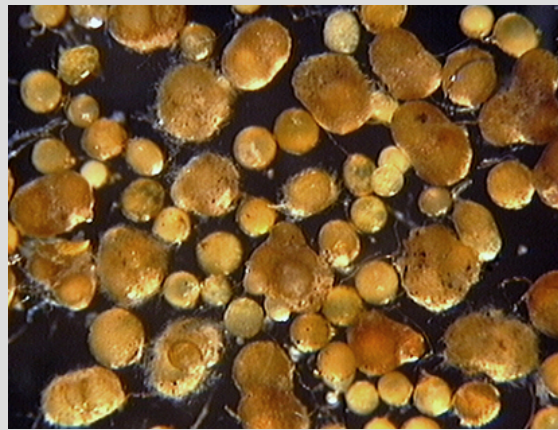


Agroecology for IPM III

Diseases



Sabine.Ravnskov@agrsci.dk

Department of Agroecology, Aarhus University

Outline



- What is a plant disease?
- Available non-chemical methods to control plant diseases
- Biological control of plant diseases
- IPM management of potato stem canker – an example
- Beneficial interactions between plants and soil microbes
- Arbuscular mycorrhiza – an example of biocontrol of diseases



Plant diseases - examples



Grey mould

Botrytis cinerea



Mildew

Podosphaera xanthii



Late blight

Phytophthora infestans



Club root

Plasmodiophora brassicae



Cavity spot

Pythium violae



Root and stem rot

Phytophthora cryptogea

Tactics for biological plant disease management



- **Conservation;optimisation of environmental conditions for beneficials**
 - Organic matter
 - Continuous mono-culture (Take all decline in wheat)
 - Crop rotation
 - Biofumigation
 - Intercropping
- **Inoculation with biocontrol agents**
 - Strategic application if niche competent
 - Pre-inoculation of transplants
 - Seed coating
 - Application to protect wound
 - Continuous massive introduction
 - Routine spray

Other non-chemical tactics to manage plant diseases



- Resistant varieties
- Mechanical control of soilborne diseases
- Thermal control of soilborne diseases
- Botanicals
- Cropping system design
- Timing of sowing
- (alternative crops)



Alternative crops



Healthy soil



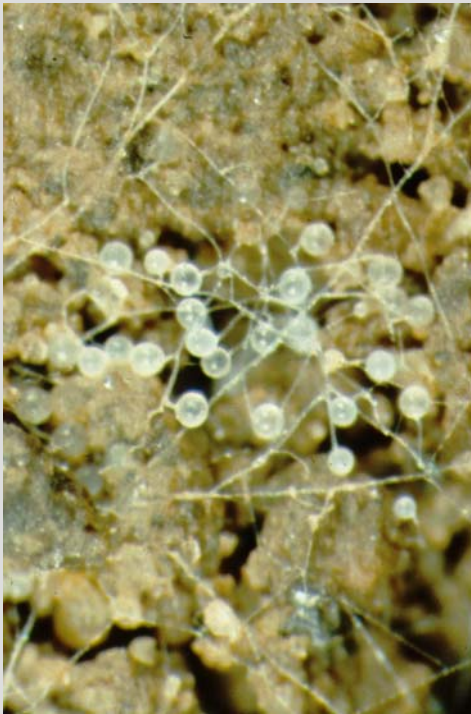
Soil infested with lupin pathogens



Soil infested with pea pathogens



Biological control of plant diseases





Outline

- Mode of action of biocontrol agents
- Biological management of root pathogens
- Biological management of foliar pathogens
- Summary

Definition of biological plant disease control



Biological control refers to the purposeful utilization of introduced or resident living organisms, other than disease resistant host plants, to suppress the activities and populations of one or more plant pathogens

Pal & Gardener, 2006, Biological Control of Plant Pathogens, APSnet



Mode of action of Biological Control Agents (BCAs)

- Competition
- Antibiosis
- Parasitism
- Grazing
- Induction of plant defense

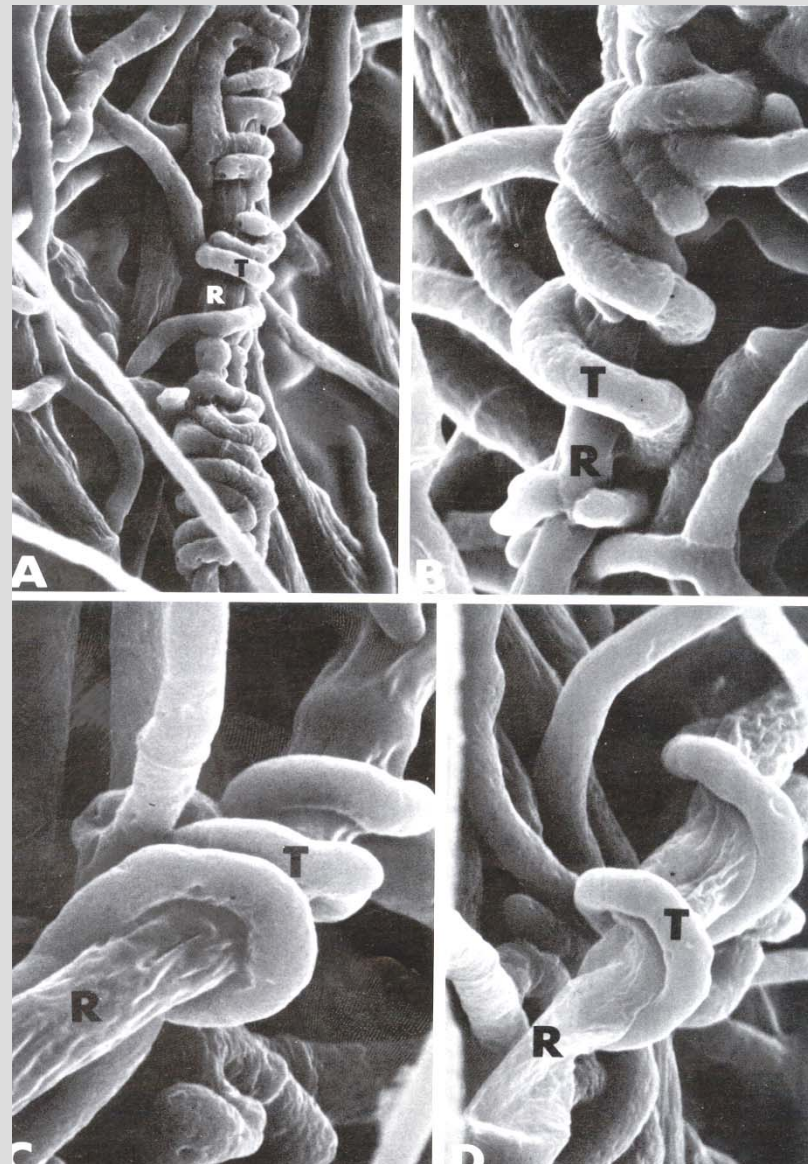
Microbial antibiotics



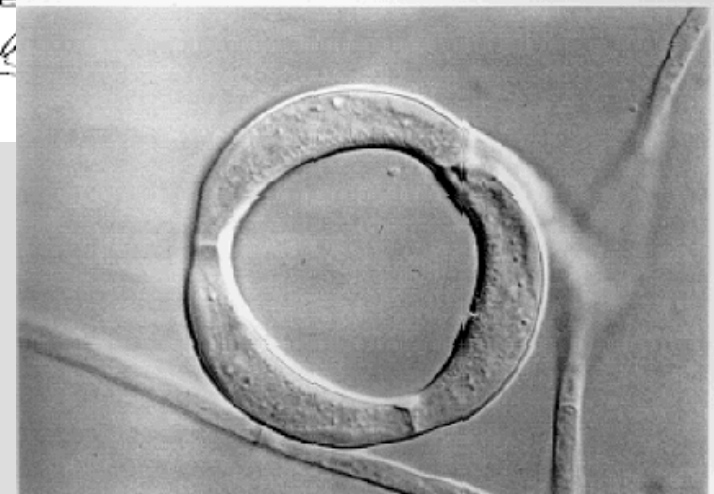
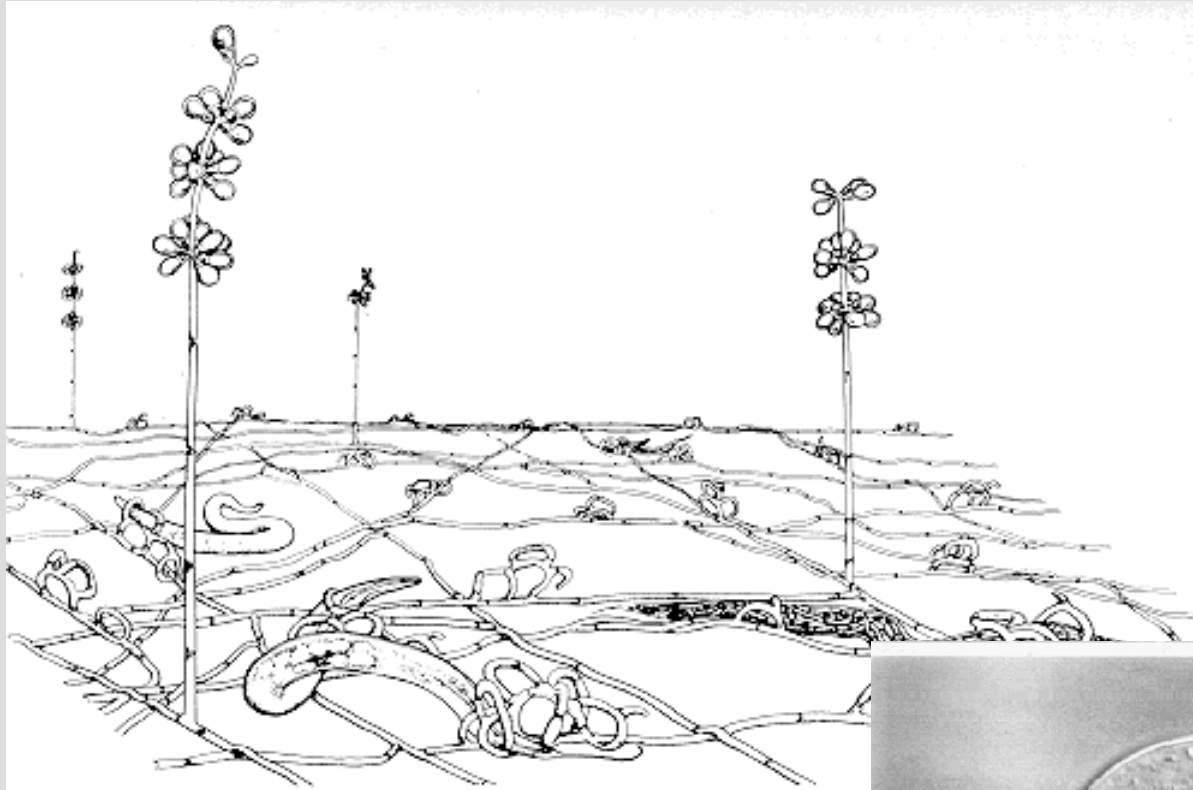
Table 2. Some of antibiotics produced by BCAs

Antibiotic	Source	Target pathogen	Disease	Reference
2, 4-diacetyl-phloroglucinol	<i>Pseudomonas fluorescens</i> F113	<i>Pythium spp.</i>	Damping off	Shanahan et al. (1992),
Agrocin 84	<i>Agrobacterium radiobacter</i>	<i>Agrobacterium tumefaciens</i>	Crown gall	Kerr (1980)
Bacillomycin D	<i>Bacillus subtilis</i> AU195	<i>Aspergillus flavus</i>	Aflatoxin contamination	Moyne et al. (2001)
Bacillomycin, fengycin	<i>Bacillus amyloliquefaciens</i> FZB42	<i>Fusarium oxysporum</i>	Wilt	Koumoutsis et al. (2004)
Xanthobaccin A	<i>Lysobacter</i> sp. strain SB-K88	<i>Aphanomyces cochlioides</i>	Damping off	Islam et al. (2005)
Gliotoxin	<i>Trichoderma virens</i>	<i>Rhizoctonia solani</i>	Root rots	Wilhite et al. (2001)
Herbicolin	<i>Pantoea agglomerans</i> C9-1	<i>Erwinia amylovora</i>	Fire blight	Sandra et al. (2001)
Iturin A	<i>B. subtilis</i> QST713	<i>Botrytis cinerea</i> and <i>R. solani</i>	Damping off	Paulitz and Belanger (2001), Kloepper et al. (2004)
Mycosubtilin	<i>B. subtilis</i> BBG100	<i>Pythium aphanidermatum</i>	Damping off	Leclerc et al. (2005)
Phenazines	<i>P. fluorescens</i> 2-79 and 30-84	<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	Take-all	Thomashow et al. (1990)
Pyoluteorin, pyrrolnitrin	<i>P. fluorescens</i> Pf-5	<i>Pythium ultimum</i> and <i>R. solani</i>	Damping off	Howell and Stipanovic (1980)
Pyrrolnitrin, pseudane	<i>Burkholderia cepacia</i>	<i>R. solani</i> and <i>Pyricularia oryzae</i>	Damping off and rice blast	Homma et al. (1989)
Zwittermicin A	<i>Bacillus cereus</i> UW85	<i>Phytophthora medicaginis</i> and <i>P. aphanidermatum</i>	Damping off	Smith et al. (1993)

Mycoparasitism of *Rhizoctonia* by *Trichoderma*



Nematode trapping fungi



Fungal grazing by Collembola



Foto: John Larsen

Plant defense induction



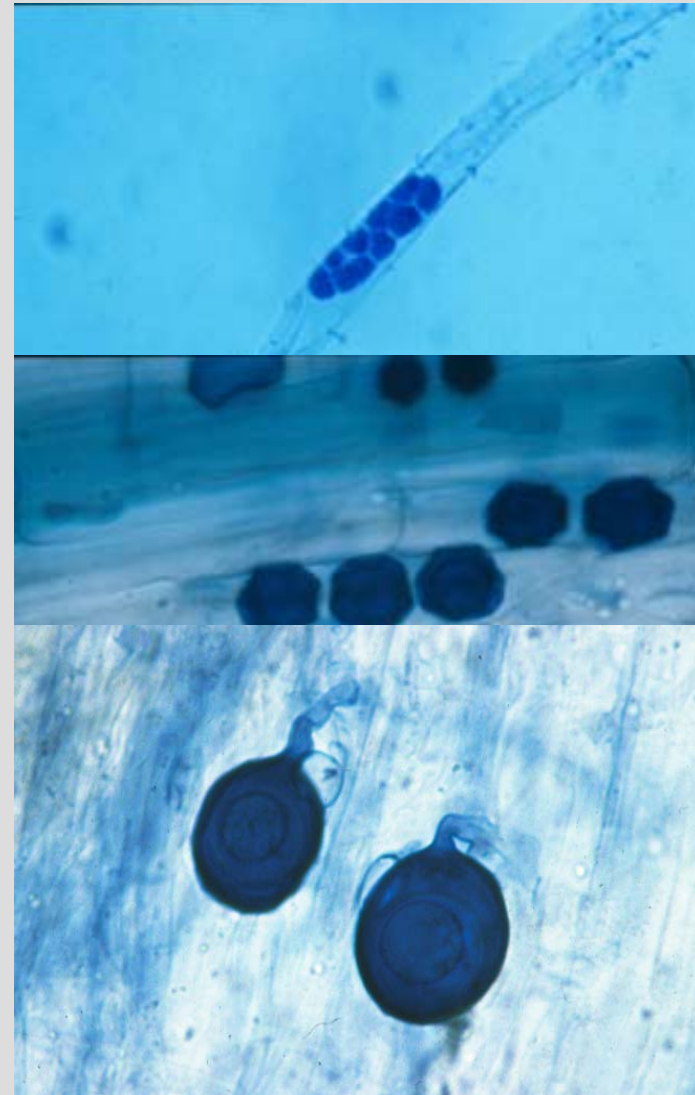
Table 3. Bacterial determinants and types of host resistance induced by biocontrol agents

Bacterial strain	Plant species	Bacterial determinant	Type	Reference
<i>Bacillus mycoides</i> strain Bac J	Sugar beet	Peroxidase, chitinase and β -1,3-glucanase	ISR	Bargabus et al. (2002)
<i>Bacillus pumilus</i> 203-6	Sugar beet	Peroxidase, chitinase and β -1,3-glucanase	ISR	Bargabus et al. (2004)
<i>Bacillus subtilis</i> GB03 and IN937a	<i>Arabidopsis</i>	2,3-butanediol	ISR	Ryu et al. (2004)
<i>Pseudomonas fluorescens</i> strains				
CHA0	Tobacco	Siderophore	SAR	Maurhofer et al. (1994)
	<i>Arabidopsis</i>	Antibiotics (DAPG)	ISR	Iavicoli et al. (2003)
WCS374	Radish	Lipopolysaccharide	ISR	Leeman et al. (1995)
		Siderophore		Leeman et al. (1995)
		Iron regulated factor		Leeman et al. (1995)
WCS417	Carnation	Lipopolysaccharide	ISR	Van Peer and Schipper (1992)
	Radish	Lipopolysaccharide	ISR	Leeman et al. (1995)
		Iron regulated factor		Leeman et al. (1995)
	<i>Arabidopsis</i>	Lipopolysaccharide	ISR	Van Wees et al. (1997)
	Tomato	Lipopolysaccharide	ISR	Duijff et al. (1997)
<i>Pseudomonas putida</i> strains				
WCS 358	<i>Arabidopsis</i>	Lipopolysaccharide	ISR	Meziane et al. (2005)
	<i>Arabidopsis</i>	Siderophore	ISR	Meziane et al. (2005)
BTP1	Bean	Z,3-hexenal	ISR	Ongena et al. (2004)
<i>Serratia marcescens</i> 90-166	Cucumber	Siderophore	ISR	Press et al. (2001)



Root pathogens

- Aphanomyces
- Pythium
- Phytophthora
- Spongospora
- Olpidium
- Gaumannomyces
- Bipolaris
- Sclerotinia
- Fusarium
- Rhizoctonia





Biocontrol of root diseases

- Organic matter
 - Compost, green manure, etc
- Biofumigation
 - Plants with allelopathic effects
- Microbial BCAs
- Pathogen grazers

Fungal grazers - an example of agroecological root disease control



Foto: John Larsen

Fungi from different niches as Collembola food items



Root pathogenic fungi

- *Fusarium culmorum*
- *Rhizoctonia solani*

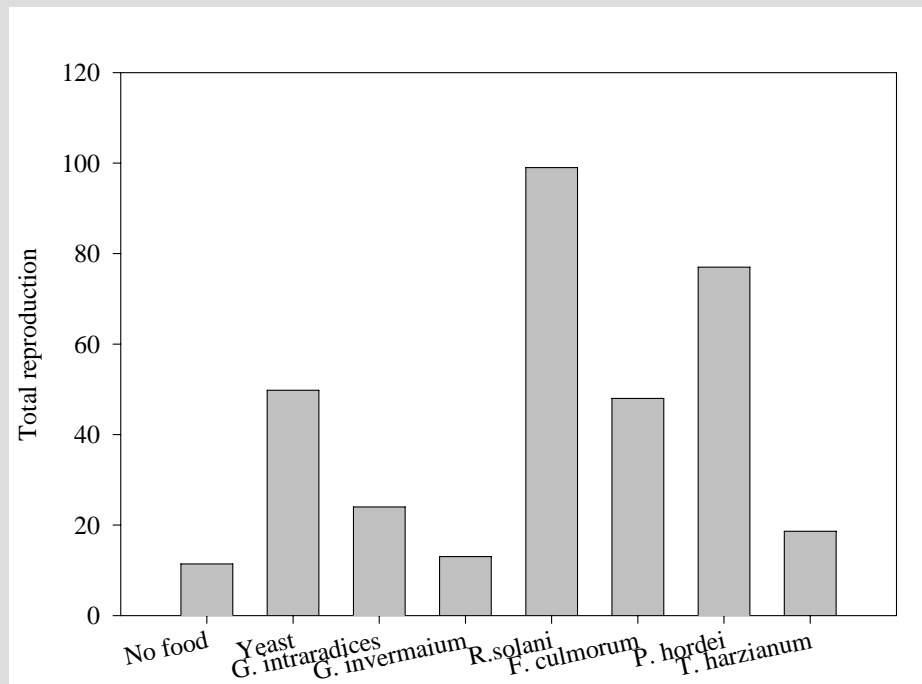
Saprotrophic fungi

- *Pencillium hordei*
- *Trichoderma harzianum*

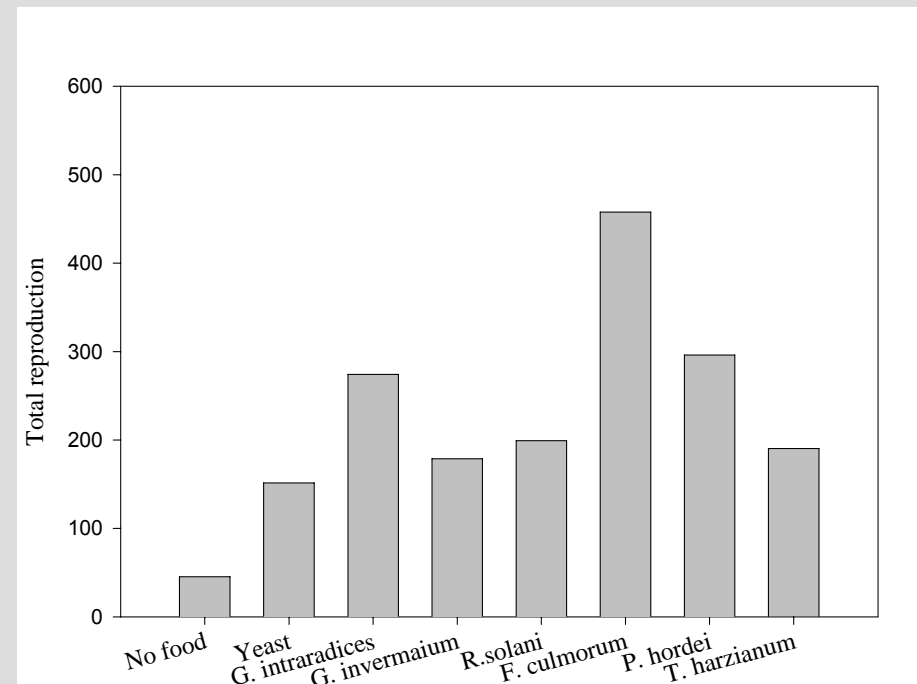
Mycorrhizal fungi

- *Glomus intraradices*
- *Glomus invermaium*

Collembola reproduction on different fungal food items



Folsomia fimetaria



Folsomia candida

Examples of foliar pathogens



- Phytophthora (Potato late blight)
- Peronospora (Onion downey mildew)
- Bremia (Lettuce downey mildew)
- Botrytis (Grey mold)
- Puccinia (Rust)
- Erysiphe (Mildew)





Biocontrol of foliar diseases

- Induction of plant defense
- BCAs applied to the foliage
- Botanicals (plant extracts)

Effects of *Ulocladium atrum* against grey mold in pot roses – an example of biological control of a foliar pathogen

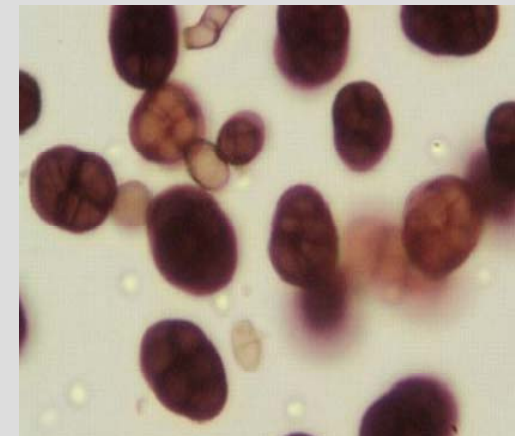
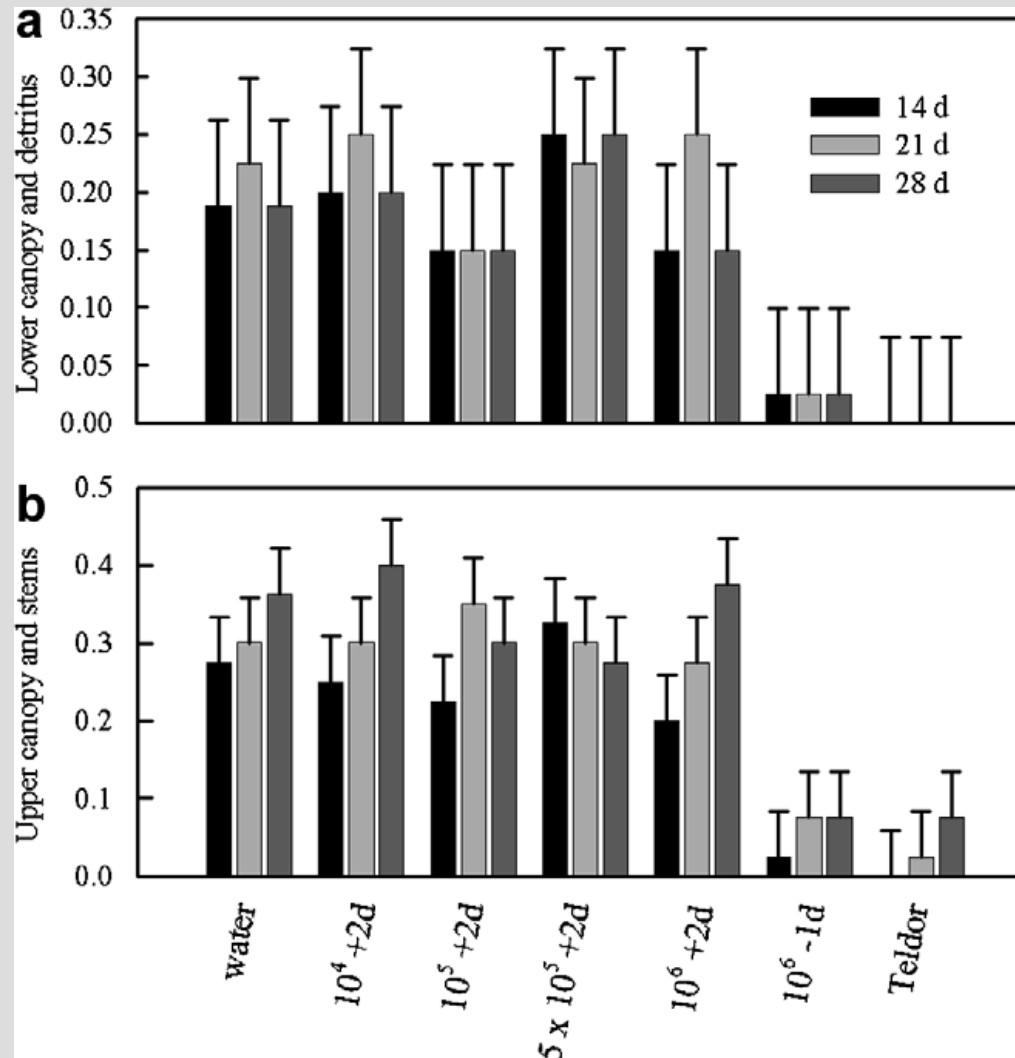


without *U. atrum*



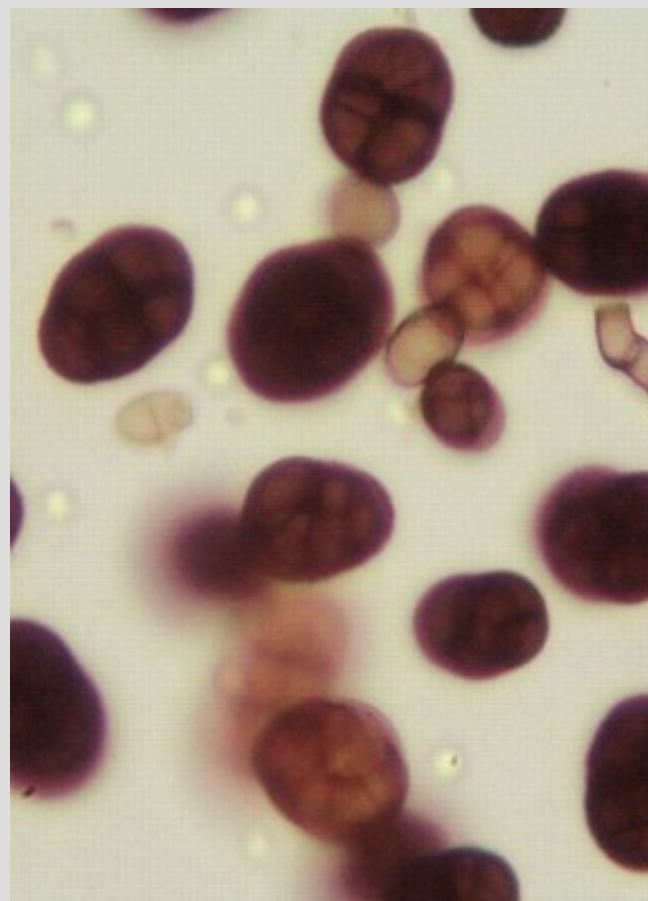
with *U. atrum*

Control of grey mould in pot roses by *Ulocladium atrum*

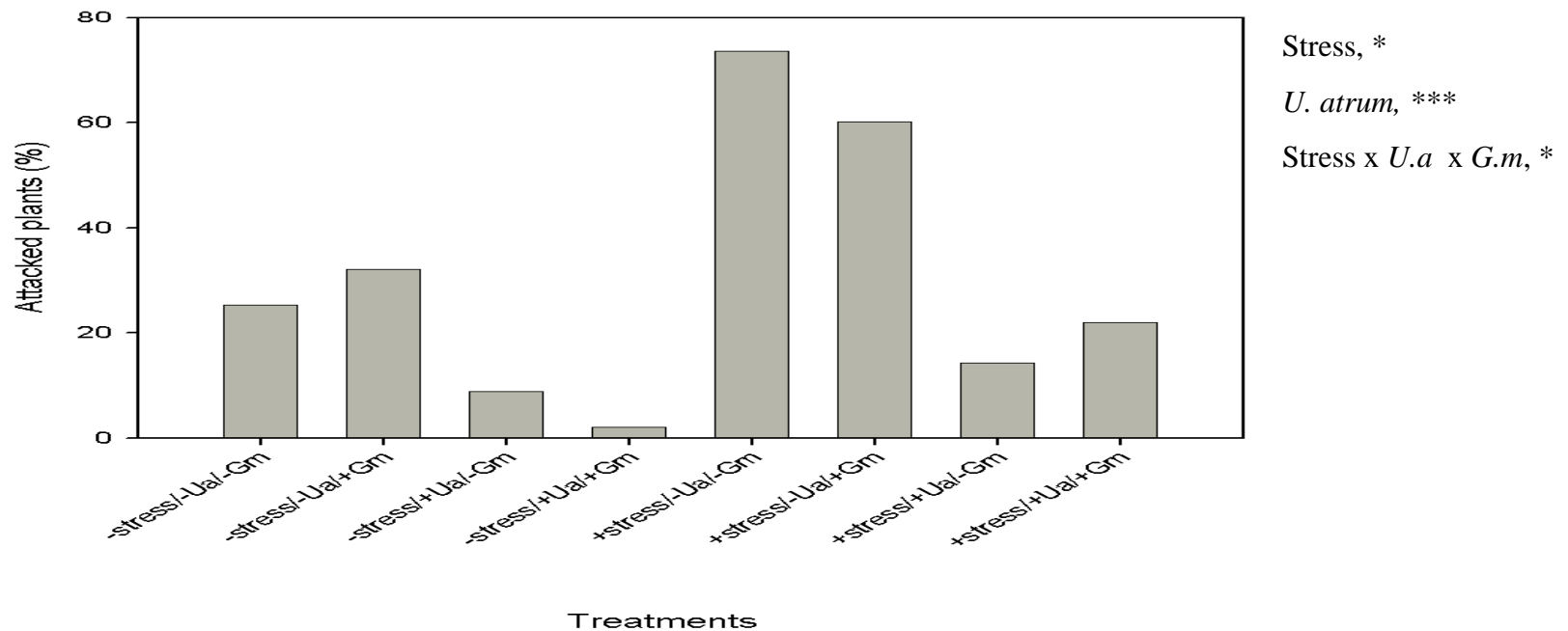




Biocontrol of grey mold by a combination of
inoculation with the arbuscular mycorrhizal
fungus *Glomus mosseae* and the BCA
Ulocladium atrum



Lowest grey mold frequency in plants inoculated with a combination of the AM fungus *G. mosseae* and the BCA *U. atrum*





Biological management of plant diseases – a summary

- There are several modes of actions in biological management of plant diseases, and this is important be aware of when developing agroecological IPM strategies
- Strategies to manage root- and foliar pathogens can be different
- Combination of more biological strategies to control a disease can be an advantage

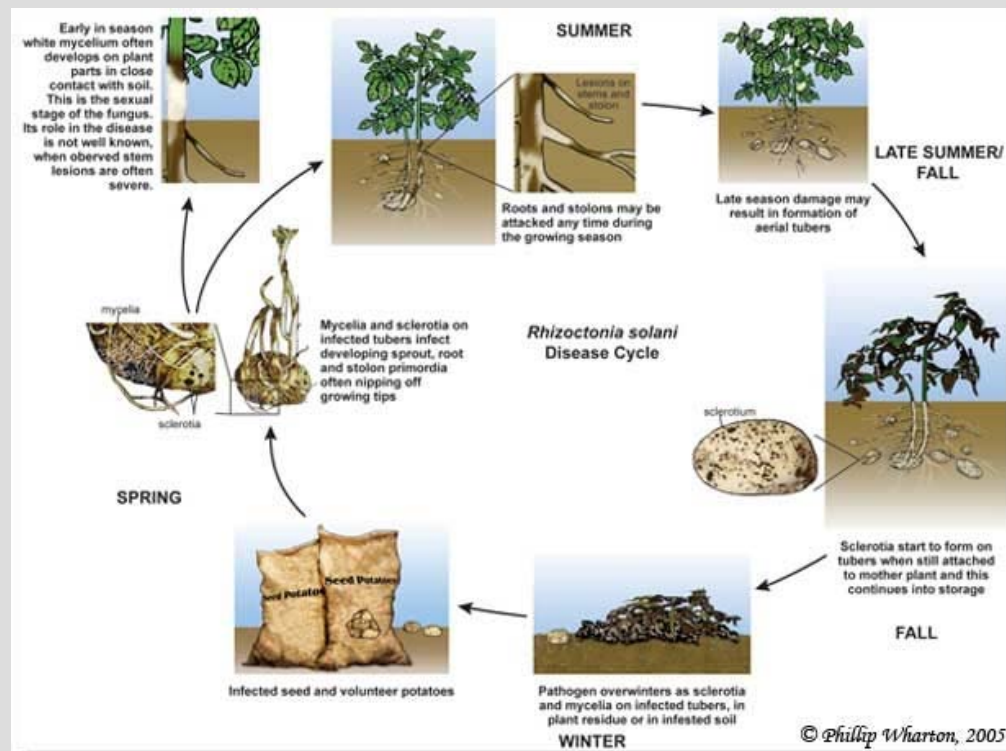
Management of potato stem canker – an attempt to develop an IPM strategy



Potato stem canker



- Caused by *Rhizoctonia solani* AG3
- Major problem in potato production
- Soil or tuber borne
- Patchy occurrence



Field experiments-objective



- To study of the effect of green manure crops, mechanical soil treatments, biological and chemical seed coating on potato stem canker caused by *Rhizoctonia solani* AG3



Experimental field site



- 96 plots 8x4.5 m
- Inoculated with vermiculite based *R. solani* inoculum August 2007
- Pre-crops grown from August 2007 and ploughed into the soil April 2008
- Sowing of seed tubers April 2008
- Potato cultivar: Agata
- Experiment repeated 2008/2009 and 2009/2010



Experimental design



Soil treatments

- Ploughing (30 cm)
- Reduced soil treatments (harrow 10 cm)



Green manure crop

- None
- White Mustard
- Oat



Seed coating

- None
- Rizolex
- Floragro based on *Bacillus* sp.
- Supresivit based on *Trichoderma harzianum*



Analyses



- Emergence of potato plants
- Detection of potato stem canker
- Yield
- Quality of potato (based on size)
- *R. solani* infection in tubers
- Nematodes in soil from selected plot
- *R. solani* in soil



Results/average incidence of disease

- **2008; low infection level**
 - Disease index plants 0,27, Disease index tubers 0,45
- **2009; high infection level**
 - Disease index plants 2,40, disease index tubers 4,41

Note inoculum potential same both years, but different weather conditions



Effects of the single tactics on disease incidence in plants



2008 Low disease incidence

- No effects



2009 High disease incidence

- Ploughing reduced 16 %
- Oat reduced 13%
- Rizolex reduced 11%



Effects of the single factors on yield of potatoes



2008 Low disease incidence

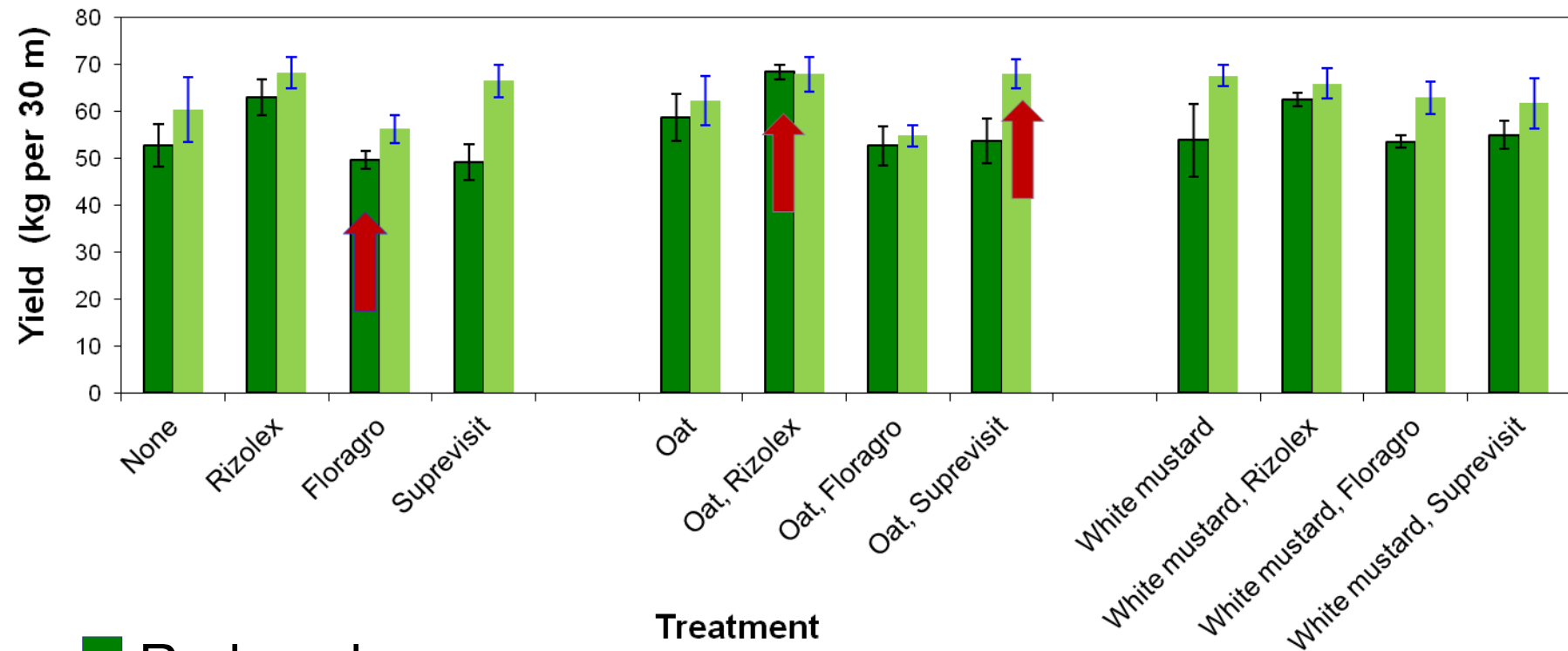
- Reduced soil treatment
12 % higher yield

2009 High disease incidence

- Ploughing 15 % higher yield
- Rizolex 14 % higher yield



Results combined treatments 2009 high disease pressure

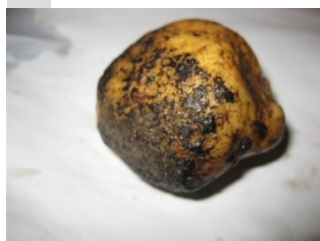
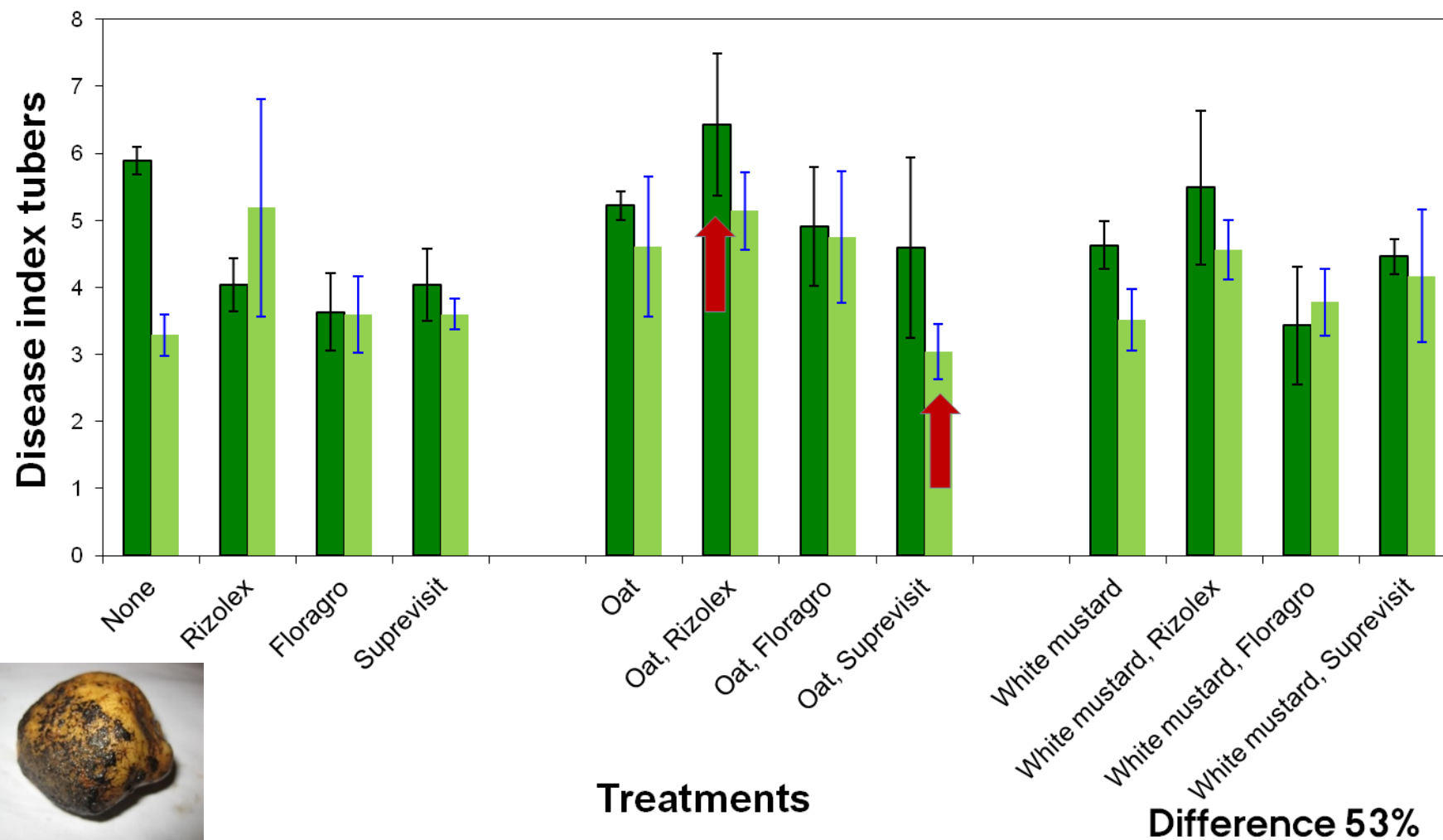


■ Reduced
■ Ploughed



28 % difference

Results combined treatments 2009 high disease pressure



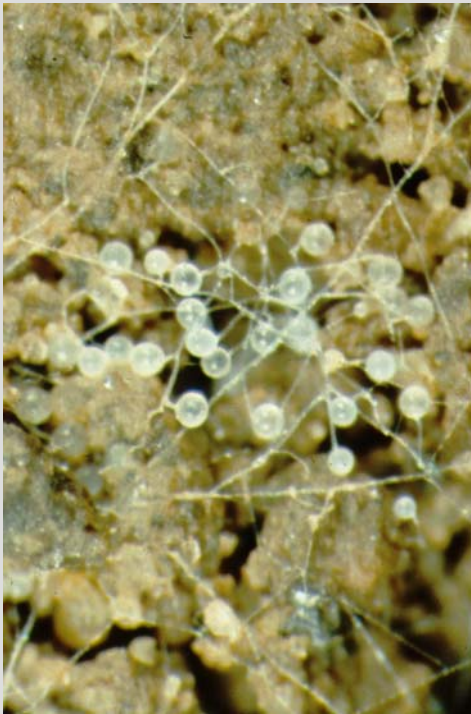
Conclusions



- Green manure crops and seed coating can reduce incidence of stem canker and increase yield, if they are included in the right IPM strategy
- Oat as green manure crop resulted in higher yield and less disease on tubers under high disease pressure
- Soil treatments had significant influence on incidence of disease but the effect depended on the disease pressure
- The most important factor for development of stem canker in soil with high inoculum potential was the weather conditions

The experiment was repeated in 2010

Beneficial interactions between plants and soil microbes



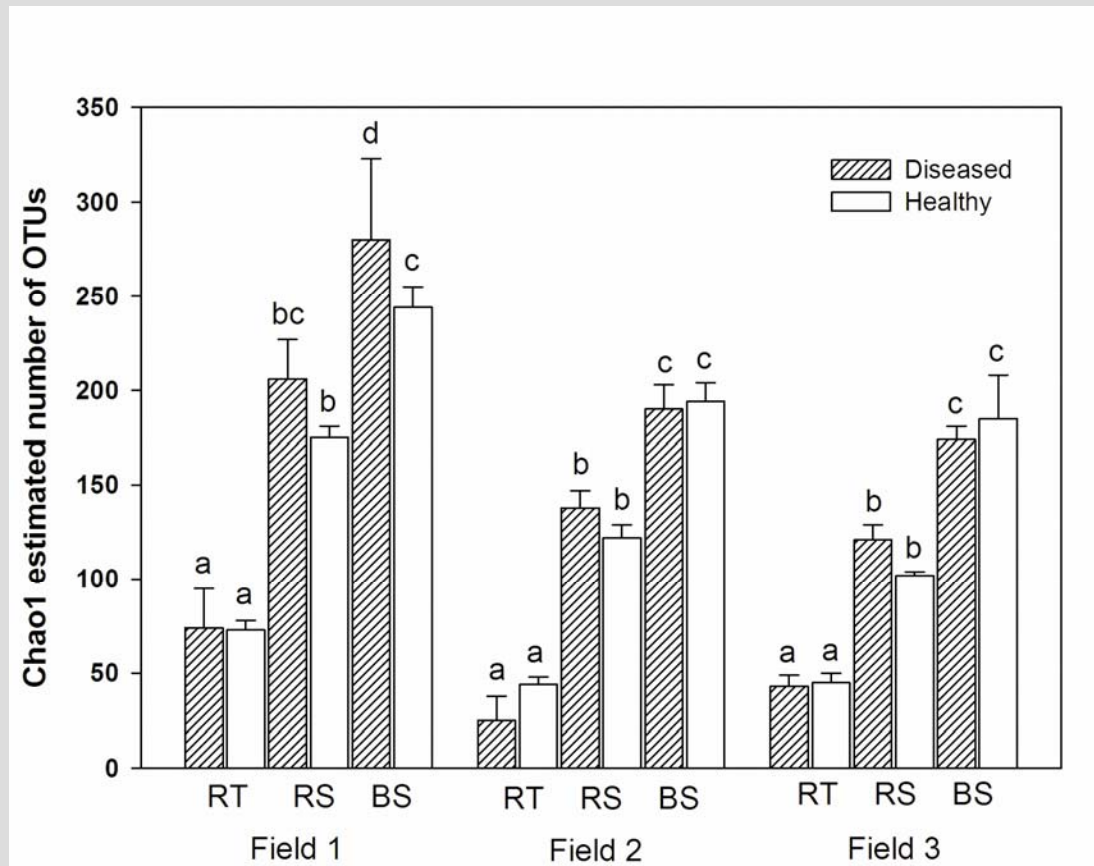


Outline

- Fungi in root and soil environments
- Arbuscular Mycorrhizal (AM) fungi – an example of a plant beneficial microorganism
- Interactions between AM and pathogenic oomycetes
- Conclusions
- (Mechanisms underlying increased AM plant tolerance against pathogens)



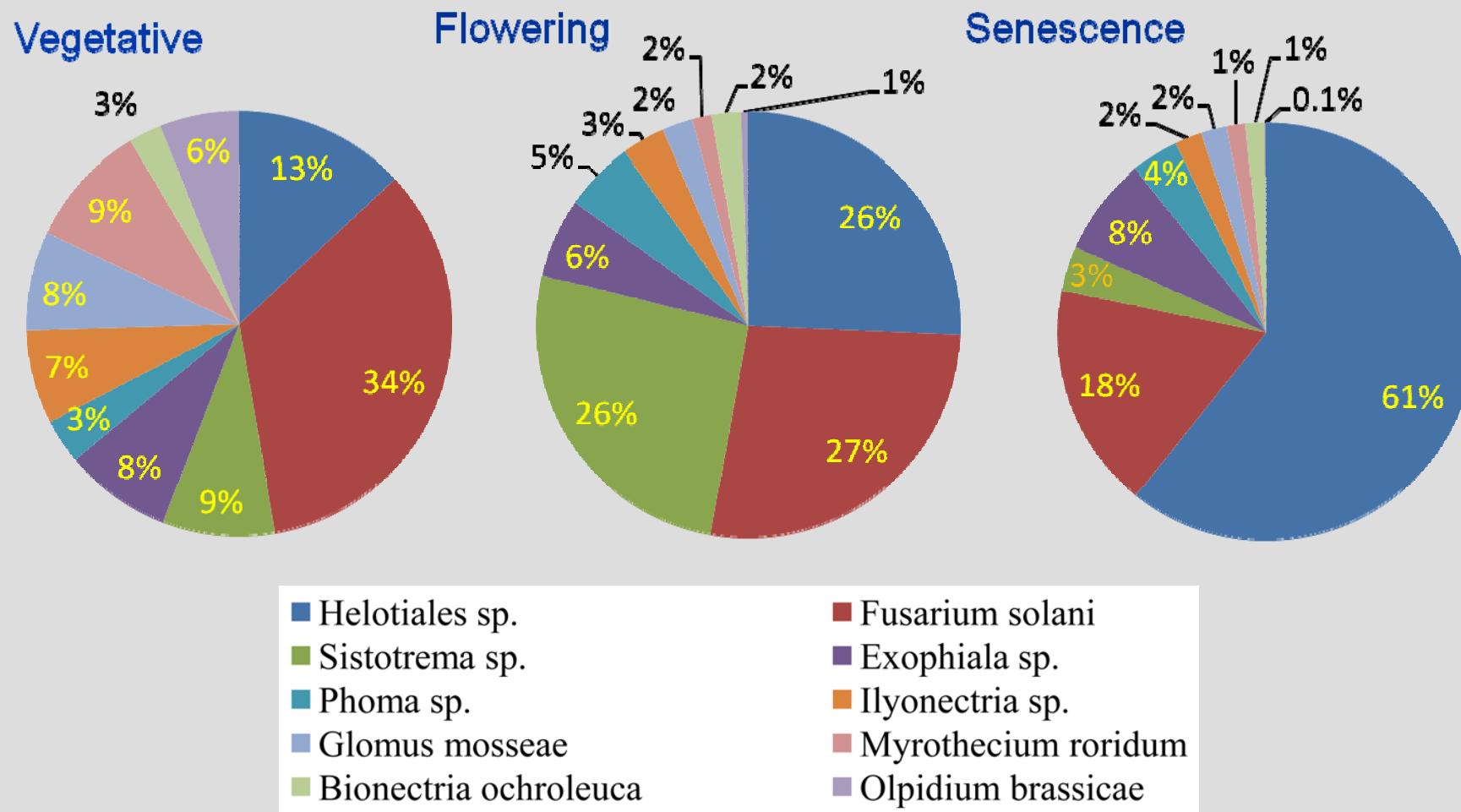
Fungal richness in soil and roots



Xu *et al.* 2012, FEMS Microbiology Ecology DOI:10.1111/j.1574-6941.2012.01445



Succession pattern of fungi in pea roots (121 OTUs)



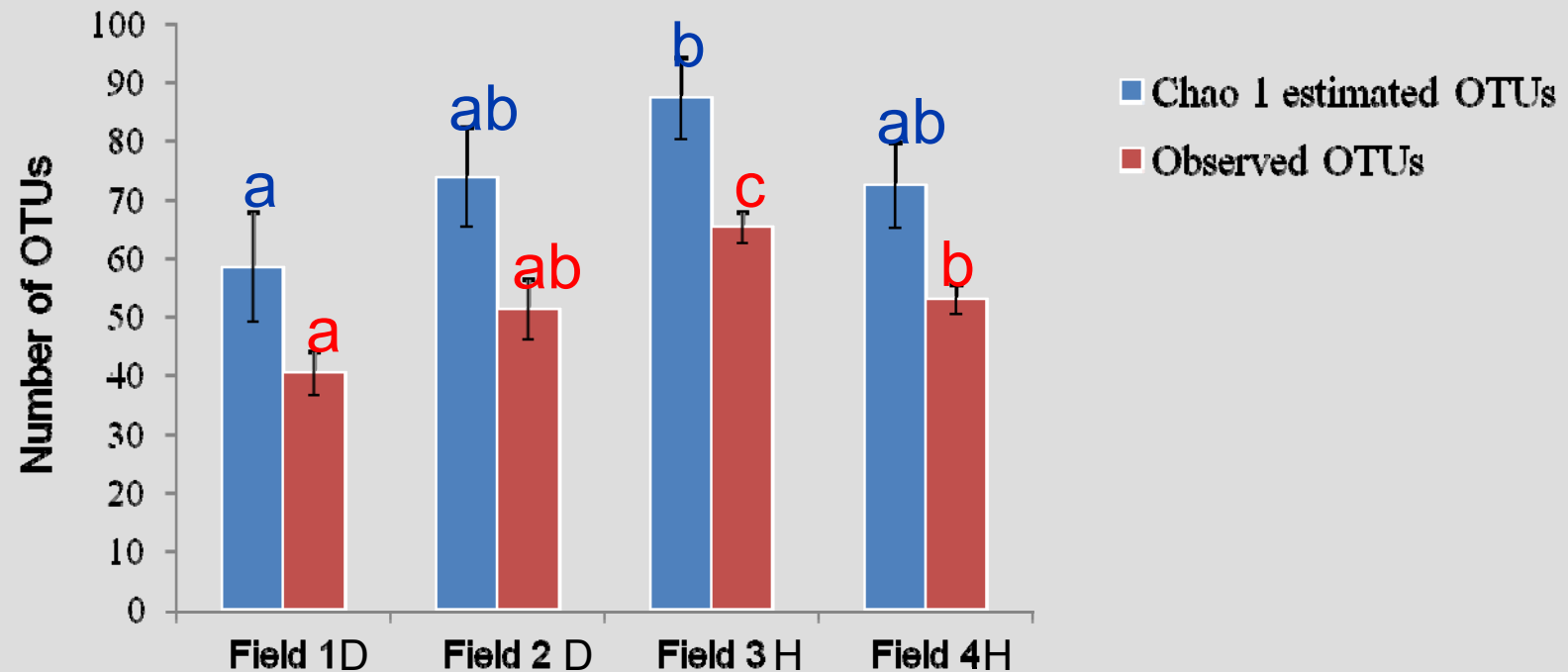


The 20 most abundant of 165 OTUs in pea roots grown with different levels of organic fertilizer

OTU No.	Best hit in GenBank	No OF	1OF	2OF	3OF	ANOVA <i>P</i> -value
Relative abundance of sequences (%)						
1	<i>Olpidium brassicae</i>	0.97 a	15.4 b	32.1 c	51.3 c	*** #
2	<i>Fusarium oxysporum</i>	29.5 a	31.9 a	24.7 a	11.2 b	**
3	<i>Archaeospora trappei</i>	13.4 a	10.3 ab	6.9 ab	5.6 b	NS
4	<i>Exophiala</i> sp.	13.9 a	7.4 b	9.2 ab	5.8 b	* #
5	Uncultured fungus	16.6 a	9.9 ab	4.9 b	1.1 b	* #
6	<i>Paraglomus</i> sp.	4.3 ab	5.6 a	3.8 ab	1.9 b	*
7	<i>Glomus mosseae</i>	0.6 a	2.7 b	3.6 b	5.6 b	** #
8	<i>G. caledonium</i>	0.5 a	1.6 b	2.7 b	7.1 c	*** #
9	<i>Trichocladium asperum</i>	3.1 a	2.3 a	2.0 a	1.0 a	NS #
10	<i>F. solani</i>	1.6 a	0.6 a	0.8 a	2.3 a	NS #
11	<i>Eucasphaeria capensis</i>	1.9 a	1.2 a	1.1 a	0.3 b	NS #
12	<i>Xylariales</i> sp.	2.2 a	0.5 b	0.3 c	0.2 c	*** #
13	<i>Plectosphaerella cucumerina</i>	0.4 a	0.7 a	0.7 a	0.6 a	NS #
14	<i>Hypocreales</i> sp.	0.6 a	0.6 a	0.5 a	0.5 a	NS
15	<i>Arthrobotrys</i> sp.	0.8 a	0.3 b	0.3 b	0.5 ab	NS
19	<i>Phoma eupyrena</i>	0.15 ab	0.53 c	0.52 bc	0.08 a	* #
20	<i>Cryptococcus terreus</i>	0.03 a	0.07 a	0.32 a	0.75 b	* #

Yu *et al.* 2012, Soil Biology and Biochemistry, accepted with revision

Richness of root-associated fungi in healthy and diseased roots



Yu *et al.* 2012, Plant and Soil 357:395-405



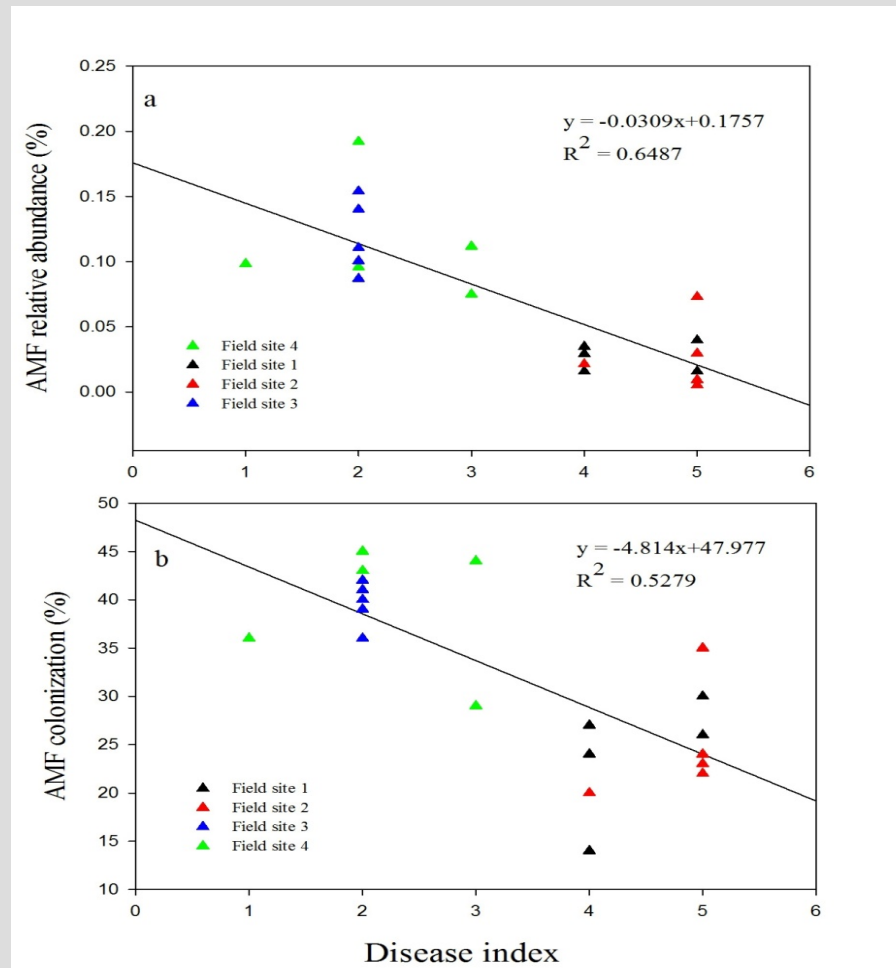
The 15 most abundant of 142 fungal Operational Taxonomic Units (OTUs) in pea roots

OTU No.	Best hit in GenBank	Pea field No.				ANOVA
		1 (D)	2 (D)	3 (H)	4 (H)	<i>P</i> value
		Relative abundance of sequences (%)				
1	<i>Fusarium</i> sp.	20.8 a	31.3 a	20.5 a	24.7 a	NS
2	<i>Olpidium brassicae</i>	25.7 a	5.6 c	14.5 b	10.8 bc	**
3	<i>Tetracladium maxilliforme</i>	9.7 ab	22.4 a	2.3 c	3.3 bc	*
4	<i>Stachybotrys chartarum</i>	0.4 a	1.4 b	18.4 c	10.1 c	***
5	<i>Glomus caledonium</i>	2.6 a	1.9 a	10.8 b	11.1 b	***
6	<i>Nectria haematococca</i>	7.2 a	7.0 ab	2.1 c	6.7 ab	NS
7	<i>Phoma sojicola</i>	7.1 a	8.0 a	1.9 b	0.7 b	***
8	Uncultured basidiomycete	13.1 a	0.2 b	0.02 b	0.0	*
9	<i>Exophiala salmonis</i>	4.2 a	2.9 ab	0.9 b	5.0 a	*
10	<i>Plectospharella cucumerina</i>	1.5 a	4.2 a	1.7 a	4.8 a	NS
11	<i>Sistotrema</i> sp.	0.8	0.5	8.3	0.1	-
12	<i>Myrothecium</i> sp.	0.1 a	0.5 b	5.3 c	1.3 b	***
13	<i>Chaetomium globosum</i>	0.2	1.8	0.5	2.2	-
14	<i>Microdochium bolleyi</i>	0.5 a	2.3 b	0.6 a	0.4 a	**
15	Uncultured fungus	2.3 a	0.3 a	0.2 a	0.4 a	NS

Yu et al. 2012, Plant and Soil 357:395-405



Abundance of AM fungi in *Pisum sativum* roots correlates with root health status



26 different
OTUs of AM
fungi in these
roots



Fungal pea root health indicators as calculated using Indicator Species Analysis (ISA)

OTU ID	Indicator value
<i>Glomus mosseae</i>	97.9
<i>G. caledonium</i>	90.3
<i>Mortierella elongata</i>	84.4
No ID	79.5
<i>Exophiala salmonis</i>	75.5
<i>Cladosporium cucumerinum</i>	72.4
<i>G. versiforme</i>	64.7
<i>G. mosseae</i>	64.5
<i>M. elongata</i>	50.1

- 123 OTU's were identified in these roots

- These nine showed significant health indicator values ($P \leq 0.005$) (Monte Carlo permutation test)



Fungi in root and soil environments - summary

- High diversity of fungi in roots and soil
- Fungal composition in roots is influenced by
 - Plant health
 - Plant growth stage
 - Root external conditions such as organic fertilizer
- Next generation sequencing may help to identify microbial plant health indicators

Plant beneficial microorganisms may 
increase plant -

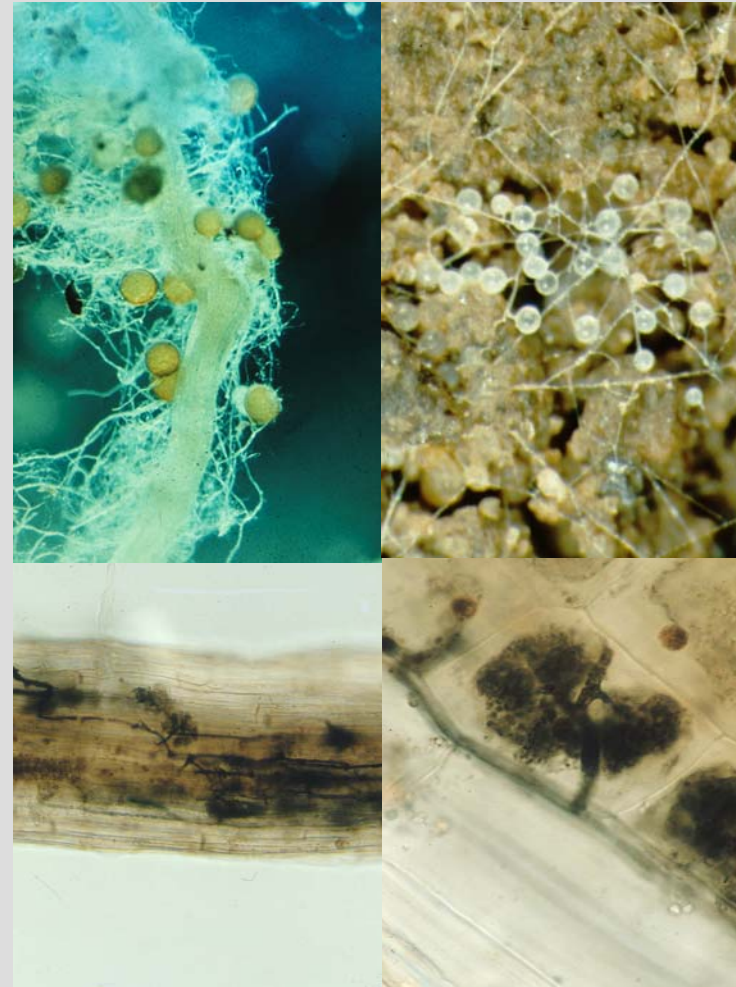
- nutrient uptake
- growth
- tolerance against abiotic stress
- tolerance against biotic stress

Some microbes cover part of these capabilities, Arbuscular mycorrhizal (AM) fungi cover all of them

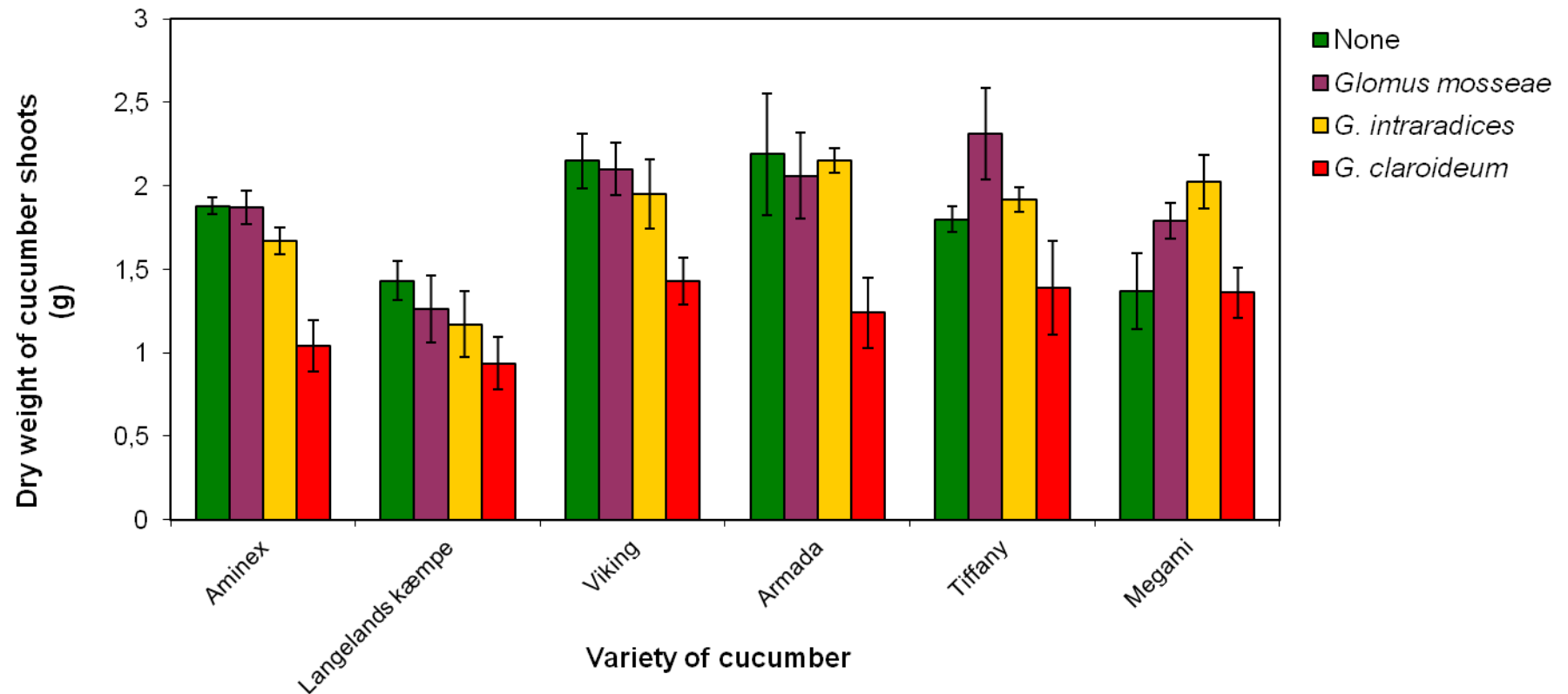
Arbuscular mycorrhizal fungi



- Obligate biotrophic
- Forms symbioses with 80-90 % of plants
- Important for plant nutrition
- Increase plant stress tolerance
- Affects rhizosphere microbial communities
- Form a mycorrhizosphere
- Considered as an ecosystem service

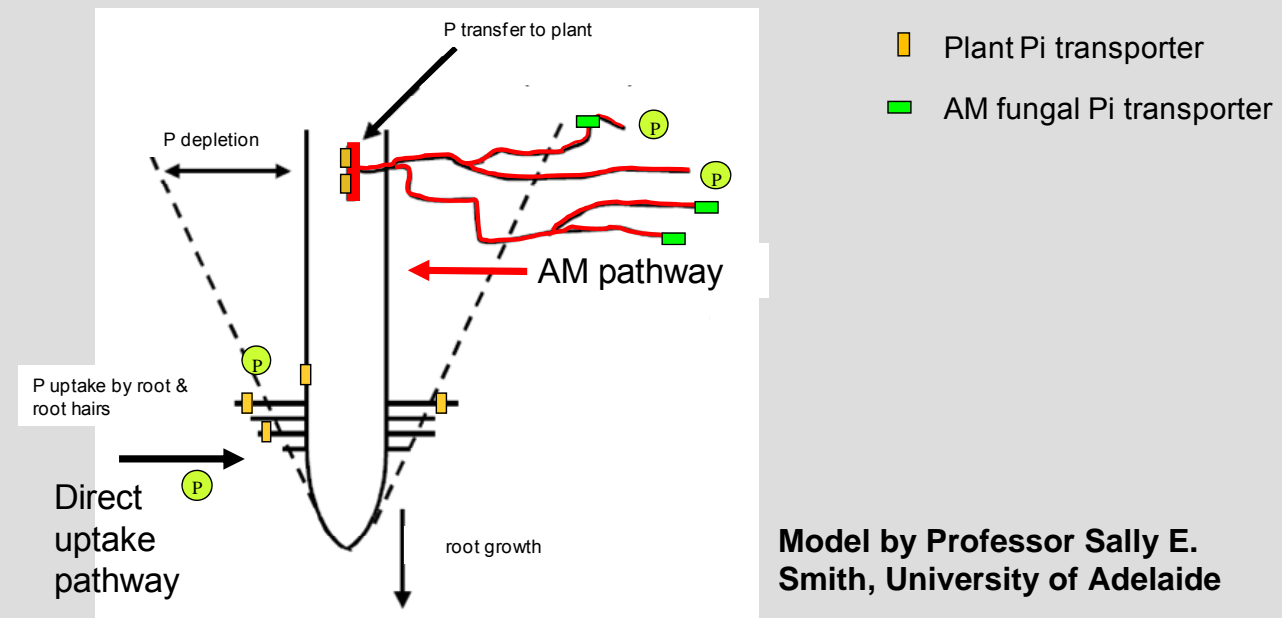


The role of AMF in plant growth



Greenhouse example – under field condition these plants will form symbiosis the more AM fungi at the same time!

The role of AMF in plant nutrition



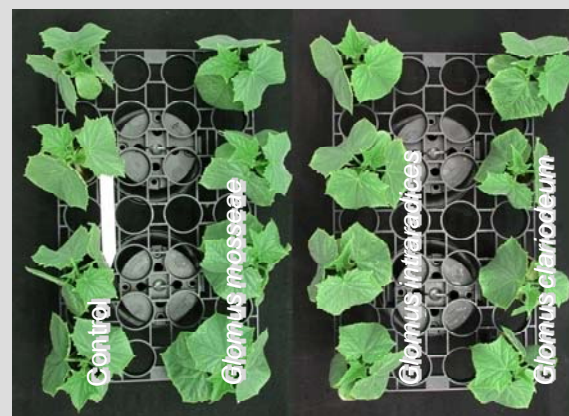
Nutrient concentrations in shoots of six varieties of cucumber inoculated with AM fungi

P values of a two-way analysis of variance * $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$										
	Tot. N	P	K	Mg	Ca	Na	Fe	Zn	Mn	Cu
Cucumber ssp. (V)	***	***	***	*	***	***	0.21	***	***	***
AM fungal species(F)	0.11	***	***	***	***	0.09	**	***	***	*
Interaction VxF	*	*	*	0.36	**	***	0.14	0.41	0.15	*



The role of AMF in alleviation of biotic stress

AM fungus	<i>Pythium ultimum</i>	Dry weight of 28-day old shoots (g)
None	-	1.63 d
	+	1,15 a
<i>Glomus mosseae</i>	-	1.64 d
	+	1.56 cd
<i>G. intraradices</i>	-	1.44 bc
	+	1.31 b
<i>G. claroideum</i>	-	1.35 b
	+	1.17 a

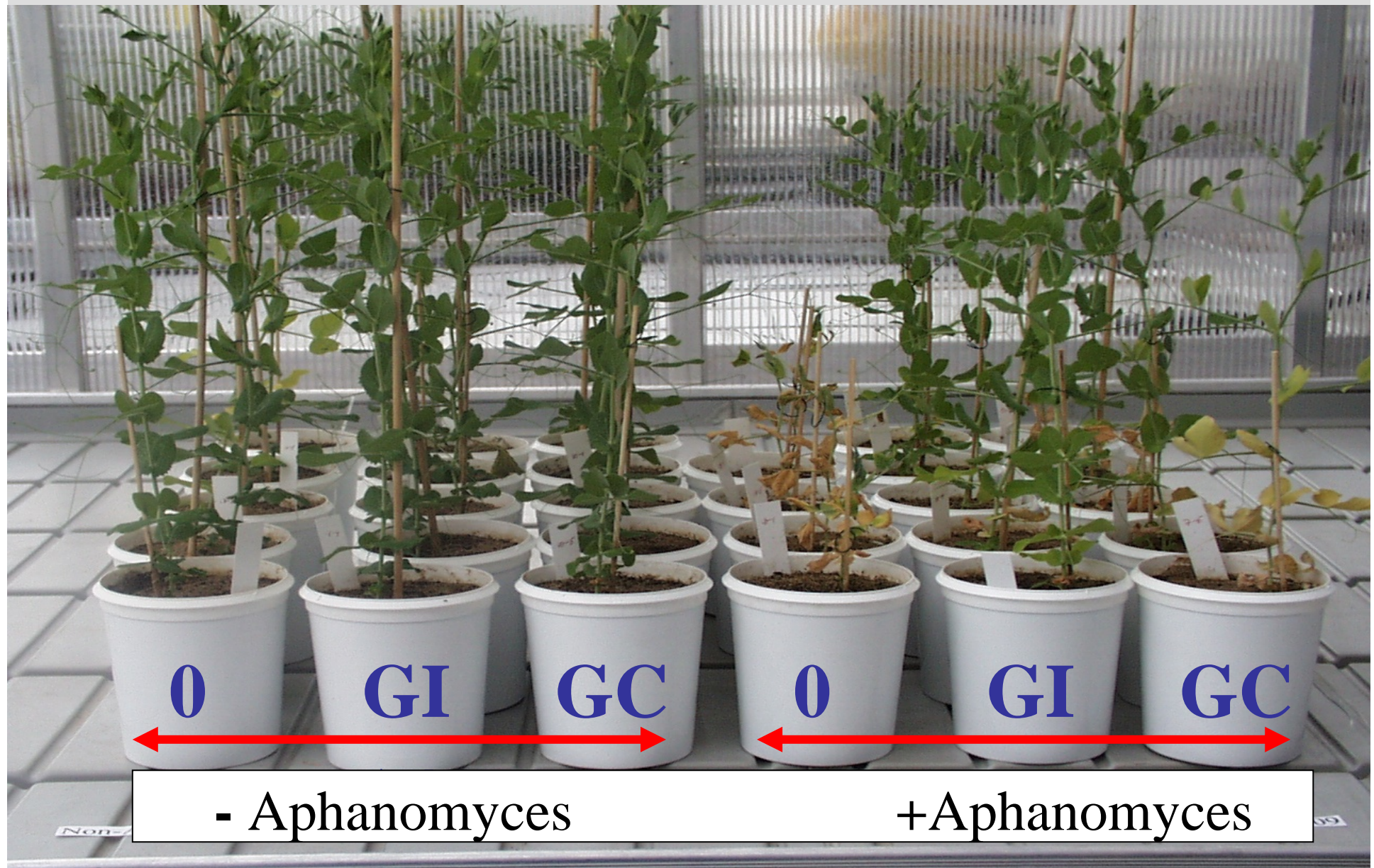


AM fungi as an example of a multifunctional plant beneficial microorganism - summary



- **AM fungi**
 - Influence growth of plants
 - Influence uptake of nutrients
 - Increase plant stress tolerance
 - Indicate plant health?

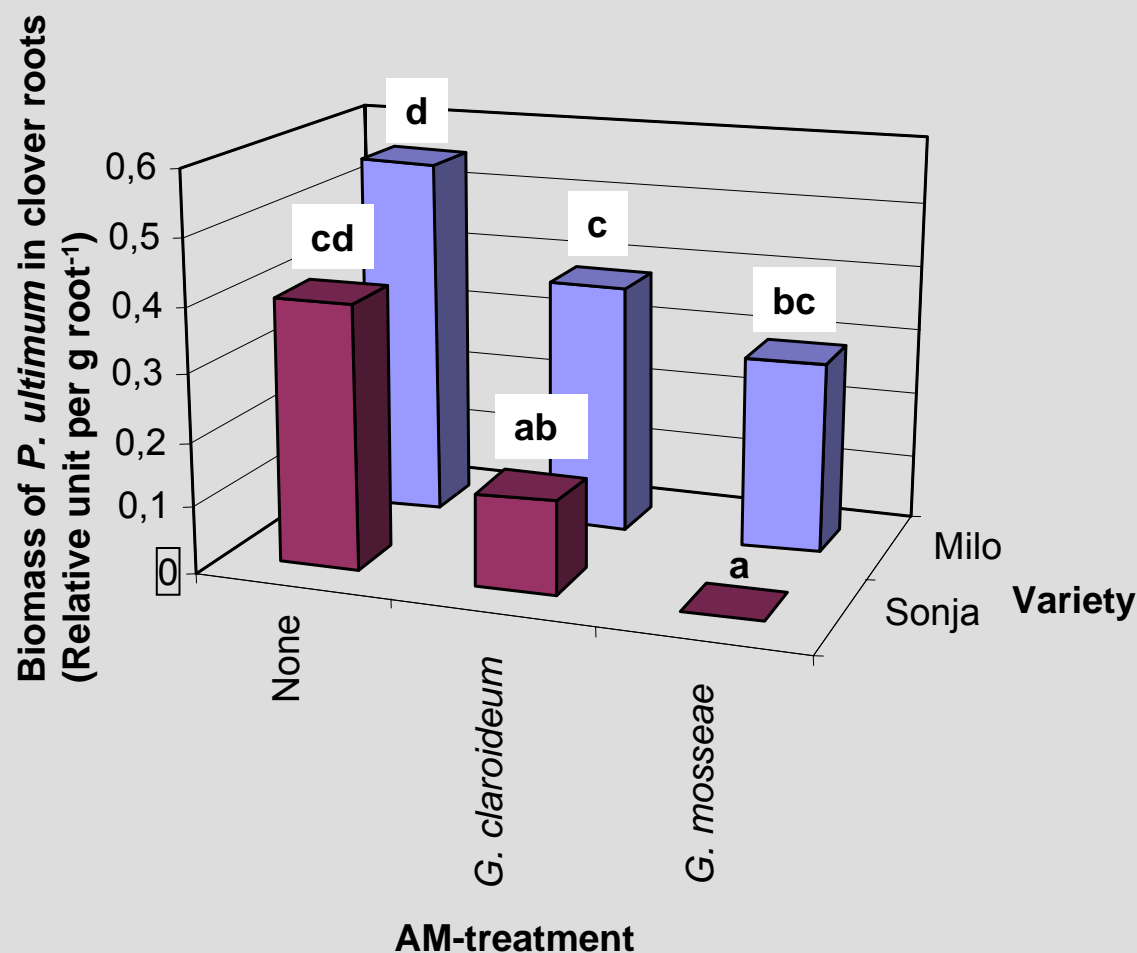
Interactions between AM and pathogenic oomycetes



Thygesen et al., 2004. European Journal of Plant Pathology 110: 411-419

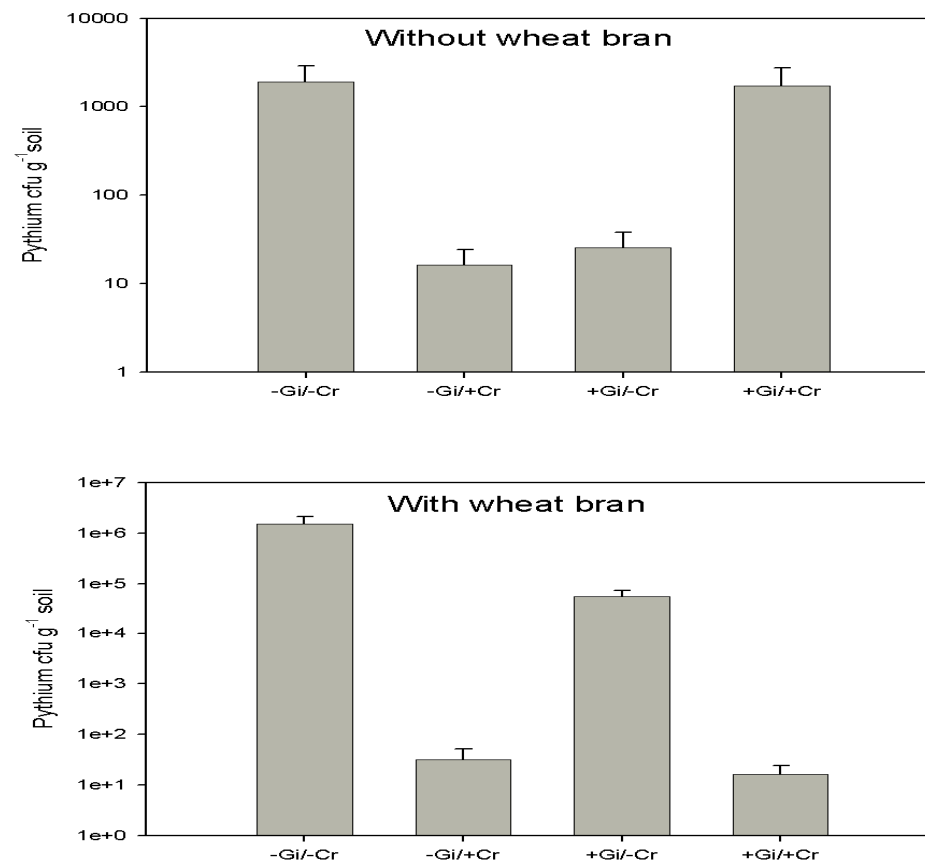


AM fungal effect on *Pythium ultimum* in white clover roots



- *Glomus mosseae* colonised 49 % of the roots
- *G. claroideum* colonised 75 % of the roots

Effects of AM fungi and *Clonostachys rosea* on *Pythium* in soil



Conclusions



- Plant beneficial microorganisms as AM fungi play a key role in growth, nutrient uptake and health of plants
- The environment influence the composition of AM fungi in soil and function of AM in plant health
- More knowledge on the agroecology of these microorganisms will enhance the exploitation of this ecosystem service for plant production
- Most plants do not have roots, they have mycorrhiza!!!