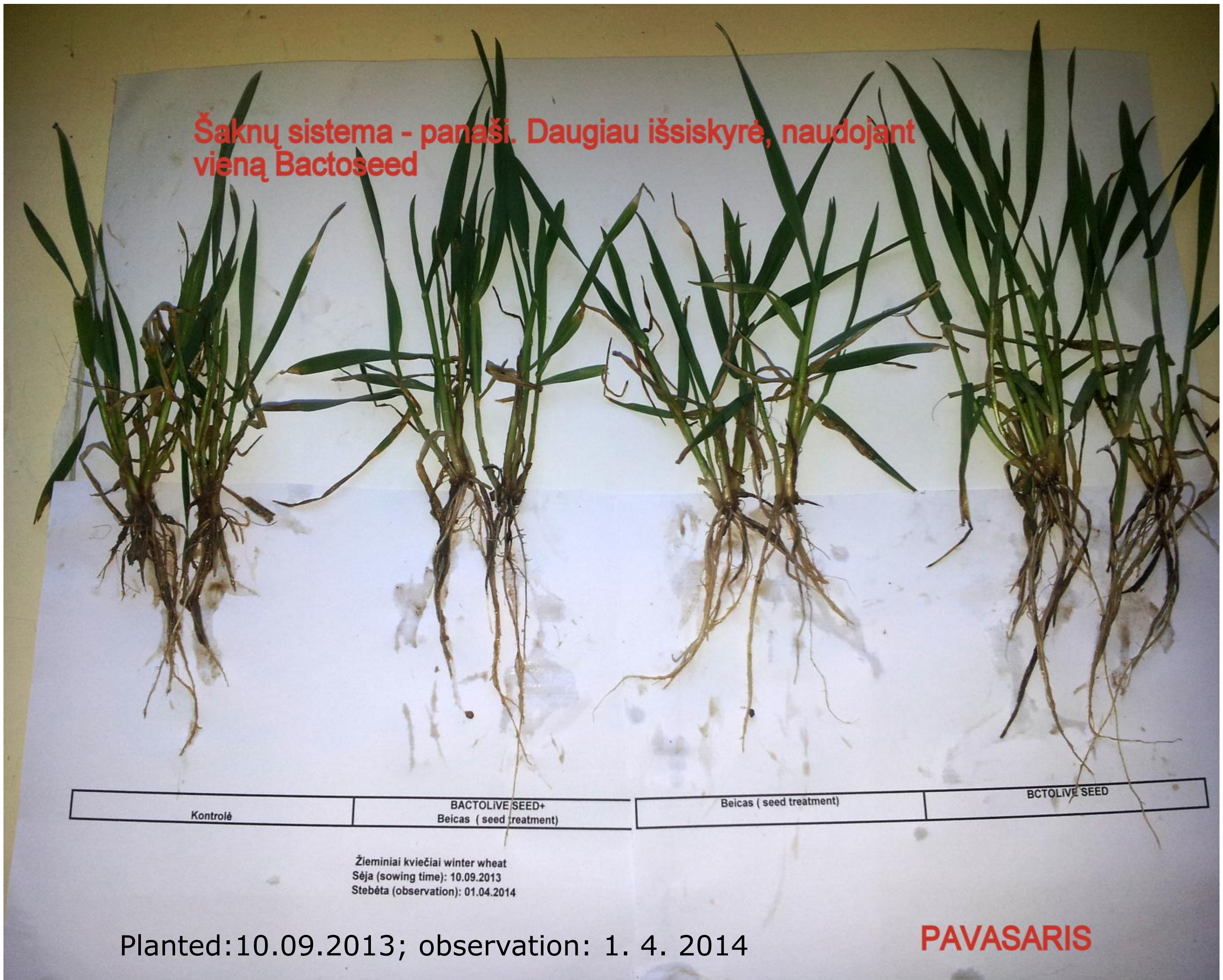
- ***Stimulation of the native mycorrhizal fungi:***
  - Manipulation/promotion of root colonization by biostimulants which can be natural products or combinations of micro-organisms with natural products
  - Biostimulants can be applied to soil or by seed treatment:

After seed treatment with BACTOLiVE SEED (right)

Parameter per plant (mean of 5 plants)	Control 30 DAT	BACTOLiVE SEED 30 DAT
Mycorrhiza Frequency (%)	54%	62%
Mycorrhiza (%) Infection Intensity	8.4%	10.3%
Mycorrhiza root biomass (mg)	2.6 mg	4.7 mg
Root fresh weight (mg)	41 mg	67 mg
Shoot fresh weight (mg)	121 mg	242 mg

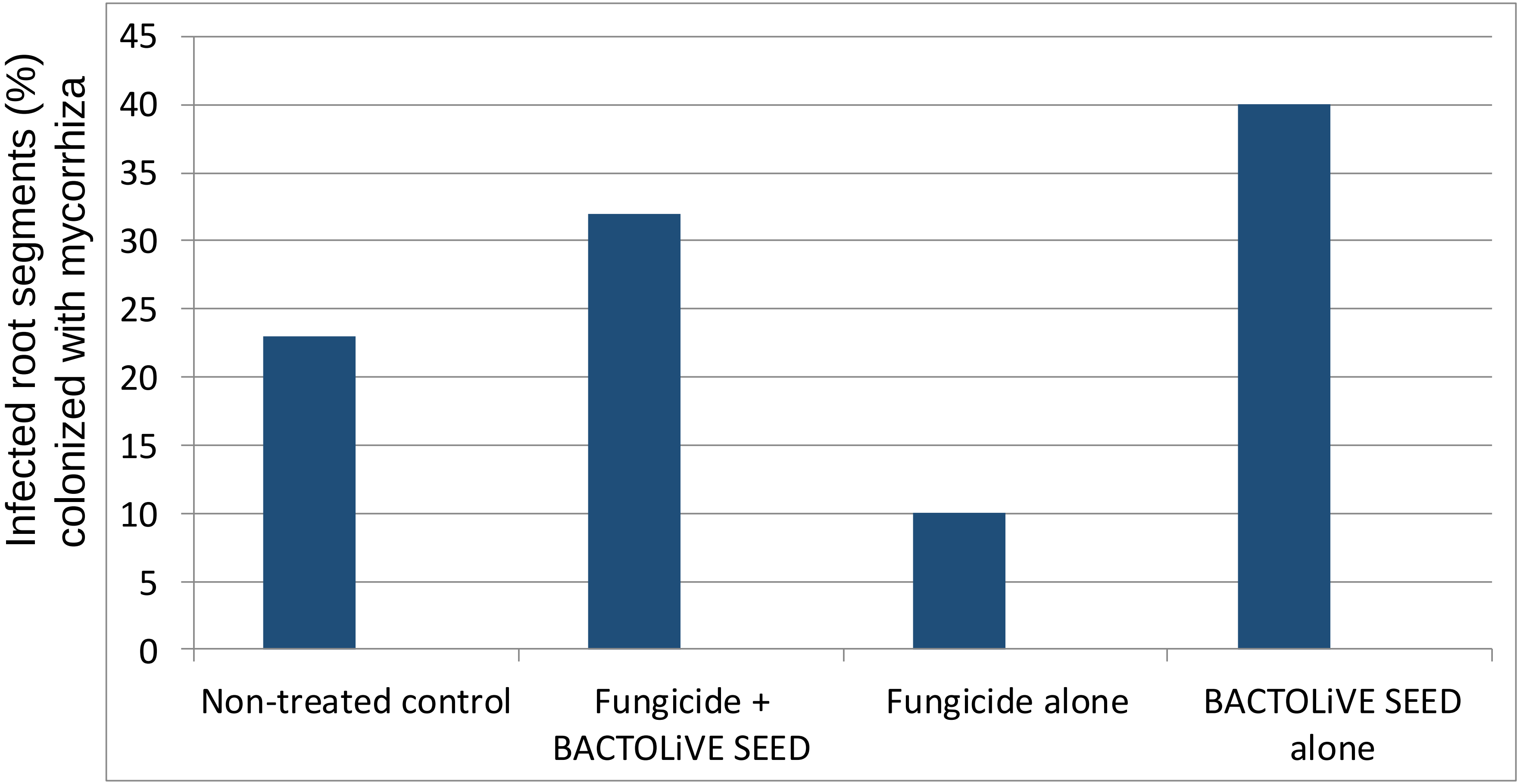
Field experiment with wheat

BACTOLiVE SEED in Winter Wheat Root development in field (LT)



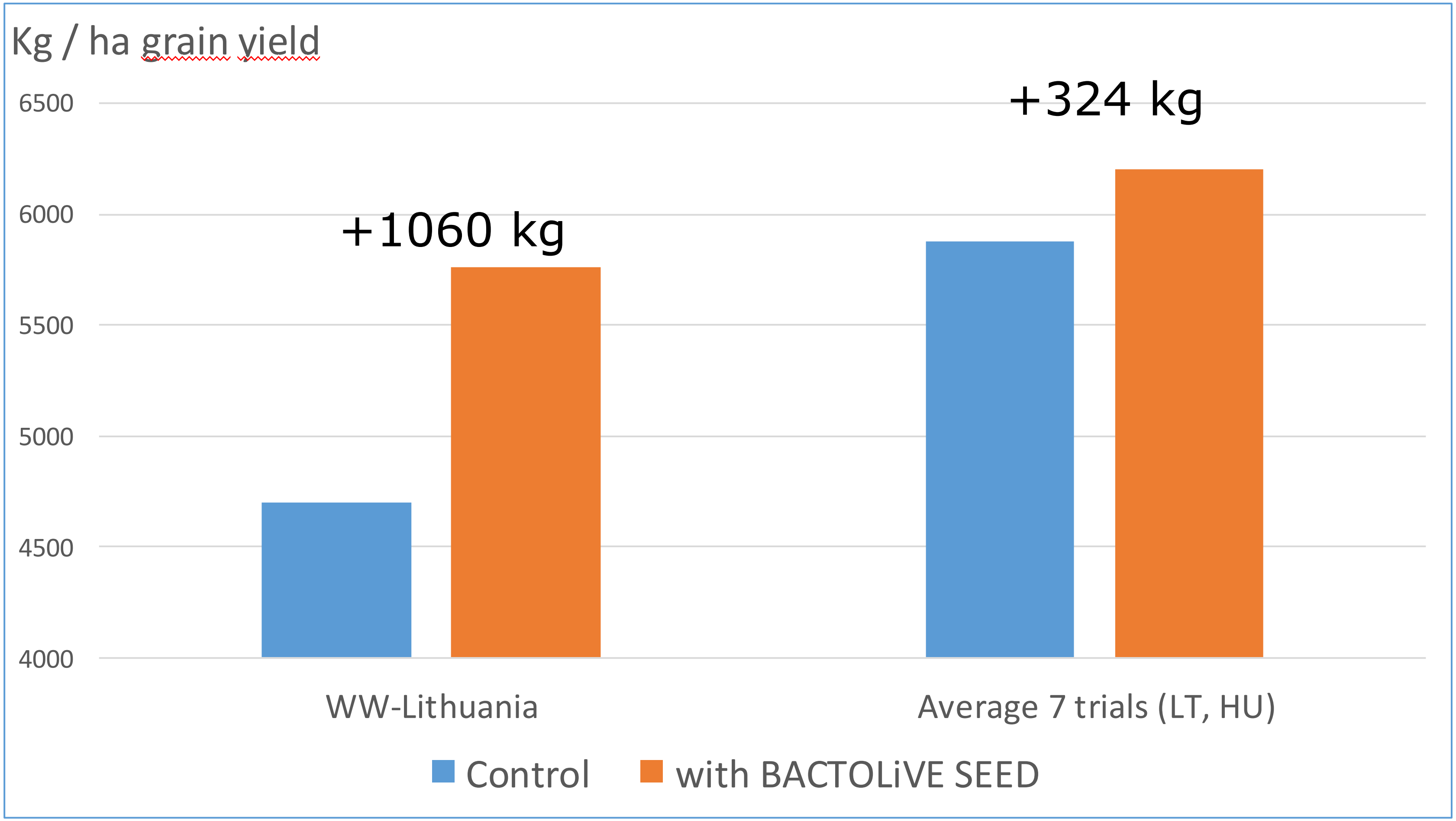
Planted:10.09.2013; observation: 1. 4. 2014

BACTOLiVE SEED in Winter-Wheat Effect on Mycorrhiza Root Colonization



Results are expected: Fungicides alone can have slight negative effects on mycorrhiza, and RHIZO-MIC SEED has positive effects, either alone or in combination with fungicide seed treatment. The higher the mycorrhiza colonization – the better potential has the root to take up phosphate and other plant nutrients. Data are from 100 root segments investigated.

BACTOLiVE SEED – Field trials Wheat
Grain yields (kg/ha) after seed treatment



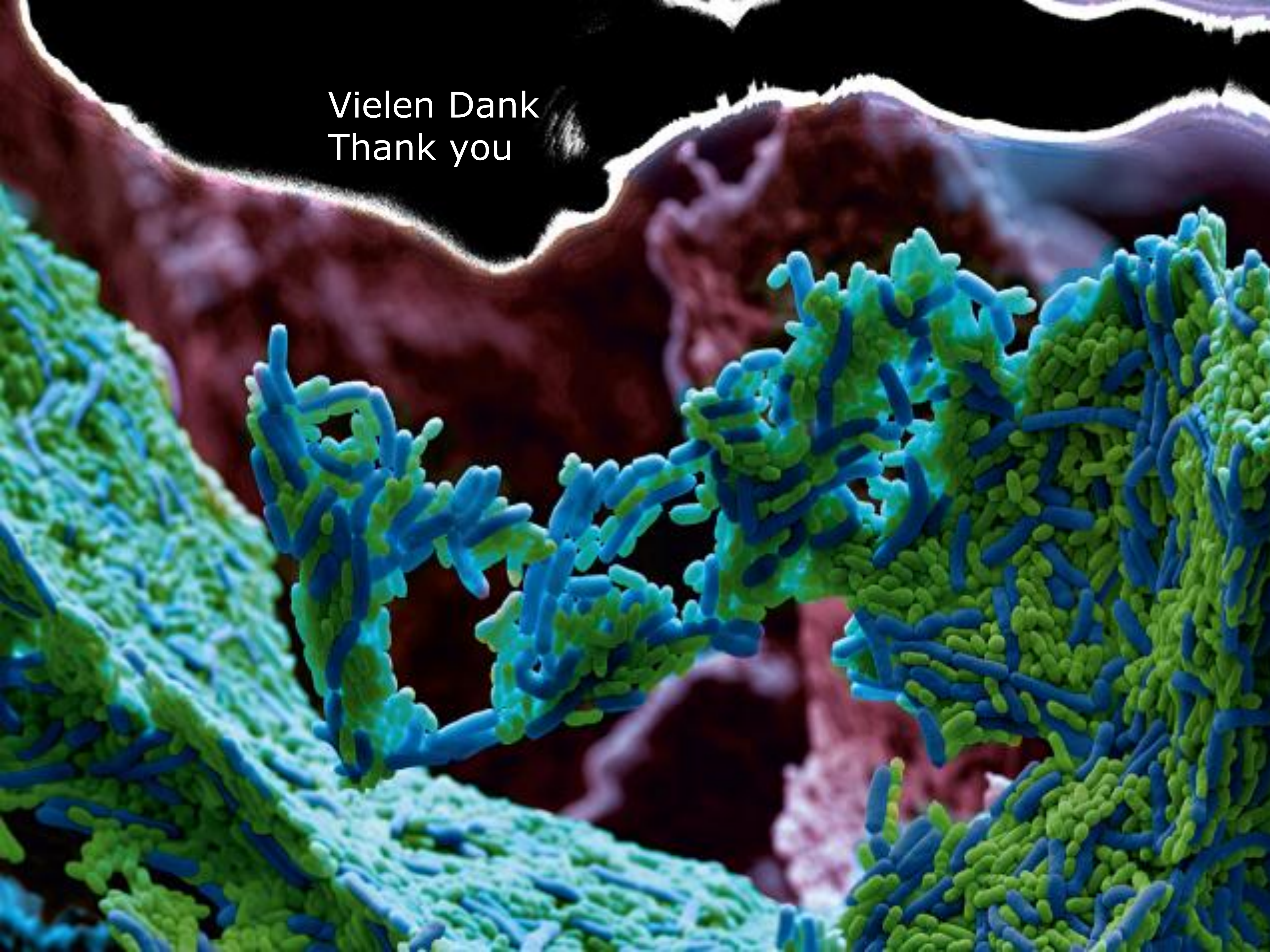
Note: In addition of stimulation of mycorrhiza,
BACTOLiVE SEED and BACTOLiVE AGRO have other effect on plants like root and soil health, root growth stimulation, nutrient recycling, etc

Where and when should native mycorrhiza be promoted with biostimulants

- Intensively used soils have low concentrations of mycorrhiza – need inoculation or biostimulants
- Sandy soils need biostimulants more than clay soils
- After time without vegetation the concentration of mycorrhiza is low – need stimulation
- After several years of mono-culturing, e.g. with wheat, the concentration of native mycorrhizal fungi is low or not effective: need stimulation
- After non-mycorrhizal crops like oil-seed-rape and sugarbeet, native mycorrhizal concentration is decreased: stimulation of native mycorrhiza needed with next crop

Conclusions –Biostimulants

- CALCITE (mineral), FORMONONETIN (plant extract), BACTOLiVE SEED (mixture of PGPR microbes plus sea weeds extracts) and some others have positive effects on early root growth and arbuscular mycorrhiza development
- Stimulation of early arbuscular mycorrhiza colonization increases early phosphate uptake which is important for grain yield formation
- Wet seed treatment is a simple and well known agricultural technology – suitable also for biostimulants
- BACTOLiVE SEED was selected for field trials as it was regularly positive for root development and stimulated root colonization with native arbuscular mycorrhizal fungi – it increased yields by 324 kg/ha (n=7)
- BACTOLiVE SEED is used at low dose (100 g/ton) and is specifically for cereals like wheat, barley, rye, oates, triticale (winter and summer cereals).
- It can be combined with fungicide (e.g. Vitavax) and insecticide seed treatment
- It is approved for ecological farming by FIBL
- It is of relative low cost for the farmer (2.5 – 3 Euro / ha at farmers level)


Vielen Dank
Thank you

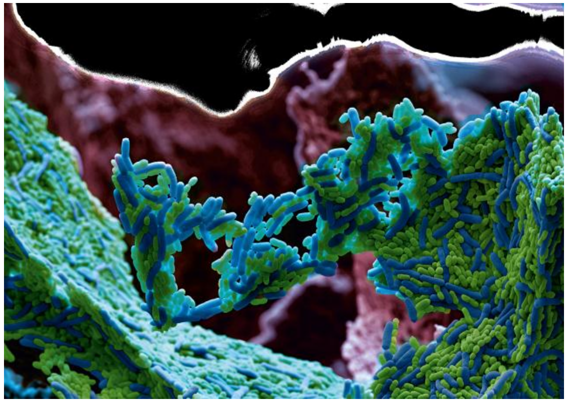
Some additional slides

- Categories of rhizosphere micro-organisms
- Methods of investigating arbucular mycorrhiza
- Advantages of BACTOLiVE SEED

Beneficial
Soil Microorganisms

Symbiotic nitrogen fixation of Rhizobia with legumes
Forming root nodules and fixing up to 200 kg N/ha y

Microorganisms for fixation of air nitrogen (N_2)



Bacillus spp., Azo-bacteria, Pseudo-monads, *Penicillium*, *Actinomycetes* etc

Microorganisms for the solubilization of nutrients like phosphates, potassium, calcium, magnesium, micro-nutrients etc.

Beneficial Non-Pathogenic Soil Microorganisms

- Microorganisms for the decomposition & transformation of organic matter and for the mineralization of organic material (extremely important)
- Microorganisms for the health of the soil and roots (rhizosphere)

Without With
BACTOLiVE **AGRO**

Accelerated Decomposition

BACTOLiVE **SEED**

Techniques to measure / identify arbuscular mycorrhizal structures

- Quantify root length per unit soil (Mycorrhiza studies are root studies)
- Stain and measure / quantify fungal structures in roots
  - Wash roots carefully, place roots in reagenz glasses, cook at 95 degree C in 10% KOH for 15-120 min., decant, neutralize/acidify with 10% HCl, add mixture Glycerol+Lactic-Acid+Water with 0.5% Trypan Blue (some people use blue ink) and heat to 95o C for 15-60 min., decant and add mix glycerol-water. To extract excess of dye
  - Plant cell nuclei are distroyed, trypan blue /dye adheres to chitin of fungal wall, structures appear blue in cleared roots under microscope at 60-400 x magnification
  - Alternatively chitin or fungal proteins can be determined chemically
- Extract from soil and stain root external fungal mycelium
  - Wash defined small volume of soil on 45 um sieve – transfer to defined volume of water – take small aliquot and pass through nitrocellulose filter with grid lines – measure length of mycelium by e.g. gridline intersection method in microscope at 200-400 x.
- Separate spores from soil, identify species (count spores)
  - Take defined volume of soil, disperse in water – wait 10 sec, pass through a series of fine sieves being the smalles 45 um opening – transfer to centrifuge glass – establish gradient with sugar solution – centrifuge – separate spores from sugar gradient – wash spores – pass to Petri dish – separtate from water and mount on glass slides – fix with PVLG and observe in microscope
- Determine Glomalin which is specific to Arbuscular Mycorrhiza
  - Glomalin is a mycoprotein (part of humus fraction which can be extracted from soil
- Use molecular biological methods to identify mycorrhizal fungus/i
  - Use specific markers for determining Glomeromycota

Advantage of BACTOLiVE SEED

- Easy to handle (wet seed treatment)
- Relative low cost
- Stimulating native mycorrhiza
- Improves soil health and root health
- Some biostimulants can induce resistance in plants against diseases
- Biostimulants (e.g. BACTOLiVE products) can also be used in other crops like potato, tubers, maize, sunflower, legumes etc. to stimulate arbuscular mycorrhiza
- Biostimulants can also be used as soil treatment, e.g. BACTOLiVE AGRO

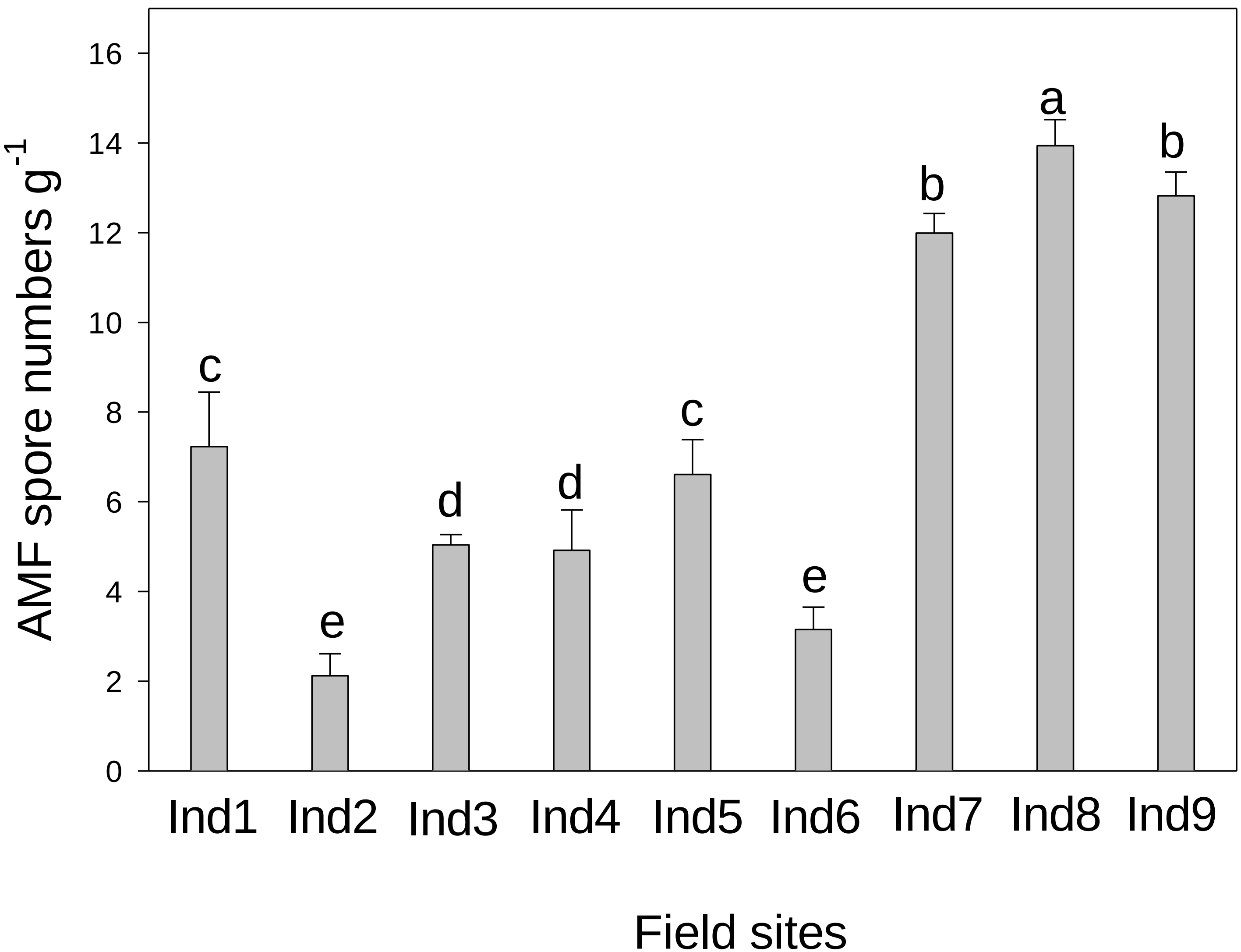
Occurrence of mycorrhizal species in european vegetable areas

Less than 5 spores / ml soil and less than 3 AMF species is a deficite situation

	Spain								Holland			GERMANY						
Fungal species	Site 1 Spain Cuellar	Site 2 Spain Huescar	Site 3 Spain Sevilla Cucumber	Site 4 Spain Sevilla PEP 1	Site 5 Spain Cadiz PEP 2	Site 6 Spain Cadiz PEP 3	Site 7 Spain Almeria	Site 8 Spain Murcia	Site 9 NL 16-03	Site 10 NL 16-01	Site 11 NL 16-02	Site 12 Germany Goch Aspeden	Site 13 Ger Stadecken Spargel	Site 14 Ger Ober-Olm Spargel	Site 15 Ger N-Sachsen Spargel	Site 16 Ger N-Sachsen Händorf	Site 17 Ger B-Württh. Pleidersh.	Frequency of Species Occurrence
Glomus caledonium															15	5		2
Glomus claroideum													10					1
Glomus coronatum	6																	1
Glomus diaphanum													10					1
P. dominikii		6																1
Glomus etunicatum	13	6								15			15	10				5
Glomus fasciculatum	13		6			6						5		10	5			6
Glomus geosporum														5			5	2
Glomus invermaium						6			5	15		5		70				5
Glomus macrocarpum					13						15							2
Glomus intraradices	25	13			6		10	25		10		5	90	100			10	10
Glomus mortonii	13			13	13	19		10										5
Glomus mosseae		13											10					2
Glomus tortuosum	6																	1
Glomus sp. 1		6																1
Glomus sp. 2			19															1
Glomus spp.													10		5		20	3
Acaulospora morrow ae	6	13							5				5					4
Acaulospora sp. 1		19																1
Acaulospora sp. 2								5										1
Entrophospora infrequens	6																	1
Scutellospora pellucida													55					1
Total spores / 100 ml soil	88	76	25	13	32	31	10	40	10	40	15	15	205	195	25	5	35	
Nr. of Species	8	7	2	1	3	3	1	3	2	3	1	3	8	5	3	1	3	22

Source: AMYKOR (Wolfen) unpublished data of 2002-2004

AMF spore numbers at Indian experimental agricultural field sites (wheat)



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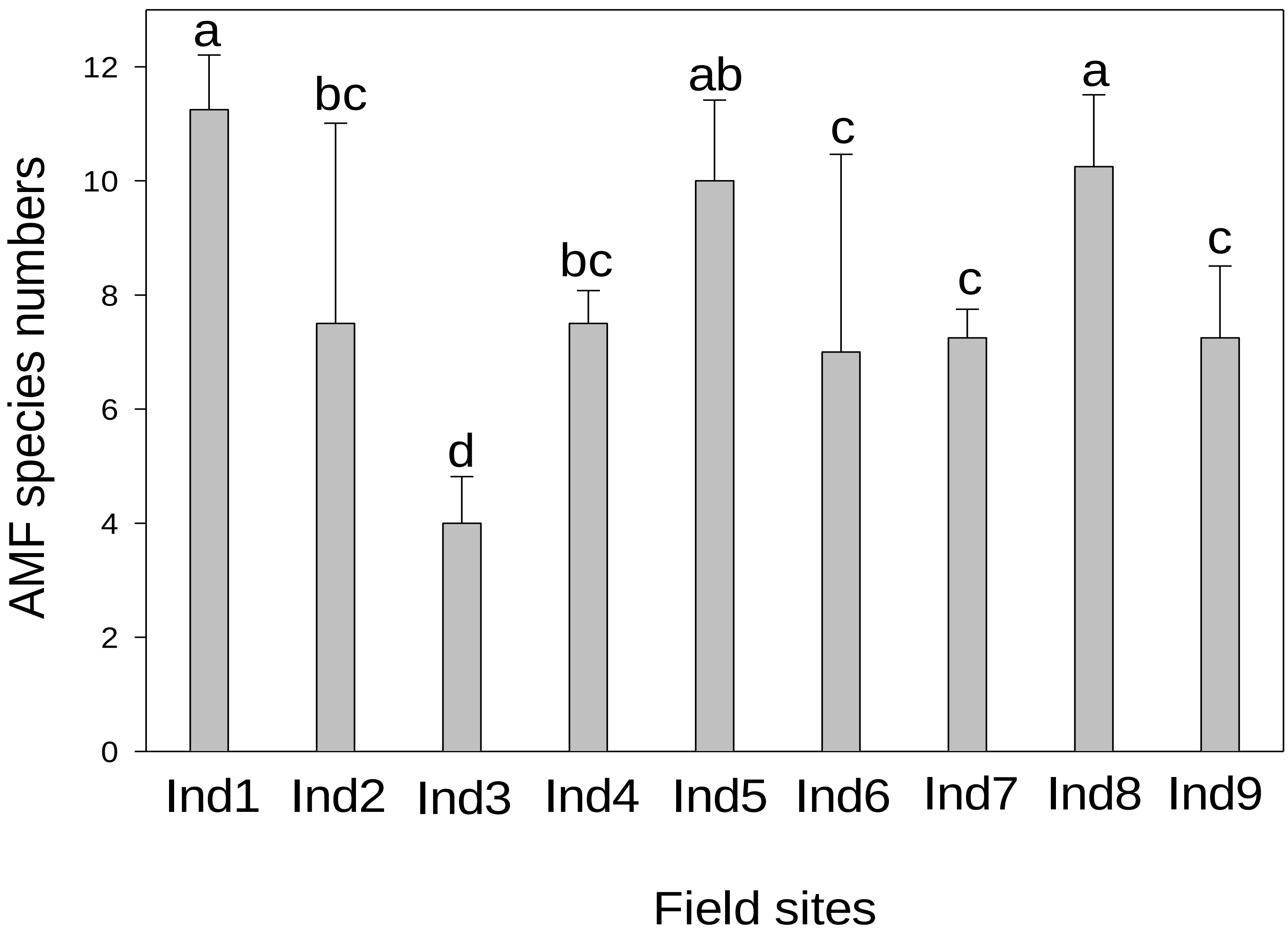

Mechanism of P uptake